2022 Belgrade

FEMS Conference on Microbiology

in association with
Serbian Society of Microbiology

30 June - 2 July

2022 • Serbia

ELECTRONIC ABSTRACT BOOK

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Message from the organizers

Dear colleagues and friends,

The 1st FEMS Belgrade Conference on Microbiology in collaboration with Serbian Society for Microbiology was held from 30 June to 2 July 2022.

A large number of high-quality scientific contributions was presented at the Conference. We are delighted to have been able to put them together and send you the FEMS Conference Abstract Book. With thanks to your contributions, we can now proudly present an abstract book that both reflects the scientific abundance of the conference and serves as a memento of an event worth remembering. We thank all participants and in particular the presenters of these abstracts for making this happen!

This conference was a pioneering endeavour, one of the largest and most important microbiology events in East Europe in 2022. As in 2020, when we had to pursue the first conference online due to the COVID-19 pandemic, this conference faced challenging times but could luckily be held both onsite and online.

Again, in 2022, we were faced with the great challenges as it was the case back in 2020, and yet again, a brave decision to move ahead has been made and it paid off.

You showed large interest to become part of the Conference and our joint history. Almost 1.000 scientific contributions were submitted, and more than 870 were approved. This showcases not only the large interest to be part of the conference, but also it is the reason this event was such a success.

We are thankful and proud to have welcomed almost 600 microbiologists from 40 European countries and another 20 countries worldwide, almost 200 more participants online. With ten core scientific sessions, including one session with the best grant alumni presentations, three plenary lecture and a COVID-19 round table, six industry lectures and a satellite symposium, the total of invited lectures amounted to 60. In addition, six thematic sessions with over 120 short oral/e-poster presentations of selected participants-authors in the main program Finally, over 400 e-posters/presentations on demand, in total over 600 presentational items, uploaded on the Conference ONLINE platform and accessible to participants until the 31 December 2022.

We thank the pharmaceutical, lab and biomedical industry partners from Serbia, the South East Europe region and worldwide for their recognition of the importance of the event, their participation and their support.

We hope that you enjoyed the content and all the other aspects of the Conference. If you missed anything, you can catch up by watching the recordings, presentations or have a detailed look at the posters.

We warmly wish you health, love and happiness and are looking forward to the new encounters, coming up next: FEMS 2023 Congress in Hamburg, FEMS 2024 Conference in Tallinn and numerous events of the SSM in Serbia and South East Europe region.

Sincerely

Prof. Hilary Lappin-Scott
Scientific Committee Chairperson,
FEMS President

Prof. Vaso Taleski
Organizing Committee Chairperson,
FEMS Director of Events and Internationalization

Prof. Dragojlo Obradović
Scientific Committee Co-Chairperson,
President of Serbian Society of Microbiology

Prof. Lazar Ranin
Organizing Committee Co-Chairperson,
Vice-President of Serbian Society of Microbiology

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Scientific Committee Chairperson, FEMS President

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Alexander Netrusov / *Russian Federation*

THURSDAY 30th June 2022

08:00-10:00 REGISTRATION

10:00 - 12:00

SESSION 1
Emerging Infections (fungal, bacterial, parasitic)
Chairpersons:
Ana Kaftandzieva / *North Macedonia*
Alexandra Nascutiu / *Romania*

Maja Stanojević / *Serbia*
Emerging arboviral infections in the Balkans

Tomasz Jagielski / *Poland*
Molecular epidemiology and drug resistance of tuberculosis in the Eastern border of the EU: a Polish-Lithuanian study

Andrea Radalj / *Serbia*
Detection and characterization of viruses circulating in wildlife populations in the Republic of Serbia

Alexandra Nascutiu / *Romania*
Emerging diseases – deeper understanding for better anticipation

Ana Kaftandijeva / *North Macedonia*
Occurrence of Carbapenem-resistant Enterobacteriaceae - a three-year study

SESSION 2
Food Microbiology
Chairpersons: **Peter Raspor** / *Slovenia*
Dragoslava Radin / *Serbia*

Peter Raspor / *Slovenia*
Education and training in food microbiology: What to do next?

Sonja Smole Mozina / *Slovenia*
Biocontrol of Campylobacter jejuni - cell-to-cell signalling inhibition and inter-species interaction as options

George Nychas / *Greece*
PLSR and SVM Applied on Spectroscopic Data for the Rapid Assessment of Microbiological Quality in Chicken Fillets

Evelyne Selberherr / *Austria*
Microbial populations transmission routes throughout meat processing

12:00-12:30 **INDUSTRY SESSION – Pfizer**
Snežana Jovanović : Sensitivity and resistance of Gram negative pathogens, the view from green and red corner .

12:30-13:00 **INDUSTRY SESSION – Biomerieux**
Daniela Talapan :Syndromic testing for the appropriate therapy decision: Team Experience at National Institute of Infectious Diseases „Prof. Dr. Matei Bals".

13:00-13:15 **INDUSTRY SESSION – Symcel**
Marta Veces Garcia :
A novel way to measure microbial activity using heat production

13:15-14:45 Lunch Break / Poster Viewings / Exhibition

14:45 - 16:45

SESSION 3

Antimicrobial Resistance - Challenges and Perspectives

Chairpersons:
Dejan Baskić / *Serbia*
Nijaz Tihic / *Bosnia and Herzegovina*

Francesca Saluzzo / *Italy*
New trends in antibiotic resistance

Edit Szekely / *Romania*
A decade with carbapenemase-producing Enterobacterales - experiences from Romanian hospitals

Svetlana Ugarcina Perovic / *China*
Challenges in metagenomic annotation of antibiotic resistome

Laura Brülisauer / *Switzerland*
Clinical Antibiotic Resistance Plasmids Affect Biofilm Formation Depending on Bacterial Host Genetic Background

Ina Gajic / *Serbia*
Molecular epidemiology of carbapen-em-resistant *Acinetobacter baumannii*

SESSION 4

Plant Microbiology

Chairpersons:
Ivica Dimkić / *Serbia*
Konstantinos Kormas / *Greece*

Kalliope Papadopoulou / *Greece*
RNA-mediated signalling of a fungal endophyte upon colonization of its host plant

Ivica Dimkić / *Serbia*
Beneficial Microorganisms in Agriculture - New generation of biological control agents and biofertilizers for sustainable agriculture

Aleksa Obradovic / *Serbia*
Challenges in crop use of bacteriophages

Jelena Zindovic / *Montenegro*
Molecular characterization of Citrus tristeza virus in Montenegro

Marie Legein / *Belgium*
Phyllosphere bacterial communities in greenhouses ecosystems and the impact of bumblebees and predatory mites

16:45-17:30 Coffee break

17:30-18:30 **OPENING CEREMONY**

18:30-19:30 PLENARY TALK 1
Surprises in science - lessons from COVID-19 - Ivan Djikic, Croatia/Germany
Chairperson: Vaso Taleski, *North Macedonia*

19:30-22:00 **Welcome Reception**

FRIDAY 1st July 2022

09:00-09:45 — **PLENARY TALK 2 - Programmable RNA antibiotics for health, disease, and ecology**
Jörg Vogel, *Germany*
Chairperson: Hilary Lappin-Scott, *United Kingdom*

09:45-10:15 — **Coffee break**

10:15 - 12:15

SESSION 5
Trends in prevention and control of Emerging and Re-emerging Infections (Vaccines and other Strategies)
Chairpersons: **Daniela Marchetti** / *Italy*
Ivana Dakić / *Serbia*

Matthew Jenner / *UK*
Bioprospecting for new antimicrobials and understanding the chemistry behind their production

Livia Leoni / *Italy*
Novel therapeutic approaches for microbial infections

Igor Mokrousov / *Russian Federation*
Highly drug-resistant and hypervirulent Mycobacterium tuberculosis strains emerging in Russia: phylogenomics and pathobiology

Miriam Latorre-Millán / *Spain*
Variation of vaccine-mediated SARS-CoV2 immunity in a cohort of health care workers

Marina Milenković / *Serbia*
Anti-virulence strategies for combating bacterial infections

SESSION 6
Environmental Microbiology
Chairpersons:
Hilary Lappin-Scott / *UK*
Jaak Truu / *Estonia*

Maja Ravnikar / *Slovenia*
Environmental epidemiology

Jaak Truu / *Estonia*
Application of machine learning methods for microbiome data analysis- current trends and challenges

Shari Wouters / *Belgium*
FMT as a promising therapeutic to remediate radiation-induced intestinal dysbiosis and colonic alterations in a CRC mouse model

Hans-Peter Grossart / *Germany*
Interaction traits of aquatic microbes

Antonio Ventosa / *Spain*
Insights into procaryotic diversity in hypersaline soils

12:15-12:45 — **INDUSTRY SESSION – Pfizer**
Aleksandra Radovanović Spurnić : Outpatients COVID -19 treatment
Nataša Opavski: Pneumococcal serotype distribution in the conjugate vaccine era in Serbia

12:45-13:15 — **INDUSTRY SESSION – East Diagnostics**
Nick Jordan : Setting a new standard in microbial sequencing with highly accurate long reads

13:15-14:15 — **Lunch Break / Poster Viewings / Exhibition**

14:15 - 16:15

SESSION 7
Advanced Microbiology Techniques, Microbiomes and Microbiota
Chairpersons: **Jelena Begović** / *Serbia*, **Özgür Kurt** / *Turkey*

Nathalie Sauvonnet / *France*
Host-microbe interactions

Dejan Vidanović / *Serbia*
A new generation of sequencing (NSG) in clinical microbiology

Ener Cagri Dinleyici / *Turkey*
Human Milk Microbiota Composition and Related Factors

Lesley Hoyles / *UK*
Microbiota-associated metabolites and their interactions with the gut–brain axis

Andriy Sibirny / *Ukraine*
Development of the SARS-CoV-2 vaccine based on the yeast cells with surface displayed RBD fragment of coronavirus spike protein

SESSION 8
Biotechnology and Industrial Microbiology
Chairpersons: **Ken Ichi Yoshida** / *Japan*, **Sinisa Markov** / *Serbia*

Ken Ichi Yoshida / *Japan*
Bacillus subtilis cell factories for production of value-added chemicals

Jan Maarten van Dijl / *Netherlands*
Engineering of Bacillus cell factories for recombit protein production

Evangelos Topakas / *Greece*
Towards the enzymatic breakdown of recalcitrant xylan: the role of GH30 xylobiohydrolases

Pavel Dvořák / *Czech Republic*
Engineering of Pseudomonas putida for fast co-utilization of glucose and cellobiose yields aerobic overproduction of pyruvate

Miomir Nikšić / *Serbia*
Type of cultivation of medicinal mushrooms and their biological potential in production of biologically active compounds

16:15-16:45 Coffee break

16:45 - 18:45

PARALLEL POSTER SESSIONS & SATELLITE SYMPOSIA

SESSION 1
Advanced Microbiology Techniques & Antimicrobial Resistance (AMR)
Chairpersons: Ina Gajic, TBA

SESSION 2
Environmental Microbiology 1
Chairpersons: Ivica Dimkic, TBA

SESSION 3
FEMS-Supported Projects & Miscellaneous
Chairpersons: Bauke Oudega, TBA

16:45 - 17:45

Satellite Symposia 1
Virological aspects of the Serbian HIV cohort

Jovan Ranin / *Serbia* – An overview of the Serbian HIV cohort

Mila Paunić / *Serbia* – Modalities of HIV testing in Serbia

Maja Stanojević / *Serbia* – Impact and implications of HIV drug resistance mutation M184V

Biljana Popovska Jovičić / *Serbia*
Association of HIF-1A gene polymorphisms with metabolic syndrome in person living with HIV on antiretroviral therapy

INDUSTRY SESSION – GSK/ViiV

Maja Stanojević / *Serbia* – The Importance of Dolutegravir High Barrier to Resistance from virological perspective

20:00-00:00 **Conference dinner / restaurant Gardoš**

SATURDAY 2nd July 2022

09:30-10:15 PLENARY TALK 3 - The Evolution and Ecology of Bacterial Warfare – Elisa Granato / *UK*
Chairperson: Lazar Ranin / *Serbia*

10:15-10:45 Coffee break

10:45 - 13:05

SESSION 9
Supported Research Projects and Impact of FEMS Grants on Professional Development and Outreach of ECS
Chairpersons: **Vaso Taleski** /*North Macedonia*
Jozef Anné / *Belgium*

Behnaz Nowrouzi / *UK*
Synthetic and Systems Biology Approaches for Improve Taxol Precursors Production in Saccharomyces cerevisiae

Isidro García-Meniño / *Germany*
Investigation of multidrug-resistance plasmids and their genetic relationship from Spanish K. pneumoniae of UTI and retail meat

Klara Filek / *Croatia*
Multi-domain investigations of microbial communities associated with loggerhead sea turtles

Anastasia Christinaki / *Greece*
Malassezia comparative mitochondrial genomics towards sensitive diagnostics tools

Olha Maslovska / *Ukraine*
Immobilization of cells of photosynthetic bacteria Rhodopseudomonas yavorovii for optimization of biologically valuable compound

Eldin Kurpejovic / *Turkey*
Genome-wide mutation analysis of the L-Tyrosine overproducer Corynebacterium glutamicum ATCC 21573

Zofia Bakuła / *Poland*
Detection of the multidrug-resistant phenotype in Mycobacterium tuberculosis by molecular assays –a Polish-Lithuanian experience

SESSION 10
Miscellaneous
Chairpersons:
Gordana Mijović / *Montenegro*
Ivana Čolovic-Čalovski / *Serbia*

Ivana Čolović-Čalovski / *Serbia*
Ivermectin for human use:
from Nobel Prize to contraverses

Vittorio Venturi / *Italy*
Cell-cell signaling among bacteria in the microbiome

Bojan Adzić / *Montenegro*
Viral diseases in animals

Mikhail Gelfand / *Russian Federation*
Evolution of bacterial genomes

Astrid Paulitsch-Fuchs / *Austria*
Cobalt-chromium-molybdenum implant surface modifications related biofilm development of S. aureus and S. epidermidis

10:45 - 13:05

Miroslav Caboň / *Slovakia*
Soil microbial community changes along elevational gradient in beech forests of Central Slovakia

Ricardo León-Sampedro / *Switzerland*
Pervasive transmission of a carbapenem resistance plasmid in the gut microbiota of hospitalized patients

Caroline Poyntner / *Austria*
Effects of season and elevation on soil (micro-)biome structure and function of two contrasting Alpine forest sites

Dhirendra Kumar Singh / *Sweden (Online)*
Role of Candida parapsilosis secreted aspartic proteases in Vulvovaginal candidiasis (VVC)

Filippo Vascon / *Italy*
Nanobody-Based Inhibitors of The SOS Response as Potential Suppressors of Antimicrobial Resistance

Sandra Godinho Silva / *Portugal*
Big Data meets Natural Products discovery: mining thousands of bacterial genomes to identify novel sources of drug leads

Damir Dermic / *Montenegro*
Regulation of ssb gene expression in Escherichia coli

Kostas Konstantinidis / *United States*
Efficiently Searching and Taxonomic Identification of the Genomic Prokaryotic Diversity with the Microbial Genomes Atlas (MiGA 1

13:05-13:25 **INDUSTRY SESSION – ProMedia**
Matilda Ivović: Beyond identification - exciting new features of the MALDI-TOF

PROMEDIA
Laboratory supply specialists

13:25-14:30 **Lunch Break / Poster Viewings / Exhibition**

14:30-17:00 **ROUND TABLE SESSION - One Health Paradigm: COVID 19 - Lessons learned**
Moderator: **Antonio Ventosa** / *Spain*
Panelists: **Stipan Jonjić** / *Croatia*, **Miroslav Perovec** / *Slovenia*, **Dimitrios Paraskevis** / *Greece*, **Aleksandra Knežević** / *Serbia*, **Alessandro Marcello** / *Italy*, **Arzu Sayiner** / *Turkey*

17:00-19:00 PARALLEL ORAL POSTER SESSIONS

Session 1
Environmental Microbiology 2
Chairpersons: Ivica Dimkic, TBA

Session 1
Food Microbiology & Plant Microbiology
Chairpersons: Peter Raspor, TBA

Session 1
Biotechnology and Industrial Microbiology & Emerging Infections (Fungal, Bacterial, Viral, Parasitic)
Chairpersons: Bauke Oudega, TBA

19:15-20:00 **CLOSING CEREMONY**

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CONFERENCE
MAIN PROGRAM
ABSTRACTS

Chairpersons: **Ana Kaftandzieva** / *North Macedonia*, **Alexandra Nascutiu** / *Romania*

LECTURE 3 / DETECTION AND CHARACTERIZATION OF VIRUSES CIRCULATING IN WILDLIFE POPULATIONS IN THE REPUBLIC OF SERBIA

Andrea Radalj

BACKGROUND

Wild boar and golden jackals are species under geographic expansion that represent reservoirs of various viruses of veterinary importance. Different porcine parvoviruses (PPVs) are being successively discovered, and while we know that PPV1 causes reproductive disease, the role of other PPVs (PPV2-PPV7) is still studied. Circoviruses infect numerous species and are associated with immunosuppression, reproductive problems, respiratory and enteric disease. Porcine circovirus 2 (PCV2) causes illnesses commonly named porcine circovirus-associated disease (PCVAD) and is divided into eight genotypes (PCV2a-PCV2h) with frequent global genotype shifts. Canine circovirus (CCV) is an emerging virus that has not been studied in Serbia. It causes various clinical symptoms in wild and domestic canids and may be transmitted between these animals.

OBJECTIVES

The study objective was to expand the knowledge on the molecular characteristics of PPVs and PCV2 in wild boar, as well as to demonstrate the presence and characterize CCV in the golden jackal population in Serbia.

METHODS

Various organs from hunted wild boar (154 samples) and organs and feces of jackals (56 samples) were tested by PCR. Positive samples were sequenced, and phylogenetic trees were analyzed.

RESULTS

The presence of PCV2 and various PPVs was confirmed in 48% and 63% of the examined samples, respectively, with frequent co-infections. PCV2 phylogenetic analysis results are in agreement with the global genotype shift, while PPVs mostly cluster with European strains. CCV was identified in 38% specimens from jackals, and representative sequences shared the highest similarities with a CCV strain detected in foxes in Croatia.

LECTURE 5 / CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE - AN ACTUAL PROBLEM IN CLINICAL PRACTICE

Ana Kaftandzieva

The aim of the study was to determine the prevalence, susceptibility, and the type of carbapenemases of carbapenem-resistant K.pneumoniae (CRKp).

During a four-year-period (2017-2020) all samples (urine, wound, blood, tubus/canila, sputum, tracheal aspirates) obtained from the hospitalized patients were analyzed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje. Determination of the susceptibility was done by disk diffusion method, Vitek 2 and E-tests. CIM method and carbapenemase set (Mast Diagnostic) were performed for detection of carbapenemase production to all strains that showed smaller inhibition zones to carbapenems according EUCAST.

Easyplex SuperBug CRE (Amplex Biosystems, Germany) was performed for detection of beta-lactamase genes. Out of the total number of Klebsiella pneumoniae - Kp 215/394/363/277, carbapenem resistant were 21%/18%/19%/21%, respectively. The percentage of CRKp, according to the origin, was as follows: 25/13/11/13 from urine, 18/30/43/28 from wound, 29/30/36/17 blood culture, 20/25/41/35 tubus/canilla and 13/19/27/27 was from sputum/tracheal aspirate. In 76 randomly selected isolates with positive phenotypic tests for carbapenemase detection, metallo-beta lactamases encoding genes were detected, such as: NDM (89.5%), OXA-48 (9.2%), VIM (1.3%). Between 90-100% of Kp strains were resistant to 9 tested beta-lactams and ciprofloxacin. The resistance to meropenem and to cotrimoxazole was between 80-89%, to imipenem 50-80%, to gentamicin 40-80% and to amikacin 30-50%. Increased MICs to colistin showed 17.8%, 4.5%, 12% and 12.3% of Kp isolates in 2017, 2018, 2019 and 2020, respectively.

The percentage of CRKp isolates is increasing and it is the right time to perform all strategies to control their spread.

SESSION 02 FOOD MICROBIOLOGY

Chairpersons: Peter Raspor / *Slovenia,* Dragoslava Radin / *Serbia*

LECTURE 3 / BIOCONTROL OF CAMPYLOBACTER JEJUNI - CELL-TO-CELL SIGNALLING INHIBITION AND INTER-SPECIES INTERACTION AS OPTIONS

Sonja Smole Mozina

Bacterial contamination is still a food safety concern even in developed countries. Campylobacter has been the most frequently reported food-borne pathogen in the EU since 2005, causing health and economic damages with associated costs of 2.4 billion €/year (EFSA, 2020). As an avian commensal, C. jejuni is mainly transmitted to humans through contaminated poultry meat, water, and cross-contamination in food processing areas. Reports of the "Campylobacter paradox" have confirmed that biofilms play an important role in bacterial survival during transmission from animals to humans. The increasing prevalence and antimicrobial resistance of this pathogen are causes of concern calling for the development of novel alternative treatment options.

Our objective was to investigate novel biocontrol approaches for C. jejuni reduction. First, plant-based preparations inhibiting cell-to-cell signalling of Campylobacter via the autoinducer-2 (AI-2)/LuxS dependent system were investigated, resulting in reduced bacterial motility, adhesion, biofilm formation and invasion of INT407 cells by C. jejuni.

Furthermore, the preparation α-pinene showed also reduction of C. jejuni in vivo in broiler chickens. Second, we tested the potential of Bacillus subtilis to control C. jejuni growth and confirmed that B. subtilis is a strong competitor for C. jejuni in vitro and in vivo as a spore preparation continuously given as a supplement to the drinking water of broiler chickens. The molecular mechanisms underlying the interactions C. jejuni - B. subtilis are being elucidated, as are their effects on C. jejuni survival, growth, and biofilm formation. We conclude that plant preparations and novel probiotics are viable options for C. jejuni control.

LECTURE 4 / MULTISPECTRAL IMAGING FOR ESTIMATING THE MICROBIOLOGICAL QUALITY OF CHICKEN FILLETS STORED UNDER DIFFERENT PACKAGING CONDITIONS

George Nychas

Quality control in industries is mainly based on chemical, microbiological, but also on molecular analyzes. These analyzes are time consuming, expensive, and tedious. The development of analytical approaches for estimating microbial load, such us multispectral imaging (MSI) coupled with machine learning (ML) methods, is very important for the rapid examination of large numbers of food samples in real time.

The aim of the study is to evaluate MSI data combined with two widely used ML methods in food science (i.e., Partial Least Squares Regression, PLSR and Support Vector Machines, SVM) for the estimation of microbial spoilage in chicken fillets

Chicken thigh fillets were stored under two packaging conditions (aerobically (air), vacuum) and at three temperatures (0, 5 and 10◦C). At predetermined time intervals, samples were subjected to microbiological analyses for the enumeration of total viable counts (TVC), as well as multispectral images were acquired. The experiment was repeated two times for each packaging condition. The MSI data were collected and stratified sampling was applied so as 70%-30% of the datasets to be used for the development-external validation of the models, respectively. Subsequently, the correlation between microbiological counts and spectral data was determined using PLSR and SVM.

PLSR and SVM regression models were developed and externally validated with the data collected from air (n=149) and vacuum (n=162) conditions. The R2, RMSE for external validation were 0.822, 0.697 (air) and 0.754, 0.659 (vacuum), respectively, when SVM was applied. The performance was similar for air packaging (R2= 0.805, RMSE= 0.736) and slightly improved for vacuum packaging (R2= 0.829, RMSE= 0.554) when PLSR was applied.

MSI spectral data showed promising results for the prediction of TVC on the surface of chicken thigh fillets regardless of packaging condition (air, vacuum) and ML method (PLSR, SVM).

SESSION 03 ANTIMICROBIAL RESISTANCE – CHALLENGES AND PERSPECTIVES

Chairpersons: Dejan Baskić / *Serbia*, Nijaz Tihic / *Bosnia and Herzegovina*

LECTURE 1 / NEW TRENDS IN ANTIBIOTIC RESISTANCE

Francesca Saluzzo

Antimicrobial resistance (AMR) represents an escalating threat to public health.

In the European Union/European Economic Area (EU/EEA) AMR is widespread and bacteria resistant to antibiotics cause more than 600 000 infections and 30 000 deaths per year.

Among EU/EEA regions differences exist concerning AMR rates for bacterial species and antimicrobial groups involved. Nonetheless, carbapenem resistance in Enterobacteriaceae, especially Klebsiella pneumoniae, and vancomycin resistance in Enterococcus faecium, showed a global increase during the last 4 years.
The dissemination of Acinetobacter species and Pseudomonas aeruginosa resistant to carbapenems is also a matter of concern, while the recent massive displacement of the Ukrainian population, is raising apprehension for the possible spreading of Multi Drug Resistant Tuberculosis (MDR-TB).

Great efforts and investments are needed to tackle AMR in EU/EEA and escalating and improving AMR surveillance strategies is a key step in this process.

The implementation of Next Generation Sequencing (NGS) techniques in AMR surveillance systems is emerging as effective and trustworthy approach to enhance the response to the AMR menace.

NGS can provide important and detailed information regarding the pathogens' epidemiology, origins and spreading. Moreover, it can determine pathogens' characteristics of public health importance, such as virulence and resistance, complementing the available phenotypic methods.

Additionally, AMR transmissibility may also be study in detail through the in-depth characterization of mobile genetic elements, as plasmids.

This substantial amount of highly specific and reliable information can contribute to inform the timely development of tailored policies and to monitor the efficacy of the applied interventions.

LECTURE 2 / A DECADE WITH CARBAPENEMASE-PRODUCING ENTEROBACTERALES - EXPERIENCES FROM ROMANIAN HOSPITALS

Edit Szekely

The first carbapenemase-producing Enterobacterales strains emerged in Romania by the end of the year 2010 and since then they have spread successfully to several hospitals, becoming a constant threat.

According to the EARS-Net database, the percentage of carbapenem resistant Enterobacterales strains among invasive Romanian isolates increased from 0 to 48% during the last decade. The most prevalent carbapenemases were OXA-48-like, followed by NDM-1. In recent years KPC carbapenemases also emerged and disseminated.

A particular situation was represented by the interhospital spread of certain successful clones of carbapenemase-producing Providencia stuartii. Due to their intrinsic resistance to colistin and the concomitant presence of resistance mechanisms to several classes of antibiotics, acquisition of carbapenemase genes often renders these bacteria extensively or even pan-resistant.

Containing the spread and managing infections caused by carbapenemase-producing Enterobacterales represented an increasing challenge over the years.

LECTURE 3 / CHALLENGES IN METAGENOMIC ANNOTATION OF ANTIBIOTIC RESISTOME

Svetlana Ugarcina Perovic

BACKGROUND

The antibiotic resistance genes (ARGs) in both host-associated and environmental microbiomes – antibiotic resistome – play an important role in the spread of antibiotic resistance. Metagenomics enables high-throughput exploration of microbiomes. One important step in these analyses is ARGs annotation within the metagenomes, for which several tools are in use. However, most of these tools are developed for genomics studies and their databases may pose certain biases.

OBJECTIVES

We aimed to compare outputs from different ARGs annotation tools for metagenomes and detect their potential challenges in microbiome studies.

METHODS

We ran >13 000 high-quality metagenomes from 14 habitats (Coelho et al., 2022; https://gmgc.embl.de/) through three ARGs annotation pipelines using DeepARG (CARD, ARDB, UNIPROT; https://bench.cs.vt.edu/deeparg), RGI (CARD; https://card.mcmaster.ca/) and ABRicate (CARD, ResFinder, ResFinderFG, NCBI, MEGARes, ARG-ANNOT; https://github.com/tseemann/abricate

RESULTS

DeepARG and RGI provide higher coverage (with potential novel ARGs and/or false positives) while ABRicate with different databases has less coverage but more well validated ARGs. The annotations did not differ only in number of hits but also in the information provided: e.g. using ABRicate, more sulfonamide resistance genes were identified using ResFinderFG version 2.0 as the database (ARGs obtained by functional metagenomics by Gschwind et al.), while using ResFinder resulted in more macrolide-resistance genes. Thus, choice of annotation tool and database should be driven by research questions and ARGs targets.

LECTURE 4 / CLINICAL ANTIBIOTIC RESISTANCE PLASMIDS AFFECT BIOFILM FORMATION DEPENDING ON BACTERIAL HOST GENETIC BACKGROUND

Laura Brülisauer

Plasmids contribute to the spread of antibiotic resistance (AR) via horizontal gene transfer among bacterial pathogens. Besides conferring resistance, AR plasmids can provide other beneficial traits to their hosts, enhancing their success colonizing the human gut. In clinical settings, biofilms are a key concern due to their potential to further increase resistance by physical and chemical protection. There is some evidence the biofilm lifestyle can promote plasmid endurance.

However, there is little consensus about the specific effects of AR plasmids on biofilm formation and how this affects the success of clinically relevant bacteria. To test this, we study the influence of clinical AR plasmids on biofilm formation in enterobacteria isolated from hospitalised patients as well as in related laboratory strains. We use five plasmids encoding some of the most problematic resistance genes, such as extended-spectrum β-lactamases (ESBL), Klebsiella pneumoaniae carbapenemases (KPC) and extended-spectrum oxaxillinases (OXA-48). First, we constructed 25 plasmid-host combinations and subsequently tested the effect of the plasmids on biofilm formation for each combination under different growth conditions. We found plasmid-mediated effects on biofilm formation in specific plasmid-host combinations which could not be explained by plasmid fitness costs. In ongoing work, using a CRISPR/Cas9 based system we are curing additional clinical isolates of their plasmids, which we re-introduce to their hosts to generate more plasmid-host combinations. Our study provides new information about how AR plasmids influence biofilm formation and other phenotypic traits in epidemiologically successful, clinical bacteria.

LECTURE 5 / MOLECULAR EPIDEMIOLOGY OF CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII

Doc. dr Ina Gajić [1]
1 Faculty of Medicine, University of Belgrade, Institute of Microbiology and Immunology

BACKGROUND

Acinetobacter baumannii has emerged as one of the most important causative agents of hospital-acquired infections, including ventilation-associated pneumonia, skin and soft tissue infections, and septicemia. Among multidrug-resistant bacteria, A. baumannii is one of the members of the “ESKAPE” group of pathogens, along with Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterobacter species. Furthermore, carbapenem-resistant A. baumannii (CRAb) are commonly reported worldwide, raising serious concerns about the limited treatment options remaining. Colistin, the drug of the last resort, has shown some promising activity against CRAb. However, an increase in the colistin resistance rate in A. baumannii has been reported globally.

OBJECTIVES

The aim of this research was to assess the molecular epidemiology of the circulating CRAb isolates.

METHODS

A. baumannii collected from 2018 to 2022 throughout Serbia were tested for the genetic basis of acquired carbapenemases and colistin resistance. Clonal relatedness was assessed by multilocus sequence typing.

RESULTS

In Serbia, the prevalence of CRAb isolates was 93%. Inter- and intra-hospital dissemination of the high-risk international clone II has been observed. The circulating CRAb clones were as follows: bla_{OXA66}/bla_{OXA23}/ST2 (32.4%), bla_{OXA66}/bla_{OXA23}/bla_{OXA72}/ST2 (2.7%), bla_{OXA66}/bla_{OXA72}/ST492 (37.8%), and bla_{OXA66}/bla_{OXA72}/ST636 (27.1%). The most common mechanism of colistin resistance (4.3%) was the chromosomal mutation in genes associated with the modification of the lipid A. Analysis of capsular loci revealed the presence of six KLs, the most common being KL9 (53.3%).

To provide a new strategy for the prevention and control of A. baumannii infections, further genetic evolutionary analysis of A. baumannii are required.

KEYWORDS: international clone II, oxacillinase, sequence type, colistin-resistance, KL9

SESSION 04 PLANT MICROBIOLOGY

Chairpersons: Ivica Dimkić / *Serbia*

LECTURE 1 / RNA-MEDIATED SIGNALLING OF A FUNGAL ENDOPHYTE UPON COLONIZATION OF ITS HOST PLANT

Kalliope Papadopoulou

The soil-borne endophyte Fusarium solani strain K (FsK) has a broad host range: it confers resistance and/or tolerance to biotic and abiotic stress in tomato and promotes plant growth in Nicotiana benthamiana.

By characterizing its core RNAi machinery, we determined that the fungus's DCLs process double stranded RNAs (dsRNAs) into short interfering RNAs (siRNAs) of predominantly 21-nt in size, which lead to the degradation of homologous mRNAs. In additions, we report on an RNA interference (RNAi)-based mechanism of communication between the endophyte and its host plant, i.e. we show that root colonization of N. benthamiana by FsK led to the induction of systemic silencing and DNA methylation of a host reporter gene. These data reflect a more general but so far unrecognized mechanism wherein fungal endophytes translocate RNAi signals to its hosts to modulate gene expression during symbiosis that may be translated to beneficial phenotypes.

LECTURE 2 / BENEFICIAL MICROORGANISMS IN AGRICULTURE - NEW GENERATION OF BIOLOGICAL CONTROL AGENTS AND BIOFERTILIZERS FOR SUSTAINABLE AGRICULTURE

Ivica Dimkić

BACKGROUND

The constant demand for new, effective agents has triggered intensive research in microbial diversity. Beneficial bacteria are widely considered a potential solution to the growing problem of resistance to conventional pesticides, fungal infection and plant diseases.

OBJECTIVES

Development of appropriate bacterial formulation, seed coating/treatment and composting material that can be distributed together with seeds during the sowing was aimed to achieve optimal performances on plants and increase the crop yields, together with advancement strategies for treatment of crops during the most sensitive growth phases.

METHODS

Differently designed products based on free-living bacterial inocula were used as liquid and solid-state fermentation products, in the treatment of different plant cultivar seeds and crops in situ and in planta in the most appropriate crop stage, with an evaluation of the reduction of the disease severity and communities' composition by using next generation sequencing techniques.

RESULTS

The bacterial isolates from the endosphere of different crop origins were selected according to plant-growth-promoting effect, biocontrol capacities, and environmental competitiveness characteristics. Advances in microbiome-enabling technologies and computational biology analyses provided an assessment of the community composition, potential function, and activity of the selected strains as co-formulants in the phytobiome. Results were indicated maintenance of the appropriate level of selected endophytes within seedlings and young plants, without affecting the "core" microbiota, and on reduction of the plant disease severity. The use of beneficial bacteria represent a good starting point for developing and patenting a product that would be utilized for plant protection on the large-scale production.

LECTURE 3 / CHALLENGES IN CROP USE OF BACTERIOPHAGES

Aleksa Obradovic

Plant diseases reduce global food production up to 20–30% annually. Those caused by plant pathogenic bacteria contribute considerably to economic losses. Due to lack of effective chemical control, bacteriophages (phages) appear to be promising natural agents for management of bacterial diseases. Phages occur naturally in the environment and generally have very narrow host range, targeting a specific bacterial species without affecting beneficial microbiota. However, phage effectiveness in the field may be limited by: phage concentration and persistence, method and timing of application, accessibility of the host bacterium, and host resistance. In order to control bacteria effectively a minimum number of phages is required. The effectiveness also depends upon host bacterial density and the phage adsorption rate.

Phage persistence in the phyllosphere is limited by UV, high temperature, desiccation, extreme values of pH, and rain-leaching. Although rhizosphere is a more hospitable niche, it can affect phage effectiveness by pH, moisture levels, soil type, presence of organic matter and biofilm. In order to enhance direct interaction with bacteria, phage application should be based upon nature of the pathogen and the type of plant. For foliar pathogens, phage suspensions are applied by spraying the leaf surface, whereas soil-inhabiting bacteria are controlled by soil drenching. Narrow host-range of bacteriophages may actually be disadvantage in case of the host population variability. Bacteria have ability to develop resistance to phages by mutation of an essentially required protein or modification of a phage adsorption receptor. Another mechanism of interrupting phage lytic life cycle is an altruistic abortive infection, where phage-infected bacterial cells commit suicide. In spite of the obstacles in “phage therapy”, the coming years are expected to be boom years for research on phages as biocontrol agents for plant bacterial diseases.

LECTURE 4 / MOLECULAR CHARACTERIZATION OF CITRUS TRISTEZA VIRUS IN MONTENEGRO

Jelena Zindovic

Citrus tristeza virus (CTV) is the most devastating virus in citrus production, able to cause different symptoms depending on strain, citrus species and scion/root combination. Tristeza, stem pitting (SP) and seedling yellows (SY) has already been recognized as main syndromes associated with CTV infection on citruses. However, with the exception of the first report on CTV (Papić et al., 2005), no survey has been carried out so far to investigate virus distribution and its genetic diversity in Montenegro. The present research has been conducted to provide this data.

During the five-year period (2016-2020), field survey has been performed in seven municipalities in the coastal and central part of Montenegro. A total of 290 randomly collected samples of Citrus species from nurseries and orchards were tested for the presence of CTV by DAS-ELISA. The results showed that 28,6% samples tested positive with polyclonal antisera against broad spectrum of mild and severe (tristeza, SP, SY) CTV strains. A one-step reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) were employed for molecular identification of CTV strains. A primer pair CTV1/CTV10 was used in RT-PCR to amplify the entire coat protein (CP) gene. Restriction analysis of PCR amplicons with KpnI and AvaII distinguished two RFLP profiles. The majority of isolates (76%) belonged to VT-like type which correspond to severe stem pitting inducing isolates. The rest of isolates (24%) belong to T-30 type which represents mild isolates. The high throughput sequencing (HTS) was used to identify difference between two groups of isolates.

LECTURE 5 / PHYLLOSPHERE BACTERIAL COMMUNITIES IN GREENHOUSES ECOSYSTEMS AND THE IMPACT OF BUMBLEBEES AND PREDATORY MITES

Marie Legein

As it is known that plant-associated microbes are important for plant health, we aimed to characterize the bacterial communities on the leaves of tomato (Lycopersicum solanum) and strawberry (Fragaria x ananassa) crops grown in greenhouses. Greenhouses are environments in which conditions are highly regulated to optimize plant growth. However, these artificial environments harbor fewer sources of bacterial diversity and also the dispersal of microbes is reduced, often resulting in less diverse plant-associated microbiomes. Furthermore, we explored the impact of two introduced biological solutions on the plant microbiome: bumblebees (Bombus terrestris), commonly introduced for pollination, and predatory mites (Phytoseiulus persimilis), introduced for the control of pests such as spider mites. We hypothesized that these invertebrates were an important source and a means of dispersal of microbes in greenhouses.

We found that the phyllosphere microbiomes were poor in abundance and in diversity. Microbiomes were often dominated by a single taxon and few taxa reoccurred over time and across departments. Secondly, bumblebees were an important source of bacteria in greenhouses. On average 20% of bacterial reads in tomato samples were bumblebee-associated ASVs and 80% of the crops were impacted. The impact of predatory mites was more difficult to quantify, as their microbiomes varied between batches.

Nevertheless, predatory mites did harbor a diverse set of plant-adapted bacteria which are currently under-explored. This study demonstrated that commonly implemented biological solutions, such as bumblebees and predatory mites, show potential to be used as a source and means of dispersal for beneficial microbes in greenhouses.

Chairpersons: Daniela Marchetti / *Italy,* Ivana Dakić / *Serbia*

LECTURE 2 / NOVEL THERAPEUTIC APPROACHES FOR MICROBIAL INFECTIONS: THE CASE OF PSEUDOMONAS AERUGINOSA

Livia Leoni

Antivirulence drugs and antibiotic adjuvants could complement classic antibiotic therapies in the fight against MDR pathogenic bacteria.

The ESKAPE Gram-negative pathogen Pseudomonas aeruginosa causes severe antibiotic-resistant infections in lungs, bladder, cornea and wounds and lethal chronic lung infections in cystic fibrosis patients. Besides being intrinsically resistant to many antibiotics, it is very prone to acquire new resistances and forms hard to treat biofilms. In fact, P. aeruginosa is included in the WHO priority list of pathogens for which new therapies are urgently needed and has become a major model organism for studies focused on the identification of antivirulence agents and antibiotic adjuvants.

This talk will retrace the pre-clinical research about novel therapeutic approaches against P. aeruginosa carried out by our group along almost one decade: from the development of target-oriented screening systems to the discovery of antivirulence activities in old drugs, through their validation against clinical strains, up to the discovery of additional and unpredicted antibiotic adjuvant activities. By giving solid examples of bottlenecks and successes, this excursus will hopefully help understand why, despites the efforts of the scientific community, antivirulence drug discovery is still at the pre-clinical stage.

LECTURE 3 / HIGHLY DRUG-RESISTANT AND HYPERVIRULENT MYCOBACTERIUM TUBERCULOSIS STRAINS EMERGING IN RUSSIA: PHYLOGENOMICS AND PATHOBIOLOGY

Igor Mokrousov

BACKGROUND

Ancient/ancestral sublineage of Mycobacterium tuberculosis Beijing genotype is endemic in East Asia and mainly drug susceptible. To our surprise, we recently discovered its highly resistant clonal clusters emerging in Russia.

OBJECTIVES

To analyze pathogenetics, phylogenomics and phylogeography of these clusters.

METHODS

The virulence and lethality were studied in C57BL/6 mouse model. The whole-genome sequencing (WGS) data were submitted to bioinformatics analysis.

RESULTS

Under phylogenetic analysis of the ancient Beijing sublineage (205 genomes from Russia and East Asia), all Russian isolates were drug resistant and formed two clusters. The largest cluster 1071-32 included isolates from different parts of Russia. All had resistance mutations in KatG315/335, RpoB450, RpoC485, EmbB497, RpsL43. The smaller cluster 14717-15 included isolates from Asian Russia; they were phylogenetically close to isolates from Korea. In murine model, the clusters demonstrated contrasting patterns. Low virulent, low lethal Beijing 1071-32-cluster is widespread across former Soviet Union but at low prevalence; multiple resistance mutations likely reduced its fitness and transmissibility. In contrast, highly lethal and hypervirulent Beijing 14717-15-cluster is predominant in Buryatia, Far East (16%), sporadically found beyond it, but not forming clusters of transmission. WGS analysis revealed 69 cluster-specific SNPs, including genes related to immune evasion and mycobacterial adaptation. We term Beijing 14717-15-cluster conditionally transmissible as it is endemically prevalent only in Buryatia. The reasons may lie in the interplay of the human immune system and the genetic background of this strain. Our analysis highlights that medically relevant research should focus on compact epidemic clusters of the genetically related isolates.

LECTURE 4 / VARIATION OF VACCINE-MEDIATED SARS-COV2 IMMUNITY IN A COHORT OF HEALTH CARE WORKERS

Miriam Latorre-Millán

BACKGROUND

Real-life studies are necessary to assess the covid vaccine effectiveness. Health care workers (HCW) are a key in the epidemiology of covid, since they are in contact with the patients, both as healers and as transmitting agents.

OBJECTIVES

To assess the variation of anti-spike IgG-mediated immunity in a cohort of hospital health workers.

METHODS AND RESULTS

109 representative HCW from a third level hospital were included in a study cohort. Peripheral blood swabs were collected, and SARS-CoV2 anti spike IgG were measured (with the Liason SARS-CoV2 kit from Diasorin®) each month between the second and third dose of SARS-CoV2 vaccination, for a total period of five months (between July and November of 2021). Student's T test was used to assess the significance of SARS-CoV2 anti spike variation through this time frame. SARS-CoV2 anti spike IgG medium values shifted down from 114.1 (+/-67.8) to 70.2 (+/-67.8) BAU/ml (p<0.001). At the initial time, 25 HCW (22.9%) showed levels of SARS-CoV2 anti-S IgG above the quantification capacity of the technique, but only 15 (13.7%) showed that values after five months (three of them showed lower values at the beginning of the study). Nine HCW increased their IgG anti spike values after SARS-CoV-2 contact.

SESSION 06 ENVIRONMENTAL MICROBIOLOGY

Chairpersons: Hilary Lappin-Scott / *United Kingdom*, Jaak Truu / *Estonia*

LECTURE 1 / ONLINE: ENVIRONMENTAL EPIDEMIOLOGY

Maja Ravnikar

Understanding the presence, diversity and survival of viruses in environmental samples, such as water, can help us to understand their transmission pathways and their roles in ecosystem cycles. We have been studying plant and human pathogenic viruses in surface waters and wastewaters. Recently, we have focused on the wastewater viromes, revealing high diversity of plant viruses and confirming their infectivity before and after conventional wastewater treatment, which is rising concerns on the uncontrolled use of reclaimed water for irrigation purposes. For studying viruses in water, we have developed different methods for efficient virus concentration, qPCR/ddPCR detection and quantification and shotgun high-throughput sequencing (HTS).

With the onset of COVID-19 pandemic, we have extended our expertise to SARS-CoV-2 wastewater-based epidemiology, which enables the studies of the trends of viral epidemics in larger populations and in specified geographic regions and is providing complementary surveillance of the spread of SARS-CoV-2 and the efficiency of adopted measures. We have assessed the stability of inactivated SARS-CoV-2 particles and patient-derived SARS-CoV-2 RNA, compared different virus concentration approaches, validated the concentration methods and virus quantification by qPCR/ddPCR. Additionally, we used HTS, followed by bioinformatic analysis, to detect and quantify the presence of key mutations belonging to SARS-CoV-2 variants of concern. Using the developed and improved methodology, we have established a wastewater monitoring of SARS-CoV-2 concentrations as well as SARS-CoV-2 variants of concern from several Slovenian wastewater treatment plants. National monitoring is helping us to follow the COVID-19 epidemics in Slovenia and has great potential to be used for other known and emerging diseases.

LECTURE 2 / APPLICATION OF MACHINE LEARNING METHODS FOR MICROBIOME DATA ANALYSIS - CURRENT TRENDS AND CHALLENGES

Jaak Truu

The number of microbiome-related studies has notably increased the availability of data on microbiome composition and function in different environmental settings and organisms. Improved data analysis tools are needed to utilize all information from these biological datasets, considering the particularities of microbiome data, i.e. compositional, heterogeneous and sparse nature of these datasets. Machine learning (ML) methodologies can overcome many of these barriers, but these methodologies need to be adapted to the specific properties of microbiome data.

The advantages of ML techniques over classical statistical models are to infer relationships between variables for automatic pattern discovery and handling of multi-dimensional data. So far, ML techniques have been mostly applied to analyze human microbiome data, harvesting the hidden knowledge to uncover and understand diversity in taxonomy and function within microbial communities and their impacts on human health. The application of ML methods for microbiome data analysis is linked to a number of challenges that range from study design, data processing, and standardization to analysis, modeling, cross-study comparison, prediction, data science ecosystems, and reproducible reporting. The full deployment of ML techniques for microbiome data analysis requires further efforts, while microbiome data accumulation will boost the application of more advanced ML technologies.

LECTURE 3 / FMT AS A PROMISING THERAPEUTIC TO REMEDIATE RADIATION-INDUCED INTESTINAL DYSBIOSIS AND COLONIC ALTERATIONS IN A CRC MOUSE MODEL

Shari Wouters

BACKGROUND

Studies have shown that colorectal cancer (CRC) patients are confronted with intestinal dysbiosis. Additionally, experimental models have shown that a dysbiotic gut community provokes enhanced CRC development. Radiotherapy plays an adjunctive role in CRC treatment, yet exposure to high cumulated doses of X-irradiation leads to collateral damage of the surrounding intestinal tissue and drives dysbiosis. Altogether, dysbiosis can lead to epithelial destruction and mucosal ulceration which might require premature treatment interruption or additional symptomatic treatment.

OBJECTIVES

Assessment of faecal microbiota transplantation (FMT) as a potential therapeutic to prevent radiation-induced gastrointestinal damage and intestinal dysbiosis.

METHODS

An established CRC mouse model receiving hypofractionated pelvic radiation and mimicking clinical radiation-induced pathology was treated with FMT or saline. A survival experiment was initiated to validate the long-term potential of FMT. Additionally, tissues were harvested to assess tumour development, radiation-induced intestinal damage and bacterial translocation. Fresh faeces were collected for microbial analysis.

RESULTS

The survival experiment shows that exceeding 60% of mice are surviving over one year post-irradiation, with no significant difference in survival between FMT- and saline-treated groups. FMT did not jeopardize the therapeutic potential of radiation when assessing tumour burden. Interestingly, bacterial diversity analysis revealed significantly distinct communities when comparing groups 10 and 38 days post-irradiation, which was no longer apparent 80 days post-irradiation.

Altogether, our study supports the potential of FMT to temporally remediate radiation-induced intestinal dysbiosis in CRC mice. Yet, a more thorough analysis of the microbiome and intestinal toxicity is warranted to validate FMT as a therapeutic candidate.

LECTURE 4 / INTERACTION TRAITS OF AQUATIC MICROBES

Hans-Peter Grossart

Marine microbes perform one-half of the global primary production, and form the base of the marine food chain. The aim of our project is to disentangle and predict how marine microorganisms growing together interact and affect each other. We performed a global analysis of the functional and taxonomic diversity of marine bacteria and their potential to interact, based on a newly developed trait-based framework.

By analysing a wide selection of complete genomes of marine bacteria, we provide a first comprehensive atlas picturing the functional diversity of these bacteria. In order to condense the huge amount of information encoded in this atlas into an interpretable map, we introduce the concepts of genome functional clusters (GFCs) and linked trait clusters (LTCs). GFC and LTC allow us to identify prevalent patterns in the functional traits of specific bacterial groups, including their capability to interact with other microorganisms both physically (e.g. through motility and adhesion to substrates) and through the exchange of vitamins, toxins and other info-chemicals (e.g. phytohormones).

Here, we show that GFCs are coherent functional units which overlap with known bacterial ecotypes, but also allow identifying new putative ones. Our framework enables to uncover mechanisms of bacteria-bacteria and bacteria-phytoplankton interactions and to extend our understanding beyond direct genome-based inferences. In particular, GFCs allow us to extrapolate present knowledge on specific model organisms to the whole diversity of genome-sequenced microbes. Conversely, the LTCs identify genetic traits that are functionally connected and may have evolved together, which allows to test for new hypotheses.

LECTURE 5 / INSIGHTS INTO PROCARYOTIC DIVERSITY IN HYPERSALINE SOILS

Antonio Ventosa

Hypersaline environments are extreme habitats that present high salt concentrations. Traditionally, microbiological studies on these habitats have been focused mainly on aquatic systems, such as saline lakes and salterns. Recent studies have revealed the large complexity and diversity of the prokaryotic (bacteria and archaea) community on saline soils. This study is focused on the prokaryotic diversity of hypersaline soils located at the Odiel saltmarshes in Southwest Spain. The aim of this work was to compare the microbial diversity determined by culturomic techniques in contrast to metagenomics.

For that purpose, three saline soil areas of the Odiel saltmarsh were selected for sampling. Total DNA was extracted from the soil and then sequenced by Illumina. Furthermore, we cultured bacteria and archaea present in the soil samples using different media and growth conditions for a period of three months. About 4,000 strains have been isolated, and 500 of them were characterized by partial or almost completed 16S rRNA gene sequence analysis. The most representative members were related to the genera Halomonas, Marinobacter, Aquibacillus, Bacillus, and Thalassobacillus within the domain Bacteria, and representatives of the genera Halorubrum, Haloarcula, and Halogeometricum within the domain Archaea. Approximately 40 isolates presented 16S rRNA gene sequence percentages lower than the 98.7 % threshold with respect to known prokaryotic species. For these strains, we carried out further analysis to determine whether they constitute new species. Among those presumed new species we find members of the haloarchaeal genera Haloarcula, Halomicroarcula, and Haloferax, and the bacterial genera Aliifodinibius, Pseudidiomarina and Aquibacillus.

SESSION 07

ADVANCED MICROBIOLOGY TECHNIQUES, MICROBIOMES AND MICROBIOTA

Chairpersons: **Jelena Begović** / *Serbia,* **Özgür Kurt** / *Turkey*

LECTURE 1 / ONLINE: HOST-MICROBE INTERACTIONS

Nathalie Sauvonnet

Online - Physical forces are essential for biological function, but their influence at the tissue level is not yet understood especially during infectious processes. The colon, organized in 3-dimension (3D), is constantly subjected to two main forces induced by the fluid-flow and the peristaltic motion, both being important for the tissue function and renewal. This epithelial monolayer forms a protective barrier facing the external side that provides a wealth of “ecological niches” permanently exposed to microorganisms that can be beneficial (microbiota) or damageable (pathogens).

We have shown using organ-on-chip (OOC) technology recapitulating the intestinal 3D architecture and its physical forces, that this gut surrogate allows the highly efficient invasion of the human pathogen Shigella, directly from the apical side of the monolayer, thereby shifting the paradigm on the early stages of invasion. Furthermore, by modulating the flow rate and peristalsis, and by reconstructing dynamic OOC imaging in 4D live, we observed that the 3D topology of the monolayer and the peristaltic motion greatly enhance the colonization rate, the bacterial virulence, and cell-to-cell spreading throughout the barrier. Our results reveal that Shigella takes advantage of the gut morphology and physical forces to disrupt the colonic barrier. More recently, we also used OOC to investigate enteric infections by two other pathogens, the parasite Entamoeba histolytica and the virus SARS-CoV-2. Once again, the 3D topology and mechanical stimulation of the gut enhance the infection, but each pathogen adapted differently to gut physical cues, highlighting the essential role of mechanical forces in host-pathogen interactions.

LECTURE 2 / A NEW GENERATION OF SEQUENCING (NSG) IN CLINICAL MICROBIOLOGY

Dejan Vidanović

The lecture explains the principles and platforms of NGS, emphasizes the importance of their use, and the possibility of application in human and veterinary clinical microbiology.

Special attention is given to the introduction and application of NGS technologies in Veterinary Specialized Institute Kraljevo, the types of pathogenic microorganisms (including SARS-CoV-2) that are being tested and the results so far.

LECTURE 3 / MICROBIOTA-ASSOCIATED METABOLITES AND THEIR INTERACTIONS WITH THE GUT–BRAIN AXIS

Lesley Hoyles

BACKGROUND

Our understanding of the contribution of the gut microbiota to mammalian health and disease has increased substantially in recent years. Gut microbes use the food we ingest as their own dietary substrates, with their end-products of metabolism often taken up from the gut into the bloodstream. Over 200 microbiota-derived metabolites are found in our systemic circulation at any one time, but our knowledge of how these microbial metabolic by-products influence host signalling systems is limited.

OBJECTIVES

The blood–brain barrier (BBB) protects the brain from harmful substances such as endotoxin and bacteria. We sought to understand a) how dietary-derived microbiota-associated metabolites (MAMs) influence the gut–brain axis, specifically at the BBB, and b) the implications for cognition and neural disease.

METHODS

Different classes of MAMs commonly found in human blood (short-chain fatty acids, methylamines, cresols) were screened at physiologically relevant concentrations in vitro (human cerebromicrovascular endothelial cell line hCMEC/D3) to assess their effects on BBB function. Transcriptomic data were generated from in vitro and in vivo (mouse brain) samples to allow us to identify the ways in which MAMs affect BBB integrity, with mechanistic work underpinned by immunohistochemistry, cell adhesion and shRNA assays. Where relevant, mouse feeding and cognition studies were undertaken to support our mechanistic findings.

RESULTS

We have demonstrated a direct interaction of MAMs with the mammalian BBB in vitro and in vivo [1,2,3]. While these interactions are metabolite-specific, several MAMs have protective effects that warrant further investigation in the context of mammalian health and healthy aging.

LECTURE 5 / DEVELOPMENT OF THE SARS-COV-2 VACCINE BASED ON THE YEAST CELLS WITH SURFACE DISPLAYED RBD FRAGMENT OF CORONAVIRUS SPIKE PROTEIN

Andriy Sibirny

Currently, many vaccines against coronavirus SARS-CoV-2 have been developed or are in the development. One of the promising vaccines could be the inactivated microbial cells with surface displayed viral antigens. Thus, it was proposed to use bacteria or yeast which expressed coronavirus proteins as oral or spray vaccines. We work with humanized strains of the methylotrophic yeasts Komagataella phaffii. In the first round of experiments, we constructed K. phaffii strains producing intracellular and secreted versions of RBD, fusion peptide with two heptad repeats and putative virus-like particles. To construct K. phaffii strains with surface displayed RBD protein, DNA sequence was expressed under control of AOX1 promoter. Binding of RBD to outer surface of yeast cell was achieved using signaling sequence of α-factor of S. cerevisiae at the 5'-terminus whereas sequence of S. cerevisiae SAG1 gene was localized at the 3'terminus of the RBD sequence. Surface displayed RBD localization was proved using double immunofluorescence. Surface displayed RBD protein was also obtained for probiotic yeast Saccharomyces boulardii. For this, RBD was expressed under control of Saccharomyces cerevisiae TEF1 promoter.

Intraperitoneal administration to mice of the purified intracellular or secreted RBD protein, fusion peptide with two heptad repeats fragment, putative virus-like particles were highly immunogenic while produced serum exerted efficient neutralization of spike protein binding with ACE2 receptor. The same results on high immunogenicity and neutralization activity were obtained after oral administration of K. phaffii cells with surface displayed RBD.

SESSION 08 BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY

Chairpersons: Ken Ichi Yoshida/ *Japan*, Sinisa Markov / *Serbia*

LECTURE 1 / BACILLUS SUBTILIS CELL FACTORIES FOR PRODUCTION OF VALUE-ADDED CHEMICALS

Ken Ichi Yoshida

Bacillus subtilis is a representative Gram-positive bacterium studied for more than half a century, ever since the dawn of molecular biology. B. subtilis is characteristic for its natural competence for DNA uptake, which has been investigated intensively during these long years to enjoy its genetic plasticity. This bacterium has evolved into an excellent platform for synthetic biological research and development.

There are various examples for B. subtilis cell factories to produce value-added chemicals. I will describe B. subtilis engineered to be cell factories for scyllo-inositol. scyllo-Inositol is an inositol stereoisomer known as a therapeutic agent for Alzheimer's disease. myo-Inositol is the most abundant in nature and thus supplied relatively cheaply. B. subtilis was modified in its inositol metabolism to convert myo-inositol into scyllo-inositol to yield a production rate of 27.6 g L-1 in 48 h. The myo-inositol biosynthesis is conserved in various organisms, but B. subtilis lacks the essential enzyme for the biosynthesis, myo-inositol-1-phosphate synthase. Therefore, a heterologous gene for myo-inositol-1-phosphate synthase was required to produce myo-inositol, which was converted further into scyllo-inositol. Thus constructed cell factory produced 2 g L−1 scyllo-inositol from 20 g L−1 glucose in 96 h, which would help us challenge the growing problem of Alzheimer's disease.

LECTURE 2 / ENGINEERING OF BACILLUS CELL FACTORIES FOR RECOMBINANT PROTEIN PRODUCTION

Jan Maarten van Dijl

Bacteria are widely used as cell factories for the production of enzymes and pharmaceutical products that, respectively, replace wasteful chemical processes and support human health and wellbeing. However, not all societal demands can be adequately met with the current bacterial production strains. Genome engineering can solve this problem through the creation of novel strains with desired properties. This view is underscored by our studies on genome-minimized strains of the bacterial cell factory Bacillus subtilis, a non-pathogenic soil bacterium that is used to produce enzymes and vitamins at industrial scale. New genome-reduced strains have a higher capacity for the synthesis and secretion of proteins, while suffering less from detrimental 'protein production stresses'. Altogether, our findings highlight the great potential of genome engineering to develop new-generation bacterial cell factories for industrial applications that satisfy the ever-increasing demands for high quality enzymes and pharmaceutical proteins.

LECTURE 3 / TOWARDS THE ENZYMATIC BREAKDOWN OF RECALCITRANT XYLAN: THE ROLE OF GH30 XYLOBIOHYDROLASES

Evangelos Topakas

Recently, accumulating data on the discovery of novel enzyme activities tend to revolutionize the way microbial lignocellulose degradation was perceived during the last 30 years. In this regard, the lecture will focus in the recent discovery of xylobiohydrolase activity found in the family of fungal GH30 glucuronoxylan-specific xylanases that changed our concept in the mode of action of hemicellulases. The novel catalytic function will be elucidated through biochemical and structural studies of a GH30 from Thermothelomyces thermophila (TtXyn30A), underpinning its dual endo- and exo- substrate specificity alone or in the presence of other hemicellulases. Cell wall recalcitrance in regards to xylan is influenced by different factors, such as the type, number and position of decorations, impairing enzymatic action and/or serving as branching points for cross-linking with lignin and/or presenting a specific pattern that affect the interaction of xylan with cellulose. The strong synergistic interaction of xylobiohydrolase with a novel xylan-active AA14 lytic polysaccharide monooxygenase (LPMO), indicates that both enzymes might have a major role in the degradation of recalcitrant xylan, contributing in the design of next-generation enzymatic cocktails for the saccharification of woody lignocellulosic biomass.

To further elucidate the mechanism underlying the dual endo- and exo- mode of action of TtXyn30A, several variants have been produced targeting both the amino acids expected to mediate MeGlcA recognition and those suggested to determine exo-activity as well as catalytic function. The biochemical results were supplemented by the high-resolution crystal structure (PDB: 7O0E), which is the second structure of a GH30_7 family member.

LECTURE 4 / ENGINEERING OF PSEUDOMONAS PUTIDA FOR FAST CO-UTILIZATION OF GLUCOSE AND CELLOBIOSE YIELDS AEROBIC OVERPRODUCTION OF PYRUVATE

Pavel Dvořák

BACKGROUND

Pseudomonas putida KT2440 is an attractive bacterial host for the bioproduction of chemicals from renewable lignocellulosic feedstocks [1]. It can valorize lignin-born aromatics or cellulosic glucose but its ability to utilize cellooligosaccharides, by-products of cellulose hydrolysis, is limited. Recently, we empowered P. putida with beta-glucosidase from Thermobifida fusca [2,3]. The recombinant showed growth on cellobiose but it preferred glucose in the mix of the two sugars. Sequential utilization of lignocellulosic substrates hinders their biotechnological valorization.

OBJECTIVES

In this study, we aimed to investigate the possibilities of glucose-cellobiose co-utilization in P. putida EM42 for the bioproduction of valuable compounds.

METHODS

A palette of experimental and computational engineering techniques was adopted including cell cultures, genome editing, enzymatic assays, and in silico analyses using a metabolic model of P. putida constrained with proteomic and kinetic data.

RESULTS

We achieved co-utilization of glucose and cellobiose after closing the periplasmic glucose oxidative pathway. Reduced growth rate of the mutant was compensated by the implanting of heterologous glucose and cellobiose transporters. Remarkably, this intervention resulted in the accumulation of pyruvate in aerobic P. putida cultures. Overproduction of this key metabolic precursor of many valuable chemicals was explained by the newly constructed metabolic model which pointed to the enzyme saturation due to unregulated substrate uptake. We then demonstrated that the excess of pyruvate can be redirected towards value-added products. Our work shed a light on the co-metabolism of cellulosic sugars in P. putida and introduced a novel strategy for pyruvate overproduction in aerobic bacterial cultures.

LECTURE 5 / TYPE OF CULTIVATION OF MEDICINAL MUSHROOMS AND THEIR BIOLOGICAL POTENTIAL IN PRODUCTION OF BIOLOGICALLY ACTIVE COMPOUNDS

Miomir Niksić

Mushrooms, part of fungi, have been valued for many centuries as a food due to the attractive sensory characteristics, optimal nutritional compositions and in medicine and pharmacy for the treatment and prevention of several diseases such as arthritis, rheumatism, bronchitis, gastritis, cancer, as well as in health and longevity as potential immunostimulator, antioxidant and antimicrobial remedy. They have a range of protein and minerals but also have a lot of strong stimulators and nutrients, which help to enhance or suppress the immune system.

The industrial cultivation of medicinal mushrooms is a difficult and complex process due to multiple external and internal parameters, resulting in an unforeseen yield and unpredicted financial outage. A lot of studies on the development of suitable growth substrates have been presented to improve as well as accelerate the growth of mycelium and fruit bodies of mushrooms. There are a set of consequential very important steps in mushroom cultivation. The cultivation process begins with composting the growing substrate, continuing with spawning, spawn running, development in different growing conditions, and with various methods and harvesting. Cultivation technologies were presented, including traditional cultivation on wood logs and beds and also on substrate-based media, like cultivation on bags, bottles, and others in the most important medicinal mushrooms species like Ganoderma lucidum, Grifola frondosa, Pleurotus ostreatus, Agaricus brasiliensis, and Lentinula edodes.

The presentation discusses cultivation techniques, processing equipment, and highlights the numerous achievements that have been made to promote higher mushroom production and use as a source of nutraceuticals.

SESSION 09 SUPPORTED RESEARCH PROJECTS AND IMPACT OF FEMS GRANTS ON PROFESSIONAL DEVELOPMENT AND OUTREACH OF EARLY CAREER SCIENTISTS

Chairpersons: **Vaso Taleski** / *North Macedonia*, **Jozef Anné** / *Belgium*

LECTURE 1 / SYNTHETIC AND SYSTEMS BIOLOGY APPROACHES FOR IMPROVE TAXOL PRECURSORS PRODUCTION IN SACCHAROMYCES CEREVISIAE

Behnaz Nowrouzi

Out of many reasons that heterologous biosynthesis of anticancer Taxol has not witnessed considerable progress, the presence of the first cytochrome P450 enzyme, CYP725A4, with promiscuous activity can be named. Attempts to optimise the activity of this enzyme and its interacting reductase such as self-sufficient protein construction, gene dosage and definitive screening design revealed the potential mediating effect of reactive oxygen species on production of main and side oxygenated taxanes in Saccharomyces cerevisiae (Nowrouzi et al., 2022). However, since the overexpression of P450-interacting reductase gene and excessive oxygenated taxanes production was at the cost of reduced cell viability and total taxanes production, long-term investment on the current yeast factory for production of later oxygenated taxanes and multiplexing was deemed infeasible. Hence, to create a robust Taxol-producing cell factory, adaptive laboratory evolution was deployed to adapt the CYP725A4 substrate, taxadiene-, producing yeast to oxidative stress to mimic the oxygenated taxanes production. Consequently, a yeast strain with approximately three-fold higher taxadiene production titre was evolved.

The performance of both non-evolved and evolved strains were evaluated using carbon-limited chemostats, with and without oxidative stress induction to mimic the oxygenated taxanes production in both strains. The initial analyses showed lower carbon uptake and ethanol, and higher carbon dioxide production rates by the evolved strain and upon applying exogenous oxidative stress. Combining these extracellular metabolite fluxes and transcriptomics results with genome-scale constraint-based modelling, we aim to provide new insights regarding the key components underlying the yeast metabolic adaptations to oxidative stress and taxanes production.

LECTURE 2 / INVESTIGATION OF MULTIDRUG-RESISTANCE PLASMIDS AND THEIR GENETIC RELATIONSHIP FROM SPANISH K. PNEUMONIAE OF UTI AND RETAIL MEAT

Isidro García-Meniño

BACKGROUND

Klebsiella pneumoniae represents a major cause of nosocomial infections and is also designated as an important member of the ESKAPE organisms due to its ability in spreading plasmids associated with antimicrobial/biocide resistance determinants. However, information on the dynamics of specific clones and/or mobile genetic elements within different ecosystems as well as their impact on the public health is lacking.

OBJECTIVES

The genetic basis of K. pneumoniae as well as the core/accessory genome of prevalent plasmid-types carrying qnr-/bla-genes were analysed in-detail. The study also provides information on the occurrence and relatedness of plasmids associated with multidrug-resistance.

METHODS

Isolates originating from human urinary tract infection (UTI, n=52) and retail meat (n=12) were recovered in Spain in February 2020 and subjected to microbiological/molecular in depth-characterization, including WGS (Illumina & ONT). Plasmid genomes were reconstructed using plasmid ID and further analysed for their resistance/virulence gene content and their transmission potential. The outcome of the in silico results was confirmed with biological data from S1-PFGE profiling, SNP-comparison and filter mating studies.

RESULTS

Here, we show the presence, diversity and phylogenetic relationship of different epidemic multidrug-resistance plasmids (pKp589-231, pG4584, p203), carrying both bla and qnr resistance determinants. All multidrug-resistance plasmids were shown to be transmissible to E. coli. The SNP-comparison revealed some overlaps between lineages isolated from the clinical and non-clinical sources, which was especially remarkably for the K. pneumoniae high-risk clones ST307 and ST147. It seems that meat represents a yet underestimated source of clinically relevant K. pneumoniae and of high-transmissible multidrug-resistance plasmids.

LECTURE 3 / MULTI-DOMAIN INVESTIGATIONS OF MICROBIAL COMMUNITIES ASSOCIATED WITH LOGGERHEAD SEA TURTLES

Klara Filek

Marine vertebrates are hosts to myriads of microorganisms. Bacteria and diatoms are known for being the first colonizers of submerged surfaces, including the skin and carapace of loggerheads where they establish multi-domain biofilms. Recent studies point to the prevalence and ecological importance of interactions between bacteria and planktonic diatoms, but to date, little is known about bacteria-diatom interactions in biofilms associated with epizoic diatoms. Furthermore, it is known that loggerheads harbour diatom taxa specific to marine vertebrate hosts; however, it is not known if this is the case for bacteria and how important diatom taxa within these communities are.

This study aimed to investigate the bacterial repertoire of diatoms associated with sea turtles, as well as to gain insight into the diatom community dynamics and host exclusivity by investigating putative epizoic and non-epizoic diatoms via co-cultivation.

We isolated diatoms from carapace and skin scrapings of loggerhead sea turtles and established xenic monocultures. Bacteria within diatom cultures were investigated by culturing and partial 16S rRNA gene sequencing, and amplicon sequencing of the V4 region of 16S for bacterial communities within diatom monocultures and their source sample. Additionally, putative epizoic and non-epizoic diatoms were co-cultivated to examine their growth both in monocultures and co-cultures.

Microbial communities differed between diatom genera and their phycospheres were enriched in Alcanivoracaceae, Marinobacteraceae, Phycisphaeraceae families. Initial co-culturing results show a faster growth of epizoic diatoms in co-culture with non-epizoics. This research provides first insights into the biology of microeukaryotes within epizoic biofilms and their associated bacteria.

LECTURE 4 / MALASSEZIA COMPARATIVE MITOCHONDRIAL GENOMICS TOWARDS SENSITIVE DIAGNOSTICS TOOLS

Anastasia Christinaki

Malassezia is a unique basidiomycetous yeast genus, currently comprising 18 species. Besides being the most dominant component of the healthy human skin mycobiome, it is also responsible for various skin disorders, like seborrheic dermatitis and pityriasis versicolor. Some species i.e., M. furfur, M. pachydermatis and M. sympodialis, are associated with bloodstream infections in immunocompromised patients and neonates.

A unique feature of this genus is that it is lipid dependent, and some clinically relevant species are fastidious, making them difficult to culture and diagnose with traditional tools. Recent advances in Malassezia genomics and genetics focused almost entirely on the nuclear genome. Here we unveiled the mitochondrial (mt) genetic heterogenicity of the genus. For this reason, knowing the 8 publicly available Malassezia mt genomes, the additional 10 were retrieved from WGS data and further used for a comparative analysis revealing significant variability in the mt genome organization. Genome size varied between approximately 28-60 kb and size variability was attributed to differences in intron abundance and intergenic region variability.

Genes of homing endonucleases were located within introns with the LAGLIDADG type as the main contributor. Intergenic region variability was observed even at the intraspecies level, most notably in M. furfur (Theelen et al., 2021). Syntenic diversity, intron abundance, intergenic region variability, and the multicopy nature of these fungal mitogenomes, make them suitable targets for the development of sensitive and robust diagnostic tools. This work revealed highly variable mt regions, applicable for detection and identification of Malassezia at the inter- and intra- species level.

LECTURE 5 / IMMOBILIZATION OF CELLS OF PHOTOSYNTHETIC BACTERIA RHODOPSEUDOMONAS YAVOROVII FOR OPTIMIZATION OF BIOLOGICALLY VALUABLE COMPOUND

Olha Maslovska

BACKGROUND

Rhodopseudomonas yavorovii IMV B-7620 are resistant to heavy metal compounds, effective exoelectrogens and producers of H2 during growth in wastewater. Immobilization of phototrophic bacteria on nanofibres will increase the surface area with optimal illumination, ensure the continuity of the process of bioremediation of wastewater with the simultaneous generation of H2 and will affect the need to separate bacterial biomass from treated wastewater.

OBJECTIVES

To design electrospun hybrid nanofibers as carriers for the immobilization of R. yavorovii IMV B-7620 for optimization of the processes of wastewater bioremediation and production of H2.

METHODS

Samples of wastewater of the alcoholic plant and infiltrates of the solid waste landfill were taken immediately before experiments. An electrospinning system from IME Technologies was used for the fabrication of nanofibers, that contain polycaprolactone (8–20%), and hydroxyapatite nanoparticles (2–6%), sulfur (1.5%). Morphology of nanofibers was examined using scanning electron microscopy. The nitrate content was determined potentiometrically, composition of the gas phase – using gas chromatography, chemical oxygen demand (COD) – by the method of the permanganate index (IMn).

RESULTS

The highest adhesion of R. yavorovii IMV B-7620 was observed for the surface of carrier that contained the highest content of polycaprilactone (20%) and hydroxyapatite nanoparticles (6%). As a result of immobilization of bacteria on nanofibers H2 production increased by 9 times during growth in the wastewater from ethanol production plant, the NO3-content in the landfill infiltrates decreased by 15–42 %, and COD – by 60 %, compared with the control.

SESSION 09

LECTURE 6 / GENOME-WIDE MUTATION ANALYSIS OF THE L-TYROSINE OVERPRODUCER CORYNEBACTERIUM GLUTAMICUM ATCC 21573

Eldin Kurpejovic

Corynebacterium glutamicum is used industrially for large-scale production of amino acids. Here, whole genome sequencing of C. glutamicum ATCC 21573 was performed to gain insight into L-Tyr overproduction. Genome-wide analysis identified 28090 mutations, of which 27611 were SNPs and 479 were InDels.

Mutations have led cells to favor PTS-independent uptake. Cell growth and concentration were low with any of the tested carbon sources, which clued impaired carbon flux. In this respect, TCA and glyoxylate cycles were the most adversely effected pathways. Furthermore, defects in anaplerotic reactions probably kept replenishing pathways from adequately operating. Nevertheless, mutations in aroE and qsuC possibly altered flux through shikimate pathway. Synthesis and export of protocatechuate was probably hindered, preventing leakage towards this by-product of shikimate pathway. Modifications downstream of the branch point molecule prephenate could be considered important in shifting flux. Despite the information on L-Phe auxotrophy, cells grew in its absence, but L-Phe supplement alone could not fully restore growth.

Mutations in genes specific for L-Phe synthesis (pheA, pat, and ilvE) clearly indicated flux change here but the overall results suggested that the overproducer is L-Phe bradytroph. Interestingly, external L-Phe dropped L-Tyr titer, possibly by inhibiting shikimate pathway enzymes. Mutations in tyrA, involved in L-Tyr synthesis, was not regarded significant. Thus, L-Tyr overproduction could be explained partly by L-Phe bradytrophy and partly by carbon flux redistribution upstream from prephenate. This study is a powerful platform for reverse metabolic engineering leading to rational design of cells for L-tyrosine production with an industrially important organism C. glutamicum.

LECTURE 7 / DETECTION OF THE MULTIDRUG-RESISTANT PHENOTYPE IN MYCOBACTERIUM TUBERCULOSIS BY MOLECULAR ASSAYS – A POLISH-LITHUANIAN EXPERIENCE

Zofia Bakuła

BACKGROUND

Worldwide in 2019, ca. 400,000 people had multidrug-resistant tuberculosis (MDR-TB). Importantly, Europe still carries about one-fourth of the global MDR-TB burden.

The aim of the study was to compare the capacity of two molecular approaches, i.e. (i) multiplex PCR combined with hybridization and (ii) whole genome sequencing (WGS), for the detection of MDR resistance of Mycobacterium tuberculosis.

METHODS

The study included 150 M. tuberculosis (75 MDR and 75 drug-susceptible; DS) isolates, recovered from as many Polish and Lithuanian patients, over a 4-year period (2018-2021). Conventional drug susceptibility testing (DST) was performed using the currently WHO-approved methods during a one-month FEMS Research and Training (RTG) grant.

For molecular determination of drug resistance, mutation profiling was performed using either GenoType MTBDRplus assay (Hain Lifescience, Germany) or WGS analysis.

Phylogenetic clades of M. tuberculosis were assigned in silico, using SpoTyping (https://github.com/xiaeryu/SpoTyping-v2.0).

RESULTS

The spoligotype-based structure of the MDR M. tuberculosis population was conspicuously compact, since more than 80% of the isolates belonged to either of two lineages (Beijing and Ural). Among DS isolates, the T lineage predominated, comprising close to a third of the isolates. The Genotype MTBDRplus and WGS-based approaches showed high performance to predict MDR-resistance.

CONCLUSIONS

The study revealed a homogenous structure of MDR M. tuberculosis population in Poland and Lithuania. Furthermore, high sensitivities of Genotype MTBDRplus assay and sequence analysis for the detection of INH and RIF resistance in TB support their use for large-scale screening of MDR phenotype in TB.

LECTURE 8 / SOIL MICROBIAL COMMUNITY CHANGES ALONG ELEVATIONAL GRADIENT IN BEECH FORESTS OF CENTRAL SLOVAKIA

Miroslav Caboň

Beech forests dominated by Fagus sylvatica are the most common managed forest type in Slovakia. This type of forest occurs, depending on slope exposition, across a wide range of elevations of 300 to 1100 m. Along this gradient, forest communities face very different climatic conditions, including increased rainfall and decreased season length due to lower average annual temperatures with at elevations. With global climate changes, Slovak beech forests seem to be an ideal model to study impact of the warming on forest soil microbial communities. Soil from four elevational classes (approximately of 300, 500, 700, 900 m a. s. l.) was sampled in beech mono-dominant forests in a volcanic area of Javorie Mts. in 2020. Fungal and bacterial soil communities are known to have strong responses to changes of ecological conditions.

This study aimed to assess changes in community composition, and its function connected to changing conditions along elevation gradients in beech forest. We used high throughput sequencing to assess fungal and bacterial communities, analyses of ergosterol and PFLA to trace microbial biomass and enzymatic analyses to understand differences in function of the ecosystem. This study is supported by the FEMS grant GO-2019-585 which allowed performing advanced methods of molecular ecology in the host institution. Original planned topic of the FEMS fellowship had changed because of postponing due to COVID-19 travel restrictions.

LECTURE 9 / PERVASIVE TRANSMISSION OF A CARBAPENEM RESISTANCE PLASMID IN THE GUT MICROBIOTA OF HOSPITALIZED PATIENTS

Ricardo León-Sampedro

Infections caused by carbapenemase-producing enterobacteria (CPE) are a major concern in clinical settings worldwide. Two fundamentally different processes shape the epidemiology of CPE in hospitals: the dissemination of CPE clones from patient to patient (between-patient transfer), and the transfer of carbapenemase-encoding plasmids between enterobacteria in the gut microbiota of individual patients (within-patient transfer).

The relative contribution of each process to the overall dissemination of carbapenem resistance in hospitals remains poorly understood. Here, we used mechanistic models combining epidemiological data from more than 9,000 patients with whole-genome sequence information from 250 enterobacteria clones to characterize the dissemination routes of a pOXA-48-like carbapenemase-encoding plasmid in a hospital setting over a 2-yr period. Our results revealed frequent between-patient transmission of high-risk pOXA-48-carrying clones, mostly of Klebsiella pneumoniae and sporadically Escherichia coli. The results also identified pOXA-48 dissemination hotspots within the hospital, such as specific wards and individual rooms within wards. Using high-resolution plasmid sequence analysis, we uncovered the pervasive within-patient transfer of pOXA-48, suggesting that horizontal plasmid transfer occurs in the gut of virtually every colonized patient. The complex and multifaceted epidemiological scenario exposed by this study provides insights for the development of intervention strategies to control the in-hospital CPE spread. During this project, the FEMS Research and Training grant provided me with the opportunity to collaborate with top international experts in a new field, significantly improving the outcome of the project. Besides, I got to acquire new skills that are helping me to become a young ambitious scientist in a prestigious institution.

SESSION 09

LECTURE 10 / EFFECTS OF SEASON AND ELEVATION ON SOIL (MICRO-)BIOME STRUCTURE AND FUNCTION OF TWO CONTRASTING ALPINE FOREST SITES

Caroline Poyntner

BACKGROUND

Alpine forests are major carbon sinks and play an important role in the global carbon cycle. Soil (micro-)biomes drive carbon cycling and are expected to be strongly affected by climate change. While effects of seasons and elevation on the (micro-)biomes have been explored with microbiological and targeted molecular methods, quantitative metatranscriptomics was only recently applied. Using this technique, active (micro-)biome members can be identified to better understand changes in ecosystem functioning. Additionally, protists and viruses can be detected, who control the microbiome structure.

OBJECTIVES

The aim of this study was to investigate seasonal and site-specific soil (micro-)biome differences and soil microbial activity at two contrasting Alpine forest sites.

METHODS

Twenty-four metatranscriptomes were investigated in spring and autumn at two forest sites, which differed in elevation, vegetation (deciduous and coniferous), and soil properties. Molecular biological work was done at the University of Innsbruck. Subsequently, sequencing and data analysis was performed at the University of Greifswald which was supported by a FEMS research and training grant.

RESULTS

The studied forest sites distinctively differed in their (micro-)biome pattern as indicated by the number of transcripts quantified in the soils. For instance, we could identify season and site-specific abundancies of eukaryotic rRNA sequences such as those of Rhizaria. Measured mRNA patterns could be related to changes in seasons and soil organic matter content. Overall, we explored active members of the soil food web of two Alpine forest sites and found dynamic changes in (micro-)biome structure and function between sites and seasons.

LECTURE 11 / ONLINE: ROLE OF CANDIDA PARAPSILOSIS SECRETED ASPARTIC PROTEASES IN VULVOVAGINAL CANDIDIASIS (VVC)

Dhirendra Kumar Singh Singh

FEMS research grant awarded to me in 2019 not only helped to complete part of my PhD work but also opened new ideas to further investigate the role of pathogenic fungi C. parapsilosis secreted aspartyl proteinases (SAPPs) in Vulvovaginal candidiasis (VVC). VVC is a common clinical condition of vaginal inflammation with at least one episode of VVC in up to 75% of reproductive age group women during their lifetime.

Using different strains of Lactobacillus, a bacterial member of the vaginal microbiome, we investigated the antifungal effect against a pathogenic strain of C. parapsilosis by the compounds released by Lactobacilli. We used different in vitro and 3D co-culture systems to investigate these effects. Findings of this study suggest that, in the context of the vaginal microbiota, the compounds secreted by Lactobacilli have postbiotic-like properties and may play a role in preventing Candida-caused Vulvovaginal candidiasis (VVC). In summary, work done with the support of the FEMS grant helped us to identify and define the complex relationship of Lactobacillus and C. parapsilosis in VVC. Findings of this study have already been submitted in a peer-reviewed journal for publication.

After finishing this project, the experience, I got to work with anaerobic bacterial culture, 3D co-culture system, neutrophil isolation, and ELISA, helped me to secure a postdoctoral position in an international laboratory. The experience, I got in the host laboratory helped me to finish and design several experiments even during my current postdoctoral period.

LECTURE 12 / NANOBODY-BASED INHIBITORS OF THE SOS RESPONSE AS POTENTIAL SUPPRESSORS OF ANTIMICROBIAL RESISTANCE

Filippo Vascon

BACKGROUND

The pro-mutagenic SOS response, regulated by the genotoxic stress sensor RecA and the transcriptional repressor LexA, is a bacterial pathway driving the evolution of antimicrobial resistance.

OBJECTIVES

The objective of this study was to develop anti-LexA nanobodies (NbSOSs) to inhibit the RecA-induced LexA autoproteolysis, crucial for SOS response activation.

METHODS

A library of NbSOSs was obtained by llama immunization with Escherichia coli LexA and phage-display selection of binders. A LexA autoproteolysis assay based on fluorescence polarization led to identify the strongest LexA inhibitors, which were characterized by Surface Plasmon Resonance and X-Ray crystallography, paving the way for their further amelioration by rational engineering.

Expression of SOS genes in bacterial cultures expressing NbSOSs and treated with DNA-damaging antibiotics was followed by RT-PCR.

RESULTS

Three NbSOSs were selected as the best hits, showing promising inhibition and affinities for LexA (IC50 and KD in the low micromolar).

X-Ray structures of these NbSOSs in complex with LexA revealed that they block it in an autoproteolysis-inactive state, sampling two different binding sites.

This observation reasoned for the construction of biparatopic nanobodies, which showed improved inhibition and binding on LexA, compared to the “parental” NbSOSs.

NbSOSs were confirmed to be potent suppressors of the antibiotic-induced SOS response by expression profiling of SOS genes, while not showing synergism with fluoroquinolones.

Computational protein design has been performed to enhance inhibition properties of NbSOSs. Engineered species are undergoing biochemical and microbiological characterization for determining whether they are able to reduce antibiotic-induced hypermutation and prevent the onset of resistance.

LECTURE 13 / BIG DATA MEETS NATURAL PRODUCTS DISCOVERY: MINING THOUSANDS OF BACTERIAL GENOMES TO IDENTIFY NOVEL SOURCES OF DRUG LEADS

Sandra Godinho Silva

BACKGROUND

Flavobacteriaceae spp. are known for their extensive carbon degradation capabilities. Yet, our view of their secondary metabolism repertoire remains narrow, limiting our ability to exploit natural products from this metabolically versatile and keystone microbial taxon.

OBJECTIVES

The goal of this study is to determine whether secondary metabolism signatures underpin the evolution of Flavobacteriaceae species.

METHODS

Advanced comparative genomic analyses were performed on 2680 publicly available genomes, including 175 different genera, 1158 unique species and 692 metagenome-assembled genomes (MAGs) from marine and terrestrial environments.

RESULTS

Automated genome mining revealed that 88.6% of the observed Flavobacteriaceae secondary metabolite biosynthetic gene clusters (SM-BGCs) presumably lead to the biosynthesis of novel natural products. Networking analysis of 9330 SM-BGCs uncovered a previously unknown phylogenetic signal dictating secondary metabolism diversification and genome evolution within the Flavobacteriaceae family. Conspicuously, three phylogenetically close genera - Aquimarina, Kordia and Tenacibaculum – presented a distinct secondary metabolic profile, breaking new ground to their future study as renewable sources of novel drug leads.

IMPACT

These results, obtained through an international collaboration enabled by a FEMS Research Training Grant, laid the foundations for my subsequent PhD studies, focused on structure elucidation and bioactivities of novel compounds from Aquimarina species. Moreover, this bioinformatics apprenticeship helped me to develop essential skills in programming and data science, and to design my own comprehensive, fully scalable genome annotation pipelines. Because of this experience, I now envision and aspire a future career as a bioinformatician, working in genomics and big data science.

SESSION 10 MISCELLANEOUS

Chairpersons: **Gordana Mijović** / *Montenegro*, **Ivana Colovic-Calovski** / *Serbia*

LECTURE 2 / CELL-CELL SIGNALING AMONG BACTERIA IN THE MICROBIOME

Vittorio Venturi

Next-generation sequencing has evidenced the different bacterial groups populating plantmicrobiomes many of which play important roles in plant health and resistance to biotic andabiotic stresses. Microbiologists have discovered and are currently studying many of themechanisms of cell-cell interactions that take place between bacteria in the microbiome.These are likely to play an important in shaping and maintaining plant microbiomes. One ofthe prevalent mechanisms for intercellular interactions is cell–cell siganling/communicationto dynamically regulate a set of metabolic and physiological activities in response to theplant host, environment, and microbial neighbours. In this lecture, interests are focusedtowards a widespread bacterial regulatory family in proteobacteria which is playing a majorrole in microbiome cell-cell interactions.

This research will have an impact on bothfundamental sciences and translational agriculture via the development of bacterialprobiotics, resulting in a more sustainable agriculture of economically important crops.

LECTURE 3 / VIVIRAL DISEASES IN ANIMALS

Bojan Adzić

BACKGROUND

Infectious viral diseases in animals represent one of the major threats not only to agriculture but often to human population. Their impact can be evidenced at local, regional, and even at the international level or worldwide. There is a large number of viral animal diseases. Some of those viruses affect both humans and animals.

OBJECTIVES

Encouraged by new findings about the coronavirus, our research team examined the presence of the human strain of coronavirus in pets of covid-19 positive owners. Testing dogs and cats living in the home community or in the immediate surroundings of infected patients, on presence of SARS-CoV2 was conducted in summer 2020.

METHODS

In this study, total number of 130 animals was tested on presence of virus that cause Covid-19. Of that number 86 were dogs while 44 were cats. The majority of the tested animals were in direct contact with COVID-19 infected patients. Some of the animals were from asylum for abandoned animals. For Real-time rt-PCR testing Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit was used, while for antibody detection ID Screen Sars-Cov-2 Double Antigen Multi-Species Kit was applied.

RESULTS

The presence of antibodies was detected in three animals (dogs) or 4.5% of tested samples. With Real-time rt-PCR, RNA sequence of SARS-CoV2 was not detected in any of 130 tested samples. For better understanding of the Covid-19 infection in animals, further monitoring is needed.

LECTURE 4 / EVOLUTION OF BACTERIAL GENOMES

Mikhail Gelfand

Evolution of bacterial genomes may be looked at from several different angles.

At the level of sequences, mutation signatures reflect noise in the replication and repair systems. In E. coli strains, lab mutations are best explained by infidelity of DNA polymerases II and III, while inefficient 8-oxoG removal by mutT and mutY strongly contributes to polymorphisms in wild strains. Analysis of sequence conservation is instrumental for the identification of regulatory regions such as transcription factor binding sites.

At the gene content level, one may study patterns of gene gain and loss during major evolutionary changes. We observe gene loss when a species becomes an obligate pathogen and then an endosymbiont; at the threshold of 400 genes, the loss of ribosomal proteins begins. Bipartite genomes such as Vibrio and Burkholderia spp. demonstrate a linear dependency between the sizes of the primary chromosome and the chromid, showing consistency between the gene gain and loss rates in the genome parts. Finally, the analysis of gene content allows for an unbiased definition of bacterial species, e.g. in the Prochlorococcus genus.

At the gene order level, we see the increased inversion rate following change of the lifestyle in Shigella spp., Yersinia pestis, and Burkholderia mallei. This is caused by bursts in IS copy numbers and weaker negative selection due to speciation bottlenecks and decreased effective population size.

Finally, comparative analyses demonstrate high evolutionary flexibility of regulatory networks, with transcription factors easily substituting each other in the regulation of functional subsystems, e.g. metabolic pathways.

LECTURE 5 / COBALT-CHROMIUM-MOLYBDENUM IMPLANT SURFACE MODIFICATIONS RELATED BIOFILM DEVELOPMENT OF S. AUREUS AND S. EPIDERMIDIS

Astrid Paulitsch-Fuchs

The number of biofilm-infection related replacement surgeries is increasing and will continue to do so. To reduce the health burden of the patients as well as the costs in the healthcare sector, new solutions for implant materials resistant to such infections are necessary. In this study different surface modifications of Cobalt-chromium-molybdenum (CoCrMo) based implant materials and their influence on the development of biofilms were studied.

Three smooth surfaces (CoCrMo, CoCrMo TiN and CoCrMo polished) and three rough surfaces (CoCrMo porous coated, CoCrMo cpTo and CoCrMo TCP) are compared. One type strain of Staphylococcus aureus and one of Staphylococcus epidermidis have been chosen as model organisms. Biofilms were grown on material disks for 48 h and cell number, polysaccharide content, and protein contend of the biofilms were measured.

Regulation of genes involved in early biofilm development (S. aureus icaA, icaC, fnbA, fnbB, clfB, atl; S. epidermidis atle, aap) was detected using RT-q-PCR. The results show a correlation between the surface roughness and the development of biofilm structures as well as the maturation state of the biofilms grown on the different surface modifications.

This is supported by the significantly different protein and polysaccharide contents of the biofilms between rough and smooth surface types as well as early phase biofilm genes being statistically significantly downregulated compared to the control at 48h on rough surfaces. CoCrMo TiN and CoCrMo polished present the two smooth surface modifications performing best when aiming at low biofilm content. Future studies should also include earlier timepoints and clinical isolates.

LECTURE 6 / REGULATION OF SSB GENE EXPRESSION IN ESCHERICHIA COLI

Damir Dermic

Bacterial SSB proteins, as well as their eukaryotic RPA analogues, are essential and ubiquitous. They bind single-strand DNA and regulate/coordinate its metabolism, hence enabling essential processes such as DNA replication, DNA transcription and DNA repair. The prototypic SSB protein, the one of Escherichia coli, is encoded by an ssb gene. Although the ssb gene promoter harbors SOS box, multiple studies during several decades failed to elucidate the mechanisms of the ssb gene regulation, i.e. whether it is inducible and SOS dependent. SOS regulon is comprised of about 40 genes, whose transcription is induced in stress conditions in a coordinated way.

Using quantitative real-time PCR, we determined the ssb gene expression in UV- and γ-irradiated E. coli and revealed that the ssb gene expression is elevated in irradiated cells in an SOS-dependent manner. Elucidating regulation of expression of the essential bacterial gene such as the ssb can be very useful for understanding regulation of the abovementioned essential DNA metabolic processes, as well as for finding ways of combating bacterial infections.

LECTURE 7 / EFFICIENTLY SEARCHING AND TAXONOMIC IDENTIFICATION OF THE GENOMIC PROKARYOTIC DIVERSITY WITH THE MICROBIAL GENOMES ATLAS (MIGA 1)

Kostas Konstantinidis

BACKGROUND

The prokaryotic diversity captured by genomes has rapidly increased during the past two decades, aided by the development of cheaper sequencing technologies and cultivation-independent genomics. Sequencing data growth has outpaced hardware, so it is imperative to make the prokaryotic genome diversity efficiently searchable and accessible.

AIMS

For that purpose, we built MiGA, a framework for data organization, exploration, quality control, taxonomic identification, and full-set whole-genome comparisons including average nucleotide and amino acid identities (ANI and AAI).

METHODS

We implemented new additions to MiGA, including faster estimators of genome identities, allowing MiGA to scale up to hundreds of thousands of genomes (FastANI and FastAAI), significant improvements of the user interfaces (graphical and command line), internal hooks for the automation of control and maintenance tasks, and extended capabilities for the preprocessing of genomes.

RESULTS

MiGA 1.0 enabled the indexing of large genomic databases (to make them searchable) including GEMS (52k genomes), GTDB-Anchors (32k), our own collection of previously unpublished culture-independent genomes (37k), and TypeMat with available genomes from type material (16k). The MiGA software and databases are available through the Rails web application MiGA-Web for manual curation (http://microbial-genomes.org/) and the Airavata application MiGA@XSEDE for high-throughput querying (https://xsede.microbial-genomes.org). We have expanded the documentation for in-house MiGA deployments and developed ready-to-use Docker and Singularity containers as well as an Amazon Web Services prebuilt machine, all of which allow the quick deployment of MiGA in local or cloud infrastructures and should facilitate research across clinical and environmental settings and education on genomics and taxonomy.

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ORAL POSTER PRESENTATIONS

Oral Poster presentations

Advanced Microbiology Techniques

Mariano Molina / *Netherlands*
In-depth insights into cyanobacterial microbial communities and hrHPV infections using high-resolution microbiome profiling

Djordje Bajic / *United States*
Mechanisms of global epistasis and the predictability of evolution in microbial metabolism

Miguel Carda Diéguez / *Spain*
The tongue biofilm metatranscriptome identifies metabolic pathways associated with halitosis and its prevention

Aleksandar Pavic / *Serbia*
Zebrafish as a model host for Serratia marcescens infection

Antimicrobial Infections

Bar Manon / *Israel*
Random Peptide Mixtures as Safe and Effective Antimicrobials in Mouse Models of Bacteremia and Pneumonia

Andres Corral Lugo / *Spain*
Development of a combined therapy to combat infections caused by multi resistant Acinetobacter baumannii strains

Eloi Littner / *France*
IntegronFinder 2.0: identification and analysis of integrons across bacteria

Gernot Zarfel / *Austria*
Effect of mutations of valine 57 of ribosomal protein S10 on antibiotic resistance, translation and ribosomal biogenesis

Eli Podnar / *Slovenia*
Bacillus subtilis inhibition of Salmonella Typhimurium in mixed species biofilms is bacillaene depended

Anna Greppi / *Switzerland*
Glycerol and Limosilactobacillus reuteri supplementation inhibits AMR strains during in vitro human fermentations

Michael Koller / *Austria*
Monitoring non-wild Type Antibiotic resistant Enterobacteriaceae in the River Danube: a Six-Year-Comparison

Aylin USKUDAR GUCLU / *Turkey*
The Lytic Activity of Various Phage Cocktails against Escherichia coli Isolates Carrying Antibiotic Resistant Gene

Dhrati Patangia / *Ireland*
IMPACT OF EARLY LIFE ANTIBIOTIC EXPOSURE AND DELIVERY MODE ON INFANT MICROBIOME

Svetlana Durica-Mitic / *Germany*
Single-cell resolution of target regulation by peptide nucleic acids

Cristina-Veronica Moldovan / *Romania*
ANTIMICROBIAL ACTIVITY OF SYNTHETIC FLAVONOIDS AGAINST MULTIDRUG RESISTANT BACTERIA

Luka Svet / *Belgium*
Competition between Salmonella and intestinal microbes leads to increased mutation rates and the emergence of resistance

Elitsa Penkova / *United Kingdom*
Context-dependent Fitness Costs of Antibiotic Resistance in Enterobacter cloacae

Milka Malesevic / *Serbia*
Quorum quenching lactonases from Burkholderia cepacia clinical isolate attenuate Pseudomonas aeruginosa quorum sensing and virul

Ashhan ÖZCAN YÖNER / *Turkey*
Screening of FDA-Approved Natural Drugs as Anti-Virulence Agents Against Pseudomonas aeruginosa and Neisseria meningitidis

John Rossen / *United States*
Precision Metagenomics Vs. Shotgun Metagenomics for Common Gram-Negative and Gram-Positive Uropathogens

Biotechnology and Industrial Microbiology

Anush Babayan / *Armenia*
Hydrogen production during glucose and formate utilization in osmotic stressed cells of Escherichia coli at alkaline pH

Angela Matanović / *Croatia*
Gene targeting efficiency in non-conventional yeasts

Meri Iskandaryan / *Armenia*
Hydrogen-oxidizing Hydrogenases activity of Ralstonia eutropha H16 during bacterial growth in organic waste materials

Christopher Riccardi / *Italy*
The robustness of bacterial core metabolism to growth at different temperatures

Jon Pittman / *United Kingdom*
Isolation of fungal strains and secreted enzymes for producing biofuels from microalgal biomass

Alex Lepevie / *Slovenia*
LEGO Microbes: The colloid biology approach to building a microbial community for successful remediation of the environment

Anna Poladyan / *Armenia*
The valorization of whey-based side-streams for microbial biomass, hydrogen and hydrogenase enzyme production

Tomaž Rijavec / *Slovenia*
Microbiological transformation of paper production waste

Fulya AY SAL / *Turkey*
The specificity of ferulic acid esterase from Geobacillus thermoglucosidasius towards model substrates by molecular docking

Alexandre Delarouzée / *France*
Genetic Engineering of Clostridium acetobutylicum for second generation substrates utilization

Lluis Banyeras / *Spain*
Carbonic anhydrase enhances bioelectro CO2 recycling by Clostridium ljungdahlii

Emerging Infections (Fungal, Bacterial, Viral, Parasitic)

Maria Desamparados Ferrer Garcia / *Spain*
Slight effect in reducing the risk of SARS-CoV-2 transmission by oral antiseptics

Mikhail Belousov / *Russian Federation*
OmpC porins of Salmonella enterica and Escherichia coli possess amyloid-forming properties

Carla Hernández-Cabanyero / *Switzerland*
Vibrio vulnificus T6SS exhibits anti-eukaryotic effect but not anti-bacterial properties and contributes to virulence

Branislava Filipić / *Serbia*
ASSESSING VIRULENCE POTENTIAL OF ACINETOBACTER BAUMANNII ISOLATES RECOVERED FROM COVID-19 PATIENTS

Pierre Nottelet / *France*
Structural characterization of a bacterial antibody-degrading system

Andjela Rodic / *Serbia*
Estimating parameter values, analyzing the role, and identifying predictors of the SARS-CoV-2 inherent transmissibility

Adriana Triga / *Greece*
Comparative genomics of Vibrio harveyi strains from marine aquaculture

Anja Vuksan / *Serbia*
Detection of SARS-CoV-2 virus using four different commercial kits without RNA extraction

Özlem Alhan Güncü / *Turkey*
Management of Carbapenem-Resistant Enterobacteriaceae Bacteremia in The Patient with Hematological Malignancies

Jeswin Joseph / *India*
Generation of diverse spike variants of SARS-CoV-2 and to elucidate the role of these mutations in viral pathogenesis

Anush Babayan / *Armenia*
Hydrogen production during glucose and formate utilization in osmotic stressed cells of Escherichia coli at alkaline pH

Sanja Mehandziska / *North Macedonia*
Advantages of molecular methods for infectious diseases in clinical care

Kamila Zdeňková / *Czech Republic*
Wastewater-based monitoring of RNA SARS-CoV-2 in local neighborhoods and selected schools in Prague

Konstantin Ermolenko / *Russian Federation*
Effects of different probiotic strains in treatment of campylobacteriosis in children

Environmental Microbiology

Juan Carlos Cambronero-Heinrichs / *Italy*
Microbes in the gut of triatomine bugs can negatively affect the growth of Trypanosoma cruzi.

Pok Man Leung / *Australia*
Multiple energy sources and metabolic strategies sustain microbial diversity in Antarctic desert soils

Marino Korlević / *Croatia*
Dynamics of microbial assemblages associated with marine macrophyte surfaces

Nikolai Ravin / *Russian Federation*
Diversity and evolution of deep subsurface microorganisms illuminated by metagenomics

Mai-Lan Pham / *Austria*
Monitoring the presence of cyanobacteria in water based on the influences of nutrients to the growth of bacteria

Federica D'Alò / *Italy*
Soil microbial responses to long-term warming experiments in alpine Dryas octopetala heath

Valentin Barberoux / *Belgium*
Spatiotemporal study along the Meuse river reveals that Bacterial diversity and community composition are driven by seasons and distance from the source

Sofija Markovic / *Serbia*
Inferring environmental drivers of COVID-19 severity by machine learning

Camilla Fagorzi / *Italy*
Unraveling the bacterial epigenome and its influence on gene transfer and transcriptional control

Leonor Pizarro / *Portugal*
The effect of cadmium on the N2O reduction potential of model marine bacteria

Elena Perrin / *Italy*
Microbial adaptation to global warming: the model system Pseudoalteromonas haloplanktis TAC125

IFRA FERHEEN / *Italy*
Plastics in aquatic ecosystems: a new route for trafficking multidrug-resistant bacteria?

Maria Bonatelli / *Germany*
How wastewater bacteria learn to feed on previously persistent xenobiotics – the case of acesulfame K

Luis Miguel Rodriguez-R / *Austria*
The SeqCode Registry: A web application to automate and assist the validation of taxon name proposals for all prokaryotes

Erwan Bourdonnais / *France*
Occurrence of potential indicator genes of antimicrobial resistance contamination in the North Sea and English Channel seawaters

Melanie Leopold / *Austria*
A quantitative study concept for investigating the spread of antibiotic resistance in the river water environment

Iris Schachner / *Austria*
Faecal pollution as potential driver of antibiotic resistance genes in the Danube River

Jon Pittman / *United Kingdom*
Adaptation and manipulation of plant-microbial interactions to enhance acid mine drainage bioremediation within natural wetlands

Louis Miguel Rodriguez Rojas /
The SeqCode: a proposed nomenclatural code for uncultivated prokaryotes with DNA sequences as type

László Kredics / *Hungary*
Towards the bacterial biocontrol of weed moulds in mushroom cultivation

Alexander Kirschner / *Austria*
Faecal Pollution and Antibiotic Resistance in the Danube River: Visions, Concepts and Lessons Learned from Joint Danube Surveys

Ioannis Kampouris / *Denmark*
Enhanced in situ oil biodegradation at deep as compared to shallow water depth in a Greenlandic fjord.

Lukas Malfertheiner / *Switzerland*
Reintegrating environmental information into species-level OTU delimitations

Lasse Z. Jensen / *Denmark*
Seasonal Variation of the Atmospheric Bacterial Community in the Greenlandic High Arctic Is Influenced by Weather Events and Local and Distant Sources

Maja Zugan / *Slovenia*
Isolation of oil degrading bacterial consortia by random electrostatic aggregation of cells using microfluidic approach

Josefien Van Landuyt / *Belgium*
Does the Alcanivorax genus play an active role in marine plastic degradation?

Sarah Haenelt / *Germany*
Monitoring the fate of sulfonamide resistance genes and the mobile genetic element intI1 in a waste water impacted river stream

Yekaterina Astafyeva / *Germany*
Microalgae and bacteria interaction – novel insights and applications

Iris Schachner / *Austria*
Faecal pollution as potential driver of antibiotic resistance genes in the Danube River

Rhiannon Mondav / *Sweden*
Taxonomy and metabolism of the global freshwater microbiome

Maria Bôto / *Portugal*
Marine Microorganisms for Bioremediation of Oil Spills: Culture-dependent and Independent Approaches

Mina Bizic / *Germany*
Phytoplankton photosynthesis: an unexplored source of biogenic methane emission from oxic environments

Maria Tomasino / *Italy*
Arctic deep-sea microbial communities as potential resources for Hydrocarbon biodegradation

FEMS-Supported Projects

Vince Chukwu / *United Kingdom*
Modelling the deactivation of Escherichia coli in Nigerian soils amended with differently treated manures

Stoimir Kolarevic / *Serbia*
Comparative in vitro evaluation of genotoxic potential of the Danube River water extracts in prokaryotic and eukaryotic assays

Tim Van Den Bossche / *Belgium*
Founding the Metaproteomics Initiative, driven by the first multi-lab benchmark effort in the field

Fiorella Masotti / *Argentina*
Performance of Pseudomonas putida KT2440 as microbial chassis for phosphonate compounds's degradation

Ildiko Imrefi / *Hungary*
Fluorescent Periconia macrospinosa strains to study hosts preferences and understand ecological functions of root endophyte

Food Microbiology

Hilal Samut / *Turkey*
Assessment of the effect of temperature on the attachment of Escherichia coli O104:H4 to the cress plant

Vita Rozman / *Slovenia*
Contribution of lactic acid bacteria and bifidobacteria to antimicrobial resistance in the agro-food chain

Bernard Gitura / *Hungary*
Natural phenolics as anti-yeast substances: effects on growth and biofilm formation of food spoilage yeasts

Arturo Blazquez Soro / *Ireland*
Application of a free modelling tool to evaluate the effectiveness of an UV-LED device to inactivate Campylobacter jejuni

Maret Andreson / *Estonia*
Isolation and identification of novel non-dairy starter cultures candidates from backslopped plant matrix

Silvestar Mežnarić / *Croatia*
Fir (Abies alba Mill.) honeydew honey and Lactiplantibacillus plantarum–a successful combination against Salmonella enterica ser

Emilia Drozłowska / *Poland*
The development of non-dairy beverage based on black cumin (Nigella sativa) press cake fermented with yogurt culture

Dragoslava Radin / *Serbia*
EFFICIENCY OF COMMERCIAL DISINFECTANTS AGAINST MIXED BACTERIAL AND VIRAL CONTAMINANTS ON FOOD-CONTACT SURFACES

Ainhoa Arrieta-Gisasola / *Spain*
Biofilm formation of Salmonella enterica serovar 4,[5],12:i:- and Pan-Genome-Wide Association Study

Miscellaneous

Gábor Bende / *Hungary*
Potential of Candida albicans to develop resistance to Neosartorya fischeri antifungal protein 2 and its peptide-derivative

Léa THORAVAL / *France*
Evaluation of the ability of C. acnes strains to trigger acute inflammation in bone and joint infection contex

Ersin Gül / *Switzerland*
Involvement of intestinal inflammation and the microbiota in the preservation of Salmonella virulence

Hugo Bisio / *France*
Evolution from icosahedral to amphora shaped giant virions revealed by CRISPR/Cas9

Mauricio Farfan / *Chile*
Correlation between gut microbiota composition and short-chain fatty acid (SCFA) levels in Diarrheogenic E. coli infections

Martina Tambassi / *Italy*
Swine-adapted Salmonella serovars undergo extensive mutations in Salmonella Pathogenicity Island 1 in their adaptation to swine

Elisa Venturini / *Switzerland*
An integrative, genomic- and transcriptomic-driven census of Salmonella small proteins to find new regulators of virulence

Aleksandra Djukić-Vuković / *Serbia*
Understanding and exploiting the impacts of low pH on micro-organisms: a COST Action

Benoit Pugin / *Switzerland*
Unveiling the function and regulation of tryptamine, a gut microbiota-derived neuroactive molecule

David A. Cisneros / *Sweden*
The minor Hcp proteins from the T6SS of Bacteroides fragilis form hierarchical interactions

Maria Tutukina / *Russian Federation*
Antisense transcription: what does it do for bacteria?

Arely Marcos-Vilchis / *Mexico*
On the role of the sorting platform in hierarchical type III secretion

Falk Ponath / *Germany*
New genetic tools enable dissection of a global stress response in the early-branching species Fusobacterium nucleatum

Andrea Colautti / *Italy*
Virulence in Bacillus spp. isolated from an ancient Roman amphora

Plant Microbiology

Shilpi Sharma / *India*
Engineering the microbiome by soil transplantation: Transforming conducive soil to disease suppressive one

Sang-Ah Lee / *Germany*
Growth-acceleration of Haematococcus lacustris by indigenous key-role bacteria identified from the algal phycosphere

Max Kolton / *Israel*
Peatmoss microbiome: the power of the obligatory methanotrophic nitrogen-fixing minority.

254 / IN-DEPTH INSIGHTS INTO CERVICOVAGINAL MICROBIAL COMMUNITIES AND HRHPV INFECTIONS USING HIGH-RESOLUTION MICROBIOME PROFILING

Keywords: *Vaginal microbiome, Human papillomavirus, Community state types, CiRNAseq*

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BACKGROUND

The cervicovaginal microbiome (CVM) correlates with women's cervical health, and variations in its structure are associated with high-risk human papillomavirus (hrHPV)-induced high-grade cervical lesions. The CVM exhibits five community state types (CSTs) based on microbial dominance and composition; however, elucidating the impact of CSTs in health and disease is challenging because current sequencing technologies have limited confident discrimination between bacterial species that shape microbial communities.

OBJECTIVES

This study aimed to apply high-resolution microbiome profiling to obtain in-depth and unambiguous insights into the composition of the CVM.

METHODS

Circular probe-based RNA sequencing (ciRNAseq) was used to profile the CVM of a cohort of healthy women from the Dutch population-based screening program (n = 341) and a second cohort of hrHPV positive women with known clinical outcomes (n = 300). CSTs were established and correlated to clinical outcomes.

RESULTS

We defined subgroups of CSTs I, III, and IV, which display dominance by L. crispatus, L. iners, and high diversity, respectively, in the CVM. We discovered that these microbial states strongly depend on the bacterial species L. acidophilus, which is highly abundant within CSTs I-B and III-B, L. iners, which has high abundance within CSTs I-B and III-A, and M. genomosp type 1, which is dominant in CST IV-B. Notably, CST I-A associated with uninfected conditions, while CST IV-A with hrHPV infections and cervical disease. In conclusion, we corroborated and further characterized new subdivisions of cervicovaginal CSTs, which may further advance our understanding of women's cervical health and hrHPV-related progression to disease.

ACKNOWLEDGEMENTS/REFERENCES

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375 / MECHANISMS OF GLOBAL EPISTASIS AND THE PREDICTABILITY OF EVOLUTION IN MICROBIAL METABOLISM

Keywords: *Genome-scale metabolic modeling, Global epistasis, Metabolism, Evolution*

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BACKGROUND

The topology of fitness landscapes is a fundamental determinant of evolutionary trajectories and outcomes. A key property observed across many empirical fitness landscapes is global epistasis, whose hallmark is the systematic scaling of mutation fitness effects with background fitness. Currently, the strength and distribution of global epistasis among loci and its mechanistic origins remain poorly understood. We do not know, for instance, which genes should be expected to exhibit strong or weak global epistasis, and why. The main reason is the combinatorial difficulty in examining meaningfully large regions of genotype space in an exhaustive manner.

OBJECTIVES

In this work, we aimed to characterize patterns and mechanisms of global epistasis in microbial metabolism.

METHODS

To do this, we used constraint-based genome-scale metabolic models, a technique that offers a biologically realistic way to explore a large-scale, empirically calibrated fitness landscape. Our landscape included the fitness of hundreds of thousands of genotypes, defined by the presence or absence of specific reactions, in several nutrient environments.

RESULTS

Using this model, we were able to reproduce known patterns of global epistasis and illustrate their mechanistic basis. We show that metabolic reactions that are key for growth in a given environment typically exhibit global epistasis patterns. These reactions are able to bring many different genotypes close to the optimum (the fitness peak). This implies that their evolution should be predictable, which we show using evolutionary simulations. Our results have implications for our understanding of global epistasis and, more generally, the predictability of evolution.

410 / THE TONGUE BIOFILM METATRANSCRIPTOME IDENTIFIES METABOLIC PATHWAYS ASSOCIATED WITH HALITOSIS AND ITS PREVENTIONM

Keywords: *microbiota, metatranscriptome, tongue, halitosis*

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BACKGROUND

The oral malodour or halitosis is a common condition in modern society which is caused by the production of volatile sulfur compounds (VSCs) by tongue-associated microbiota. Fusobacterium, Prevotella, Leptotrichia are known VSCs producers that have been previously shown in higher concentration in tongue of individuals diagnosed with halitosis. Studies in vitro have proven that these microbes degrade amino acids to produce VSCs. However, there are no reports of which genes are these bacteria expressing in vivo in the tongue of halitosis patients.

OBJECTIVES

The aim of this metatranscriptomic study was to understand which bacteria and metabolic pathways were actively responsible for VSCs formation. In addition, potential bacteria and pathways were evaluated as halitosis-free biomarkers.

METHODS

The concentration of VSCs in the breath of 40 individuals was measured and the total RNA in the tongue-associated biofilm was sequenced using NextSeq Illumina technology.

RESULTS

The activity of Streptococcus (including S. parasanguinis), Veillonella (including V. dispar) and Rothia (including R. mucilaginosa) was associated with halitosis-free individuals while Prevotella (including P. shahi), Fusobacterium (including F. nucleatum) and Leptotrichia were associated with halitosis. Additionally, gene expression profiles showed a significant over-expression of genes involved in L-cysteine and L-homocysteine synthesis, as well as nitrate reduction genes, in halitosis-free individuals; and an over-expression of genes responsible for cysteine degradation into hydrogen sulfide in halitosis patients. This is the first confirmation that specific microbes are actively degrading amino acids and producing VSCs whereas nitrate reduction is significantly reduced in the tongue of individuals with halitosis.

540 / ZEBRAFISH AS A MODEL HOST FOR SERRATIA MARCESCENS INFECTION

Keywords: *zebrafish, Serratia marcescens, infection, nan*

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BACKGROUND

Once considered as an innocuous saprophyte, Serratia marcescens is now recognized as an important multi-resistant opportunistic pathogen that poses a risk of healthcare-associated infections. S. marcescens causes nosocomial infections in intensive care and pediatric units, including pneumonia, meningitis, and endocarditis as well as infections of the urinary tract, wounds and soft tissues.

OBJECTIVES

To establish zebrafish (Danio rerio) embryo model of infection with S. marcescens in order to enable assessment of novel drugs efficacy, other Serratia strains virulence, and mechanisms of host-bacteria interactions.

METHODS

Wild type zebrafish embryos at the stage of 26 hours post-fertilization were exposed to different doses of cells of S. marcescens reference or clinical strains. In order to establish systemic and localized infections, bacteria were administered through various routes (circulation, hindbrain, yolk). Survival, inner organ colonization and neutrophil chemotaxis in infected embryos together with bacterial burden and dissemination were assessed over the period of four days. To validate the newly established model of infection, S. marcescens-infected embryos were treated with four antibiotics (linezolid, meropenem, penicillin, gentamicin) and assessed for survival and infection eradication.

RESULTS

Both S. marcescens strains were capable of causing systemic and localized infection in zebrafish embryos. The infective doses leading to the mortality of 50% of embryos have been determined. Bacterial cells injected into circulation caused a much higher mortality rate than ones injected locally into the hindbrain. Meropenem and gentamicin successfully rescued zebrafish embryos of lethal bacterial infection, while linezolid and penicillin were ineffective.

247 / RANDOM PEPTIDE MIXTURES AS SAFE AND EFFECTIVE ANTIMICROBIALS IN MOUSE MODELS OF BACTEREMIA AND PNEUMONIA

Keywords: *Antimicrobial peptide, infectious disease, nan, nan*

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Antibiotic resistance is a daunting challenge in modern medicine, and novel approaches that minimize the emergence of resistant pathogens are urgently needed. Antimicrobial peptides are newer therapeutics that attempt to do this; however, they fall short because of low-moderate antimicrobial activity, low protease stability, susceptibility to resistance development and high cost of production. The recently developed random peptide mixtures (RPMs) are promising alternatives. RPMs are synthesized by incorporating a defined proportion of two amino acids at each coupling step rather than just one, making them highly variable but still defined in their overall composition, chain length and stereochemistry. Because of RPMs' extreme diversity, it is unlikely that bacteria would be capable of rapidly evolving resistance. However, their efficacy against pathogens in animal models of human infectious diseases remained uncharacterized. Recently, we have demonstrated that RPMs have strong safety and pharmacokinetic profiles. RPMs rapidly killed both Pseudomonas aeruginosa and Staphylococcus aureus efficiently, and disrupted preformed biofilms by both pathogens. Importantly, RPMs were efficacious against both pathogens in mouse models of bacteremia and acute pneumonia. Our results demonstrate RPMs are potent broad-spectrum therapeutics against antibiotic-resistant pathogens.

ACKNOWLEDGEMENTS/REFERENCES

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422 / DEVELOPMENT OF A COMBINED THERAPY TO COMBAT INFECTIONS CAUSED BY MULTI RESISTANT ACINETOBACTER BAUMANNII STRAINS

Keywords: *Acientobacter buamannii, combination therapy , LOS inhibitors, nan*

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Acinetobacter baumannii is a gram-negative bacterium that causes serious nosocomial infections. Due to their broad-spectrum resistance to antibiotics, it has been categorized by the WHO within the pathogens for which it is essential to research and develop new therapies (1). To address the problem, combination therapy has shown good results, even when one of the antibiotics has lost its effectiveness. A. baumannii has the ability to survive without LOS, these cells are characterized to be more permeable to small molecules, and have greater sensitivity to compounds to which they were resistant before losing LOS, including: detergents (deoxycholate), antibiotics (rifampicin, vancomycin, and azithromycin), and iron chelators (BIP, DFP)(2-4). Considering this sensitivity, in the present work we use A. baumannii cells with and without LOS as a study model. Bacterial growth was screened in the presence of a 1520 compounds for medical use (Prestwick Chemical Library®). We select several compounds with antimicrobial effect in A. baumannii without LOS, among which are antibiotics, antifungals, antianginal and antihyperparathyroid. Newly determined antimicrobial compounds were evaluated in vitro in monotherapy and combined therapy against A. baumannii, using LOS inhibitors, LpxC4 and Chir-90 (4). To determine the optimal concentration of the two compounds in the combined therapy, the checkerboard methodology, were performed. While to see the effect on growth, lethality and death curves in monotherapy and combined therapy, were developed. The results obtained may be used to support and carry out preclinical trials in the future, as well as to combat multi-resistant A. baumannii infections.

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469 / INTEGRONFINDER 2.0: IDENTIFICATION AND ANALYSIS OF INTEGRONS ACROSS BACTERIA

Keywords: *integron, bioinformatics, genomics, AMR*

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Integrons are flexible gene-exchanging platforms comprising an integrase followed by a series of gene arrays called cassettes. Through recombination, the integrase mediates cassette excision and integration, enabling a dynamic shuffling. Integrons were first identified as embedded in mobile genetic elements, playing a key role in the dissemination of antibiotic resistance genes. The later discovery of sedentary chromosomal integrons, containing up to several hundred cassettes, often with unknown function, shed light on the complex evolutionary processes involving integrons.

IntegronFinder is a widely used software that can quickly identify mobile as well as sedentary chromosomal integrons in bacterial genomes. Here, we present the second version of IntegronFinder, developed specifically to handle draft genomes and metagenomes and showing improved efficiency and usability. We describe the consequent changes and illustrate the potential of the new version by studying the distribution of integrons across bacterial genomes and in several thousand genomes of Klebsiella pneumoniae, an important nosocomial pathogen. Our results further reveal the evolutionary mechanisms at work in mobile and sedentary integrons.

516 / EFFECT OF MUTATIONS OF VALINE 57 OF RIBOSOMAL PROTEIN S10 ON ANTIBIOTIC RESISTANCE, TRANSLATION AND RIBOSOMAL BIOGENESIS

Keywords: *Escherichia coli , tetracyclin, ribosomal biogenesis, nan*

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Bacterial ribosomal protein S10 and the eukaryotic homologue Rps20 in have a conserved internal loop that contacts helix 31 of the small ribosomal subunit rRNA (16S rRNA in bacteria). Mutations in this loop lead to a reduced sensitivity to tetracyclins. On the other hand, the eukaryotic Rps20 internal loop plays an important role in ribosome biogenesis. Up to now it remains elusive whether the S10 internal loop of bacteria plays a similar role in bacterial ribosome biogenesis. To address this question, Escherichia coli S10 was mutated at the tip of the loop (position V57) to investigate possible effects on translation, ribosome biogenesis and antibiotic resistance and to show possible links between these effects.

Nine (deletion of valine 57, exchange for alanine, glycine, lysine, arginine, leucine, isoleucine, aspartic acid and asparagine) different exchanges were made at position 57. The mutations were tested for their susceptibility to various antibiotics using microdilution and Etests. Growth was evaluated at 25°C, 37°C and 42°C. Translation and ribosome biogenesis defects were investigated by polysome profiles and northern blotting.

The results showed that most exchanges affected the sensitivity to tetracyclins. Also, all tested mutants showed a ribosome biogenesis defect as well as slight translation defects.

Our results strongly suggest that besides its influence on tetracycline sensitivity, the bacterial S10 loop also plays an important role in bacterial ribosome biogenesis and translation.

522 / BACILLUS SUBTILIS INHIBITION OF SALMONELLA TYPHIMURIUM IN MIXED SPECIES BIOFILMS IS BACILLAENE DEPENDED

Keywords: *biofilm, probiotic, pathogen , nan*

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Biofilms represent a prevalent lifestyle of bacteria, which provide protection against a variety of stresses, including antibiotics. Salmonella enterica is one of the most common foodborne pathogens with biofilm formation as an important virulence factor. Probiotic bacteria, such as B. subtilis, present a promising strategy to limit Salmonella infections and thus reduce the potential spread of antimicrobial resistance.

To address interactions between probiotic B. subtilis PS-216 and pathogenic S. Typhimurium SL1344 in static biofilms we estimated fitness change of S. Typhimurium and B. subtilis WT strain or its mutants impaired in antimicrobial synthesis or biofilm formation by determining the colony forming units. We also studied spatial distribution of the two species in a biofilm by confocal microscopy and indirectly monitored the production of antibiotics produced by B. subtilis.

The results showed that B. subtilis PS-216 inhibited the growth and adhesion of S. Typhimurium and reduced the pathogen's biofilm thickness. The antagonistic potential of B. subtilis was dependent on the synthesis of the polyketide antibiotic bacillaene. The activity of PpksC promoter, which controls bacillaene production, was strongly induced in the pairwise interactions, and low in nutrient poor condition. However, ΔepsA-O mutant, that does not produce polysaccharide component of biofilm matrix, has lost responsiveness of PpksC to low nutrients and the pathogen, implying the regulatory role of this polysaccharide in bacillaene synthesis. Overall, this work reveals the mechanism of B. subtilis and S. Typhimurium interactions in biofilms and draws attention to the potential use of B. subtilis PS-216 as a probiotic.

ACKNOWLEDGEMENTS/REFERENCES

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582 / GLYCEROL AND LIMOSILACTOBACILLUS REUTERI SUPPLEMENTATION INHIBITS AMR STRAINS DURING IN VITRO HUMAN FERMENTATIONS

Keywords: *glycerol, antimicrobial resistance, Limosilactobacillus reuteri , reuterin*

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Via consumption of contaminated food, antimicrobial resistance (AMR) genes can be transferred to bacteria inhabiting the dense gut microbial ecosystem. To decrease AMR occurrence in the human gut, a novel microbial approach based on in situ reuterin production, a broad spectrum antimicrobial produced from glycerol by Limosilactobacillus reuteri, was tested.

Anaerobic batch fermentations were conducted for 24 h at 37°C in McFarlane medium. Fermentations were inoculated with 1% artificial microbiota from a continuous PolyFermS model, and with or w/o: 107 CFU mL-1 VRE Enterococcus faecium CCUG59168 (vanA) and ESBL Escherichia coli HV292.1 (blaCTX-M-1) AMR strains, L. reuteri SD2212 (reuterin producer) or PTA8-11 (non-reuterin producer), 100 mM glycerol. Strains were enumerated on KFS (+ 6 µg µL-1 vancomycin) and MacConkey (+ 10 µg µL-1 cefotaxime) plates and qPCR. 16S rRNA sequencing and HPLC used to asses microbial composition and activity.

A significant growth inhibition (- 3 Log) of HV292.1 and CCUG59168 was observed after 24 h in presence of SD2112 and glycerol, compared to w/o glycerol, and associated with in situ reuterin production (7.4 ± 0.6 mM 3HPA and 1.0 ± 0.0 µM acrolein). In the same condition, a decrease of relative abundance of Bifidobacteriaceae (- 4 %), Coriobacteriaceae (- 2 %), Enterobacteriaceae (- 2 %), and Veillonellaceae (- 3 %), and a significant reduction of acetate (- 16.8 mM), butyrate (- 8 mM), propionate (- 20 mM) and formate (-3 mM) were measured. Reuterin appeared a promising antimicrobial to inhibit AMR E. faecium and E.coli strains in the gut.

636 / MONITORING NON-WILD TYPE ANTIBIOTIC RESISTANT ENTEROBACTERIACEAE IN THE RIVER DANUBE: A SIX-YEAR-COMPARISONM

Keywords: *Enterobacteriaceae, Danube, Antimicrobial Resistance, nan*

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Human induced antibiotic resistant bacteria (ARB) are not only found in clinical surroundings: large rivers are of great concern as regards their spreading. This ongoing study´s aim is to analyse the major propagation pathways and sources of ARB in the Danube, and to compare the results with data obtained in 2013. Escherichia coli and Klebsiella spp. isolated during the 4th Joint Danube Survey were tested for their antibiotic susceptibility. 23.2% of 1635 E. coli were resistant and 11.7% were multiresistant. There was a significant increase in resistances to augmentin, moxifloxacin and piperacillin/tazobactam and a significant decrease regarding tetracycline. 23 expressed an extended-spectrum-beta-lactamase phenotype. 15.87 % of 630 Klebsiella spp. were resistant and 0.95% were multiresistant. Resistances to moxifloxacin and augmentin were the most common resistances detected.

Two isolates showed an ESBL-phenotype, and two isolates were resistant to the last line antibiotic tigecycline. In comparison the data of 2013 and 2019 show a similar proportion for multiresistant E. coli, but regarding resistances to some single antibiotics significant changes were determined. In contrast, the Klebsiella isolates showed a very similar distribution of resistances. Despite, 30% of non-wild type resistant E. coli in the environment is a very high number when compared to clinical settings with approximately 50%. However, the relevance of these findings concerning human health and the environment is yet unclear as there is only a very limited number of studies for comparison and studies on the significance of pathways from the river back to humans are largely missing.

648 / COMPARISON OF THE LYTIC ACTIVITY OF VARIOUS PHAGE COCKTAILS AGAINST ESCHERICHIA COLI ISOLATES CARRYING ANTIBIOTIC RESISTANT GENE

Keywords: *bacteriophage cocktails, Escherichia coli, carbapenem resistant, ESBL*

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BACKGROUND

Bacteriophages, bacterial viruses infecting and replicating in bacteria, have been proposed as an alternative therapy for the treatment of infections caused by antibiotic-resistant bacteria.

OBJECTIVES

Differences in lytic activity of commercially available bacteriophage cocktails against ESBL-positive, ESBL-negative and carbapenem-resistant Escherichia coli (CRE) were evaluated.

METHODS

A total of 88 clinical E.coli isolates were included in the study. ESBL and carbapenemase encoding genes CTX-M, SHV, TEM, OXA-48, KPC, and NDM were detected by PCR. The activity of bacteriophage cocktails (ENKO, SES, INTESTI, PYOphage George Eliava Institute) against E.coli were detected by spot test. Lysis zones were evaluated from confluent to opaque lysis, in which lysis zones contain many bacteria. Bacteriophages with confluent and opaque lysis zone were purified separately with different strains of E.coli, and optimum multiplicity of infection (MOI) was calculated for each. Phage stability in various pH (2-8) and temperature (10-80°C) were evaluated.

RESULTS

The susceptibility of CRE carrying OXA-48 gene to ENKO, SES, INTESTI, and PYO cocktails were 21%, 26.3%, 10.5%, and 5.3%; while the susceptibility of ESBL-positive (CTX-M) E.coli were 33.3%, 36.4%, 24.2% and 12.1%, respectively. The susceptibility of CRE for all tested cocktails was lower than those calculated for ESBL-negative and ESBL-positive strains. One-step growth curve revealed that the latent period was 25 min, and the burst size was 286 PFU/cell. MOI for the phage with confluent lysis zone was calculated as 10, while with opaque lysis zone was 0.01. The tested phage was thermally stable and tolerant to a wide pH range.

ACKNOWLEDGEMENTS/REFERENCES

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866 / IMPACT OF EARLY LIFE ANTIBIOTIC EXPOSURE AND DELIVERY MODE ON INFANT MICROBIOME

Keywords: *Microbiome, resistome, early-life, metagenomics*

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BACKGROUND

Infant gut microbiota is strongly influenced by delivery mode and uptake of antibiotics. Infant gut is a reservoir of antibiotic resistance genes (ARGs).

OBJECTIVE

The longitudinal impact of antibiotic uptake in early life together with mode of delivery on infant gut microbiota is not extensively studied. Given the high abundance of ARGs, longitudinal studies are needed to understand the impact of these factors and long term presence of ARGs on functionality of the microbiota.

METHODS

Based on shotgun metagenomics, we compared gut microbiota and resistome in early life of infants divided into 3 groups based on mode of delivery and antibiotic exposure: vaginal delivery/no antibiotic (VG-noab); C-Section/no antibiotic (CS-noab); C-Section/antibiotic (CS-ab). Using read and assembly based approaches, we analysed 208 samples from 45 infants collected over week 1, 4, 8, 24, year 2.

RESULTS

Gentamicin and benzylpenicillin were the most commonly administered antibiotics at week 1. New-born gut microbial composition differed in all 3 groups with higher diversity and stable composition seen at year 2. CS-ab group had low microbial diversity during first week of life. Alpha diversity is similar across the 3 groups in all time points after week 1, while beta diversity is significantly different during the first 3 timepoints. Overall relative abundance of Actinobacteria and Bacteroidetes was significantly higher in VD-noab while Proteobacteria was higher in CS-ab. ARGs associated with beta lactams and aminoglycosides were found to be highest in the genus Klebsiella and Escherichia in all 3 groups during first 2 weeks of age.

ACKNOWLEDGEMENTS/REFERENCES

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883 / SINGLE-CELL RESOLUTION OF TARGET REGULATION BY PEPTIDE NUCLEIC ACIDS

Keywords: *Peptide nucleic acid (PNA), Species-specific antibiotics, nan, nan*

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Targeted manipulation of complex microbial communities may be accomplished by species-specific antisense oligomers such as peptide nucleic acid (PNA) directed against mRNAs of essential genes, and coupling them to cell penetrating peptides (CPPs) to allow their cellular uptake. Despite the growing number of CPP-PNA conjugates that efficiently silence their target genes, fundamental insights into their mechanism of action are lacking. For example, it is unknown whether one PNA can regulate multiple mRNAs in the cell. Should this be the case, designing PNAs with dual binding sites may boost their efficiency. To probe the potential of PNAs for multiple targeting, we have utilized fluorescent reporters that provide single-cell resolution of target regulation. We first show that PNAs are able to repress chromosomally- and plasmid-encoded fluorescent reporters in a sequence-specific manner. Next, we established a dual-reporter system consisting of chromosomal superfolder GFP and plasmid-derived mCherry fused to the same target sequence in E. coli.

Flow cytometry analysis combined with confocal microscopy demonstrated that a PNA complementary to the target sequence effectively represses both reporters in individual cells. To follow up, we designed PNAs with a target sequence shared between mRNAs of two essential genes and found that they can efficiently induce cell death in treated bacteria. Subsequent whole transcriptome analysis confirmed that the levels of both targeted transcripts are significantly decreased upon PNA treatment. Taken together, these results reveal that a single PNA can regulate multiple cellular targets simultaneously – a feature that can be exploited to increase the PNA treatment efficiency.

942 / ANTIMICROBIAL ACTIVITY OF SYNTHETIC FLAVONOIDS AGAINST MULTIDRUG RESISTANT BACTERIA

Keywords: *synthetic flavonoid, antibacterial activity, antibiotic resistance, MDR strains*

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According to the World Health Organization, antibiotic resistance represents one of the biggest threats to global health. Because the number of new efficient antibiotics development decreased in the last years, several alternative solutions are being explored to fight antibiotic resistance. For this purpose, a potential solution would be the use of flavonoids, well known for their antimicrobial properties.

In this context, we investigated the antimicrobial activity of a new tricyclic flavonoid with bromine and chlorine as halogen substituents (Br-Cl), against several multidrug resistant (MDR) bacterial strains. The antibacterial activity was assessed using minimum inhibitory and minimum bactericidal concentrations (MIC/MBC). The influence of Br-Cl flavonoid on cell viability was also assessed. The mechanism of action was investigated using anti-biofilm formation ability and cell membrane permeability tests as well. Our results revealed a high antibacterial activity of Br-Cl flavonoid against Staphylococcus aureus and Escherichia coli clinical isolates (MICs values of 0.48 µg mL-1, 1.95 µg mL-1 and 3.9 µg mL-1 respectively). In the presence of flavonoid at MBC concentrations, Acinetobacter baumannii and S. aureus cells lost viability within only 0.5 and 2 hours respectively. The mechanism of action is related to impairment of cell membrane integrity.

Our results suggests that Br-Cl flavonoid has a very good antibacterial activity against different MDR strains representing a reliable alternative for fighting antibiotic resistance phenomenon.

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1045 / COMPETITION BETWEEN SALMONELLA AND INTESTINAL MICROBES LEADS TO INCREASED MUTATION RATES AND THE EMERGENCE OF RESISTANCE

Keywords: *antimicrobial resistance, mutation rate, competitive interactions, biofilms*

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Social interactions are predicted to influence the emergence and spread of resistant strains in microbial communities, with the role of competitive interactions presumed as highly prominent. Nevertheless, the knowledge about the impact of such interactions on the evolution of antimicrobial resistance (AMR) is limited, particularly regarding biofilms (habitats microbes commonly inhabit and where competition prevails).

Overall, our study aims to understand how competitive interactions affect the emergence of resistance in biofilms and deliver novel approaches that interfere with these interactions in microbial communities in order to halt the development of AMR.

We chose to employ the principal food-borne pathogen Salmonella Typhimurium as a model organism since it forms biofilms both in the environment and in the host and encounters and competes with various intestinal species. Moreover, the need for novel antimicrobial strategies is high as multiple drug resistance is a major concern for this pathogen.

After selecting the relevant microbial species exhibiting different levels of competition with Salmonella, we proceeded with studying the impact of these interactions on the development of AMR via competition experiments, microscopy, omics approaches to measure the activity of stress-response systems, fluctuation tests, experimental evolution and mathematical modelling.

Preliminary results indicate that competition profoundly influenced microbial physiology which in turn led to significantly increased mutation rates in Salmonella. This enhanced the probability for resistance development and resulted in a heightened number of new mutants resistant to treatment compared to communities where competition was weaker.

The all-encompassing AMR crisis therefore undeniably warrants further research into competitive interactions.

ACKNOWLEDGEMENTS/REFERENCES

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The figure was created with BioRender.com.

1100 / CONTEXT-DEPENDENT FITNESS COSTS OF ANTIBIOTIC RESISTANCE IN ENTEROBACTER CLOACAE

Keywords: *fitness costs, antibiotic resistance, nan, nan*

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The emergence and spread of antibiotic resistance mechanisms is a growing crisis globally [1]. The fitness cost of antibiotic resistance is considered an important parameter in evaluating the clinical relevance and capacity for persistence of resistant bacterial infections [2]. Costs are most commonly reported as growth rates or relative fitness in direct competition, quantified in vitro [3]. These measurements, however, ignore environmental factors that could further affect bacterial fitness within a live infection, such as population size, colonisation rate and shedding, or host defences [3, 4]. To bridge this gap, this study created a library of characterised Enterobacter cloacae mutants, resistant to either cefotaxime, nalidixic acid or rifampicin. Relative fitness in competition, was assessed both in vitro and in vivo, using a Lepidopteran insect model. Genotypes with varying competitiveness within and between the two environments were selected for further work, aiming to assess each genotype's potential for horizontal transmission in vivo. This is currently being quantified by determining the size of colonising populations within the gut of infected hosts, bacterial shedding rate, as well as direct transmission from infected to susceptible hosts. Results so far indicate competitive fitness can be environment-dependent, and increased in vivo but may be a poor predictor of bacterial transmission. This work highlights that fitness costs in competition may not be a reliable basis for antimicrobial resistance management.

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1173 / QUORUM QUENCHING LACTONASES FROM BURKHOLDERIA CEPACIA CLINICAL ISOLATE ATTENUATE PSEUDOMONAS AERUGINOSA QUORUM SENSING AND VIRUL

Keywords: *quorum quenching, AHL lactonase, Burkholderia cepacia, Pseudomonas aeruginosa*

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BACKGROUND

Pseudomonas aeruginosa, one of the most significant opportunistic pathogens, uses quorum sensing (QS) system to regulate virulence factors expression, biofilm development and interaction with hosts. Silencing of QS represents one of the most promising strategies for antivirulence therapy.

OBJECTIVES

Main aim of our study was the identification and functional characterization of quorum quenching (QQ) enzymes from Burkholderia sp. BCC4135 clinical isolate which shares the same ecological niche with P. aeruginosa during infections.

METHODS

Burkholderia sp. BCC4135 was selected using AHL biosensor strain Chromobacterium violaceum 026. Whole genome sequencing of BCC4135 was performed using Illumina HiSeq platform. Cloning of candidate QQ gene, expression, and purification was performed using pQE30 expression system. Potential of BCC4135 lactonases for degradation of N-acyl-homoserine lactones (AHLs) was investigated by HPLC analysis. Functional characterization of QQ enzymes on P. aeruginosa MMA83 clinical isolate was done monitoring their effect on biofilm formation and virulence factors production.

RESULTS

Burkholderia sp. BCC4135 displayed strong QQ activity. Whole genome sequencing unveiled this isolate as B. cepacia with unique sequence type ST1485 and genes belonging to resistome and virulome. QQ lactonases YtnP and Y2-aiiA were active against a broad substrate spectrum of AHLs. Both enzymes were able to attenuate virulence potential of P. aeruginosa MMA83 declining its biofilm formation and virulence factors production. BCC4135 lactonases interfered with the las, rhl, and pqs QS circuit of MMA83 transcription and the effect of combined enzymes was even more prominent.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (No. 451-03-68/2020-14/200042).

1196 / SCREENING OF FDA-APPROVED NATURAL DRUGS AS ANTI-VIRULENCE AGENTS AGAINST PSEUDOMONAS AERUGINOSA AND NEISSERIA MENINGITIDIS

Keywords: *Anti-virulence therapy, Type 4 pili, Inhibitor, nan*

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BACKGROUND

Antimicrobial resistance is a major health concern that poses a serious threat to the prevention and treatment of an ever-growing range of infections caused by bacteria, pathogens, viruses, and fungi that are no longer susceptible to available drugs. Anti-virulence therapy emerges as an alternative to conventional drug treatment. In this respect, type 4 pili may be regarded as attractive targets since they are known to play a key role in the virulence of a variety of bacterial diseases. Type 4 pili are polymerized or depolymerized by ATPases to facilitate fiber extension and retraction, respectively.

OBJECTIVES

Our aim was to find common inhibitors for the elongation ATPases of the type 4 pili of Pseudomonas aeruginosa and Neisseria meningitidis. These microorganisms are listed by WHO as pathogens for which new therapeutics are urgently needed.

METHODS

Homology models of the two proteins from P. aeruginosa and N. meningitidis were constructed and their binding pockets were selected as inhibitor targets. FDA-approved natural compounds were then screened for their binding to these ATPase. The dynamics of the binding of the identified inhibitor candidates were investigated using molecular dynamics simulations.

RESULTS

13 common inhibitors were selected from the list of ligands with binding affinities less than -8.0 kcal/mol for two ATPases. Among these, reserpine and glyburide were further experimentally investigated for their inhibitory potential. The results of this work will guide anti-virulence approaches in the fight against infectious diseases.

ACKNOWLEDGEMENTS/REFERENCES

This work has been supported by the Scientific and Technological Research Council of Turkey (Project no: 120M225).

1661 / PRECISION METAGENOMICS VS. SHOTGUN METAGENOMICS FOR COMMON GRAM-NEGATIVE AND GRAM-POSITIVE UROPATHOGENS

Keywords: *Precision Metagenomics, urinary tract infections, nan, nan*

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BACKGROUND

Recurrent urinary tract infections are difficult to treat and decrease quality of life. Improved methods of detection, treatment and prevention are needed.

OBJECTIVES

Compare shotgun metagenomics (SM) and target-enriched Precision Metagenomics (PM) to detect eight common bacterial causes of UTI.

METHODS

Urine cultures with growth ≥ 10,000 CFU for eight common uropathogens were studied. DNA was extracted (Quick-DNA Urine Kit, Zymo), for targeted metagenomics, libraries were enriched in 3-plex with the research use Urinary Pathogen ID/AMR panel (UPIP, IDbyDNA), sequenced (1x147bp, NextSeq550, Illumina) to a depth of 10 M (SM) and 1 M (UPIP) reads/sample, and automated data analysis performed with research use Explify® (IDbyDNA). UPIP detects, quantifies and genotypically characterizes >170 uropathogens and >2,000 AMR markers.

RESULTS

Of 355 total urine specimens selected, 180 (51%) were culture-positive, and 175 (49%) were culture-negative for the eight targeted pathogens. Of culture-positive specimens, 178/180 (99%) and 173/180 (96%) were PM-positive, and SM-positive, for the eight targeted pathogens, respectively. Of culture-negative specimens, 90/175 (51%) and 56/175 (32%) were PM-positive and SM-positive for the eight targeted pathogens, respectively. Of specimens that were both culture-positive and PM positive for the eight targeted pathogens, PM detected 3 Klebsiella pneumoniae, 1 Proteus mirabilis, 1 Pseudomonas aeruginosa, 5 E. faecalis, 1 E. faecium, 2 S. aureus, and 2 S. saprophyticus that SM did not detect. UPIP precision metagenomic testing improves detection of common Gram-negative and Gram-positive causes of UTIs compared to a shotgun metagenomic workflow.

ACKNOWLEDGEMENTS/REFERENCES

We are grateful to the entire Microbiology Unit at PathGroup Labs for coordination of sample selection, de-identification and shipment, and to Illumina for reagents for library preparation and enrichment in support of this study. We also thank the IDbyDNA Laboratory, R&D, MSA and Bioinformatics Teams.

543 / HYDROGEN PRODUCTION DURING GLUCOSE AND FORMATE UTILIZATION IN OSMOTIC STRESSED CELLS OF ESCHERICHIA COLI AT ALKALINE PH

Keywords: *osmotic stress, hydrogen production , Escherichia coli , nan*

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Studies on the production of molecular hydrogen (H2), as an alternative energy source, are currently ongoing. The production of H2 during glucose and formate fermentation was studied in E. coli BW25113 and the single and double mutants lacking the formate transporter(s). Bacteria were exposed to hyper-osmotic stress with 0.8 M sucrose and were compared with values obtained from stress-free experiments.

H2 production assays were done by using redox potential determination via pair of titanium-silicate (Ti-Si) and platinum (Pt) redox electrodes. H2 production rate (VH2) was calculated as the difference between the initial decreased in readings of the Pt- and Ti-Si-electrodes over time.

Without hyper-stress when glucose was added to the assays focA mutant showed no significant effect on H2 production, compared to wild type, but VH2 was inhibited in focB ~ 1.5 fold. Analysis of the focA-focB essentially confirmed the data obtained for the focB single mutant, suggesting when glucose was added in the assays focB acted to import formic acid at alkaline pH. Meanwhile, without hyper-stress in the formate assays no significant difference was observed in the mutants, compared to wild type. Interestingly, in osmotic stressed focA H2 production decreased ˜1.5 fold compared to wild type, when formate was added as exogenous electron source, while no effect of focB was noted. These data suggest that addition of formate under hyper-osmotic stress focA mainly imports the acid.

These findings are significant for understanding the mechanisms of cell osmoregulation in application for developing H2 production technology.

ACKNOWLEDGEMENTS/REFERENCES

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Ministry of Education, Science, Culture and Sports of Armenia to AP (21AG-1F043).

555 / GENE TARGETING EFFICIENCY IN NON-CONVENTIONAL YEASTS

Keywords: *gene targeting, non-conventional yeasts, transformation, homologous recombination*

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Gene targeting is a genetic technique that allows the modification of an endogenous gene by homologous recombination. It is achieved by the introduction of linear, non-replicative DNA fragments that should replace the homologous (targeted) sequence in the genome. It is generally assumed that gene targeting in the conventional yeast Saccharomyces cerevisiae almost exclusively occurs by homologous recombination and that it is usually higher than 90 %. Non-conventional yeast species are isolated from different niches and have interesting properties such as utilization of different carbon sources, fermentation potential, tolerance to different growth inhibitors and they are emerging as potential producers in different biotechnological processes. However, the efficiency of gene targeting in most of those species is still unknown. Using linear transformation cassette, a DNA that contains antibiotic resistance gene in the middle flanked by regions homologous to the target sequence in the genome, we have determined the efficiency of gene targeting in Brettanomyces/Dekkera bruxellensis, Kluyveromyces marxianus and Scheffersomyces stipitis. Depending on the yeast species gene targeting efficiency, obtained using transformation cassettes with flanking homologies longer than 1 kb, varied from less than 0,1 % to 75 %. Our results suggest that in some species (e. g. K. marxianus) use of flanking homologies, longer than 1 kb, allows the introduction of targeted modifications in the genome, while in some other yeast species, advanced gene-editing techniques e.g. CRISPR/Cas system might be mandatory to allow precise modifications of the genome.

ACKNOWLEDGEMENTS/REFERENCES

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568 / HYDROGEN-OXIDIZING HYDROGENASES ACTIVITY OF RALSTONIA EUTROPHA H16 DURING BACTERIAL GROWTH IN ORGANIC WASTE MATERIALS

Keywords: *Ralstonia eutropha H16 , Hydrogenases, biocatalysts, organic waste materials*

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Oxygen-tolerant Hydrogenases (Hyds) of facultative chemolithoautotrophic Ralstonia eutropha β-proteobacterium are efficient candidates as anode biocatalysts in enzymatic fuel cells (EFC). Hence, stimulation of Hyds production of R. eutropha by using cheap carbon sources (e.g. organic waste) is an attractive strategy for biotechnology. One of the promising waste materials for bacterial growth is whey: whey is an important by-product of the dairy industry, e.g. cheese and curd production: it represents a valuable source of proteins and sugars, which can be consumed by this bacterium. The effects of cheese whey (sweet whey (SW)) and curd whey (acid whey (AW)) on bacterial growth and H2-oxidizing Hyd activity were investigated. The pretreatment of whey has been done: filtered and unfiltered samples with various dilutions were studied. The H2-oxidizing Hyd activity of bacterial whole cells was measured by monitoring H2-dependent methylene blue reduction at 570 nm. After 72 h, compared to control (Fructose-Nitrogen (FN) medium), bacterial growth was ~3-4 fold stimulated 4 times and 2 times diluted and filtered AW samples. H2-oxidizing Hyd activity of R. eutropha whole cells was not observed in control cultures, while it was recorded upon bacterial growth on 2 times diluted and filtered AW and 4 times diluted and filtered SW, after 24 h. Moreover, 2.5 times stimulated (0.1U(mg·cell dry weight-1)) H2-oxidizing Hyd activity was detected in 4 times diluted and filtered SW samples. Therefore, the utilization of dairy industry organic waste by microbes may efficiently support the economical generation of both bacterial biomass and biotechnologically relevant Hyds.

ACKNOWLEDGEMENTS/REFERENCES

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812 / THE ROBUSTNESS OF BACTERIAL CORE METABOLISM TO GROWTH AT DIFFERENT TEMPERATURES

Keywords: *NGS, Antarctic Bacteria, genome-scale metabolic reconstruction, nan*

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BACKGROUND

The cervicovaginal microbiome (CVM) correlates with women's cervical health, and variations in its structure are associated with high-risk human papillomavirus (hrHPV)-induced high-grade cervical lesions. The CVM exhibits five community state types (CSTs) based on microbial dominance and composition; however, elucidating the impact of CSTs in health and disease is challenging because current sequencing technologies have limited confident discrimination between bacterial species that shape microbial communities.

OBJECTIVES

In this work we have investigated the metabolic and transcriptional changes that support its growth at two different temperatures, i.e., 0 and 15 °C.

METHODS

We have quantified the intracellular and extracellular metabolism, together with the changes occurring at the transcriptomic level in the same growth conditions. This information was then combined with both its gene regulatory network (obtained through computational modelling of omics data from condition-dependent datasets) and genome-scale metabolic reconstruction to provide a systemic understanding of cellular adaptation to growth in a range of temperatures.

RESULTS

We suggest that the transcriptomic profile can buffer the temperature shift so that the metabolome is not deeply affected and, in some cases, not affected at all. We found that the intracellular concentration of nearly 90% of the analysed metabolites correlates positively in the two conditions, across five time points; the least correlated were ethanol and thymidine, while PEP and ribose showed the strongest anticorrelation.

862 / ISOLATION OF FUNGAL STRAINS AND SECRETED ENZYMES FOR PRODUCING BIOFUELS FROM MICROALGAL BIOMASS

Keywords: *Fungal enzymes, Microalgae, Biofuels, Saccharification*

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Microalgae are of interest as a source of chemicals and metabolites for various industrial uses, including biofuel production. One challenge is the ability to efficiently hydrolyse and release products from the biomass, particularly for algal species with thick cell walls. The carbohydrates derived from starch and from the cell walls are a potential feedstock for bioethanol production via fermentation, while released lipids can be used for conversion into biodiesel. This work aimed to identify and isolate fungal strains for their ability to degrade microalgal biomass, and then characterise the fungal secretome to identify the enzymes involved. Compost bioreactors containing algal biomass were used to screen for fungal isolates at different temperatures. Crude enzyme extracts from the most dominant fungal strains were used to quantify the saccharification of intact or lipid-extracted Chlorella vulgaris biomass. An extract from Doratomyces nanus released up to 76% of carbohydrates from the algal biomass, while an Aspergillus fumigatus could release up to 94% of carbohydrates. Since these saccharification reactions were efficient at 37°C, we demonstrated simultaneous saccharification and fermentation of the biomass to produce bioethanol. Proteomic characterisation of the A. fumigatus secretome identified enzymes that were up-regulated or uniquely expressed in the presence of algal biomass and included cellulases, glucanases, hemicellulases, chitinases, pectinases and amylases, which are candidates for the degradation of complex carbohydrates in the microalgae biomass. Outcomes of this study can led to improved rational design of microbial enzymes to efficiently degrade microalgal biomass for various industrial applications.

ACKNOWLEDGEMENTS/REFERENCES

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867 / LEGO MICROBES: THE COLLOID BIOLOGY APPROACH TO BUILDING A MICROBIAL COMMUNITY FOR SUCCESSFUL REMEDIATION OF THE ENVIRONMENT

Keywords: *colloid biology, cross-feeding, bioremediation, biotechnology*

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Structures such as flocs, mats and biofilms are extremely well internally organized due to the microenvironmental conditions allowing the establishment of different niches. Synthetically producing and maintaining these structures is very important for different biotechnological applications. Hence, we have thus set out to test different approaches to manipulate the physical properties of the cells' surfaces to spatially order them into 3D structures and through this shape their intra- and interspecies interactions. In order to achieve this, we treated cells as colloid particles with a net negative charge. Besides, cells are not just particles and in biotechnological applications they need to stay viable. To fulfil these two criteria, namely, controlling the surface charge and keeping them metabolically active, prior to forming the spatial multicellular structures, we need to provide three main components: (i) a special type of "glue", which modifies the cell surface and attaches specific cells together, (ii) forming particular metabolic niches and (iii) forcing the stability of the constructed artificial structures resulting in increased scalability. As a result, we have shown how the charged polyelectrolytes electrostatically deposited onto the counter-charged surface of the bacterial cells cause various biological effects, such as controlling cellular metabolism and growth, spatial distribution in a consortium and intercellular interactions. This approach was developed in our group in order to manipulate structures and processes useful in the remediation of contaminated water with pesticides and metals, the transformation of lignin into high added value products, prevention of biocorrosion and enhancing different bacterial based therapies.

ACKNOWLEDGEMENTS/REFERENCES

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910 / THE VALORIZATION OF WHEY-BASED SIDE-STREAMS FOR MICROBIAL BIOMASS, HYDROGEN AND HYDROGENASE ENZYME PRODUCTION

Keywords: *Side-streams of the dairy industry , Biohydrogen, Hydrogenase, nan*

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Side-streams of the dairy industry is rich in proteins, ion of microorganisms, production of enzymes and high-value chemical compounds. The heterotrophic Escherichia coli and chemolithoautotrophic Ralstonia eutropha have biotechnological interest: R. eutropha is a model organism for the production of polyhydroxyalkanoates and O2-tolerant [NiFe]-Hydrogenases (Hyds), which are valuable biocatalysts in Biological Fuel Cells, whereas E. coli is a popular expression platform for the production of recombinant proteins, as well as, hydrogen (H2). H2 is known as ecologically clean, alternative energy of the future. The pretreatment (filtration, dilution, and pH adjustment) of cheese (sweet) whey (SW) and curd (acid) whey (AW) with ß-glucosidase [1] has been performed. Growth parameters (oxidation-reduction potential (ORP), pH changes, specific growth rate (μ), biomass formation (OD, cfu)) of E. coli BW25113 and R. eutropha H16 on various media (dilution up to 5-fold) was monitored during cultivation on filtered and non-filtered SW and AW at 37°C, pH 7,5 and 30°C, pH 7, respectively. Microbial growth, pH decrease, drop of ORP from positive to low negative values were stated. Maximal cell yield (OD600 4.0), stimulated μ, and H2-oxidizing Hyd activity of R. eutropha was achieved after 72 h and 24h, respectively. Biomass (OD600 2.0) and ~ 1.5 mmol/L H2 formation were observed during cultivation of E. coli BW25113 on whey with ß-glucosidase treatment. Our study evaluates the possibility of side-streams of the dairy industry as a potential feedstock for microbial (R. eutropha, E. coli) biomass, hydrogen, and Hyds formation.

ACKNOWLEDGEMENT

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REFERENCE

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934 / MICROBIOLOGICAL TRANSFORMATION OF PAPER PRODUCTION WASTE

Keywords: *waste valorization, bioremediation, recalcitrant compounds, polyhidroxyalkanoates*

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Pulp and paper production generates large amounts of waste rich in organic polymeric substances as well as recalcitrant environmentally harmful compounds. Engineered microbial consortia help us combine together different metabolic traits and keep the interspecies cooperative compatibility in order to perform the biotransformation of wastewater components.

Our first aim was to find an effective bio-based strategy for increasing the efficiency of compound degradation in whitewater using a selection of bacterial isolates originating from different microenvironments in the paper production line. We obtained a large collection of bacterial isolates capable of degrading either a single pollutant or combination of them, i.e, carbohydrates, resin acids, alkyl ketene dimers, polyvinyl alcohol, latex, and azo and fluorescent dyes. Of the 318 bacterial isolates, we selected out metabolic specialists or generalists to finally form a consortium of four strains that degraded the entire spectrum of additives. A proof-of-concept study on a pilot scale was then performed by immobilizing this artificial consortium within a 33-liter tubular flow-through reactor, to achieve 88% reduction in the COD in whitewater.

Our second aim was to transform the cellulose and hemicellulose waste into polyhydroxyalkanoates (PHA) that are used for the production of bioplastics. To see if the production of PHA can be sped, we spatially organized different combinations of environmental bacterial isolates cross-feeding on glucose. In conclusion, we showed that the synthetic community with particular microgeographic placement of cells increases the efficiency of degradation and shortens the time of water treatment.

ACKNOWLEDGEMENTS/REFERENCES

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03

1013 / THE SPECIFICITY OF FERULOYL ESTERASE FROM GEOBACILLUS THERMOGLUCOSIDASIUS TOWARDS MODEL SUBSTRATES BY MOLECULAR DOCKING

Keywords: *feruloyl esterase, docking, Geobacillus, nan*

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Feruloyl esterases (FAEs) are hydrolase enzymes, (EC 3.1.1.73) a subclass of carboxylic acid esterases (EC 3.1.1), that catalyze the formation and breakdown of ester bonds between the ferulate ester groups required for crosslinking between hemicelluloses and hemicellulose-lignin.

The aim of this study is to investigate the affinity of FAE for four different model substrates (methyl/ethyl ferulat, methyl caffeine, methyl-p-coumarate, and methyl sinapat) and to support these datas by molecular docking. For this, FAE from Geobacillus thermoglucosidasius DSM 2542T which was cloned into pET28a(+) and expressed in Escherichia coli was used. Expressed enzyme was purified by heat shock and anionic ion exchange column chromatography. Four model substrates of FAE were used and determined which substrate the enzyme has more affinity for. Molecular docking studies were performed using AutoDock 4.2 software to investigate possible interactions of methyl and arabinose forms of model substrates. In this study, eight different compounds were docked to the 3D structure of feruloyl esterase from Geobacillus thermoglucosidasius DSM 2542T.

The catalytic domain of the enzyme was determined as Ser114-His232-Asp202, taking into account the literature studies in previous studies. In the docking studies performed according to this region, it was observed that all substrates could bind to this region. According to these results, the substrate with the lowest binding energy (the substrate that forms the most stable complex with the enzyme) was determined as arabinose ferulate (-7.32 kcal/mol).

1096 / GENETIC ENGINEERING OF CLOSTRIDIUM ACETOBUTYLICUM FOR SECOND GENERATION SUBSTRATES UTILIZATION

Keywords: *CRISPR-Cas9, 2G subtrates, Clostridium acetobutylicum, Carbon Catabolite Repression*

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BACKGROUND

The solventogenic bacterium Clostridium acetobutylicum is naturally able to produce chemicals of interest by fermentation, such as acetone, butanol, and ethanol. Currently, carbon catabolite repression (CCR) mechanisms found in this micro-organism impede the efficient assimilation of pentoses in the presence of hexoses. Since both types of sugars are commonly found in second generation (2G) substrates such as lignocellulose hydrolysates, CCR mechanisms lead to poor carbohydrate utilization when fermenting them by C. acetobutylicum.

OBJECTIVES

The aim of this study was to tackle CCR mechanisms in C. acetobutylicum by genetic engineering, to obtain a micro-organism able to simultaneously assimilate hexoses and pentoses, which could therefore be utilized to efficiently produce chemicals of interest from 2G substrates.

METHODS

A CRISPR/Anti-CRISPR genetic tool was employed to delete genes encoding several putative transcriptional repressors involved in the assimilation pathway of pentoses. Mutants were characterized by analyzing the kinetics of carbohydrate assimilation when grown on media containing sugar mixtures. The effect of genes deletion was precisely assessed by transcriptomic analyses.

RESULTS

We show that the inactivation of the targeted genes leads to an increased growth rate on a mixture of carbohydrates, as well as a lift of CCR, resulting in an increased sugar consumption rate and an improved assimilation of pentoses along with hexoses. We also show a cumulative effect of deletions. predictability of evolution.

03

1122 / CARBONIC ANHYDRASE ENHANCES BIOELECTRO CO2 RECYCLING BY CLOSTRIDIUM LJUNGDAHLII

Keywords: *Bioelectrochemistry, Clostridium ljungdahlii, carbonic anidrase, Carbon capture*

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Gas mass transfer, mainly carbon dioxide (CO2) and hydrogen (H2) bioavailability, is one of the main challenges of bioeletro CO2 recycling into carbon-neutral products. While H2 can be continuously produced at the (bio) cathode surface, CO2 can become limiting depending on cell consumption and the chemical properties of the catholyte. Here we aimed at coupling a carbon capture/solubilisation process based on an engineered carbonic anhydrase (CA) and the microbial bioelectrosynthesis of acetate in a lab-scale reactor. H-type bioelectrochemical reactors were assembled (nominal capacity of 155 mL) using carbon cloth (24 cm2 surface area) and graphite as cathode and anode respectively, and inoculated with C. ljungdahlii DSM 13528T. Cultures were poised at -1.0 V vs Ag/AgCl and let to stabilize until consistent acetate production was observed, followed by repetitive replacements of the catholyte (75%) with fresh medium. Acetate, pH and current demand were monitored daily. Acetate production rates were calculated as specific rate activities for every batch test. Once CA was added to the reactor, batch tests were repeated, and acetate production was calculated and compared to control conditions. The addition of carbonic anhydrase caused a significant increase in acetate production rates (from 2 to 10-fold). Collectively, our results established a proof-of-concept for the combination of an enzyme-based carbon capture method with the in-situ transformation of CO2+H2 into acetate, as a model product. Despite the promising results, the stability of the process in the long-term remains to be elucidated.

ACKNOWLEDGEMENTS/REFERENCES

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348 / SLIGHT EFFECT IN REDUCING THE RISK OF SARS-COV-2 TRANSMISSION BY ORAL ANTISEPTICS

Keywords: *SARS-CoV-2, oral antiseptics, Viral infectivity, Clinical trial*

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BACKGROUND

In vitro studies have consistently shown that several oral antiseptics have virucidal activity against SARS-CoV-2. Based on this, mouthwashes have been proposed as an easy to implement, inexpensive strategy to reduce viral transmission. In addition, oral rinses are recommended worldwide in order to reduce infection risk. However, in vivo evidence of mouthwash efficiency in reducing the levels of salivary SARS-CoV-2 is limited and viral viability after mouthwashes has not been measured.

OBJECTIVES

The effect in salivary viral load of SARS-CoV-2 and its infectious capacity have been evaluated before and after antiseptic mouthwash.

METHODS

The in vivo effect of several antiseptics was evaluated by a randomized double-blind, five-parallel-group, placebo-controlled clinical trial. Four oral antiseptics were studied: Chlorhexidine (CHX), Cetylpyridinium chloride (CPC), Hydrogen Peroxide (H2O2) and Povidone-Iodine (PVP-I); and were compared to a placebo group (distilled water) with measures of total (qPCR-based) and infective (infection in Vero6 cells) salivary viral load.

RESULTS

Contrary to in vitro evidence, salivary viral load was not affected by any of the four tested mouthwashes. Viral culture of the saliva samples indicated that CPC significantly reduced viral infectivity, but only at one-hour post-mouthwash.

These results indicate that some of the mouthwashes currently used to reduce viral infectivity are not efficient in vivo and furthermore, this effect is not immediate. More studies are needed to evaluate the effect of CPC and its potential contribution to reduce disease progression within an individual or viral infectivity between individuals.

433 / OMPC PORINS OF SALMONELLA ENTERICA AND ESCHERICHIA COLI POSSESS AMYLOID-FORMING PROPERTIES

Keywords: *amyloid, porin, Omp, bacterium*

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BACKGROUND

One of the groups of bacterial proteins, the amyloid properties of which are currently being actively studied, is the porins of the outer membrane. These proteins are among key virulence factors and have a beta-barrel structure enriched with beta sheets. Amyloidogenesis of outer membrane porins may represent an important physiological mechanism that allows bacterial cells to survive more efficiently in the aggressive internal environment of the host organism.

OBJECTIVES

The Escherichia coli and Salmonella enteritidis species are important pathogens in humans and animals, which makes it relevant to study the properties of their virulence factors. The choice of OmpC for the experimental verification of amyloid properties can be explained by its important role in bacterial virulence, and by the lack of detailed information on the amyloid properties of this protein and the biological role of its amyloidogenesis.

METHODS

The amyloid properties of OmpC porins of enterobacteria E. coli and S. enteritidis in vitro were studied in detail using SDS-PAGE, transmission electron microscopy, binding of amyloid-specific dyes.

RESULTS

We found that OmpC porins of Salmonella enterica and Escherichia coli possess amyloid-forming properties. In these experiments, the morphology of fibrils of the OmpC proteins, their resistance to treatment with ionic detergents and proteases, as well as their thermal stability were studied in detail. We have also studied the binding of amyloid-specific dyes thioflavin T and Congo Red by the fibrils. The obtained data are important for understanding the mechanisms of amyloidogenesis of outer membrane proteins and their role in "pathogen-host" interactions.

ACKNOWLEDGEMENTS/REFERENCES

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Petersburg State University (SPbSU) for the equipment provided for use in this work.

478 / VIBRIO VULNIFICUS T6SS EXHIBITS ANTI-EUKARYOTIC EFFECT BUT NOT ANTI-BACTERIAL PROPERTIES AND CONTRIBUTES TO VIRULENCE

Keywords: *Vibrio vulnificus, T6SS, zoonosis, emerging pathogen*

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BACKGROUND

Vibrio vulnificus is an emerging marine zoonotic pathogen whose distribution and incidence is expanding due to global warming. The species causes a disease (vibriosis) whose most severe form is a hemorrhagic septicemia that leads to death in less than 24 h regardless the host, humans or fish. T6SS is a toxin secretion system present in many gram-negative bacteria that has been associated with pathogenicity towards other bacteria or eukaryotic cells, but it remains poorly studied in V. vulnificus. Previous experiments performed in our laboratory revealed that VgrG (the main toxin of T6SS) is over-expressed in V. vulnificus when it is incubated in presence of fish erythrocytes.

OBJECTIVES

To characterize the role of the VgrG toxin in V. vulnificus pathogenesis.

METHODS

We have constructed mutant strains in VgrG and other toxins, and used them, together with the parental strain, in in vitro, ex vivo, and in vivo experiments, to determine the anti-bacterial and anti-eukaryotic effect of the T6SS of V. vulnificus and its role in virulence towards its main host, the eel.

RESULTS

We found strong evidence that iron could activate vgrG transcription. V. vulnificus VgrG does not exhibit anti-bacterial activity, but it is involved both in resistance to natural predators in the aquatic ecosystem (i.e. amoeba) and bacterial cytotoxicity towards fish erythrocytes, contributing to V. vulnificus virulence. Therefore, VgrG could be considered a survival factor for V. vulnificus both outside and inside the host.

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533 / ASSESING VIRULENCE POTENTIAL OF ACINETOBACTER BAUMANNII ISOLATES RECOVERED FROM COVID-19 PATIENTS

Keywords: *Virulence potential, Acinetobacter Baumannii, ICU, COVID-19*

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BACKGROUND

From the start of COVID-19 pandemic, many patients suffering severe clinical presentation of COVID-19 and acute respiratory failure have been administered to intensive care units (ICUs). Need for noninvasive or invasive mechanical ventilation represents a high-risk factor for these patients to develop Acinetobacter baumannii superinfection.

OBJECTIVES

The main goal of this study was to examine virulence potential of A. baumannii isolates recovered from COVID-19 patients admitted to ICU as well as to compare differences in virulence among isolates originated from male and female patients or from different sample types.

METHODS

64 A. baumannii isolates were recovered from COVID-19 patients from December 2020 to February 2021. The isolates were originated from different sample types: blood, tip of the central venous catheter, tracheal aspirate, tip of the aspirator and sputum. Genetic relatedness of ApaI digested A. baumannii isolates, was determined by pulsed-field gel electrophoresis (PFGE). Affinity to mucin binding was tested using in vitro model and isolates were examined for two types of motilities: swarming and twitching.

RESULTS

The obtained results revealed that 93.75% of isolates had an identical ApaI profile indicating the dominant presence of one pulsotype among the isolates, while only 6.25 % isolates were different according to PFGE. Mucin adhesion test showed that isolates recovered from male patients had higher binding affinity to mucin compared to isolates from female patients ($p<0.05$). Relative to the type of the sample, the number of motile strains were higher for isolates from blood, compared to isolates originated from the respiratory tract.

ACKNOWLEDGEMENTS/REFERENCES

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557 / STRUCTURAL CHARACTERIZATION OF A BACTERIAL ANTIBODY-DEGRADING SYSTEM

Keywords: *Mycoplasma, Antibody, CryoEM, Immunity*

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Mycoplasmas cause various chronic diseases in animals and humans. They have evolved strategies to evade the host immune response, including the Mycoplasma Ig Binding (MIB)- Mycoplasma Ig Protease (MIP) antibody degrading system. The Fab domain of many types of immunoglobulins is recognized by MIB. This interaction mediates antibody-antigen dissociation and the targeted activity of the serine protease MIP, which cleaves the VH domain of the antibody.

To understand the molecular basis of this system, we solved the structure of the MIB-MIP antibody degrading system in complex with a Fab at different state of the mechanism using single particle cryo-electron microscopy.

The structure allowed us to understand the architecture of the complex in a previously unseen “hug of death” mechanism. The structure shows that MIB interacts mainly with the Fab’s light chain leading to a drastic change in the structure of the Fab’s antigen binding site. The complementarity determining regions of VL and VH are separated resulting in the dissociation of the antigen- antibody complex. MIP interacts with the Fab’s heavy chain and contains a serine protease catalytic triad that is ideally placed to cleave this chain. In addition, surface plasmon resonance experiments highlighted MIB’s unexpected behaviour which is the first identified protein that actively dissociate various immune complexes.

Solving this cryoEM structures reveals the molecular basis of the antibody binding, dissociation and degradation mechanism and further our understanding of the MIB-MIP system. MIB and MIP are emerging as new therapeutic targets to fight against the immune evasion mechanism of mycoplasma.

ACKNOWLEDGEMENTS/REFERENCES

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606 / ESTIMATING PARAMETER VALUES, ANALYZING THE ROLE, AND IDENTIFYING PREDICTORS OF THE SARS-COV-2 INHERENT TRANSMISSIBILITY

Keywords: *epidemiological model, COVID-19, transmissibility predictors, feature selection and regularization*

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BACKGROUND

In the first COVID-19 epidemic wave, strikingly larger numbers of cases and deaths were reached in Hubei than in all other China provinces together, raising a question if this can be explained reasonably by transmissibility and intervention effects. Inherent virus transmissibility in a fully susceptible population is measured by the Basic Reproduction Number, R0, which is often improperly determined from the counts registered under control measures. Furthermore, simple correlations of different socioeconomic and meteorological factors with R0 may not represent causal relationships but rather artifacts of extensive mutual factor correlations.

OBJECTIVES

We aimed to construct a simple model that can explain the epidemic progression in China provinces, use it to accurately estimate R0 values for different world countries, and identify direct R0 predictors among numerous variables.

METHODS

We set a dynamic model of the COVID-19 spread in a population, which mechanistically describes social distancing measures. By fitting the model to the cumulative counts data, we estimated the parameters of virus transmission and control measures effectiveness for each China province. From the countries' pre-intervention periods, we determined their R0 values, which we correlated using feature selection and regularization methods with various variables, mutually decorrelated by a Principal Component Analysis.

RESULTS

We show that different epidemic outcomes in Chinese provinces can be explained by interplays of virus transmissibility and control measures effectiveness. While many factors are strongly correlated with R0, the few selected as direct predictors include the prosperity level, indoor crowdedness, the epidemic onset, net migrations, and unhealthy lifestyle indicators.

632 / COMPARATIVE GENOMICS OF VIBRIO HARVEYI STRAINS FROM MARINE AQUACULTURE

Keywords: *aquaculture, fish deseases, Vibrio harveyi, genomics*

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BACKGROUND

Vibrio harveyi is one of the most important bacterial pathogens for a wide range of aquatic organisms and it is responsible for severe economic losses in aquaculture worldwide. In Greece, it is one of the leading cause of mortalities in marine cultured species and its management has become a top priority for the sector.

OBJECTIVES

The main objective of this work is the study of the genetic diversity of several strains of the pathogen that have been isolated from diseased fish over the past 6 years. Clinical isolates from 5 hosts including, European seabass, gilthead seabream, greater amberjack, common dentex and tilapia from Greece and Saudi Arabia have been included in the study.

METHODS

Paired-end sequencing was performed using the DNBSEQ platform. Phylogenetic and pangenome analysis were carried out and the genomes were compared to investigate the number of genomic islands, the distribution of antibiotic resistance and virulence factors.

RESULTS

Comparative genomics of the strains revealed great diversity. The pangenome included 46% core, 26% dispensable, and 28% singleton genes. Analysis of the genomes uncovered several antibiotic resistance genes and putative virulence genes. More than 20 genomic islands per genome were predicted, accounting for total 10% of the genome length. The pathogenicity mechanisms involved hemolysins, enterotoxins, metalloproteases, TonB-dependent receptors and iron transporters. The use of genomic data obtained for the identification of conserved antigenic proteins towards the development of a universal vaccine for the Mediterranean fish species is discussed.

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748 / DETECTION OF SARS-COV-2 VIRUS USING FOUR DIFFERENT COMMERCIAL KITS WITHOUT RNA EXTRACTION

Keywords: *SARS-CoV-2, RT-qPCR, RNA extraction, nan*

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Detection of SARS-CoV-2 virus using four different commercial kits without RNA extraction

The current COVID-19 pandemic (over 359 million infected in the world) demanded urgent development of rapid and massive detection tools for identification and isolation of SARS-CoV-2 virus.

Extraction of RNA combined with real-time PCR has become method of choice for detection of SARS-CoV-2 virus from different types of samples (nasopharyngeal swabs, oral swabs, etc.). Beltran-Pávez et al (2021) suggested new approach for the detection of SARS-CoV-2 without RNA extraction.

Our objective was to compare modified Beltran-Pávez et al (2021) approach with magnetic beads- based RNA extraction using four different commercially available SARS-CoV-2 detection kits.

We collected 60 samples which were confirmed as positive by National reference laboratory. Extraction was performed on the automatic nucleic acid extraction system (Natch 48, Sansure Biotech, PRC). Modified Beltran-Pávez et al (2021) approach involved 10 minutes incubation on 95°C. Analysis was performed using four different commercially available SARS-CoV-2 nucleic acid detection kits: Sansure Biotech, GeneFinder, Liferiver and Bioeksen on Azure Cielo 6 Real Time PCR system (Azure Biosystems, USA).

Our findings suggest that there is no statistically significant difference ($p=0.85$) between modified Beltran-Pávez approach and magnetic beads-based RNA extraction which implies that modified Beltran-Pávez approach can be used as a method of choose for detection of SARS-CoV-2 virus without RNA extraction.

824 / MANAGEMENT OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE BACTEREMIA IN THE PATIENT WITH HEMATOLOGICAL MALIGNANCIES

Keywords: *Hematological malignancies, Carbapenem-Resistant Enterobacteriaceae, Bone marrow transplantation, Bacteremia*

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BACKGROUND

Carbapenem-resistant-Enterobacteriaceae (CR-E) bacteremia is associated with high mortality in neutropenic patients with hematological malignancy, due to ineffective empirical therapy and delayed initiation of appropriate antibiotics. Antibiotic management will be discussed in two episodes of CRE bacteremia in our patient.

OBJECTIVES

The 45-year-old female patient was diagnosed with acute myeloid leukemia and chemotherapy was started. Neutropenic fever developed in the patient with CR-K.pneumoniae in urine culture and CRE in rectal swab and meropenem and colistin were started. Tongue numbness and facial hemiparesis were observed after colistin therapy and it was stopped. Meropenem treatment also was stopped on the 5th day because of erythematous maculopapular rash compatible with drug eruption. Gram-negative-bacillus was reported in his blood culture in the other neutropenic fever episode and the treatment was started with imipenem+fosfomycin+tigecycline on the same day. CR-K.pneumoniae and E.coli were detected in the blood culture (Table-1). The treatment was completed with imipenem+gentamicin according to the antibiogram.

The patient was hospitalized again for allogeneic bone marrow transplantation (BMT) 8 months later. After BMT, meropenem and polymyxin-B were started when neutropenic fever occurred. CR-K.pneumoniae isolated from blood culture and treatment was changed to fosfomycin+gentamycin+imipenem (Table-1). In the patient with resistant hypokalemia, fosfomycin was discontinued and treatment was completed with tigecycline+gentamycin+imipenem.

RESULTS

Mortality in CRE bacteremia developing in neutropenic patients with hematological malignancy is around 50%, and this rate decreases significantly when appropriate antibiotic therapy is started early (1). In our case, the patient with CRE bacteremia that developed during the neutropenic period was successfully treated.

04

929 / GENERATION OF DIVERSE SPIKE VARIANTS OF SARS-COV-2 AND TO ELUCIDATE THE ROLE OF THESE MUTATIONS IN VIRAL PATHOGENESIS

Keywords: *SARS-CoV-2, Pseudotyped viruses, Coronavriuses, Antibody escape*

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BACKGROUND

The ongoing pandemic severe acute respiratory syndrome coronavirus-2 and its diverse variants has affected the vaccine efficacy and inculcates the need for development of proper tools to understand the after effects of the mutational phenotypes. .

OBJECTIVES

Here, we aim to develop SARS-CoV-2 spike variants belonging to alpha, beta, gamma, delta and omicron lineages.

METHODS

In this study, we generated multiple spike variants of SARS-CoV-2 including the most frequent mutations such as D614G, E484K, L452R, P681R, N501Y, T478K and multiple combinations in the D614G backbone (D614G+E484K, D614G+L452R, D614G+P681R, D614G+N501Y, D614G+T478K) using site directed mutagenesis followed by VSV based pseudotyped virus (PV) production. We assessed the entry of PVs in different cell lines and analysed the antibody escape phenotype in a BSL-2 laboratory.

RESULTS

First, we showed that high titre PVs can be produced for SARS-CoV-2 variants in a short time and found that D614G and L452R mutants showed 3-4 fold higher infectivity compared to WT and other variants. In addition, E484K in beta, gamma and omicron lineages conferred antibody escape phenotype against SARS-CoV-2 polyclonal antibodies. Moreover, soluble form of SARS-CoV-2 receptor, Angiotensin converting enzyme -2 inhibited the entry of SARS-CoV-2 variants in a dose dependent manner. The data suggests that effective vaccine strategies has to be developed to combat current or future SARS-CoV-2 variants. In summary, the developed SARS-CoV-2 variants can be used for understanding the cellular entry and for analysing the key mutations which can minimise the efficacy of vaccine candidates against SARS-CoV-2.

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1035 / ADVANTAGES OF MOLECULAR METHODS FOR INFECTIOUS DISEASES IN CLINICAL CARE

Keywords: *infectious diseases, molecular analyses, pathogens, nan*

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In the past couple of years, the number of molecular analyses for infectious microorganisms has pivoted, especially with the emergence of the COVID-19 pandemics and the Nucleic acid testing for SARS-CoV-2.

Nevertheless, the molecular analyses for various respiratory, gastrointestinal, and other infectious diseases should not be neglected since they were and are heavily being used for identification of known infectious pathogens.

Their speed and sensitivity can greatly affect the patient treatment and clinical outcome.

In our laboratory since 2018, a comprehensive panel for 33 respiratory pathogens, comprised of 21 viruses, 10 bacteria and 1 fungus, is extensively used for screening of both hospitalized and out-patients. More than 3000 patients have been investigated in the time frame from 2018-2022.

We followed the progress of the influenza A, inlfuenza A (H1N1) and Influenza B virus infection waves, in autumn-winter and winter-spring seasons, and more rare pathogens throughout the year. Moreover, in combination with the SARS-CoV-2 screening, these tests are used for all the hospitalized COVID and non-COVID patients in the clinic.

As a rare case example, we were able to identify Streptococcus pneumoniae in the cerebrospinal fluid of a meningeal patient, where it tested negative on the classical microbiological test.

With our standard protocols, these molecular tests, in a matter of few-to-24 hours, identify if the patient is infected, and whether it is a virus, bacteria or a combination of both, and thus give an appropriate antibiotic or other therapy, which prevails the possibility of a false-positive result which can sometimes occur.

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1119 / WASTEWATER-BASED MONITORING OF RNA SARS-COV-2 IN LOCAL NEIGHBORHOODS AND SELECTED SCHOOLS IN PRAGUE

Keywords: *SARS-CoV-2, wastewater-based epidemiology, Prague , RT-qPCR*

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In our study, we compared the occurrence of SARS-CoV-2 RNA in municipal wastewater treatment plants (WWTPs) with COVID-19 epidemic data in the same area. It is obvious that the presence of SARS –Cov-2 RNA in WWTPs corresponds with higher number of covid-positive people. The presence of SARS-CoV-2 in wastewater at selected places in Prague sewer network was monitored using RT-qPCR since August 2020 to May 2022. Beside small sub-sewersheds, wastewater was collected in individual buildings such as schools, nursing homes and university college dormitories since the autumn 2021. The correlation between the concentration of viral RNA in wastewater and data from clinical trials was good especially for residential areas with more than 7000 registered inhabitants and for the main Prague's sewers.

Although the data obtained by sampling wastewater from the main Prague sewers were more consistent than those obtained from the small sewers, the correlation between wastewater-based and clinical-testing data was also good for the residential areas and individual building.

It was shown that monitoring SARS-CoV-2 RNA in wastewater sampled from small sewersheds could indicate the occurrence of COVID-19-positive cases in local neighbourhoods. This find could help in tracking COVID-19 hotspots within large cities.

ACKNOWLEDGEMENTS/REFERENCES

This work was financially supported by Czech Technological Agency TA CR grant no. SS01020112 and by the financial support from all institutions included in this research.

1125 / EFFECTS OF DIFFERENT PROBIOTIC STRAINS IN TREATMENT OF CAMPYLOBACTERIOSIS IN CHILDREN

Keywords: *Campylobacteriosis, Probiotics, Treatment, nan*

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BACKGROUND

Campylobacteriosis is the leading cause of bloody diarrhea in children worldwide. The growing resistance of Campylobacter spp. determine the need to find alternative therapeutics.

OBJECTIVES

to study the effects of different probiotic strains in children with campylobacteriosis.

METHODS

Material and methods 150 children aged 1 to 7 years were examined. The average age of the patients was 2,9±1,3 years, among which there were 79 (52.7%) boys and 71 (47.3%) girls. Diagnosis was confirmed by PCR detection. Patients received complex therapy including oral and parenteral rehydration, enzymes, sorbents and symptomatic drugs, excluding antibiotics. Patients were divided into two groups according to probiotic given: group 1 (n=75) - Saccharomyces boulardii, group 2 (n=75) - Bifidobacterium longum, Enterococcus faecium. Microbiota changes were estimated using both PCR-RT and bacterial seeding methods on days 3 and 14.

RESULTS

Microbiota disorders were characterized by decrease in content of symbiotic microorganisms: Bifidobacterium spp., Lactobacillus spp., B. thetaiotaomicron and F. prausnitzii. Duration of diarrhea was significantly lower in patients from group 1 (2,2±1,1 and 3,7±1,1 days; p=0,02). The choice of probiotics didn`t affect on duration of fever (p=0,1), vomiting (p=0,12) and hospital stay (p=0,09). On the 14th day 23 patients (30,7%) in group 2 and 9 (12%) in group 1 had dysbiosis according to feces study (p=0,01).

Treatment of campylobacteriosis with Saccharomyces boulardii probiotics had led to quicker diarrhea resolution. Both probiotics were effective. Further studies are needed to establish probiotics effects in children with campylobacteriosis.

1385 / WASTEWATER-BASED MONITORING OF RNA SARS-COV-2 IN LOCAL NEIGHBORHOODS AND SELECTED SCHOOLS IN PRAGUE

Keywords: *RNA SARS-CoV-2 , wastewater-based epidemiology, Prague , nan*

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It was shown that monitoring SARS-CoV-2 RNA in wastewater sampled from small sewersheds could indicate the occurrence of COVID-19-positive cases in local neighbourhoods. This find could help in tracking COVID-19 hotspots within large cities.

ACKNOWLEDGEMENTS/REFERENCES

This work was financially supported by Czech Technological Agency TA CR grant no. SS01020112 and by the financial support from all institutions included in this research.

326 / MICROBES IN THE GUT OF TRIATOMINE BUGS CAN NEGATIVELY AFFECT THE GROWTH OF TRYPANOSOMA CRUZI

Keywords: *Medical Entomology, Microbial Ecology, Antimicrobial compounds , Tropical neglected diseases*

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Chagas disease is an endemic zoonosis in Latin America, and it is mostly concentrated in rural and poor areas. It is considered a neglected tropical disease, and the main vector in Central America is Triatoma dimidiata. In this study, we investigated the effect of Trypanosoma cruzi infection on the microbiota and the localization of important microbial taxa in different sections of the gut of T. dimidiate, using a metabarcoding approach.

Studies on the microbiota of triatomine bugs traditionally link microbes to nutritional associations, where bacteria provide vitamins to the host. On the other hand, we conducted isolation of microbes from the gut of T. dimidiate and assayed their functional capacities to produce antimicrobial compounds against protozoa, fungus, and bacteria.

Our study gives relevant ecological information on microbiota compartmentalization and microbial interactions in the gut of T. dimidiata. We showed that some of our isolates produce an anti-Trypanosoma significant effect. Such effect is produced by microbes identified as yeast specimens and uncommon genera of Actinobacteria (different from Streptomyces and Pseudonocardia). The bacteria are between the most abundant amplicon sequence variants in our study and their antimicrobial activity seems to not affect bacteria or fungus. We highlight that further investigation on interactions and localization of important microbial taxa in the gut of T. dimidiata and other triatomine bugs may help to develop new strategies and/or improve current control efforts.

ACKNOWLEDGEMENTS/REFERENCES

The project was financed by Vicerrectoria de Investigación, Universidad de Costa Rica. Project C0186

402 / MULTIPLE ENERGY SOURCES AND METABOLIC STRATEGIES SUSTAIN MICROBIAL DIVERSITY IN ANTARCTIC DESERT SOILS

Keywords: *Trace gas oxidation, Metagenomics, Metabolic water, Antarctica*

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Numerous diverse microorganisms reside in the cold desert soils of continental Antarctica, though we lack a holistic understanding of the metabolic processes that sustain them. Here, we profile the composition, capabilities, and activities of the microbial communities in 16 physicochemically diverse mountainous and glacial soils. We assembled 451 metagenome-assembled genomes from 18 microbial phyla and inferred through Bayesian divergence analysis that the dominant lineages present are likely native to Antarctica. In support of earlier findings, metagenomic analysis revealed that the most abundant and prevalent microorganisms are metabolically versatile aerobes that use atmospheric hydrogen to support aerobic respiration and sometimes carbon fixation. Surprisingly, however, hydrogen oxidation in this region was catalyzed primarily by a phylogenetically and structurally distinct enzyme, the group 1l [NiFe]-hydrogenase, encoded by nine bacterial phyla. Through gas chromatography, we provide evidence that both Antarctic soil communities and an axenic Bacteroidota isolate (Hymenobacter roseosalivarius) oxidize atmospheric hydrogen using this enzyme. Based on ex situ rates at environmentally representative temperatures, hydrogen oxidation is theoretically sufficient for soil communities to meet energy requirements and, through metabolic water production, sustain hydration. Diverse carbon monoxide oxidizers and abundant methanotrophs were also active in the soils. We also recovered genomes of microorganisms capable of oxidizing edaphic inorganic nitrogen, sulfur, and iron compounds and harvesting solar energy via microbial rhodopsins and conventional photosystems. Obligately symbiotic bacteria, including Patescibacteria, Chlamydiae, and predatory Bdellovibrionota, were also present. We conclude that microbial diversity in Antarctic soils reflects the coexistence of metabolically flexible mixotrophs with metabolically constrained specialists.

ACKNOWLEDGEMENTS/REFERENCES

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417 / DYNAMICS OF MICROBIAL ASSEMBLAGES ASSOCIATED WITH MARINE MACROPHYTE SURFACES

Keywords: *marine epiphytic microbes, marine macrophytes, temporal community change, Illumina MiSeq 16S rRNA sequencing*

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BACKGROUND

Marine macrophyte surfaces are inhabited by diverse microbial communities. Studies of these epiphytic microbes typically do not account for temporal changes or use low sampling frequency approaches, making it difficult to describe in detail how communities change over time.

OBJECTIVES

Our objective was to conduct monthly sampling of microbial epiphytes inhabiting the surfaces of the seagrass Cymodocea nodosa and the invasive macroalga Caulerpa cylindracea to provide a detailed description of how communities change over time.

METHODS

Epiphytic microbial communities were sampled at monthly intervals in a meadow of C. nodosa invaded by C. cylindracea and in a monospecific settlement of C. cylindracea. The ambient prokaryotic picoplankton community was also sampled on the same dates. The community structure was characterized using Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene.

RESULTS

Community structure analysis at the OTU level revealed differences between communities in ambient water and host associated communities, as well as between communities associated with different host species. Temporal changes associated with the hosts' growth cycle were also observed. Taxonomic analysis revealed similar high rank taxa (phyla and classes) in ambient water and epiphytic communities, with the exception of Desulfobacterota, characteristic of C. cylindracea. At lower taxonomic levels (families and genera), taxa present throughout the sampling period accounted for the majority of sequences, while less abundant groups exhibited temporal patterns associated with the host's growth cycle. In conclusion, epiphytic microbial communities of marine macrophytes appear to be host-specific and contain groups that exhibit temporal changes.

ACKNOWLEDGEMENTS/REFERENCES

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423 / DIVERSITY AND EVOLUTION OF DEEP SUBSURFACE MICROORGANISMS ILLUMINATED BY METAGENOMICS

Keywords: *deep subsurface, metagenomics, microbial diversity, evolution*

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Deep subsurface ecosystems are among the largest habitats and can remain isolated from the surface for thousands to millions of years. The majority of deep subsurface microorganisms belonged to poorly characterized uncultured lineages, many of them are specific for deep habitats. Using metagenomic approach, we studied the deep (2-3 km) subsurface thermal aquifers in Western Siberia (Russia), located in the Mezosoic sedimentary rocks. Aquifers harbored diverse microbial communities comprising methanogenic archaea, sulfate-reducers, fermentative organisms, aerobic heterotrophs and anaerobic respirers in different combinations. The availability of buried organics, spatial heterogeneity of the subsurface aquifers and the long term inflow of meteoric water enabled co-existence of different metabolic groups. Deep subsurface ecosystems harbored novel uncultured prokaryotic lineages expanding our knowledge of microbial diversity. Several examples of genome-based metabolic reconstruction of members of such new lineages will be presented. Metagenomic analysis revealed the presence of Desulforudis audaxviator, a unique chemolithoautotrophic bacterium previously found in 2.8 km deep gold mine in South Africa. Analysis of metagenome-assembled genomes of Desulforudis audaxviator from Siberia and South Africa, as well as single-cell genomes from various deep subsurface sites revealed that average nucleotide identities of all pairwise genome comparisons exceeded 99.5% and did not correlate with geographic distance. Comparative genomics of Desulforudis audaxviator will be discussed in evolutionary context. Desulforudis audaxviator seems to be a living microbial fossil that underwent only minimal evolution since the breakup of Pangea more than 50 million years ago. Alternatively, unknown mechanisms enable fast global dispersion of microorganisms between remote deep subsurface sites.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by the Russian Science Foundation (projects 19-14-00245 and 22-14-00178).

453 / MONITORING THE PRESENCE OF CYANOBACTERIA IN WATER BASED ON THE INFLUENCES OF NUTRIENTS TO THE GROWTH OF BACTERIA

Keywords: *Cyanobacteria, Water, Biosensor, nan*

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BACKGROUND

Cyanobacteria are phototrophic prokaryotes in both marine and water habitats which can conduct oxygenic photosynthesis. They play important roles in aquatic systems because of their abilities to decompose organic wastes, detoxify heavy metals and suppress the growth of pathogens in water. However, harmful bloom formed by cyanobacteria has become a global concern due to its effects to the water quality. A high number of toxic compounds, so-called cyanotoxins as products of their secondary metabolism also result in high risk to aquatic organism and human.

OBJECTIVES

Due to the important role of cyanobacteria to water environment, this study aims to monitor the presence of filamentous cyanobacteria in water.

METHODS AND RESULTS

The influences of growing parameters and essential elements including C-, N-, and iron sources to the growth of two filamentous cyanobacteria in water: Anabaena sp. CY036 and Aphanizomenon sp. BL5 were investigated based on their productions of natural fluorescent pigments. Fluorescent intensities of each pigment were analyzed by flow cytometry and fluorescence spectroscopy. The chlorophyll-a biomass was measured by chlorophyll-a assay to evaluate the growth of cells. The iron availability showed a significant effect to the growth of cells and the production of chlorophyll-a biomass. The utilization of other mineral elements was also investigated. Under standard growing conditions, both strains can grow well in the pH from 6.0 to 8.0. Morphological characteristics of cyanobacteria were observed by confocal microscopy using natural fluorescent pigment. These obtained results are then utilized for developing a biosensor for detection of cyanobacteria in water.

ACKNOWLEDGEMENTS/REFERENCES

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05

510 / SOIL MICROBIAL RESPONSES TO LONG-TERM WARMING EXPERIMENTS IN ALPINE DRYAS OCTOPETALA HEATH

Keywords: *Alpine soils, Microbial communities, warming effect, climate change*

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During the last century, high altitude and latitude regions on Earth experienced rapid climate warming at rates much higher than other areas. Rising temperatures are causing vegetation changes in alpine and arctic areas, such as shrub encroachment into the tundra, an increase in plant species richness towards mountain summits and higher latitudes, and an increase in the dominance of warm-adapted plant species. As a result, these changes may induce changes in soil microbial diversity and functioning, as well as on biogeochemical processes, such as nutrient cycling and C fluxes and storage. In this study we examined how two decades of experimental warming by open top chambers (OTCs) have affected the microbial community structure and function in an alpine Dryas octopetala heath near Finse, in Norway. OTCs passively increase the ambient temperature inside them to simulate future warming scenario; the paired plots outside the OTCs act as control plots, representing the current conditions. Soil from twenty plots(10 OTCs and 10 controls) was collected and analysed for physicochemical properties, microbial biomass, extracellular enzyme activity and subjected to amplicon sequencing of 16S rDNA and ITS region in order to assess changes in bacterial and fungal community composition. Previous research reported that one or two decades of warming are required before pronounced changes in soil parameters occur, while microbial enzyme activity and biomass become visible on a shorter time scale. Two decades of experimental warming will therefore offer a good model study to provide clear responses to the warming effects on soil microbial ecosystems

528 / SPATIOTEMPORAL STUDY ALONG THE MEUSE RIVER REVEALS THAT BACTERIAL DIVERSITY AND COMMUNITY COMPOSITION ARE DRIVEN BY SEASONS AND DISTANCE FROM THE SOURCE

Keywords: *BCC (Bacteria community composition), FL (free living) bacteria, PA (particle associated) bacteria, nan*

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Among the organisms that inhabit rivers, bacteria have a crucial role in self-purification and recycling of elements. This study is the first to analyze on a broad scale the Bacterial Community Composition (BCC) of the Meuse river, which has a length of 925 km and crosses three European countries (France, Belgium and Netherlands).

We carried out spatial studies of the Meuse during spring and summer 2019, in order to identify driving parameters of the BCC from upstream to downstream.

Those aspects have been considered separately for two different bacterial types: free living (FL) and particle attached (PA) bacteria. We analyzed 30 stations from the river source to its mouth, using 16S rRNA gene amplicon sequencing. We measured several physicochemical and biological parameters, i.e. temperature, dissolved oxygen, total suspended matter, nitrate, phosphate, chemical oxygen demand, chlorophyll a, bacterial production and abundance.

Our study showed that the BCC was different between seasons and bacterial types, and PA bacterial diversity (Shannon index) was always higher than FL. Unexpectedly, for both seasons and bacterial types, diversity increased along the river course as we observed a progressive decrease of the most abundant typical freshwater taxa along the river course, such as the genera Pseudarcicella and Limnohabitans. Surprisingly as well, for FL bacteria, diversity was higher during summer than spring, whereas there was no significant difference for PA bacteria. This was not expected as several studies showed a negative correlation between temperature and diversity. Finally, temperature and dissolved oxygen were the main parameters driving BCC.

533 / INFERRING ENVIRONMENTAL DRIVERS OF COVID-19 SEVERITY BY MACHINE LEARNING

Keywords: *COVID-19, Machine learning, Epidemiological model, nan*

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Understanding the main environmental determinants of Coronavirus disease 2019 (COVID-19) is crucial for understanding the health and economical risks of the ongoing pandemic. So far, statistical analyses based on the case and death counts proved to be highly sensitive to testing policies and the phase of the epidemic. Furthermore, simple regression methods, used in the majority of the studies, are unable to single out relevant severity predictors among high number of mutually correlated environmental factors and do not account for their possibly complex interactions. By employing the combination of epidemiological modeling and machine learning-based regression techniques, we aim to overcome these limitations. We show that our severity measure, inferred directly form the epidemiological model is an independent observable of COVID-19, as it does not depend on the transmissibility of the disease. We next use this measure as the response variable in four machine-learning based regression techniques and show, on the data from the United States, that these models successfully retained main COVID-19 severity predictors, known from the clinical studies, while uncovering some of the new environmental risk factors, such as air pollution and population density. On the global level, by analyzing both disease severity and the excess deaths during the pandemic, we aim to explain the puzzling association of Global Health Security Index (GHSI) and COVID-19 severity and show that the unintuitive positive association of COVID-19 severity and GHSI diminishes as the analysis progresses from simpler to more complex methods.

574 / UNRAVELING THE BACTERIAL EPIGENOME AND ITS INFLUENCE ON GENE TRANSFER AND TRANSCRIPTIONAL CONTROL

Keywords: *Bacterial epigenome, gene transfer, multipartite genomes, nan*

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BACKGROUND

In prokaryotes DNA methylation is known to play roles during cell cycle and gene transfer with few data only on the role in transcriptional control and in the population structuring. Indeed, bacterial genomes are often very diverse within the same species and characterized by a large fraction of horizontally transferred genes (the so called open pangenome structure). May this genomic variability relate to genome-wide methylation patterns and have epigenetic impact on transcriptional control?

OBJECTIVES

By investigating the genome-wide methylation patterns in a group of Sinorhizobium meliloti strains, a nitrogen-fixing plant symbiotic species with a classical open pangenome structure, we aimed at understanding the phylogenetic signature and functional relevance of epigenomic modifications.

METHODS

The genomes of 23 strains were sequenced using PacBio SMRT sequencing to identify genome-wide methylations. A subset of strains was also tested for pairwise gene transfer efficiency and RNA-Seq data were used to identify putative methylation-related transcriptomic signatures.

RESULTS

A computational pipeline has been developed to analyze methylation pattern, to parse the methylated positions within the genome and detect methylated-enriched genome regions.

Results obtained showed the presence of a core and of a strain-specific set of DNA methylation patterns, showing differential abundance with respect to gene position (e.g. promoter vs. coding sequence) and genomic locations, suggesting that they may either act as barrier to gene transfer and have a role on gene expression regulation.

Experimental validation of the link between methylated motifs, genome-wide horizontal gene transfer events and transcriptome variation is ongoing.

616 / THE EFFECT OF CADMIUM ON THE N2O REDUCTION POTENTIAL OF MODEL MARINE BACTERIA

Keywords: *Nitrous Oxide, Deep-sea, nosZ, nan*

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Deep-sea bacteria have high environmental importance, due to their active role on nutrient cycling in a place where time appears to run slower. Some of these bacteria are responsible for maintaining low natural levels of nitrous oxide (N2O), a powerful greenhouse gas, by reducing it to dinitrogen gas (N2) with the action of N2O reductase, encoded by the nosZ gene.

The growth of deep-sea mining activities expected for the next decades may increase the exposure of marine bacteria to toxic concentrations of metals, such as cadmium, copper, zinc, and lead. However, the susceptibility of the N2O-reducing pathway to metal exposure, is relatively unknown, especially in deep-sea conditions.

The aim of this study is to understand potential impacts of cadmium exposure on N2O reduction by using model denitrifying marine bacteria, including a pelagic Alphaproteobacteria (Ruegeria pomeroyi) and piezotolerant deep-sea strains.

The potential inhibitory effect of cadmium in N2O reduction is being investigated in controlled experiments by adding dissolved cadmium to growing cultures. In these experiments, N2O reduction activities are being quantified by gas chromatography coupled with electron capture detection. We are also evaluating nosZ relative expression through real-time quantitative PCR. To get closer to deep-sea conditions, some of these experiments are being performed in high-pressure bioreactors.

We will present results regarding the effect of cadmium in N2O reduction and nosZ expression in the different strains. Also, we look forward to exploring and understanding the role of high hydrostatic pressure in N2O reduction activities.

ACKNOWLEDGEMENTS/REFERENCES

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101038095.

672 / MICROBIAL ADAPTATION TO GLOBAL WARMING: THE MODEL SYSTEM PSEUDOALTEROMONAS HALOPLANKTIS TAC125

Keywords: *Global warming, Antarctic bacteria, nan, nan*

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BACKGROUND

Global warming affects ocean ecosystems in several ways, including alteration of temperature, acidification, oxygen content and nutrient inputs. Since microbes play a dominant role in global biogeochemical cycles that are crucial for planet's sustainability, their response to climate changes affect the overall ecosystem functioning. The Antarctic strain Pseudoalteromonas haloplanktis TAC125 (PhTAC125) is one of the model organisms of cold-adapted bacteria, and its ability to growth in a wide range of temperature (-4°C/25°C) makes it also an ideal model for studying the effects of climate change.

OBJECTIVES

The main objective of this work is the systems-level understanding of the adaptation process of a model Antarctic bacterium to environmental fluctuations imposed by the on-going global climate change.

METHODS

We combined experimental evolution, -omics data (including Tn-seq experiments) and mathematical metabolic modelling to provide an outlook on the adaptation of PhTAC125 to global warming, evaluating the impact of projected changes in some key environmental chemico-physical parameters (and/or combination thereof) and describing the molecular, evolutionary and ecological strategies that PhTAC125 may adopt to face such conditions.

RESULTS

Our results shed light on the consequences of global warming on a model organism, and on how changes at the microbial level could reverberate also in higher parts of the ecosystem.

687 / PLASTICS IN AQUATIC ECOSYSTEMS: A NEW ROUTE FOR TRAFFICKING MULTIDRUG-RESISTANT BACTERIA?

Keywords: *Microplastics , Antimicrobial resistance, Human health , nan*

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Microplastics (MPs) are emerging environmental pollutants that are potential vectors for accumulating multidrug-resistant bacteria (MDR). Although MPs have drawn considerable attention from researchers, minimal data are available on their role in trafficking antimicrobial-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs). Our study comparatively investigated the ARB's accumulation on the surface of microplastic and in raw water. Plastic substrates (polypropylene) were placed in seven geographical points of central Italy's volcanic, caldera-type lake. Raw water and plastic substrates were collected from all seven sites in two different seasons: late summer and early winter. A total of 110 MDR were isolated from freshwater and plastic substrates. Biochemical identification showed that the diversity and abundance of cultivable ARBs are higher in microplastics than in raw water, with a predominance of Enterobacteria's like Escherichia coli and Salmonella spp as well as Staphylococcus spp.

All isolated strains were tested against six antibiotics, namely Tetracycline, Gentamycin, Chloramphenicol, Ciprofloxacin, Meropenem, and Oxacillin, commonly used in humans and animal's therapeutics. Results showed that these strains are resistant to three to four antibiotics accounting for much higher proportions in the isolates obtained from microplastics. In a nutshell, the positive detection rate of ARBs was up to 89.5% in microplastics and just 71% in freshwater samples. These results revealed that microplastics are hazardous pollutants for the enrichment of superbugs and can aid the trafficking of ARGs; hence they should be regarded and evaluated as a potential threat to human health.

727 / HOW WASTEWATER BACTERIA LEARN TO FEED ON PREVIOUSLY PERSISTENT XENOBIOTICS – THE CASE OF ACESULFAME K

Keywords: *Bacterial genomics, Xenobiotic degradation, nan, nan*

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The artificial sweetener acesulfame K (ACE-K) is a widespread micropollutant in freshwater due to its persistency and mobility in aquatic environments. It has long been considered recalcitrant to microbial attack, but recent studies indicated the emerging potential of ACE-K removal in wastewater treatment plants (WWTP) and suggested a catabolic process leading to complete mineralization. We isolated several bacterial strains growing on ACE-K as sole carbon source from several WWTP in Germany. To elucidate the genetic background of ACE-K degradation, the genomes of six Chelatococcus strains and three Bosea strains (including an ACE-K degradation-defective mutant obtained by multiple transfers in non-selective medium) were sequenced using a long- and short-reads hybrid strategy, except for the mutant strain (short-reads only).

High-quality draft genomes were assembled ranging from 5.8 Mb to 7.3 Mb. Importantly, wild type Bosea sp. 100-5 shares about 98% of the genome with its degradation-defective mutant, and a gap of ~22 Kb was found. Gene prediction and annotation of this region revealed 28 coding sequences (CDS), most of them related to mobile genetic elements, but four CDS related to transporter genes and two CDS encoding a metal-dependent hydrolase and an amidase might be involved in ACE-K degradation. Almost 70% of the gene cluster (>19 CDS), including both hydrolase genes, was identified in the other seven genomes as well as in a public genome of an ACE-K-degrading Chelatococcus strain isolated from a WWTP in Hong Kong. Our results suggest that the ACE-K degradation trait has been distributed worldwide by horizontal gene transfer.

734 / THE SEQCODE REGISTRY: A WEB APPLICATION TO AUTOMATE AND ASSIST THE VALIDATION OF TAXON NAME PROPOSALS FOR ALL PROKARYOTES

Keywords: *Nomenclature, Databases, Taxonomy, Uncultivated*

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BACKGROUND

The number of prokaryotic species on Earth is likely in the millions. Of these, ~100,000 are uncultivated and represented by genomes. Problematically, less than 20,000 species are classified under the current nomenclatural system, which requires axenic cultures as type strains. Therefore, most known prokaryotic species, the "uncultivated majority", are currently unnamed or their names lack standing in nomenclature. This disparity is expected to grow as culture-independent genomics increasingly outpace the cultivation capacity.

METHODS

To address this gap in scientific communication, an international group of microbiologists have proposed a path for name validation with a new code of nomenclature for prokaryotes allowing valid publication of names with high-quality genomes as type material, the SeqCode
(https://www.isme-microbes.org/seqcode-initiative)

METHODS

To simplify name registration and validation, we developed the SeqCode Registry, a web application incorporating the SeqCode and adapting its rules and recommendations into unitary tests. This effectively translates a nomenclatural code into computer code and augments it with an interface guiding the user through the process of name registration. The SeqCode Registry evaluates uniqueness across nomenclatural codes, implements checks for name quality, genome quality, and the automated collation and formatting of etymologies, and provides a freely accessible API.

RESULTS

We anticipate the SeqCode will help democratize the naming of prokaryotes and further advance FAIR principles in prokaryotic nomenclature by facilitating taxonomic descriptions, ultimately improving scientific communication in microbiology. The application is released under the MIT license, its data under the CC-BY-4.0 license, and it can be accessed at https://seqco.de/.

759 / OCCURRENCE OF POTENTIAL INDICATOR GENES OF ANTIMICROBIAL RESISTANCE CONTAMINATION IN THE NORTH SEA AND ENGLISH CHANNEL SEAWATERS

Keywords: *Antimicrobial resistance genes, Indicator genes, Marine environment, Environmental variables*

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Water environment is known as a natural reservoir of antimicrobial resistance genes (ARGs), and a route of exposure and transmission of these resistances to humans. Marine environment can be strongly subject to anthropogenic influences particularly near the coasts and in areas with important fishing activities and maritime traffic. Recently, indicators of environmental contamination by ARGs and mobile genetic element have been proposed. In this study, we investigated the abundance and geographical distribution of four indicator genes (tetA, blaTEM, sul1 and intI1) in the English Channel and North Sea areas, and the interactions with environmental variables. Seawater samples were collected during oceanographic campaign, filtered and bacterial DNA was extracted. The tetA, blaTEM, sul1 and intI1 indicator genes were quantified by qPCR and normalized with the bacterial tuf gene. Possible correlations between the abundances of the genes and the environmental variables were determined. No quantification of the blaTEM gene was observed. The tetA gene was quantified in one sample. The sul1 and intI1 indicator genes were the most detected in 42% and 31% of samples, respectively, with an apparent co-occurrence in 19% of samples confirmed by correlation analysis. The abundances of these two genes were correlated with the microbial community and some environmental variables such as oxygen saturation and salinity. Moreover, the three indicators were quantified in the same seawater sample, near Dutch coasts. This work provided the first data in the English Channel and North Sea on the occurrence of indicators of environmental contamination by ARGs.

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05

796 / A QUANTITATIVE STUDY CONCEPT FOR INVESTIGATING THE SPREAD OF ANTIBIOTIC RESISTANCE IN THE RIVER WATER ENVIRONMENT

Keywords: *river, antimicrobial resistance, fecal pollution, nan*

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River water is used for a variety of human applications (e.g. drinking water production, recreation, irrigation). Microbiological contamination of rivers from point sources like wastewater treatment plants (WWTPs) or diffuse inputs such as run-off from agriculture can pose a threat to human health. Via faecal inputs, human-induced (acquired due to human activities) antibiotic-resistant bacteria (ARB) and antibiotic resistant genes (ARGs) can enter rivers, in addition to faecal transmitted pathogens.

Many studies to assess antimicrobial resistance in aquatic environments use either cultivation-based or DNA-based approaches and very often only in a qualitative way. Thus, we developed a more comprehensive study design covering both methodologies in a quantitative manner and including important hydrological, chemical and physical environmental factors to comprehensively investigate the distribution patterns, dispersal pathways and influencing factors of human-induced ARB and ARG. Over one year, water and biofilm samples of four rivers were taken in quarterly intervals along gradients and different sources of faecal pollution and river size and analyzed for phenotypic resistances via antimicrobial susceptibility testing and for specific ARGs via qPCR. In addition, at a representative site downstream of a WWTP, a comparison of the resistances found in Escherichia coli with resistances from a related clinic was made.

This holistic quantitative approach will provide much more comprehensive insights into the occurrence and spread of human-induced antimicrobial resistance in rivers, which are important for deeper understanding and sustainable management of microbiological water quality in the future.

800 / FAECAL POLLUTION AS POTENTIAL DRIVER OF ANTIBIOTIC RESISTANCE GENES IN THE DANUBE RIVER

Keywords: *River, Antimicrobial resistance, Faecal pollution, nan*

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Human-induced antimicrobial resistance is an emerging concern in aquatic environments. Faecal pollution sources represent potential propagation pathways, however large-scale quantitative studies in whole river systems are missing. In the course of the Joint Danube Survey, the Danube River was investigated for the fourth time in summer 2019 from its headwaters to the delta. Microbial-faecal pollution patterns and the occurrence of selected antibiotic resistance genes (ARGs) were studied. Subsequently, a one-year time series analysis at selected relevant sites allowed to assess temporal variability.

The intestinal bacterium Escherichia coli was quantified according to standard cultivation methods as indicator for total faecal pollution. In general, longitudinal patterns of faecal pollution showed a comparable picture to previous Joint Danube Surveys, with low to moderate pollution in the upper reaches (Germany, Austria) and critical to strong pollution in the middle and lower sections, especially in Serbia. Genetic microbial source tracking methods, determined via quantitative PCR, allowed us to define human faecal pollution as dominant pollution source along the whole Danube. This trend could be generally confirmed during the annual sampling campaign. To estimate the potential of permanent colonialization of faecal microorganisms within the Danube River ecosystem, biofilms as potential reservoirs were studied along the course of the entire river. Here, E.coli were ubiquitously present but highly variable. Ongoing analyses on the occurrence and quantity of selected ARGs will identify hotspots of antimicrobial resistance and their relationship to faecal pollution along the Danube River and thereby contribute to guide future monitoring and management strategies.

ACKNOWLEDGEMENTS/REFERENCES

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05

818 / ADAPTATION AND MANIPULATION OF PLANT-MICROBIAL INTERACTIONS TO ENHANCE ACID MINE DRAINAGE BIOREMEDIATION WITHIN NATURAL WETLANDS

Keywords: *Bacterial community, Microbial bioremediation, Metagenomics, Wetlands*

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Natural wetlands are known to play an important role in pollutant remediation, such as the decontamination of acid mine drainage (AMD) from abandoned mine sites. However, many aspects of the biological mechanisms underlying AMD remediation by wetlands are unclear, including the role of rhizosphere microbial communities. We aimed to examine whether the presence of wetland plants, pollution gradients and amendments such as organic carbon determine changes in microbial biodiversity and function associated with AMD remediation. We used an AMD-polluted river-wetland system [1] for metagenomic analyses of microbial communities that mediate metal immobilisation. Sequencing of environmental DNA from sediment samples identified substantial variation in bacterial community structure and species diversity on the basis of water/sediment chemistry parameters and wetland plant presence. This indicates the critical role that wetland plants play in maintaining diversity and function of sediment microbial communities despite high levels of acidity and metal pollution. Analysis of functional genes from sediment samples showed increased abundance of specific genes for iron and sulfur metabolism within the middle of the wetland, coincident with increased metal precipitation. Finally, we examined how manipulation of the sediment microbiome, by controlling organic carbon or oxygenation, can further improve water quality. The addition of organic carbon (with or without aeration) produced major changes in the abundance of microorganisms related to iron and sulfur metabolism and increased particulate metal concentrations via sulfate reduction. This demonstrates the importance of organic carbon utilisation by the bacteria for AMD treatment, yet the presence of oxygen did not inhibit these activities.

ACKNOWLEDGEMENTS/REFERENCES

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819 / THE SEQCODE: A PROPOSED NOMENCLATURAL CODE FOR UNCULTIVATED PROKARYOTES WITH DNA SEQUENCES AS TYPE

Keywords: *nomenclature, systematics, type sequence, metagenomics*

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BACKGROUND

Over the last fifteen years, genomics has become fully integrated into prokaryotic systematics. The genomes of most type strains have been sequenced, genome sequence similarity is widely used for delineation of species, and phylogenomic methods are commonly used for classification of higher taxonomic ranks. Additionally, environmental genomics has revealed a vast diversity of as-yet-uncultivated taxa.

OBJECTIVES

In response to these developments, a new code of nomenclature, the International Code of Nomenclature of Prokaryotes Described from Sequence Data (or colloquially the SeqCode), has been developed to allow naming of Archaea and Bacteria using DNA sequences as the nomenclatural types.

RESULTS

The SeqCode will allow naming of the enormous uncultivated biodiversity discovered through the aid of metagenomic sequencing and single-cell genomics, fastidious prokaryotes that cannot reasonably be maintained and distributed by culture collections, as well as organisms that cannot be legally transferred between countries. The SeqCode also recognizes the priority of ICNP names provided they do not violate the priority of SeqCode names, thus minimizing divergence between the systems. The SeqCode also provides an online self-registration system called the SeqCode Registry (https://seqco.de/) for curation and recording of names. The start date of the SeqCode was January 1, 2022. In conclusion, the SeqCode will facilitate the naming of taxa in every biome on Earth and promote synergies between the field and laboratory disciplines of microbiology.

ACKNOWLEDGEMENTS/REFERENCES

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833 / TOWARDS THE BACTERIAL BIOCONTROL OF WEED MOULDS IN MUSHROOM CULTIVATION

Keywords: *white button mushroom, biocontrol, mushroom pathogenic molds, nan*

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Cultivated mushrooms like white button mushroom (Agaricus bisporus) and oyster mushroom (Pleurotus ostreatus) have their pathogens which can have detrimental effects on the crop such as the weed moulds Lecanicillium fungicola, Hypomyces perniciosus, Cladobotryum mycophilum, Trichoderma aggressivum, Trichoderma pleuroticola, Trichoderma pleuroti and the bacterium Pseudomonas tolaasii. Among them, Trichoderma aggressivum its the most devastating, especially the subspecies Trichoderma aggressivum f. sp. aggressivum.

In this study, biocontrol bacteria were selected from strains belonging to the genus Pseudomonas and the Bacillus group, which were isolated from the casing layer and compost of white button mushroom growing medium. The isolated strains were tested against 2-2 strains of Lecanicillium fungicola and Hypomyces perniciosus. Preselected strains were further tested against the other, aforementioned fungal pathogens, as well as against white button mushroom and oyster mushroom. The tests were also performed with the cell-free supernatants of the bacteria. The pH-, salinity- and temperature-dependence and fungicide resistance properties of the selected bacteria were also recorded. Extracellular enzyme activities were measured in the cell-free supernatant of the bacteria for proteases, lipases, and chitinases.

Additionally, the concentrations of three groups of antibiotics (iturins, fengycins, surfactins) were also determined from the cell-free supernatants, and tests were performed on agar plates to detect the production of siderophores by the selected biocontrol strains. The characterisation of the selected strains are important for planning their application in a targeted environment, such as the growing medium used on mushroom farms.

ACKNOWLEDGEMENTS/REFERENCES

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847 / FAECAL POLLUTION AND ANTIBIOTIC RESISTANCE IN THE DANUBE RIVER: VISIONS, CONCEPTS AND LESSONS LEARNED FROM JOINT DANUBE SURVEYS

Keywords: *faecal pollution, antimicrobial resistance, Danube River, nan*

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Sustainable and target-oriented microbiological water quality management of rivers needs information on whole river systems, especially if catchments are large and international. The microbiological water quality of rivers is of uttermost relevance for human health as river water is used for several purposes (recreation, drinking water production, irrigation). Besides information on faecal pollution levels, the origin of faecal pollution and the assessment of associated infection- and health risks, e g. from antimicrobial resistant bacteria (ARB) are of increasing importance. This contribution summarizes the concepts and main results from the Joint-Danube-Surveys (JDS) and discusses future challenges and perspectives (pollution-scenario modelling, infection-risk assessment) for the Danube River concerning its faecal and AMR pollution status.

Between 2001 and 2019, the whole Danube was sampled four times during the JDS. Beside standard faecal indicator analysis, cutting-edge molecular detection concepts were applied, including microbial source tracking (MST) markers by quantitative PCR (qPCR) and high-throughput amplicon-sequencing of bacterial communities. For AMR-profiling, clinically relevant bacterial species were isolated and tested for resistances and resistance genes were determined via qPCR.

With this, we could impressively demonstrate that the JDS create the required multi-national “big picture” of the microbiological pollution status of large rivers. Harmonised trans-border microbiological water quality maps for the whole navigable Danube were established. MST marker analysis elucidated that the main faecal pollution source along the whole river is human wastewater. Combined analysis of faecal indicators, MST and AMR-profiles provided a solid basis for assessing the potential health impacts of AMR associated with faecal pollution.

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869 / ENHANCED IN SITU OIL BIODEGRADATION AT DEEP AS COMPARED TO SHALLOW WATER DEPTH IN A GREENLANDIC FJORD

Keywords: *Oil Pollution, Biodegradation, Marine Microbiology, Arctic Ocean*

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Due to climate change, trans-arctic shipping will increase, elevating the risks for marine oil spills. Oil removal in marine waters heavily depends on biodegradation; however its controlling factors remain incompletely resolved, especially in the Arctic region. In the highly stratified and already oligotrophic Arctic Ocean, photosynthetic activity depletes inorganic nutrients from the upper water-column. Hence, we hypothesized that the higher amount of inorganic nutrients at deeper water level could increase oil-biodegradation rates. Thus, we performed a unique in situ experiment in Godhabsfjord (West-Greenland), where we deployed oil probes at 20 m and 650 m water depth, for a one-year period. Oil components were analyzed by gas chromatography-mass spectrometry. The natural biofilm grown on our oil probes was analyzed by performing 16S rRNA gene amplicon quantification and sequencing. More than 95% of the highly-biodegradable compounds (e.g. alkanes) were removed after three months, while polycyclic aromatic hydrocarbons (e.g. Chrysene) persisted even after one year. Most hydrocarbon compounds degraded faster at 650m (e.g. Chrysene displayed a half-life time of 158 days at 650m and 208 days at 20m). The bacterial community composition of the oil biofilms differed between the two depths and strongly correlated with the hydrocarbon profile. Moreover, oil biofilms at 650m contained higher absolute abundance (16S rRNA copies/cm2) of several known hydrocarbon degrading bacteria, indicating that the higher concentration of nutrients promoted their growth. Thus, deep waters represent a better environment for oil-biodegradation compared to shallow waters in the studied Arctic Fjord system.

ACKNOWLEDGEMENTS/REFERENCES

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05

878 / REINTEGRATING ENVIRONMENTAL INFORMATION INTO SPECIES-LEVEL OTU DELIMITATIONS

Keywords: *taxonomic profiling, database, microbial ecology, nan*

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In order to distinguish biological units on a species level in taxonomic profiling of microbial communities, the current gold standard is to cluster marker genes sequences based on sequence similarity into Operational Taxonomic Units (OTUs), most prominently 97% in the case of 16S rRNA. However, it has been shown that this threshold is not always ideal[1]. Furthermore, by only considering sequence similarity, other important pieces of information such as their ecological niches and metabolic capabilities are largely ignored.

To reintegrate environmental information into OTU delimitations we are using millions of publicly available samples from all global environments, reprocessed and analyzed in the same way to ensure comparability in between different studies. In a novel approach - not relying on the often-lacking metadata annotations in public databases- 10000 OTU pairs are analyzed for each major microbial phylum. As a proxy for the similarity of their ecological niches, an in-house tool is used to calculate the average beta-diversity of all samples in which two respective OTUs are found. By observing how these metrics change based on the phylogenetic relatedness of an OTU-OTU pair we could already show that more closely related microbes tend to be found in more similar environments in all 25 investigated microbial phyla. Additionally, the strength and shape of this trend is varying in different phyla as well as in environments, demonstrating the potential of this approach to more accurately define the most adequate sequence similarity thresholds for species level OTU-delimitations in different Phyla.

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892 / SEASONAL VARIATION OF THE ATMOSPHERIC BACTERIAL COMMUNITY IN THE GREENLANDIC HIGH ARCTIC IS INFLUENCED BY WEATHER EVENTS AND LOCAL AND DISTANT

Keywords: *Bioaerosol, High Arctic, Aeromicrobiology, Microbiome*

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To get a better understanding of how the atmospheric microbiome in the high Arctic is shaped on a temporal scale and how the atmospheric microbiome may influence cloud and precipitation formation as well as the Arctic climate we need to study these systems in-situ.

We studied air samples in a time series spanning the end of the Arctic haze period and snow samples collected after a snowstorm in the spring of 2015 together with air samples collected in the summer, from Northeast Greenland. 16S rRNA amplicon sequencing of DNA and cDNA together with qPCR were utilized to process the samples. We found that the bacterial abundance is higher in the spring air compared to summer air. Community analysis revealed a clear separation between the seasonal air microbiomes as well as the snow microbiome. Interestingly, the air sample collected just before the snowstorm was more similar to the snow microbiome than with the other air samples. 134 ASVs (~35%) were shared between the air sample and the snow samples and especially Cyanobacteria were abundant in both and showed a high activity potential. After the snowstorm, we were not able to detect airborne bacteria for the next 3 weeks, which we suggest was due to a washout of the microbial community from the air into the snow. These results show that the high Arctic bioaerosol community in spring is tightly linked to long-range transport and weather patterns whereas the summer air microbiome assemble from local available sources.

912 / ISOLATION OF OIL DEGRADING BACTERIAL CONSORTIA BY RANDOM ELECTROSTATIC AGGREGATION OF CELLS USING MICROFLUIDIC APPROACH

Keywords: *oil pollution, bacterial isolation, electrostatic aggregation, microfluidics*

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Bioremediation of oil polluted environments is a crucial step when addressing the issues of oil spills. To avoid further contamination, bacterial ability to degrade hydrocarbons via strain isolation, cultivation and application to specific medium was acknowledged as an effective and eco-friendly solution. Since recalcitrant substrates, such as hydrocarbons in petroleum oil are highly resistant to degradation, bacterial consortia are often used. Classical isolation techniques often include cultivation of inoculum from polluted site with the addition of hydrocarbons as the sole carbon source, which in turn generates a consortium of bacteria where along with (i) degraders with various specificity, (ii) co-metabolic degraders, (iii) nutrient suppliers, (iv) producers of electron shuttling molecules and (v) dispersant producers, (vi) cheaters or even (vii) competitors can be found. In situ, such communities of bacteria can perform degradation less effectively since bacterial cells are randomly dispersed and growth and metabolism of most effective bacteria cannot be controlled. In this regard, an electrostatic method for oil degrading consortia was developed, where cells are aggregated to achieve a close contact to the oil substrate as well as to other bacterial cells. Encapsulation of such aggregates in microdroplets allowed for an enormous combination of cells to be produced in a short period of time via microfluidics. Each randomized bacterial community within a microdroplet was analyzed by flow cytometry, microscopy based methods and on selected the most efficient consortia the genomic methods were applied in order to determine the stability, growth and ability of pollutant removal, respectively.

ACKNOWLEDGEMENTS/REFERENCES

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925 / DOES THE ALCANIVORAX GENUS PLAY AN ACTIVE ROLE IN MARINE PLASTIC DEGRADATION?

Keywords: *Biodegradation, plastic contamination, marine microbial ecology, nan*

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Plastic pollution is a worldwide problem, affecting both organisms living in the marine and terrestrial environment. Plastics are polymers generally derived from crude oil, the sole carbon source of hydrocarbonoclastic bacteria such as Alcanivorax. When investigating the marine plastisphere, Alcanivorax are often very abundantly present . Despite their presence, the exact role of the Alcanivorax in plastic degradation is not well understood.

Seawater enrichments on nylon and biodegradable plastics - both in batch as well as in a trickling filter system - were amplicon sequenced, showing an abundance between 10 and 25% of Alcanivoraceae. Subsequently, these plastic-degrading enrichments were used for selective isolation. Two Alcanivorax isolates, Alcanivorax sp. DG881 and Alcanivorax borkumensis, were obtained. Moreover, we determined the growth and metabolic activity of the isolated strains on nylon12 compared to a mixture of cyclododecane and dodecane. The Alcanivorax borkumensis isolate showed almost no growth on the nylon12 after seven days. The metabolic activity of the isolated strains was measured through bioorthogonal non-canonical amino acid tagging (BONCAT).

Second, the ecological response to exposure of fresh polymers of these plastic degrading communities, comprising Alcanivorax, will be followed over time. Metabolic activity will be determined with BONCAT, after which the active fraction will be sorted. These sorted samples will be sent out for amplicon sequencing to identify in what part of the degradation process Alcanivorax comes in play.

During my presentation I will further elucidate the role the Alcanivorax genus plays in degradation of plastics in the marine environment.

ACKNOWLEDGEMENTS/REFERENCES

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954 / MONITORING THE FATE OF SULFONAMIDE RESISTANCE GENES AND THE MOBILE GENETIC ELEMENT INTI1 IN A WASTE WATER IMPACTED RIVER STREAM

Keywords: *antibiotic resistance, mobile genetic elements, intI1, aquatic ecosystems*

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Even though antibiotics are known to cause antibiotic resistance, currently there are no regulations regarding there discharge from wastewater treatment plants (WWTP) into river systems, making surface waters a reservoir for antibiotics and antibiotic resistance genes (ARGs) [1].

Several indicator genes, including sulfonamide resistance genes sul1 and sul2 and the integrase gene intI1, were proposed to better understand the fate of ARGs in aquatic ecosystems. Both intI1 and sul1 are part of the class 1 integron, a mobile genetic element (MGE) accumulating various ARGs [2].

Here we monitored the abundance of those genes compared to 16S rRNA in a river from pristine source to WWTP-impacted water using quantitative real-time PCR. We sequenced the gene cassette inserts of class 1 integron to determine their alteration along the stream. The dynamics of the microbial communities were studied. In addition, the concentration of sulfamethoxazole (SMX) and conventional water parameters were measured using standard analytical methods.

Our results show the highest abundancy of sul1 and sul2 in WWTP effluent. While the frequency of sul1 and sul2 is decreasing downstream the WWTP and overall water quality is improving, the relative abundance of intI1 is increasing. This observation contradicts previous studies, where sul1 and intI1 abundance were directly correlated due to their co-occurrence in the class 1 integron [3]. Apart from that, the variety of integron gene cassette inserts constantly decreased downstream the WWTP too.

Our findings confirm the knowledge gap regarding factors driving the fate and spread of ARGs linked to MGE in aquatic ecosystems.

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978 / MICROALGAE AND BACTERIA INTERACTION – NOVEL INSIGHTS AND APPLICATIONS

Keywords: *symbiosis and host, microalgae-bacteria interaction, nan, nan*

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BACKGROUND

Tight associations between microalgae and bacteria caused the evolution of a complex network of cross-kingdom interactions. Orchestrated nutrient exchange, quorum sensing mediation and quorum quenching of the signals defined a wide spectrum of associations at highly complex assemblages of unicellular microalgae and associated bacteria. However, it remains unknown if the associated microbiota is specific for the microalga and which role individual bacterial taxa play.

OBJECTIVES

Our goal is to identify the key mechanisms and signals involved in the interaction of bacteria and microalga.

METHODS

We sequenced the microbiome of the microalga Scenedesmus quadricauda MZCH10104 and isolated various bacterial strains affiliated with the alga. One of the obtained isolates, HH091, which is affiliated with the phylum Bacteroidetes strongly stimulated growth and photosynthesis of the alga Scenedesmus quadricauda and Micrasterias radians in co-culture. For this, the whole-genome of the strain HH091 was established.

RESULTS

The metatranscriptome analysis indicates that the transcriptionally most active bacteria with respect to key genes commonly involved in plant–microbe interactions in the Scenedesmus strain belong to the phylum of the Proteobacteria and Bacteroidetes. The genome analysis of the isolate HH091 revealed several fascinating genetic features, such as multiple secretion systems, QQ mechanisms, polysaccharide utilizing gene clusters, unique signal transduction systems and different mechanisms of secondary metabolites, which may explain the molecular basis of bacteria-alga interactions. These data expand our understanding of species interactions within aquaculture and identify several genes involved in the molecular basis of bacteria-alga interactions that can serve as an established synthetic plant-bacteria system.

989 / TAXONOMY AND METABOLISM OF THE GLOBAL FRESHWATER MICROBIOME

Keywords: *aquatic, community, taxonomy, function*

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The continual cataloging and investigation of environmental microbial communities is critical as a basis for understanding our planet and its biogeochemical cycles. But in order to better contextualise past and future data we need to know what is a/typical for a given habitat or if there are differences between habitats.

To explore these questions we taxonomically and functionally characterised approximately one thousand publicly available freshwater microbial metagenomes Kraken2 and humann3 with refseq, GT, and pfam databases. We then compared their metabolic gene content and community membership to sample habitat descriptions. In general, similar taxa and functions were found throughout, though differences between habitats increased as taxonomic level decreased (e.g. fig 1). To identify potential consortia we also visualised taxonomic and functional co-occurrence networks. These analyses will assist researchers in making well founded comparative statements about the microbial community of freshwaters in the future.

1023 / MECHANISMS OF GLOBAL EPISTASIS AND THE PREDICTABILITY OFMARINE MICROORGANISMS FOR BIOREMEDIATION OF OIL SPILLS: CULTURE-DEPENDENT AND INDEPENDENT APPROACHES

Keywords: *Oil spills, Bioremediation, Next-generation sequencing, Microbial cultivation*

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Microorganisms with the capability to degrade petroleum hydrocarbons have been widely studied to improve bioremediation techniques for the mitigation of oil spills. The development of culture-independent methods allowed insights into the structure and composition of microbial communities with the ability to degrade petroleum compounds.

However, culture-dependent methods are still an important tool to recover and characterize the microbial strains of interest. For this work, a combination of these two approaches was applied to study the potential of marine microbial communities to degrade hydrocarbon compounds. Seawater samples were collected in 21 sites, in four delimited areas, along the NW coast of the Iberian Peninsula, and kept in microcosms spiked with crude-oil and nutrients, for 15 days. Then, the samples from the microcosms were filtered through SterivexTM, for 16S rRNA gene next-generation sequencing and platted in three culture media: Marine Agar, M1 and Bushnell-Haas. Microbial isolates were later identified through Sanger sequencing. A total of 319 isolates were cultivated, where 48 genera were identified from the 21 sampling sites. These included the most abundant genera of the enriched communities, according to the taxonomic characterization by next-generation sequencing: Alcanivorax (composing 49% of all communities from the NGS analysis), Thalassospira (9%), Pseudomonas (5%), Marinobacter (2%), Joostella (1%), Cobetia (0.4%) and Thioclava (0.4%). Results also showed that only these 7 genera were common in the four delimited areas, potentially indicating that a prototype microbial consortium could be developed to implement as a bioremediation technique to mitigate oil spills using native microorganisms.

ACKNOWLEDGEMENTS/REFERENCES

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1046 / PHYTOPLANKTON PHOTOSYNTHESIS: AN UNEXPLORED SOURCE OF BIOGENIC METHANE EMISSION FROM OXIC ENVIRONMENTS

Keywords: *oxic methane production, photosynthesis-associated methane production , global methane budget, phytoplankton*

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Methane is the second most significant anthropogenically influenced greenhouse gas with a global warming potential 85 times higher than carbon-dioxide, over a period of 20 years. Atmospheric methane has been steadily increasing, yet its natural sources are not well constrained, with the largest uncertainties occurring in aquatic ecosystems. Significant methane concentrations in oxic surface waters have been long witnessed worldwide and shown to significantly contribute to the aquatic methane fluxes. This phenomenon that contradicts traditional concepts of strictly anoxic archaeal methanogenesis was termed the "Methane Paradox".

By now, plants, algae, saprophytic fungi and mammals were evidenced to emit methane under oxic conditions. Thus, methanogenic Archaea lost their exclusivity on methanogenesis. Recent studies show that all evaluated Cyanobacteria, diatoms and haptophytes, major constituents of freshwater and marine phytoplankton, emit methane during photosynthesis. This is independent of external methylated precursors such as methylphosphonates, methylamines and Dimethylsulfoniopropionate, whose demethylation by prokaryotic phytoplankton (and other microorganisms) was shown to be a potent methane source. Given the global distribution of phytoplankton these organisms likely significantly contribute to methane fluxes from oxic environments and accordingly to the global methane budget. Furthermore, the recently observed increase in cyanobacterial and algal blooms likely due to global warming and anthropogenic eutrophication, may result in a feedback loop where blooms release methane, leading to further warming and further increase in blooms. Current data are evidence of photosynthesis-associated methane production and its potential rates, yet further research is required to provide emission estimates at a global scale.

05

1188 / ARCTIC DEEP-SEA MICROBIAL COMMUNITIES AS POTENTIAL RESOURCES FOR HYDROCARBON BIODEGRADATION

Keywords: Arctic, microbial bioremediation , deep-sea , metabarcoding

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The rapid Arctic sea decline is opening new shipping routes, increasing the risk of accidental oil spills in this environment. The present work aims to explore the capabilities of autochthonous deep-sea microbial communities to degrade petrol hydrocarbons as potential resources that could contribute to the recovery of the arctic environment.

Sampling was performed during MarineMine cruise in summer 2016 along the Arctic Mid-Ocean Ridge (AMOR). A core device was used to collect four deep-sea sediment samples at around 2600 meters depth. Samples were preserved in LifeGuard solution till further processing. At CIIMAR laboratories a microcosm experiment was set up, incubating deep-sea samples for 15 days in presence of crude oil. At the end of the experiment, three different culture media were used to isolate hydrocarbons degrading microorganisms (HD) with the ability to use crude oil as carbon source. Isolated strains were identified by sanger sequencing. The abundance of hydrocarbon-degrading bacteria at the beginning and at the end of the experiment was evaluated through the Most Probable Number (MPN) method. Moreover, culture-independent approach allowed the characterization of the microbial communities by V4-V5 16S rRNA amplicon sequencing (Illumina MiSeq) at the beginning and end of the microcosm experiment.

Preliminary results showed a drastic decrease in richness and diversity after the enrichment with crude oil, which was accompanied by an increase in the abundance of genera already known to degrade hydrocarbons. HD microorganisms isolated in the present study were cryopreserved in a georeferenced microbial bank for future biotechnological application in bioremediation context.

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231 / MODELLING THE DEACTIVATION OF ESCHERICHIA COLI IN NIGERIAN SOILS AMENDED WITH DIFFERENTLY TREATED MANURES

Keywords: *Cattle manure, E. coli, Weibull survival functions, soil*

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BACKGROUND

There is less focus on the impact of the treatments on the deactivation of the faecal indicator organisms once added to the soil.

OBJECTIVES

In this study, the Weibull survival function was used to describe the deactivation of the indicator organisms E. coli in soil amended with cattle manure that had been treated by burning, anaerobic digestion, composting or without treatment.

METHODS

The Weibull parameters for each treatment were determined using E. coli measurements following application of the treated wastes to soils. The model was then evaluated using E. coli measurements from independent plots at the same site with different application rates of treated wastes. RESULTS: A statistically significant correlation and high coincidence between the simulated and measured values was obtained, demonstrating that the model was suitable to provide a comparative assessment of the effectiveness of different treatments methods at reducing E. coli loads at this site. The simulations revealed that anaerobic digestion or burning of cattle manure were equally effective at reducing the E. coli loads to background levels.

However, burning retained very little nitrogen, so the ash residue was ineffective as an organic fertiliser. The E. coli remaining in ash was more persistent in the soil than from other treatment methods, so care should be taken to avoid disposing of ash in large piles. Anaerobic digestion was effective at reducing the E. coli levels while retaining a high proportion of nitrogen in the bioslurry residue, but the persistence of the E. coli was higher than in compost.

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421 / COMPARATIVE IN VITRO EVALUATION OF GENOTOXIC POTENTIAL OF THE DANUBE RIVER WATER EXTRACTS IN PROKARYOTIC AND EUKARYOTIC ASSAYS

Keywords: *genotoxicity, Danube River, SOS/umUC, zebrafish*

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BACKGROUND

Prokaryotic assays are of great interest in eco/geno-toxicology as they are short-term, simple and cost effective. Application of strains of Salmonella typhimurium with modifications such as increased cell wall permeability and lack of excision repair system is common for the detection of genotoxicity. Various eukaryotic models have been developed lately, such as fish derived cell lines, which can mimic the response of the aquatic organisms. Considering different level of cell organization among these models, difference in sensitivity is expected, yet only few studies address this topic.

OBJECTIVES

The major objective was to investigate genotoxic potential of extracts of surface water of the Danube and to compare the results obtained in applied prokaryotic and eukaryotic models.

METHODS

Surface water samples from 24 sites along the Danube River were extracted by LVSPE Horizon sampler providing relative enrichment factor (REF) of the extracts of 25,000x. For representative prokaryotic model the SOS/umuC assay with S. typhimurium TA1535/pSK1002 was used. Eukaryotic model comprised assessment of cytotoxicity by MTS test and assessment of genotoxicity by comet assay in ZFL (ZebraFish Liver) cells.

RESULTS

While none of the tested samples has induced SOS response, the result of MTS assay indicated that 11 of 24 samples were cytotoxic. In comet assay, genotoxic potential was found in 9 of 24 samples. Results indicated higher sensitivity of used eukaryotic model in comparison with prokaryotic. Applied prokaryotic assay is often suggested to be used as prescreening tool in diagnostics of genotoxic potential which by our opinion could be misleading.

ACKNOWLEDGEMENTS/REFERENCES

This research was supported by FEMS Research Training Grant program. Analyzed river water extracts were prepared within the Joint Danube Survey 4 and assessment of genotoxicity was supported by International Commission for the Protection of the Danube River.

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585 / FOUNDING THE METAPROTEOMICS INITIATIVE, DRIVEN BY THE FIRST MULTI-LAB BENCHMARK EFFORT IN THE FIELD

Keywords: *metaproteomics, multi-omics, benchmarking, scientific community*

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During my FEMS RTG-funded research stay at the BAM institute in Berlin, Germany, I had the opportunity of closely collaborating with Dr. Thilo Muth. That collaboration (although in COVID times) turned out to be so successful that he was later appointed as co-supervisor of my PhD dissertation.

At the BAM institute, I worked on the first ever, community-driven, multi-lab comparison in the rapidly expanding field of metaproteomics. This publication includes detailed analyses of the impact of the different wet-lab and dry-lab workflows used in this multi-lab study, and found that the functional profiles obtained from these diverse analyses were highly similar across workflows, while differences were observed for the taxonomic composition in both the metaproteomic and multi-omic comparison.

This publication had a considerable impact on my PhD trajectory, and turned out to be an important cornerstone of my PhD dissertation - but even more importantly, it allowed me to closely collaborate with several leading PIs in the metaproteomics field.

Motivated by those fruitful collaborations, I took the opportunity to gather a team of motivated junior and senior researchers in the field to co-found the Metaproteomics Initiative (www.metaproteomics.org). Since February 2021, this Initiative is even officially recognized by the European Proteomics Society (EuPA). Currently, this Initiative gathers over 140 members in 60+ labs from 17 countries.

In conclusion: this research stay, and the resulting publication and Initiative, already had a major impact on my career and life, and I'm FEMS therefore very grateful to fund this amazing opportunity.

ACKNOWLEDGEMENTS/REFERENCES

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610 / PERFORMANCE OF PSEUDOMONAS PUTIDA KT2440 AS MICROBIAL CHASSIS FOR PHOSPHONATE COMPOUNDS'S DEGRADATION

Keywords: *Phosphonates, Glyphosate, biosensor, nan*

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BACKGROUND

Phosphonates (Phn) are a class of organophosphorus molecules characterized by a chemically stable carbon-to-phosphorus (C–P) bond (1). The growing global demand of Phn, particularly the herbicide glyphosate (GP), call for alternative solutions for their bioremediation. We had previously isolated 62 bacterial strains that can degrade Phn. When the genome of one isolate (Agrobacterium tumefaciens CHLDO) was sequenced, a phn polycistronic gene cluster was identified. This cluster (phnGHIJKLO-phnCDE2E1-duf104phnMN) encodes a C-P lyase complex and transporters, which explains the Phn-degradation capability of strain CHLDO (2).

OBJECTIVES

A synthetic biology strategy was adopted to engineer the C-P lyase pathway of A. tumefaciens CHLDO into the well-known bioremediation chassis Pseudomonas putida towards GP degradation. The transcriptional regulation of the phn cluster was likewise explored for constructing a Phn transcriptional-based biosensor.

METHODS

In a “top-down” approach, a minimal complement of phn genes was expressed in a standard plasmid (3) with its native structure and promoters. A synthetic glycine auxotroph of P. putida (ΔGLY strain) was used to couple growth with GP assimilation. In parallel, a Golden Gate toolkit was used to construct plasmids for Phn detection and quantification with orthogonal control.

RESULTS

P. putida ΔGLY carrying the phnG-phnN cluster grew faster compared to the control in the presence of GP at low glycine concentration, indicating that the Phn enzymes are active but not able to complement the auxotrophy. A. tumefaciens CHLDO carrying reporter fluorophores fused to phnG, phnC, and DUF1045 promoters could sense different Phn compounds, laying the basis towards efficient GP degradation.

ACKNOWLEDGEMENTS/REFERENCES

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ACKNOWLEDGEMENTS

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1079 / FLUORESCENT PERICONIA MACROSPINOSA STRAINS TO STUDY HOSTS PREFERENCES AND UNDERSTAND ECOLOGICAL FUNCTIONS OF ROOT ENDOPHYTES

Keywords: *endophytes, transformation, nan, nan*

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Dark septate endophytic (DSE) fungi represent one of the groups of microorganisms that colonize plant roots. Periconia macrospinosa is a common DSE that extensively colonizes gramineous plants on both agricultural and natural habitats, without causing any immediate, overtly negative effects. Although P. macrospinosa is a well-studied species, its interaction with the host plant is still not completely understood.

In this study we aimed to carry out Agrobacterium-mediated transformation on four P. macrospinosa strains isolated at Martonvásár Agricultural Institute, from long term monocultures of two crops: wheat and maize.

Agrobacterium strains were carrying one of the two binary vectors: pCBCT16 for green fluorescence and pBTRtdTOM for red, to allow simultaneous visualization of different P. macrospinosa strains in further research. Insertion of transfer DNA in the transformants' genomes was checked by digital droplet PCR (ddPCR). Saprotrophic growth capacity of transformants was measured during growth tests. Plant colonization ability was tested with wheat in an in vitro inoculation test, the colonization of the roots was visualized by confocal laser scanning microscopy.

The transformation resulted in 38 stable transformants, 16 carrying green and 22 carrying red fluorescent proteins. The tagged strains could colonize the inner tissues of the roots, and the visualization was successful in most cases. With the transformants that did not significantly differ from the wild-type in above mentioned characteristics further experiments will be set up. This experiment will contribute to our understanding of host-adaptation in the interaction between gramineous plant roots and DSE fungi.

ACKNOWLEDGEMENTS/REFERENCES

The research was supported by the Federation of European Microbiological Societies (FEMS-GO-2020-256) and National Research, Development and Innovation Office, Hungary (NKFIH K139026).

372 / ASSESSMENT OF THE EFFECT OF TEMPERATURE ON THE ATTACHMENT OF ESCHERICHIA COLI O104:H4 TO THE CRESS PLANT

Keywords: *attachment , contaminated irrigation, food safety, Escherichia coli biofilm*

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The undeniable temperature fluctuations due to climate change are recognized all over the world. Food safety is one of the areas that could be affected by the changes in temperature with changes in the survival of the foodborne pathogens on fresh produce. Prior to consumption, fresh produce is minimally processed. Therefore, they are prone to cause foodborne diseases. This study aimed to investigate the impact of climate change on the attachment of Shiga toxin producing Escherichia coli O104:H4 on cress plant grown under controlled conditions. The plant materials were inoculated at different stages of growth at a level of 8 log MPN/ml to analyze the effect of inoculation time (7, 14, 21 and 28 days post sowing; dps) on the attachment level of E. coli O104:H4 at harvest (30 dps) at temperature increase scenarios designed according to different greenhouse gas emission mitigation scenarios (+2, +4 and +6oC). This present study revealed that different climate change scenarios did not lead to a significant increase or decrease in the pathogen attachment levels (between 4.5 to 7 log MPN/g) on leaf tissue at 30 dps. The plant that was inoculated at a later stage (21 dps) showed higher pathogen load at all climate change scenarios studied. Besides, the pathogen load on the leaves were tightly bound to the surface (~3.5 to 7 log MPN/g), therefore, they could not be eliminated by washing steps.

ACKNOWLEDGEMENTS/REFERENCES

Graphical abstract was created with BioRender.com

389 / CONTRIBUTION OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA TO ANTIMICROBIAL RESISTANCE IN THE AGRO-FOOD CHAIN

Keywords: *antimicrobial resistance , lactic acid bacteria, bifidobacteria, agro-food chain*

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BACKGROUND

Antimicrobial resistance of bacterial pathogens presents a serious threat. Lactic acid bacteria (LAB) and bifidobacteria may increase the gene pool from which pathogens can acquire antimicrobial resistance genes (ARGs).

OBJECTIVES

Our aim was to determine antimicrobial susceptibility and genotypic resistance traits of LAB and bifidobacteria deliberately introduced into the agro-food chain and to evaluate their contribution to the spread of antimicrobial resistance in the agro-food chain.

METHODS

Phenotypic susceptibility was determined for 474 strains (157 strains deliberately introduced into the agro-food chain as starter, protective, or probiotic cultures or as feed additives (SPPF group), 154 isolates from fermented products, 90 from human intestinal mucosa/faeces, and 73 from human milk/colostrum). Minimum inhibitory concentrations were determined using broth microdilution in accordance with the standard ISO 10932. Whole genome sequences obtained by sequencing the selected representatives (n = 75) and publicly available genomes (n = 1039) were screened in silico for ARGs and mobile elements (MGEs).

RESULTS

The results revealed that 13.8 % of strains in the SPPF group contained acquired ARGs, most frequently for tetracycline. We confirmed that ARGs and MGEs were not as abundant in the SPPF group as in strains from human gut, suggesting that this group does not pose a significant threat. However, attention should be paid especially to individual probiotic strains containing elements that have been shown to have a high potential for transferability in the gut microbiota. These elements were uncommon in isolates of the natural microbiota from fermented products and were atypical of starter cultures.

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390 / NATURAL PHENOLICS AS ANTI-YEAST SUBSTANCES: EFFECTS ON GROWTH AND BIOFILM FORMATION OF FOOD SPOILAGE YEASTS

Keywords: *bioactive phenolics, antimicrobial activity, food-contaminating microorganisms, antibiofilm*

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BACKGROUND

Natural antimicrobials have become popular due to the negative perception associated with synthetic food preservatives. Therefore, the activity of natural phenolics against food-contaminating microorganisms deserves increased attention, but data on the effects of such phenolics on spoilage yeasts are still limited.

OBJECTIVES

To determine the antimicrobial efficacy of different classes of natural phenolics, i.e., hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, flavonoids and phenolic aldehydes, against the planktonic growth, biofilm formation, and adhesion of Pichia anomala, Saccharomyces cerevisiae, Schizosaccharomyces pombe and Debaryomyces hansenii.

METHODS

Microdilution approaches were used to assay the activities of studied yeasts in the presence of phenolics. Combined effect of phenolics with or without synthetic food preservatives was also studied by using checkerboard methodology. Biofilms formed were visualized by fluorescence microscopy. Molecular docking analysis was also performed for selected phenolics.

RESULTS

In general, cinnamic acid and vanillin had the highest anti-yeast activity in the growth inhibition tests. In S. pombe, minimum inhibitory concentrations of epicatechin and cinnamic acid decreased 16- and 4-fold, respectively, in the presence of vanillin. Synergistic effect was found between sodium benzoate and vanillin when P. anomala, S. cerevisiae and S. pombe were used as test organisms. Both microdilution and microscopy analyses showed reduced D. hansenii, P. anomala and S. pombe biofilms in the presence of many phenolics. In addition, cinnamic acid and vanillin presented strong antiadhesion activity against the studied yeasts. In silico studies revealed high binding potential for some phenolics to certain protein targets in S. cerevisiae.

ACKNOWLEDGEMENTS/REFERENCES

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408 / APPLICATION OF A FREE MODELLING TOOL TO EVALUATE THE EFFECTIVENESS OF AN UV-LED DEVICE TO INACTIVATE CAMPYLOBACTER JEJUNI

Keywords: *Campylobacter, Ultraviolet light, Modeling, nan*

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UV-LEDs have emerged as potential disinfection technologies and offer a new opportunity for combatting Campylobacter in the food industry. The objective of this study was to assess and predict the inactivation of C. jejuni populations in a liquid medium using a modelling tool when the UV-LED technology was applied at different wavelengths and treatment times. C. jejuni NCTC 11168 was cultured and incubated microaerobically in Mueller Hinton broth at 42˚C for 48 h. From this suspension, serial dilutions were carried out in maximum recovery diluent (MRD) until a concentration of ~5 log CFU/mL was obtained in a volume of 10 mL.

Suspensions were poured into glass petri dishes and treated inside the device using wavelengths of 280, 300 and 365 nm and combinations (280 + 300, 280 + 365 and 280 + 300 + 365 nm) for 1 to 150 s. Untreated samples were used as controls. After treatment, a ten-fold dilution series was prepared from samples, plated on to modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated microaerobically for 42˚C for 48 h. The GInaFit tool was employed and four models were selected: Log Linear, Log Linear and Shoulder, Weibull and Double Weibull. The most effective wavelengths were 280 + 300 and 280 nm which showed a reduction of ~4 log CFU/mL (Dt) after 5 and 7 s, respectively. Although all the selected models observed a good fit, Log Linear model was found to better describe the efficacy of the UV-LED technology for the inactivation of Campylobacter.

ACKNOWLEDGEMENTS/REFERENCES

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452 / ISOLATION AND IDENTIFICATION OF NOVEL NON-DAIRY STARTER CULTURES CANDIDATES FROM BACKSLOPPED PLANT MATRIX

Keywords: *Non-dairy starter cultures, Backslopping, 16S rRNA sequencing , MALDI-TOF*

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Currently, the majority of starter cultures on the market are originally isolated from milk and therefore do not provide the most optimal fermentation for plant matrices. Developing plant-derived starter cultures would be an enormous step to create good dairy alternatives. The aim of this study was to isolate and identify novel non-dairy starter cultures candidates using backslopping.

The evolution of bacterial consortia was studied in two oat and two pea drinks during backslopping at three different temperatures. The inoculum for the experiments was a natural consortium originating from horseradish, blackcurrant leaves, oat florets, and green peas. The samples were fermented at 42 °C, 34 °C, 25 °C and renewed for seventeen backslopping cycles.

Four samples were selected for strain isolation based on 16S rRNA sequencing and aroma characteristics. The bacterial strain isolation from samples were performed by using out plating technique and colonies were identified by MALDI-TOF mass spectrometry. The results showed that 1-3 species dominated in the consortia. We were able to identify and isolate nine lactic acid bacteria species from plant origin. The isolated species belonged to the genera Leuconostoc, Enterococcus, Lactobacillus and Lactococcus.

ACKNOWLEDGEMENTS/REFERENCES

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481 / FIR (ABIES ALBA MILL.) HONEYDEW HONEY AND LACTIPLANTIBACILLUS PLANTARUM–A SUCCESSFUL COMBINATION AGAINST SALMONELLA ENTERICA SER

Keywords: *oneydew honey, Probiotic bacteria, Salmonella enterica, nan*

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There has been a renewal of interest in the use of probiotics as biotherapeutic agents. Various probiotic strains have been identified and the most known probiotic bacteria is Lactiplantibacillus plantarum (former Lactobacillus plantarum). The prebiotic effect of honey on probiotic bacteria, which as part of the body's natural microbiota, plays an important role preventing diseases by various pathogenic microorganisms in the gut. At the same time, studies showed superior antibacterial efficacy of honeydew honeys to nectar honeys.

The aim of this study was to determine potential combined antimicrobial effect of fir (Abies alba Mill.) honeydew honey (HS) and probiotic bacteria L. plantarum on Salmonella enterica serotype Typhimurium. The effect of fir honeydew honey on the growth of L. plantarum in different media (MRS and BHI)] and the potential synergistic effect of HS and L. plantarum on the growth of S. Typhimurium in the BHI medium were examined. As control, 10% glucose instead of HS was used.

The results showed that concentrations up to 5% of HS sample stimulated the growth and metabolic activity of L. plantarum. Combined HS (5%) and L. plantarum significantly inhibited the growth of S. Typhimurium in BHI broth compared to the application of individual components. Fir honeydew honey showed potential prebiotic properties and antimicrobial activity.

724 / THE DEVELOPMENT OF NON-DAIRY BEVERAGE BASED ON BLACK CUMIN (NIGELLA SATIVA) PRESS CAKE FERMENTED WITH YOGURT CULTURE

Keywords: Nigella sativa L., fermented beverages, black cumin, diary alternatives

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BACKGROUND

New consumer behaviors have strongly affected the market for alternative dairy products. Demand for new plant-based products is driving strong growth in this sector. The need to develop modern food based on by-products of the agri-food industry is driven by consumer expectations for ecological solutions that are compatible with the circular economy concept. Black cumin oil press cake (BCPC) is a by-product of pressing oil from whole seeds and its potential for developing new types of functional foods is not fully exploited.

OBJECTIVES

In this study, attention was given to the development of new semi-solid yogurt-like products based on an unconventional matrix such as BCPC. Two trial variants containing 15% and 20% (w/w) of BCPC were fermented with commercial yogurt starter culture and refrigerated for up to 28 days.

METHODS AND RESULTS

Several parameters such as pH, total free amino acids, reducing sugars content, microbial population level, bioactivity, viscosity, textural parameters, and color were evaluated. Throughout the storage period, the number of lactic acid bacteria exceeded the recommended minimum level. The study showed numerous changes in the studied parameters compared to the unfermented products. Moreover, significant changes in the antioxidant activity (FRAP, DPPH, ABTS, and reducing power), polyphenols, and flavonoids contents were observed. Presented results open the possible pathways to obtain a novel black cumin-based product.

ACKNOWLEDGEMENTS/REFERENCES

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799 / EFFICIENCY OF COMMERCIAL DISINFECTANTS AGAINST MIXED BACTERIAL AND VIRAL CONTAMINANTS ON FOOD-CONTACT SURFACES

Keywords: *antimicrobial, norovirus, disinfectants, surfaces*

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BACKGROUND

Well-known fact is that biofilm forming bacteria are significant source of repeated problems in food processing environments. The problem is multiplied if foodborne viruses are embedded in the matrix or attached to the food-contact surfaces. Attachment of norovirus to stainless steel could be achieved after 10 min contact time. The knowledge of antimicrobial efficiency of different disinfectants allows improvement of decontamination strategies and reduction of the incidence of illness caused by these foodborne pathogens.

OBJECTIVES

To evaluate the effectiveness of commercial disinfectants based on sodium hypochlorite (4.5 g/100 g) and dimethyl benzyl ammonium chloride (0.75 g/100 g) on mixture of human norovirus (106 GE/μL) and Escherichia coli (107 CFU/mL) attached to stainless steel and plastic board.

METHODS

The effectiveness of disinfection was tested after 15 min contact time using Lightning MVP ICON method. For the quantification of norovirus RT-qPCR method was used.

RESULTS

After disinfection without rinsing for both tested surfaces, MVP results obtained in seconds were in a red warning zone, meaning that the cleaning must be repeated. After rinsing the surface were detected in a clean zone. The results indicated that adhesion of selected bacteria suspension was weak and adherent cells could be removed by additional rinsing. The number of detected noroviruses after sanitizing with sodium hypochlorite without rinsing were 2.2 for stainless steel and 2.3 log10 GE/μL for plastic board, while after rinsing 1.2 and 1.9 log10 GE/μL, respectively. The reduction of norovirus using chlorine-free disinfectant was not significant.

1166 / BIOFILM FORMATION OF SALMONELLA ENTERICA SEROVAR 4,[5],12:I:- AND PAN-GENOME-WIDE ASSOCIATION STUDY

Keywords: *S. 4,[5],12:i:-, biofilm, pan-GWAS, nan*

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BACKGROUND

Salmonellosis is the second most commonly reported bacterial zoonotic infection in humans in Europe. Since 2015, the prevalence of Salmonella 4,[5],12:i:- has increased significantly. This could be associated to different factors such as more invasiveness and persistence of this serotype in the host, and/or its ability to develop and persist inside biofilms.

OBJECTIVES

This study aims to evaluate the ability of S. 4,[5],12:i:- to form biofilms and to search for genotypes associated with this capability.

METHODS

A total of 92 isolates from serovars S. 4,[5],12:i:- and S. Typhimurium from human, food or pig origins were selected. Biofilms were developed in polystyrene microtiter plates, at 37 ºC for 24 hours, and measured by the Specific Biofilm Formation index (SBF). Illumina MiSeq was used for Whole Genome Sequencing, and pan-Genome-Wide Association Study (pan-GWAS) was performed using Prokka, Roary and Pyseer.

RESULTS

All strains produced biofilm, but it was less abundant ($p<0.001$) in S. 4,[5],12:i:- (SBF=0.44±0.25) than in S. Typhimurium (SBF=0.76±0.46). Pan-GWAS gave 127 significant clusters of orthologous groups and genes associated with SBF, including the iroBCDEN operon. This operon is required for the synthesis, secretion and uptake of the salmochelin siderophore and was interrupted in S. 4,[5],12:i:-.

CONCLUSION

Using pan-GWAS we identified different genes for the first time associated with biofilm formation. Our results suggest that interruption of salmochelin system could be involved in the S. 4,[5],12:i:- reduced biofilm. Further studies on biofilm composition, gene expression and the effect of gene-knockout will be conducted to confirm this finding.

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386 / POTENTIAL OF CANDIDA ALBICANS TO DEVELOP RESISTANCE TO NEOSARTORYA FISCHERI ANTIFUNGAL PROTEIN 2 AND ITS PEPTIDE-DERIVATIVE

Keywords: *Candida spp., antifungal protein, resistance, nan*

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BACKGROUND

Limited number of antifungal drugs are available to treat fungal infections in the first-line therapy of life-threatening mycoses. Fungi are able to develop resistance mechanism to these drugs and as a consequence, the incidences of fatal mycoses dramatically increased in the last years. Therefore, there is a substantial demand for development of new antifungal compounds with minimal potential for resistance development. The features of Neosartorya (Aspergillus) fischeri antifungal protein 2 (NFAP2) and its γ-core peptide derivative (NFAP2impγ) render them to be promising candidates.

OBJECTIVES

One of the requirements in the reliable application of NFAP2 and NFAP2impγ as antifungals drugs is an understanding the potential of fungi to develop resistance/tolerance to them. In the present study, we investigated it in Candida albicans CBS 5982 in comparison with fluconazole (FLK), a widely used antifungal drug to treat Candida infections. We also investigated whether the developed resistance/tolerance is broad spectrum or specific to FLK, NFAP2 or NFAP2impγ.

METHODS

We addressed these questions by a micro-evolution experiment; and an antifungal susceptibility testing with different licensed antifungal drugs (CLSI-M27A3 broth microdilution assay).

RESULTS

We observed that C. albicans has a limited potential to develop resistance to NAFP2 and NFAP2impγ in comparison to FLK. The NFAP2-, NFAP2impγ- and FLK-resistance/tolerance development increased or decreased the susceptibility of C. albicans to licensed antifungals depending on the class of the drug.

ACKNOWLEDGEMENTS/REFERENCES

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398 / EVALUATION OF THE ABILITY OF C. ACNES STRAINS TO TRIGGER ACUTE INFLAMMATION IN BONE AND JOINT INFECTION CONTEXT

Keywords: *Bone and Joint Infection, Inflammation, Host-pathogen interaction, Cutibacterium acnes*

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BACKGROUND/OBJECTIVES

Cutibacterium acnes is a commensal skin bacterium, involved in shoulder prosthesis infections that are difficult to diagnose [Boisrenoult et al., 2018]. C. acnes infections mainly present low-grade clinical symptoms [Portillo et al., 2013]. This led us to hypothesize that C. acnes could escape immune system detection. We investigated the ability of C. acnes strains from distinct origins to trigger human primary neutrophils (PMNs) response.

METHODS

Healthy donor's neutrophils were cultivated in contact with 96-hour planktonic (MOI 20:1 and 2:1) or 120-hour biofilms C. acnes cultures (CIP53.117, facial acne; pathogenic -Ep4- and -BPI22- and commensal -C5- clinical strains), during 4h. IL-8 and TNF-α concentrations, and MMP-9 activity, were determined by ELISA and zymography respectively. mRNA levels of these mediators were evaluated by RT-qPCR. Cell viability was assessed by lactate-dehydrogenase assay.

RESULTS

We demonstrated that neutrophils detect C. acnes both in planktonic and biofilm culture. All strains induced a significant MOI-dependent increase in PMNs cytokines production, as well as MMP-9 activity ($p<0.05$). Clinical strains triggered higher PMNs response (more than a 2-fold increase for IL-8) than the CIP strain, except for TNF-α which production remained stable with C5 and Ep4. In contact with biofilms, cytokines production by PMNs was further increased (2 to 6-fold for IL-8 and 2-fold for TNF-α compared to planktonic conditions). These data were reinforced with similar mRNA expression patterns. Finally, C. acnes biofilm led to an increase in LDH signal. This work suggests that C. acnes did not escape the PMN-mediated acute inflammation during inflammation set-up.

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473 / INVOLVEMENT OF INTESTINAL INFLAMMATION AND THE MICROBIOTA IN THE PRESERVATION OF SALMONELLA VIRULENCE

Keywords: *Salmonella, gut inflammation, microbiota, virulence*

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The gut microbiota confers colonization resistance (CR) to discourage pathogen blooms during homeostasis. Previous work identified that enteropathogenic bacteria like Salmonella Typhimurium exploit virulence-elicited host immune responses to overcome CR. In S. Typhimurium, virulence factors controlled by the HilD regulon trigger gut inflammation which creates an environment favoring pathogen growth and survival. Notably, previous work showed that HilD regulon-induced inflammation is a public good and that hilD mutants with attenuated virulence (i.e., cheaters) emerge during S. Typhimurium infections in mice. However, hilD mutants are infrequent among natural isolates. Therefore, how S. Typhimurium can benefit from the inflammation without the rise of hilD mutants is not well understood. Here, we investigated the role of gut inflammation and the host microbiota in controlling the maintenance of S. Typhimurium virulence.

To asses this, we used multiple mouse models featuring different degrees of inflammation and microbiota complexity. Under hygienic isolation, antibiotic pre-treatment plus severe gut inflammation led to the rise of mutants with damaged HilD virulence regulons that were shed over 160 days at very high densities (>108 CFU/g). In contrast, infection models triggering a lower degree of gut inflammation permitted substantial fecal shedding of S.Tm wild-type and limited the rise of such mutants. Furthermore, we assessed the role of gut inflammation, microbiota composition, and how they affect hilD mutant rise and pathogen shedding in more detail. Our results suggest that the intensity of the gut inflammation and the degree of microbiota disruption are important determinants of within-host evolution of S. Typhimurium virulence.

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505 / EVOLUTION FROM ICOSAHEDRAL TO AMPHORA SHAPED GIANT VIRIONS REVEALED BY CRISPR/CAS9

Keywords: *Pandoravirus, Mollivirus, CRISPR, Evolution*

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Giant viruses have been a hotspot of unresolved controversies since their discovery, including the definition of “Virus”, their involvement in eukaryogenesis, their origin and evolution. While increasing viral familie s and genome diversity has accumulated during recent years, functional genomics has been largely neglected. Here we describe an experimental framework to genetically modify nuclear Giant Viruses and their host Acanthamoeba castellanii using CRISPR/Cas9. We utilize this technology to trace the evolution from icosahedral to amphora-like giant viruses. Particularly, we show the importance of hijacking the host cellulose synthase during evolution of icosahedral to ovoid shape. Importantly, while host-derived cellulose can be identified in the particle of these viruses, host cellulose-binding proteins are strikingly absent. Our current work is being directed to the identification of viral cellulose-binding proteins that would have accompanied the incorporation of such polymer into the particle. Finally, transitioning from ovoid to amphora shape involved the emancipation from an essential major capsid protein; hallmark of icosahedral particles. Overall, we demonstrate the possibility of emergence of a novel viral morphogenetic module by recruitment of cellular proteins and introduce a battery of tools to allow manipulation of the up-to 70% ORFan-containing genome of giant viruses.

551 / CORRELATION BETWEEN GUT MICROBIOTA COMPOSITION AND SHORT-CHAIN FATTY ACID (SCFA) LEVELS IN DIARRHEOGENIC E. COLI INFECTIONS

Keywords: *gut microbiota, Short-chain fatty acids, Diarrheogenic E. coli, nan*

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BACKGROUND

The gut microbiota and its metabolites are responsible for the maintenance of intestinal homeostasis and the prevention of colonization by enteric pathogens, such as diarrheagenic Escherichia coli (ECD). Short-chain fatty acids (SCFA) are the most abundant products of bacterial fermentation, and their role in the development of diarrheal disease is controversial. We determined SCFA levels and gut microbiota composition in stool samples from children under 5 years of age, with and without diarrhea, and their correlation with the presence of SCFA-producing bacteria.

METHODOLOGY

40 diarrheal samples positive for a DEC pathotype and 43 stool samples from healthy children were analyzed. Gut microbiota composition and SCFA concentration were determined by 16S rRNA sequencing and HPLC, respectively.

RESULTS

A distinctive composition of the gut microbiota was observed in both groups. In diarrhea samples, higher levels of acetate, propionate, and butyrate compared to samples from healthy children were found. Correlation analysis showed that acetate production is positively correlated with Blautia genus, while propionate and butyrate production is positively correlated with Coprococcus genus in children with diarrhea by ECD. Butyrate production was positively correlated with Faecalibacterium and Roseburia genera in both the ECD and control groups, and Bacteroides was negatively correlated with acetate and propionate production in the control group.

CONCLUSION

We found high levels of SCFA in stool samples from children with DEC infection compared to samples from healthy children. The SCFA levels correlate with the presence of specific genera of SCFA-producing bacteria in both groups.

ACKNOWLEDGEMENTS/REFERENCES

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560 / SWINE-ADAPTED SALMONELLA SEROVARS UNDERGO EXTENSIVE MUTATIONS IN SALMONELLA PATHOGENICITY ISLAND 1 IN THEIR ADAPTATION TO SWINE

Keywords: *Salmonella, host-adaptation, mutations, nan*

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BACKGROUND

Salmonella pathogenicity island 1 (SPI-1) encodes the major mechanism used by Salmonella to colonize enterocytes and ultimately induce gut inflammation. We previously demonstrated that a widespread lineage of the swine-adapted Salmonella serovar Derby carries mutations in SPI-1 genes (hilD, hilC, sipA) reducing its in-vitro colonization of human more than swine enterocytes. These findings indicate that SPI-1 is nonessential for S. Derby circulation in swine and that the fixation of the observed mutations represents a step in the progressive adaptation of S. Derby to this host and a reduced risk to humans.

OBJECTIVES

to verify if other swine-adapted Salmonella serovars accumulate deleterious mutations in SPI-1 in their adaptation process to swine.

METHODS

We analysed S. Rissen, another swine-adapted Salmonella, by a gene-by-gene and a SNP-based analysis of the genomic reads of 73 S. Rissen isolates from Sequence Read Archive and seven isolates from our laboratory. We searched for loss of SPI-1 genes and/or their missense mutations.

RESULTS

We found five S. Rissen genomes lacking 9-12 out of 41 SPI-1 genes. Overall, we observed a higher percentage of missense mutations in sipA, possibly affecting the protein function, than the other SPI-1 genes. This finding is in line with what observed in the studied lineage of S. Derby which carries a nonsense mutation in sipA. Overall our data further support the hypothesis that SPI-1 is nonessential for Salmonella circulation in swine and that swine-adapted serovars undergo converging evolution in their adaptation to their host through the loss of function of SPI-1.

08

603 / AN INTEGRATIVE, GENOMIC- AND TRANSCRIPTOMIC-DRIVEN CENSUS OF SALMONELLA SMALL PROTEINS TO FIND NEW REGULATORS OF VIRULENCE

Keywords: *small proteins, Salmonella Typhimurium , dual RNA-seq, TraDIS*

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Small proteins, here defined as shorter than 100 amino acids (aa), are difficult to annotate and functionally characterize. Furthermore, their potential functions in bacterial pathogenesis are largely obscure. We sought to address these knowledge gaps in the well-characterized model enteric pathogen Salmonella Tpyhimurium. In a first step, we generated an updated annotation of the Salmonella small proteome by combining in silico predictions from the sPepFinder algorithm (Li et al., 2020), with ribosome profiling data.

We then reanalysed pre-existing datasets that inform on intracellular gene expression during infection (dual RNA-seq; Westermann et al., 2016) and on gene requirement for macrophage infection (transposon-directed insertion sequencing, TraDIS) with a focus on our extended small open reading frame (sORF) annotation. Based on the integrative results, we were able to prioritize small protein candidates with a likely role in Salmonella virulence for follow-up studies. These are the PhoPQ regulator MgrB (Venturini et al., 2020) and YjiS, a 54 aa-long protein that negatively affects Salmonella fitness during the infection of macrophages.

Follow-up characterization of YjiS suggests that its absence prevents bacterial escape from host cells and reinfection of bystanders, likely via a mechanism that relies on interaction with other proteins. Our work illustrates how several genes are still unknown even in well studied bacteria, and how the wealth of existing global datasets can be exploited to identify genes with a virulence-related function. This approach provides a blueprint for characterizing the small protein complement of diverse bacterial pathogens.

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653 / UNDERSTANDING AND EXPLOITING THE IMPACTS OF LOW PH ON MICRO-ORGANISMS: A COST ACTION

Keywords: *infection, food microbiology, biotechnology, industrial and environmental microbiology*

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Understanding the impact of low pH on bacteria and yeasts is clearly of critical importance for a range of pure and applied microbiology disciplines, including clinical/veterinary, industrial/environmental, and food/drink microbiology. Research in this area has made great progress in recent years, with much improved understanding of the molecular events which enable micro-organisms to sense and respond to low pH, and synergies between fundamental and applied research in the disciplines above have begun to develop. Despite this, there is considerable unrealised potential for people working in these different disciplines to interact more fully, and to share concepts, methods, and data. With this in mind, we established a COST Action (CA18113) entitled "EuroMicropH: Understanding and exploiting the impacts of low pH on micro-organisms", which was launched in Spring 2019 and will run until 2023. We have used this Action to develop databases of relevant literature and of Action participants and their diverse expertise. In addition, we have specific working groups looking at applications in all the above fields, working to develop more and better links between fundamental and applied research. Funding is available from the Action to enable lab exchanges and conference visits for people working in this area. Details of the work of the Action to date, and of the available funding opportunities that we can offer, will be presented.

ACKNOWLEDGEMENTS/REFERENCES

The work of all authors was done within the framework of EuroMicropH-COST Action CA18113

750 / UNVEILING THE FUNCTION AND REGULATION OF TRYPTAMINE, A GUT MICROBIOTA-DERIVED NEUROACTIVE MOLECULE

Keywords: *Tryptamine, Gut microbiota, Inhibition, nan*

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Tryptamine is a neurotransmitter produced by tryptophan decarboxylase. Approximately 10% of the human population harbors intestinal bacteria encoding tryptophan decarboxylase. Gut microbiota-derived tryptamine can modulate host cells by inducing colonic secretion and accelerated transit; a mechanism that could be exploited to treat constipation-associated disorders. However, the diversity, function and regulation of microbiota-derived tryptamine production, or its impact on other members of the gut microbiota remain unexplored.

We aimed at identifying novel intestinal tryptamine-producers in vitro and characterizing tryptamine production kinetics with/without environmental stressors using culture, metabolic (UPLC-DAD), and viability (flow cytometry) assays. We evaluated the impact of tryptamine on adult and infant gut microbiota composition (16S rRNA metabarcoding) and function (HPLC-RI) using anaerobic batch fermentations. The minimum inhibitory/bactericidal concentration (MIC/MBC) of tryptamine against various intestinal commensals was tested.

We identified Ruminococcus gnavus, Blautia hansenii, Enterocloster asparagiformis, Clostridium sporogenes, and Clostridium nexile as tryptamine-producers, with tryptamine concentration reaching up to 3383±110µM in vitro. We showed growth-associated tryptamine production in all producers. Contrary to other amino acid-decarboxylases, tryptophan decarboxylation was not promoted under acidic stress, nor did it mitigate acid-induced cellular damage in R. gnavus and C. sporogenes. Oxidative (O_2, H_2O_2) and osmotic (KCl, NaCl) stress did not promote tryptamine production. In adult and infant batch fermentations, tryptamine supplementation (3000µM) delayed carbohydrates consumption and succinate production (t=5h), leading to altered short-chain-fatty-acids and branched-chain-fatty-acids profiles (t=48h). Changes were reflected on the community structure and composition. MIC/MBC assays confirmed tryptamine's inhibitory effect against Bacteroides thetaiotaomicron, Bacteroides uniformis, Faecalibacterium prausnitzii, Roseburia intestinalis.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by own resources (ETH Zürich and University Children's Hospital Zürich) and an ETH Zürich Research Grant ETH-38 20-1.

962 / THE MINOR HCP PROTEINS FROM THE T6SS OF BACTEROIDES FRAGILIS FORM HIERARCHICAL INTERACTIONS

Keywords: *Bacterial secretion, Microbiota, T6SS, Structural biology*

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BACKGROUND

The biodiversity of the human gut microbiota has been shown to be directly linked to homeostasis and physiological regulation of distant tissues. The Bacteroides genus is one of the most abundant in the intestinal microbiota and contain members that use a type VI secretion system (T6SS) to actively kill competitors.

OBJECTIVES, METHODS AND RESULTS

We performed a genetic screen of protein-protein interactions between structural genes of the T6SS from Bacteroides fragilis and we discovered that minor subunits of the Hcp1-like superfamily, interact with each other. Biochemical purification, pull-down assays and structural studies using Cryo-EM and X-ray crystallisation showed the assembly of a hierarchical structure depending on heteromeric interactions. We hypothesise that this structure could be important for inner tube assembly of the T6SS.

1027 / ANTISENSE TRANSCRIPTION: WHAT DOES IT DO FOR BACTERIA?

Keywords: *antisense transcription, small RNAs, exoRNAs, E. coli*

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BACKGROUND

Thousands of antisense transcripts were mapped to bacterial genomes and many attempts were undertaken to reveal their functions. It seemed easy; however, we are only starting to understand their role, and the most popular concept says that such RNAs are just by-products of transcriptional noise.

OBJECTIVES

We aim to reveal the function of antisense and divergent transcription in Escherichia coli, if any.

METHODS

Promoter mapping; sequencing of mRNA, small RNAs and exoRNAs; pull-down followed by LC/MS; reporter assays.

RESULTS

Promoters for antisense transcription were predicted by the PlatProm algorithm, and their ability to interact with RNA polymerase (RNAP) was tested in vitro and in vivo. It was very similar to that of known and co-directed promoters, however, correlation between the PlatProm score and the percentage of the RNAP-bound DNA both in vitro and in vivo was higher for antisense promoters. This might be due to their enrichment with A/T tracts which can be bound with either RNAP or H-NS, also explaining low transcriptional activity of antisense promoters in vivo and suggesting their role as SOS-depots. However, there can be other scenarios. The fraction of secreted exoRNAs was significantly enriched with aRNAs suggesting their possible signal role. Besides, many short bacterial RNAs, like eukaryotic microRNAs, can form complementary duplexes. Thus, if an aRNA needs a complementary strand for functioning, then it might be also crucial for its synthesis. Finally, antisense and divergent transcription may interfere not only with H-NS but with other regulatory proteins suggesting their functional overlap.

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1078 / ON THE ROLE OF THE SORTING PLATFORM IN HIERARCHICAL TYPE III SECRETION

Keywords: *type III secretion system, EPEC, hierarchical secretion, nan*

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Enteropathogenic Escherichia coli (EPEC) is one of the causative agents of infant diarrhea in developing countries. To accomplish the intestinal infection, EPEC relies on the Type III Secretion System (T3SS). The T3SS is a nanomachine assembled in the bacterial envelope, which extends extracellularly to reach the host cell. Virulence effector proteins are translocated through this structure in order to hijack host signal pathways, creating a favorable niche for the bacterium.

The T3SS is composed of several proteins that are assembled in a hierarchical order. This process is assisted by protein complexes known as molecular switches, which determine the order of secretion among three different T3-substrates, early, middle and late substrates. The second molecular switch is composed of the SepL, SepD and CesL protein complex and regulates the secretion between middle and late substrates.

Another protein complex that has been involved in recognizing and hierarchizing T3-substrates is the sorting platform, which is made up of the proteins EscK, EscQ and EscL. We have previously shown that the sorting platform is dispensable when T3-substrates are overproduced; however, the molecular mechanisms behind this phenomenon are poorly understood. In this work, we used a combination of biochemical and genetic approaches to describe the role of the sorting platform in T3SS biogenesis and orderly secretion. Overall, our results show a functional interconnection between the sorting platform and the second molecular switch for hierarchical secretion.

ACKNOWLEDGEMENTS/REFERENCES

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1086 / NEW GENETIC TOOLS ENABLE DISSECTION OF A GLOBAL STRESS RESPONSE IN THE EARLY-BRANCHING SPECIES FUSOBACTERIUM NUCLEATUM

Keywords: *Fusobacterium, small RNA, envelope stress, genetic tools*

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Fusobacterium nucleatum, long known as a common oral microbe, has recently garnered attention for its ability to colonize tissues and tumors elsewhere in the human body. Clinical and epidemiological research has now firmly established F. nucleatum as an oncomicrobe associated with several major cancer types.

However, with the current research focus on host associations, little is known about gene regulation in F. nucleatum itself, including global stress response pathways that typically ensure the survival of bacteria outside their primary niche. This is due to the phylogenetic distance of Fusobacteriota to most model bacteria, their limited genetic tractability and paucity of known gene functions. Here, we characterize a global transcriptional network involved in F. nucleatum's sensing of envelope stress mediated by the stress regulator σE. To this aim, we developed a set of new genetic tools for this anaerobic bacterium, including inducible gene expression, scarless gene deletion as well as transcriptional and translational reporter systems. Using these tools, we identified a striking functional conservation of the σE response with phylogenetically distant Proteobacteria. Although F. nucleatum lacks canonical RNA chaperones such as Hfq, we uncovered conservation of the non-coding arm of the σE response in form of the non-coding RNA FoxI. This regulatory small RNA acts as an mRNA repressor of several membrane proteins, thereby supporting the function of σE. In addition to the first characterization of a global stress response in F. nucleatum, the genetic tools developed here will enable further discoveries and dissection of regulatory networks in this early-branching bacterium.

1170 / VIRULENCE IN BACILLUS SPP. ISOLATED FROM AN ANCIENT ROMAN AMPHORA

Keywords: *Bacillus spp., paleomicrobiology, Antibiotic-resistance, Genome-sequencing*

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Eight still viable bacterial strains belonging to the Bacillus genus have been isolated from an ancient Roman amphora dated to the IV-V century AC, found still intact and sealed in an archaeological site of Aquileia, Friuli Venezia Giulia Region, Italy. The extracted DNAs were sequenced on MiSeq in paired-end 300 bp mode and assembled with WGA-LP pipeline (1). The genomes thus obtained were annotated with Prokka in order to reconstruct and analyze their metabolisms (2), and further analyzed in vivo through the use of API tests. Through functional annotation and the use of specific databases (3, 4), possible virulence and antibiotic resistance traits were searched in bacteria dating back to an era in which the use of antibiotics was not yet widespread with the aim of reconstructing the evolution of these bacterial systems over time. Finally, an accurate taxonomic identification was made using TYGS (5), by evaluating ANI and dDDH, and by comparing the genes present in the strains under analysis with those of the genomes currently deposited on NCBI using Roary (6). While 3 strains were found to be close with modern era isolated bacteria, 5 strains were found to be unclassifiable with current standard strains. On the basis of these results, these strains can be considered new species related to the Bacillus genus, or be identified as the "progenitors" of the current bacteria, from which they differ for substantial genetic differences.

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404 / ENGINEERING THE MICROBIOME BY SOIL TRANSPLANTATION: TRANSFORMING CONDUCIVE SOIL TO DISEASE SUPPRESSIVE ONE

Keywords: *Agricultural sustainability, Disease suppressiveness, Amplicon sequencing, Organic farming*

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BACKGROUND

Agriculture sustainability is a pressing issue to feed the ever-increasing world population without adversely impacting soil health. Organic farming exerts positive effects on microbial diversity, thus restoring soil health. Disease suppressiveness, well established under organic farming, is a natural ability of soil to minimize the impact of soil-borne plant pathogens. However, there is no consensus on the contribution of microbial members towards general disease suppressiveness in organic farming.

OBJECTIVES

The objective of the study was to compare the disease suppressive ability of soil under different farming practices along with identifying the microbial members associated with it, and whether this disease suppressive property of the soil can transform a conducive soil into suppressive soil.

METHODS

A unique experimental setup was selected where conventional and organic farming were practiced for >1.5 decades. 16S rRNA based amplicon sequencing of soil samples was performed for characterisation of the bacterial diversity under the two farming practices. This was followed by planta-assays to assess the disease suppressiveness of the two soil types. To check for transferability of suppressiveness to otherwise conducive soil, soil transplantation experiment was performed.

RESULTS

Higher abundance of various biocontrol genera was observed under organic farming compared to conventional farming. Similarly, higher disease suppressive potential of soil microbiomes was observed against two fungal broad host range pathogens i.e., Rhizoctonia solani and Fusarium oxysporum using in planta-assay. The transfer of disease suppressive ability of soil to otherwise conducive soil can be utilized as a microbiome-based sustainable strategy to control plant diseases.

420 / GROWTH-ACCELERATION OF HAEMATOCOCCUS LACUSTRIS BY INDIGENOUS KEY-ROLE BACTERIA IDENTIFIED FROM THE ALGAL PHYCOSPHERE

Keywords: *Haematococcus lacustris, Astaxanthin, NGS, Phycosphere*

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Human society is rapidly becoming aging. Following this aging society, anti-aging-related materials are also drawing attention. Astaxanthin (AX) is a reputable source of various functions among the materials. A microalga, Haematococcus lacustris, is a worthy candidate to produce the AX. They have AX in intracellular up to 3-7% depending on the bio-processes. However, H. lacustris has the critical problem of slow growth, a significant hurdle for commercialization. This study focused on analyzing bacterial communities of algal phycosphere by next-generation sequencing (NGS) and identified/isolated the key-role bacteria to accelerate algal growth. As a result, algal biomass was sharply increased up to 2.1 fold higher than control. Biomass before stages of AX biosynthesis significantly influences the pigment production stage; therefore, biomass obtained through this study could finally produce 1.6 times higher AX. Thus, the industrial possibility of AX, which has been challenging to secure mass production, could be enabled by metagenome assessment.

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1111 / PEATMOSS MICROBIOME: THE POWER OF THE OBLIGATORY METHANOTROPHIC NITROGEN-FIXING MINORITY.

Keywords: *Sphagnum moss, Chip-SIP, Microarray stable isotope probing, Metagenomics*

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Peat mosses of the genus Sphagnum often dominate the primary productivity of northern peatland ecosystems and play a crucial role in global nitrogen and carbon cycles. Moreover, Sphagnum-associated diazotrophic-methanotrophs represent a possible “missing link” between the carbon and nitrogen cycles. Nevertheless, their functional contributions remain in question. A combination of omics (DNA and RNA characterization) and dual-isotope incorporation approach illuminated the functional diversity of Sphagnum-associated microbiomes.

Specifically, twelve bacterial genera in its core microbiome comprise 60% of the relative microbial abundance at the continent scale. In addition, nearly 15% of the prokaryotic communities have the potential for nitrogen fixation. While cyanobacteria comprised the vast majority (>95%) of diazotrophs detected in amplicon and metagenome analyses, obligate methanotrophs of the genus Methyloferula (order Rhizobiales) accounted for one-quarter of transcribed nifH genes. Furthermore, in dual-isotope tracer experiments, members of the Rhizobiales showed substantial incorporation of 13C-CH4 and 15N-N2 isotopes into their rRNA. These analyses suggest a critical role for methanotrophic nitrogen-fixing bacteria in Sphagnum physiology and methane emission mitigation. Furthermore, these functional interactions proved vulnerable to climate change drivers. The whole ecosystem warming experiment was shown to substantially alter the community composition and diversity of diazotrophs, and nitrogen fixation activity of the Sphagnum microbiome, which may ultimately impact host fitness, ecosystem productivity, and carbon storage potential in peatlands.

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Bacterial community composition of crown gall tumors on rhododendron

Nadia Behzadi / *Islamic Republic of Iran*
Investigating Cyanobacterial diversity in wetlands with different salinity and oil pollution

296 / INHIBITION OF EFFLUX PUMP IN MULT-DRUG RESISTANT BACTERIA WITH ROOT EXTRACT OF ONOSMA VISIANII CLEM

Keywords: *Onosma visianii Clem, antimicrobial effect, pump efflux, multiresistant bacteria*

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BACKGROUND

Numerous multidrug-resistant bacteria use efflux pumps to rapidly transport antibiotics out of the cell, increasing the dose of antibiotics they tolerate, thereby affecting the failure of antibiotic treatment.

OBJECTIVES AND METHODS

Vancomycin-resistant Enterococcus faecalis (VRE) and methicilin-resistant Staphylococcus aureus (MRSA) were used. For determination of efflux pump activity, untreated and strains treated with 50% MIC or 1000 µg/ml were examined. The slides was examined on fluorescence microscope and photographed in real time at three time points. The accumulation rate of EB/AO was determined by ImageJ-sofware as the fluorescence index relative to the baseline, for treated and untreated samples.

RESULTS

Test results have shown that extract at a concentration of 50%MIC showed a positive inhibitory effect on efflux pump of G+ bacteria in range of 18-26% relative to untreated cells. The examination of the time dependence of the extract effect showed that inhibition of efflux pumps was different in MRSA and VRE. While in MRSA isolates, a high degree of inhibition was measurable after 1 hour of incubation (61%) in VRE isolates, this effect is slower, and inhibition of efflux pumps was achieved only after 24-hour incubation. The confirmation of the inhibitory effect of Onosma visianii Clem extract directs further research towards investigating the synergistic use of this extract with other antibiotics in order to reduce resistance of multi-drug resistant strains.

324 / THE EPIGENETIC LANDSCAPE OF THIOTHRIX FRUCTOSIVORANS QT (ATCC 49748) USING SMRT, RIMS AND ONT SEQUENCING METHODS

Keywords: *Genome sequencing, DNA methyltransferases, Genome modifications, nan*

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BACKGROUND

The role of DNA modification in restriction-modification mechanisms of prokaryotic cells was established more than 50 years ago (1, 2). The bacterial DNA methyltransferases create an epigenetic landscape in DNA by modification of specific motifs and in concert with their cognate restriction endonucleases determine the boundaries between bacterial species and protecting against bacteriophages. Recently, we published a complete genome analysis of several Thiothrix species (3)

OBJECTIVES

Next generation sequencing technologies such as SMRT, RIMS-seq and ONT allowed not only the sequence and assembly of bacterial genomes, but also the analysis of epigenetic modification patterns and the identification of methyltransferase genes responsible for the modified motifs.

METHODS

SMRT sequencing technology has facilitated the sequencing and assembly of a wide variety of genomes and also clarified the modification repertoire. Detection of the modification status of bases is possible for m6A, m4C (4, 5). However, because the signal associated with m5C bases is weaker than for m6A or m4C and very similar to the IPD of unmodified cytosine. Therefore, we used recently developed Illumina based “rapid identification of methylase specificity (RIMS-seq) method to identify m5C motifs in bacterial genomes (6). Moreover, we also tested the recently developed ONT base software Nanodisco (7) to detect previously identified motifs. We applied these methods to detect the modification pattern in T. fructosivorans QT.

RESULTS

The complete methylome analysis of T. fructosivorans QT detected four m6A and three 5mC modified motifs which were assigned to appropriate DNA methyltransferases genes and deposited in REBASE (8)

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350 / HOST METABOLIC CONSEQUENCES OF INTRACELLULAR INFECTION BY MEMBERS OF THE ESKAPE PATHOGENS

Keywords: *ESKAPE, metabolomics, stable isotope, MRSA*

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BACKGROUND

The members of ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) are leading causes of hospital -acquired infections. Many are facultative intracellular pathogens and are thus able to invade both phagocytic and non-phagocytic cells. The emergence of multidrug-resistant strains, e.g. methicillin-resistant S. aureus (MRSA) has made treating infections by ESKAPE significantly more complicated. Therefore, there is an urgent need to find novel therapies.

OBJECTIVES

Our study aimed to identify and target host molecular pathways exploited by MRSA, Acinetobacter baumannii and Klebsiella pneumoniae during intracellular infection with a view of identifying possible host-directed therapies.

METHODS

We used stable-isotope (carbon-13) labelling coupled to mass spectrometry and nuclear magnetic resonance as well as molecular biology techniques to investigate the impact of bacterial infection on host carbon metabolism. Identified pathways were targeted by pre-approved inhibitors.

RESULTS

We found extensive metabolic re-routing and detected several distinct metabolic changes in infected cells. Our data suggested that both S. aureus as well as A. baumannii induced starvation-based autophagy. In agreement, drug-induced inhibition of AMPK, the host kinase triggering autophagic flux, hampered bacterial intracellular proliferation. In contrast, invasion by K. pneumoniae only led to modest changes in host metabolism.

CONCLUSION

Our findings show that targeting host pathways essential for bacterial survival is a viable therapeutic strategy against ESKAPE pathogen.

400 / STOCHASTICITY-DRIVEN MICROBIAL ASSEMBLY IN THE GUT OF DAIRY CALVES DURING EARLY LIFE

Keywords: *neutral theory, community assembly, calf, stochasticity*

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The neutral theory highlights that both deterministic and stochastic factors affect microbial community assembly in the environment, however, whether this theory applies to the microbial community in the gut of animals is unknown. We hypothesized that both deterministic and stochastic factors can affect the gut microbial community assembly during early life, which can subsequently influence the establishment of the microbiota in the gut of dairy calves. This study aimed to investigate deterministic and stochastic dynamics of fecal microbiota in preweaned calves using 16S rRNA amplicon sequencing generated from 384 fecal samples collected from 84 calves at ages week 1 to week 7.

The standardized effect size and Raup-Crick distance were adopted to evaluate the neutral theory and determine if microbial community assembly was driven by stochastic/deterministic factors. Our results revealed that stochasticity was the dominant force driving community assembly for calves aged w1 and w2, and the deterministic process was the leading factor for gut microbial community assembly in calves at w3, w5, and w7. Furthermore, partial correlation analysis revealed that the abundance of phyla Bacteroidetes was negatively correlated with stochasticity ($R=-0.9$, $P<0.01$), while the abundance of phyla Actinobacteria was positively correlated with the deterministic process ($R=1$, $P<0.01$). Overall, our study suggests that the microbiota assembly in the gut of dairy calves (at the phylum level) during early life can be affected by both deterministic and stochastic factors. Whether varied deterministic and stochastic dynamics affect the functionality of gut microbiota and its responses to interventions warrants future research.

437 / OXIDATIVE STRESS AND DETERMINATION OF MALONDIALDEHYDE CONCENTRATIONS: COMMENSAL GUT ESCHERICHIA COLI STRAINS

Keywords: *malondialdehyde, E. coli, lipid peroxidation , sample preparation*

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Malondialdehyde (MDA), the most popular marker for the assuming of oxidative stress, is an endogenous genotoxic product for both enzymatic and non-enzymatic lipid peroxidation (LPO) processes. The different approaches are used for the sample preparation to monitor the concentrations of MDA for bacteria, including homogenates of fresh and freeze-dried bacterial cultures and bacterial culture broths.

The aim of this study was to evaluate the LPO levels in bacterial homogenates, suspensions and cell-free supernatants of commensal strains of predominated commensal Escherichia coli from the healthy and diseased gut microbiota.

LPO levels in bacterial suspensions, homogenates and cell-free supernatants were studied by thiobarbituric reaction at 535 nm according to Vladimirov and Archakov (1). The concentration of MDA was calculated per mg of protein in the homogenates according to Lowry assay (2) and in the cell suspension according to Yakovleva and co-authors (3).

Depending on the concentration of bacteria in the suspension, the impact of intercellular interactions on the amount of MDA in bacterial suspensions and homogenates of E. coli was revealed in this study, which may affect the “correct” assessment of LPO levels. A diverse community of commensal E. coli from the human and animals’ mucosal and epidermal surfaces plays a crucial role in host life (4-8). The differences between the MDA concentrations in bacterial suspensions, homogenates and cell-free supernatants for E. coli strains from the healthy gut microbiota compared to strains from the diseased microbiota, possibly important for the bacteria - host interactions, were also described.

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471 / STUDY OF THE EXPRESSION OF RELEVANT BACTERIAL GENES IN HALITOSIS AND THE EFFECT OF NITRATE

Keywords: *Halitosis, Tongue, volatile sulfur compounds, Biofilm*

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BACKGROUND

The intraoral halitosis is a health condition that originates in the dorsal tongue, caused by the bacterial fermentation of sulfur-containing amino acids and the production of volatile sulfur compounds (VSC). In halitosis-free individuals, the tongue microbiota has a high overrepresentation of bacteria capable of reducing nitrate to nitric oxide, which inhibits the growth of VSC-producing bacteria. Previous metatranscriptomic studies suggest that there is a negative correlation between halitosis and the expression of genes involved in nitrate reduction.

OBJECTIVES

The main objective was to demonstrate the beneficial effect of nitrate to prevent halitosis through the study of the expression of genes associated with VSCs production.

METHODS

The tongue biofilm of 10 individuals was grown in vitro with and without 6.5 mM of nitrate. The expression of 4 halitosis-related genes (cysK, mccB, mdeA and metY) was quantified using qPCR in Rothia (halitosis-free biomarker) and Fusobacterium (halitosis biomarker). The final levels of nitrate, nitrite and ammonium were also measured.

RESULTS

The results showed that the added nitrate was completely reduced to nitrite and most nitrite further reduced to nitric oxide. The supplementation with nitrate increased the concentration of DNA in the biofilm. In addition, no significant differences were observed in the expression of genes associated with the amino acid fermentation and synthesis. However, the expression of cysK was decreased when nitrate was added. Our results showed that both mdeA, mccB and to a lesser extent metY were poorly expressed genes in vitro tongue-biofilms.

488 / ECAL FILTRATE TRANSPLANTATION (FFT) AS A NEW THERAPEUTIC STRATEGY TO REGULATE ATOPIC DERMATITIS

01

Keywords: *fecal filtrate transplantation, gut microbiota, viral components, atopic dermatitis*

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BACKGROUND

Atopic dermatitis (AD), which is a common chronic inflammatory skin disease, has been an increasing interest in the role of the gut microbiota. The gut microbiota (including bacterial and viral components) has been known to plays an important role host's health and their dysbiosis is known to be associated with pathogenesis of immune-mediated diseases, such as asthma, AD, and metabolic diseases.

OBJECTIVES

The aim of the present study was to investigate the potential therapeutic effect of fecal filtrate transplantation (FFT) is accompanied by regulation of gut microbiota, short-chain fatty acid, type 2 immune response in allergic and AD.

METHODS

FFT was performed using the filtrated fecal supernatant from healthy donor mice. The potential therapeutic of FFT was investigated using ovalbumin-sensitized AD Balb/c mouse model.

RESULTS

The results obtained from the present study showed that the gut microbiota restored after FFT. In addition, FFT led to up-regulated short chain fatty acid (SCFA) and down-regulate type 2 (Th2) immune response, IgE level, number of mast cells, percentage of eosinophil and basophil, and calprotectin level. Present findings clearly indicate that FFT showed suppressed AD-associated responses with the restoration of gut microbiota. Therefore, these results suggest that FFT can be a new therapeutic strategy for alleviating allergic and AD.

ACKNOWLEDGEMENTS/REFERENCES

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654 / EFFECTS OF SEQUENCING THE WHOLE 16S RRNA GENE OR V3-V4 REGION ON THE COMPOSITION OF ORAL AND FECAL HUMAN MICROBIAL SAMPLES

Keywords: Microbiome, 16S rRNA gene, Long-read sequencing, nan

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BACKGROUND

Sequencing variable regions of the 16S rRNA gene (≈300bp) with Illumina technology is commonly used to study the human microbiota. Unfortunately, short reads are unable to discriminate between highly similar species. Considering that species from the same genus can be associated with health or disease it is important to differentiate them. Third-generation sequencing platforms such as PacBio SMRT increase read lengths allowing now to sequence the whole 16S rRNA gene.

OBJECTIVES

To evaluate the taxonomic annotation of microbiome samples when sequencing either the full length or the V3-V4 region of the 16S rRNA gene.

METHODS

DNA from saliva, subgingival (dental) plaque and feces of 9 volunteers were extracted. In parallel, regions V3-V4 and V1-V9 were amplified and sequenced by Illumina Miseq and PacBio Sequel II sequencers, respectively. Clean reads were annotated against the Silva138 database using DADA2 and QIIME2 pipelines.

RESULTS

With PacBio sequencing, a higher number of reads were assigned to the genus (99.41% vs 93.58% for traditional Illumina V3-V4 sequencing) and to the species level (75.92% vs 55.44%). Regarding the composition, more than 85% of abundant genera (those at >0.1% abundance) were shared between platforms in all sample types. However, relevant genera such as <i>Fusobacterium</i> (6.13% vs 10.14%) was significantly reduced in saliva (adjusted p-value<0.05) using PacBio technology whereas <i>Streptococcus</i> (19.85% vs 14.03%) was increased. Our results suggest a high similarity of the overall microbial composition between platforms at the genus level but with some inconsistencies that will need to be evaluated with mock communities.

698 / PARALLEL CRISPR-CAS GUIDED PROGRAMMABLE MUTAGENESIS IN SHIGELLA FLEXNERI BY CYTOSINE BASE EDITING

Keywords: *Virulence plasmid, high-throughput mutagenesis, rapid, gene characterization*

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BACKGROUND

Shigella is a Gram-negative bacterium that causes bacterial dysentery, which is the deadliest bacterial diarrheal disease. We recently published the genome sequence of Shigella flexneri 5a M90T and analysed the differential gene expression during intracellular infection. We observed that many of chromosomal genes that were upregulated had no assigned function. To characterise these genes, a high-throughput targeted mutational analysis is necessary but the current methods for Shigella fall short of this objective.

OBJECTIVES

We decided to optimize CRISPR-Cas9 base-editing to develop a one-step, programmable method to mutate multiple Shigella genes in parallel arrays.

METHODS

A two-plasmid system for expressing an effector, nickase Cas9 and deaminase fusion (nCas-AID), and allowing high-efficiency cloning of computationally determined guide RNA was designed. As a proof of principle, chromosomally encoded fluorescent gene mCherry was inactivated and the classic fluctuation assay was used to determine mutagenic effect of nCas9-AID. Well characterized genes, both on Shigella chromosome and the virulence plasmid, were inactivated using this method and their phenotype was assessed.

RESULTS

The one-step mutagenesis resulted in gene inactivation with high efficacy (upto 100%), in both Shigella and E. coli. Fluctuation assay revealed low background mutagenic effect but high specificity at the same time. We were able to generate marker-less loss-of-function mutants with no polar effects. Several Shigella genes were mutated in a single experiment and their phenotypes conformed to those in the literature. The method can be used to construct multiple mutants in members of Enterobacteriaceae, using 96-well plates, in a matter of few days.

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01

706 / MULTIPLEX PCR IN THE ACCURATE DIAGNOSIS OF SEVERE SARS-COV-2 AND RSV CO-INFECTION IN CHILDREN

Keywords: *SARS-CoV-2, RSV, co-infection, multiplex PCR*

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BACKGROUND

The Coronavirus Disease 2019 (COVID-19) affects both adults and children. In pediatric patients, the disease may be presented as symptomatic infection with serious manifestations. We report two cases of children with COVID-19, simultaneously infected with respiratory syncytial virus (RSV), aiming to put an accent on the possibility of co-infection with a severe course and underline the significance of multiplex polymerase chain reaction (mPCR) as an accurate diagnostic tool.

RESULTS

The children are part of a larger, still ongoing study on the potential of mPCR for the rapid diagnosis of acute respiratory infections. A 2-month-old boy was hospitalized for severe bronchiolitis. His rapid antigen test for SARS-CoV-2 was negative. At first, he was given supplemental oxygen due to low saturation, but in the next few days, atelectasis appeared and he was transferred to the Intensive Care Unit (ICU).

His nasopharyngeal swab was tested with the mPCR Respiratory panel. Surprisingly, except RSV it also detected SARS-CoV-2. After 14 days of adequate treatment, he recovered successfully and was discharged.

A previously healthy 16-month-old girl was admitted to the ICU due to severe COVID-19 pneumonia. She rapidly deteriorated and was placed on mechanical ventilation the following day. In her case, the mPCR confirmed SARS-CoV-2, already diagnosed in another hospital, but also revealed RSV. Unfortunately, despite the comprehensive treatment, her condition continued to decline and she died of multiorgan failure.

CONCLUSION

The mPCR assay provided an early and accurate diagnosis, aiding in the implementation of timely contact precautions and adequate management.

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839 / BACTERIAL VIABILITY PCR AND VIRAL CAPSID INTEGRITY PCR

Keywords: *PCR, Bacterial Viabiltiy, Viral Viability, Legionella*

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BACKGROUND

PCR-based microbial enumeration often leads to an overestimation of viable cells due to the amplification of DNA from lysed, dead, and dying organisms. To overcome this disadvantage, DNA intercalating dyes such as propidium monoazide (PMA) have been used in combination with qPCR to discriminate between viable and non-viable bacteria and viruses; a technique known as viability PCR (vPCR). These dyes selectively photocrosslink dead-cell DNA, inhibiting subsequent qPCR amplification and effectively eliminating signal from non-viable/non-infectious microbes. Drawbacks of using PMA and EMA include inhibition of photocross-linking in turbid samples and lack of consistent photo-crosslinking protocols.

OBJECTIVES

Synthesize a novel viability PCR crosslinker, which is composed of a cell-impermeable DNA crosslinking dye that spontaneously crosslinks and prevents qPCR amplification of dead-cell DNA without the need for photo-crosslinking.

METHODS

Gram-negative (Pseudomonas, Legionella) and Gram-Positive (Listeria) bacteria and AAV reference virus were heat-killed and treated with the novel viability crosslinker. qPCR was then performed to assay the extent of dead cell signal suppression.

RESULTS

The crosslinker significantly reduces qPCR signal from heat-killed bacteria. The assay combines a wide dynamic range with high sensitivity, as decreasing log-dilutions of live bacteria can be detected in a large background of dead cells. A significant decrease in qPCR signal was also observed when heat-inactivated virus was treated with the vPCR crosslinker. This approach, termed Viral Capsid Integrity PCR may be applicable to a wide variety of other pathogenic viruses.

871 / CATIONIC POLYELECTROLYTES DEMONSTRATE DIFFERENT ARCHITECTURES OF CELLULAR ENVELOPES IN GRAM NEGATIVE BACTERIA

Keywords: *polyelectrolytes, antimicrobial surfaces, encapsulation, multicellular synthetic structures*

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Polyelectrolyte (PE) based layer-by-layer (LBL) electrostatic modification of bacterial cell surfaces is important for the construction of artificial multi-cellular structures, but some PEs have an unpredictable toxic nature. How the PEs are deposited onto the cell surface to form nano-thick shells around the cell is not clear.

Exposing two gram-negative bacterial strains, Escherichia coli and Pseudomonas stutzeri, to cationic PEs with a high positive charge densities such as polyethyleneimine (PEI) or poly(allylamine) hydrochloride (PAH) resulted in increased death rates of only P. stutzeri, even though the cell wall architecture of both strains should be structurally similar. Consequently, the LBL modification of the cell surface was for E. coli performed at high PE concentration without complications, while for P. stutzeri a modified protocol with lower PE concentration had to be developed to preserve cell viability. Super-resolution stimulated emission depletion (STED) microscopy helped us visualize that the PEs did not form a capsule around the cells of P. stutzeri, as observed for E. coli. Instead, they penetrated into the cell and disintegrated the cell wall and the membrane. This insight will help us further examine the architectural differences of cell envelopes in bacteria and most importantly, it will ensure high survival of bacterial strains in biotechnological and biomedical applications, when selecting the appropriate combinations of strains and PEs as well as the protocols for cell surface modification. These insights also help us to advance the development of targeted approaches for antibacterial surface treatments against specific bacteria using physical characteristics of polyelectrolytes.

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945 / PROBIOTIC BACTERIA FROM ORAL BIOFILMS: CHARACTERISTICS AND DELIVERY APPROACHES

Keywords: *cell immobilization, oral pathogens, nan, nan*

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Oral biofilms of healthy individuals are a source of bacterial strains that have beneficial traits like the inhibition of several periodontopathogens and can therefore be used in combination with other interventions in disease states, e.g. physical removal of biofilms and antibiotic treatment. Since the succession of an oral biofilm is dependent on key species, populating the oral epithelia with these species, prevents the cocolonization of pathogens and helps restore the healthy oral microbiota.

In several isolation campaigns we have collected a large collection (up to 500 strains) of aerobic, facultative and anaerobic strains, some of which have shown a biocontrol potential against periodontopathogens like Aggregatibacter actinomycetemcomitans, Fusarium nucleatum and Porphyromonas gingivalis.

By comparative genomic approach we determined the potential causes of specific antimicrobial activities against gram-negative bacteria and assessed the safety aspects for these strains for their use in clinical applications. For this las purpose we have developed several entrapment based delivery systems, such as nanofibers and microcapsules (Zupančič et al. 2018, Mirtič et al. 2018), which are enable us to combine together different bacterial strains as well as help them efficiently populate target surfaces. We discuss the importance of delivering a larger number of probiotic strains simultaneously and see the engineering of artificial communities as a superior approach to reestablishing a healthy oral microbiome.

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986 / FEATURES DEFINING HUMAN GUT MICROBIOME STABILITY

Keywords: *gut microbiome, stability, responders, nan*

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BACKGROUND

The human gut microbiome composition can be considered relatively stable, meaning that in general its inter-individual variability exceeds intra-individual variability over time. The stability of the human gut microbiome is fundamental to maintaining a symbiotic relationship with the host organism. However, the microbiome stability itself is also characterised by interindividual differences. The parameters determining these differences are yet to be elucidated.

OBJECTIVES

The current work is aimed at identifying microbiome stability markers under various external interventions. Understanding the stability determinants will promote the development of intervention schemes aimed at modulating the microbiome composition in order to shift it from unhealthy stable states and prevent destabilizations leading to such conditions.

METHODS

We analysed which microbiota parameters determine its stability using published 16S rRNA data from various types of interventional as well as large populational studies. The investigated parameters included genomic characteristics and abundance levels of microbial taxa, properties of their interaction networks and diversity estimates. Contribution of the parameters was assessed using machine learning.

RESULTS

We revealed that there exists a landscape of microbiome stability that influences the magnitude of microbiome shift regardless of the intervention type. This landscape also affects the distribution of samples from different individuals in the space of taxa' abundance in population-scale microbiome datasets. The proposed determinants of the microbiome stability are the average number of genes in the community members, community diversity, prevailing types of interspecies interactions, and certain taxa groups' abundance.

This work was supported by the Russian Science Foundation [grant 22-24-00683].

1126 / DRUG DELIVERY SYSTEMS ASSOCIATED WITH QUERCETIN: AN EFFICIENT APPROACH TO TREAT GUT DYSBIOSIS IN MOUSE MODEL

Keywords: *Dysbiosis, Quercetin, 16S amplicon sequencing, Mouse model*

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BACKGROUND

Antibiotic administration alters the composition and diversity of gastrointestinal bacteria leading to dysbiosis. Drug delivery systems (DDS) represent a promising tool for gut microbiota associated diseases, especially for target therapy. Prebiotic administration is known for its valuable contribution to dysbiosis and particularly Quercetin (QUE), noted as natural antioxidant with prebiotic effects, commonly present in human diet.

OBJECTIVES

The main objective of the study is to investigate, in vivo, novel developed DDS associated with Quercetin, as an effective therapy in gut microbiota related diseases such as dysbiosis.

METHODS

A batch of CD-1 mice, in biological replicates, were used as model organisms. A combination of DSS with AMX and/or QUE was orally administrated for 14 consecutive days. Gut dysbiosis was successfully induced in mice by treatment with Amoxicillin (AMX). In the fifteenth day, all mice were sacrificed and faeces from the intestinal tract have been collected aseptically. DNA extraction was performed using QIAamp DNA Stool Mini Kit and the gut microbial composition was characterized by 16S amplicon sequencing on Nanopore MinION device.

RESULTS

The results show that antibiotic administration significantly alter the mice gut microbiota, enhancing the opportunistic pathogenic bacteria which replace the commensal ones. In contrast, the administration of QUE enriches the gut microbiota diversity and furthermore, specific bacteria species (Barnesiella sp.), known to have the capacity to regulate gut microbiota after its colonization with antibiotic resistant bacteria, are present in a higher concentration.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by Grants of the Ministry of Research, Innovation and Digitalization, CNCS/CCCDI – UEFISCDI, Project Number PN-III-P2-PED 524/2020 and Competitiveness Operational Programme (COP) A1.1.4. ID: P_37_798 MyeloAL-EDiaProT, Contract 149/26.10.2016, (SMIS: 106774) MyeloAL Project.

1128 / THE GUT MICROBIOTA ASSEMBLAGES OF THE GIANT PANDA (AILUROPODA MELANOLEUCA) IS SHAPED BY THE BIOGEOGRAPHY NOT THE HOST GENETICS

Keywords: *Giant Panda, gut microbiota, genetic diversity, geography*

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BACKGROUND

The giant panda (Ailuropoda melanoleuca), an enigmatic carnivore, consumes large-quantities of low-nutrition and low-energy content bamboo diet. Therefore, it becomes significant to examine composition and functions of panda's gut microbiota for its health status and future survival. Several factors have been reported to play significant role in shaping the variation in composition at taxonomic scales and diversity of the gut microbiota.

OBJECTIVES

Henceforth, we must determine such factors and processes regulating microbiota composition in the giant panda. Therefore, we examine the genetic diversity, gut microbiota in wild giant panda, and correlation of structural composition of gut microbiota with host genetics and geography.

METHODS

78 fecal samples of wild giant pandas were collected from wild population to assess genetic diversity using primers for Cytochrome-b gene and D-loop region, and examine the structural profile of the panda's gut microbiome comprehensively using pyrosequencing of V4-V5 region of 16S rDNA.

RESULTS

The gut bacterial composition was dominated by Proteobacteria, Bacteroidetes, and Firmicutes (contributing 57%, 22%, and 18% of the total sequence, respectively) and revealed within-population differences in wild pandas. We assumed this variation could be correlated with genetics or geography of panda. Furthermore, we observed moderate to high genetic diversity in 28 individual fecal samples. Whereas, genetic distances displayed weak/no influence on the gut microbiota diversity (weighted and unweighted UniFrac distance; P=>0.05). However, we found a significant influence of the geographic locations on the variation in gut microbiota (weighted and unweighted UniFrac distance; P= <0.05). Therefore, conservation imperative should focus on restoration of specific geographic location.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by the "1000-Talent Program" in Sichuan and Science Foundation for Youths of Sichuan Province (2013JQ0014) and The National Natural Science Foundation of China (31471997) to YiL and the Innovative Research Team in University of Sichuan Bureau of Education. Sudhanshu Mishra was supported by Post-doctoral Bureau of China to conduct his research. We are thankful to the Wolong Nature Reserve for providing focal samples of giant panda.

1139 / SEQUENCING OF THE WHOLE GENOME OF ACINETOBACTER BAUMANNII: MONITORING OF ANTIBIOTIC RESISTANCE IN HOSPITAL DURING THE SARS-COV-2

Keywords: *Acinetobacter baumannii, Antibiotic resistance, Whole genome sequencing, Mash Index*

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BACKGROUND

The importance of antibiotic-resistance phenomenon and its worldwide spread have given rise to the activation of numerous surveillance systems. To make the data collected by these systems homogeneous and interpretable and to facilitate comparison between the various countries, a European surveillance network was created in 2000 which in 2010 assumed institutional characteristics becoming the European network European Antimicrobial Resisitance Surveillance Network (EARS-Net) coordinated by the ECDC.

OBJECTIVES

The aim of this work was to evaluate the antibiotic-resistance profile analyzing the whole genome of 24 A. baumannii strains isolated from different departments of Alessandria (Italy) hospital, in the Covid-19 era. It was a relevant project for determining the genomic proximity between strains isolated during the period considered, especially to trace their origin.

METHODS

The isolation of A. baumannii strains took place in the period of the Sars-CoV-2 pandemic. Genomic DNA was extracted from over-night cultures in Mueller-Hinton Broth, using the DNeasy® UltraClean® Microbial kit. The DNA was then quantified and shotgun libraries built using the Nextera XT DNA Library Prep kit. After product purification, the libraries were normalized and sequenced using MiSeq. The obtained sequences were bioinformatically analysed using different softwares: TrimmoMatic, PhRed, SPAdes, CheckM, Mash, Prodigal, EggNOG, FiloFlan, and RGI.

RESULTS

Here, we propose a new work-flow in order to map epidemic clusters at the hospital level. Furthermore, the proposed parameter of Mash Index, and the relative trashold of 0.0052, is reliable and validable in determining the similarity between strains and in monitoring their diffusion.

1158 / COMPARATIVE TRANSCRIPTOMICS REVEALS NEW GENES PUTATIVELY IMPLICATED IN THE AIEC PHENOTYPE

Keywords: *Crohn's disease, Adherent-Invasive Escherichia coli, Comparative transcriptomics*

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Adherent invasive Escherichia coli (AIEC) have been related to Crohn's disease. No molecular tools are available for AIEC identification. We hypothesise that differential gene expression may drive to the AIEC phenotype. To test this hypothesis we investigated differentially expressed genes (DEGs) between AIEC and non-AIEC strains and evaluated their suitability as molecular markers.

Comparative transcriptomics was performed between two AIEC/non-AIEC strain pairs during Intestine-407 cells infection. Each pair displayed identical pulsotypes and similar genomes. DEGs obtained were quantified by RT-qPCR in the same samples to confirm the results, and in a strain collection of 13 AIEC and 23 non-AIEC. Binary logistic regression was used to identify DEGs whose quantification could be used as AIEC biomarker.

Comparative transcriptomics revealed 67 DEGs between AIEC and non-AIEC in the strain pairs. In the whole strain collection, 29 DEGs were found between the two phenotypes ($p < 0.042$). Notably, six DEGs were implicated in the synthesis of two virulence determinants, and five in a metabolic pathway that could be involved in acid resistance. Mutants are being performed in order to study the implication of DEGs in the AIEC phenotype. Moreover, binary logistic regression revealed three DEGs able to predict the AIEC phenotype with an accuracy of ≥ 85%.

No previous comparative transcriptomic studies have been performed using AIEC-infected cell cultures. We have identified DEGs that could be involved in AIEC pathogenicity and used as biomarkers. These results open the door to further research in AIEC biomarkers and in new therapeutic targets against AIEC colonisation.

ACKNOWLEDGEMENTS/REFERENCES

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1159 / DEVELOPMENT OF TAQMAN PROBES FOR THE SPECIFIC QUANTIFICATION OF C. CELLULOVORANS, C. CARBOXIDIVORANS AND C. ACETOBUTYLICUM

01

Keywords: *Clostridium co-culture, TaqMan probes*

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Nowadays, the production of butanol and other added-value compounds from biomass through fermentative processes using Clostridium spp. is of great interest in the biotechnological industry. Recently, co-culturing of acetogenic and solventogenic clostridia harbouring distinct physiological properties has emerged as an alternative to increasing production rates and product spectrum. Perfect functioning of the mixed tandem culture mainly depends on the stability and fitness of each species, therefore an specific monitoring of the two (or more) members in the consortia is crucial. Quantitative PCR (qPCR) with specific TaqMan probes =is a rapid and an effective option for such an objective. In this study, a multiplex Real-Time PCR using TaqMan probes targeting the 16S rRNA gene was developed for the specific detection and quantification of Clostridium cellulovorans, Clostridium carboxidivorans and Clostridium acetobutylicum. Designed probes have been tested using synthetic co-cultures engineered for a high yield conversion of cellulose into butanol. qPCR assay validation of TaqMan probes was performed using species-specific DNA, mixtures of the selected species, and other bacterial strains, in order to test for specificity. Our method showed a high specificity with no cross-reactions between the three Clostridium strains used or to other bacteria present in the sample. The probes have been tested in actively growing axenic cultures and culture mixtures and have allowed to detect significant changes in growth kinetics in tandem cultivation that should be considered for biotechnological applications.

ACKNOWLEDGEMENTS/REFERENCES

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01

1160 / IDENTIFICATION OF MICROBES FROM HUMAN BODY FLUIDS BY RAMAN SPECTROSCOPY

Keywords: *Raman spectroscopy, diagnostics, bacteria*

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BACKGROUND

Raman spectroscopy has become a valuable tool in many fields in the last decades, allowing a non-destructive analysis of various samples. Based on inelastic (Raman) scattering of monochromatic light, it provides a fingerprint of chemical bonds present in the sample. This can be subsequently used, among other things, to identify and characterize microbial cells.

OBJECTIVES

This study aims to assess the performance of Raman spectroscopy in the identification of microorganisms causing bloodstream and urinary tract infections. Additionally, we investigated the possibility of capturing single microbial cells from human serum and urine and further characterized them by a combination of optical tweezers and Raman spectroscopy.

METHODS

We acquired Raman spectra of 400 microorganisms (30 microbial species) causing urinary tract and bloodstream infections directly from colonies grown on Mueller-Hinton agar plates (37°C/24h) by a Raman spectrophotometer (exposure time: 15 s, laser wavelength: 785 nm). For the pilot single-cell analyses, we used the combination of optical tweezers and Raman spectroscopy to capture microorganisms spiked into the human urine and serum and subsequently acquire their Raman spectra.

RESULTS

The microbial Raman fingerprints allowed to distinguish among 30 analyzed species reliably. The single-cell microbial Raman spectra pilot study showed differences between bacterial and serum/urine fingerprints and suggested that Raman tweezers can differ among various microbial species on a single-cell level. In the future, this might allow direct identification of microorganisms directly from human body fluids, which would significantly shorten the time necessary to identify causative agents.

ACKNOWLEDGEMENTS/REFERENCES

The work was supported by grants MUNI/A/1291/2021 (Grant Agency of Masaryk University) and AZV NU20-05-00166 (Czech Health Research Council).

01

1384 / EVALUATION OF LISTERIA INNOCUA VIABILITY WITH RAMAN MICROSPECTROSCOPY AFTER INCORPORATION OF HEAVY WATER (D2O)

Keywords: *Listeria, Raman microspectroscopy, viability, Deuterium Isotope Probing*

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BACKGROUND

Listeria innocua is a Gram-positive widely distributed in food-processing environments. Different environmental stresses can induce the viable but non-culturable (VBNC) state of this bacteria such as cleaning and disinfection procedures. VBNC bacteria are not detectable on conventional microbiology culture media. To detect these VBNC bacteria, an innovative approach is to use Raman microspectroscopy coupled with deuterium isotope probing (Raman-DIP). This technique allow evaluating the metabolism of bacteria, but need to treat the bacterial cells with heavy water. This treatment could cause a metabolic stress that can induce VBNC or death state of bacteria

OBJECTIVES

In this study, we evaluated the impact of heavy water incorporation on the viability state of L. innocua cells (VC, VBNC, dead) by Raman microspectroscopy.

METHODS

We exposed the L. innocua bacterial suspension to different heavy water concentrations (0%, 25%, 50% and 75%) during different times of incubation (from 0h30 to 72h). For each heavy water concentrations, total, viable (VC and VBNC) and VC populations were quantified by qPCR, PMA-qPCR and plate count agar respectively. In parallel, we analyzed heavy water absorption by Raman microspectroscopy.

RESULTS

We showed that exposure to heavy water does not affect the viability of L. innocua cells. The height of the C-D peak was correlated with the concentration of heavy water in the medium. As the concentration of heavy water increased, the height of the peak increased. The most optimal exposure condition for measuring the metabolism of L. innocua is 2h of exposure at 75% of heavy water.

1477 / RESISTOME AND XENOBIOME TEMPORAL DYNAMICS IN POULTRY AND SWINE PRODUCTION CHAINS: THE CIRCLES PROJECT

Keywords: *microbiome, food production, xenobiome, resistome*

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BACKGROUND

The CIRCLES project aims to discover and translate innovative microbiomes-tailored circular actions into concrete applications that will ultimately enhance the EU's food system performance and sustainability.

OBJECTIVES

In the CIRCLES project, real-world labs in the field for poultry and swine food systems were created, allowing to increase knowledge on the importance of microbiomes in these food systems. Specifically, we focused on the xenobiome and resistome as microbiome functionalities to provide resistance to xenobiotics and antibiotics.

METHODS

A total of 400 fecal and 160 environmental samples (feed, litters, wastewater, air, and soil) were collected at three time points from poultry and swine production sites. DNA was extracted and whole-genome shotgun sequenced, yielding 5.2 billion metagenomic reads (total sequencing depth 1.6 TeraBases). Reads were analyzed by HUMANn3. The xenobiotic-degrading genes and antibiotic-resistance genes were recruited and used for the reconstruction of the temporal dynamics of the xenobiome and resistome of the microbiomes along the whole production chain.

RESULTS

Beside the reconstruction of the compositional and functional dynamics of the animal gut microbiome in the poultry and swine production cycles, we were able to reconstruct the temporal dynamics of the respective xenobiomes and resistomes as well as to infer connections between the gut-borne and environment-borme microbiome functionalities. According to our preliminary findings, in both production chains, the xenobiome and the resistome evolve over time and across space, with changes in complexity and diversity, showing specific patterns for each production chain. Nonetheless, core profiles shared by both production systems were also identified.

ACKNOWLEDGEMENTS/REFERENCES

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1518 / INVESTIGATION OF BACTERIAL ADDICTION MODULES BY CRISPR-CAS INTERFERENCE-BASED MODEL SYSTEM

Keywords: *Post-segregational killing, Restriction-modification system, Plasmid maintenance, nan*

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Plasmids containing restriction-modification (RM) or toxin-antitoxin (TA) systems can behave as “addiction modules”, i.e., show enhanced stability in bacterial populations. One of the mechanisms of this phenomenon is post-segregational killing (PSK) of cells that lose plasmids. PSK is thought to depend on different stabilities of cognate antitoxin and toxin molecules. If toxic molecules (i.e., a toxin encoded by a TA system or restriction endonuclease encoded by an RM system) are more stable than, respectively, cognate antitoxins or methyltransferases, plasmid-free cells die since they cannot express inhibitory antitoxic molecules. In this work, we used inducible CRISPR-Cas interference to eliminate plasmids containing various TA and RM modules from the E. coli cells. We demonstrate that in most cases tested plasmid stabilization is not mediated by the killing of plasmid-free cells. However, for two Type II RM systems (EcoRV and Esp1396I), the role of the PSK in plasmid maintenance was confirmed. Using live microscopy and single cell analysis we show that in the case of EcoRV, cells losing a plasmid carrying the RM system die upon plasmid loss. For Esp1396I, plasmid loss resulted in temporal growth inhibition followed by recovery of plasmid-free cells population. Our results provide insights into the mechanism of PSK by Type II RM systems and show that efficient and rapid clearance of plasmid by CRISPR interference can be used to study PSK and related phenomena.

1594 / MULTIPLE PARS CENTROMERE-LIKE SITES IN REPABC REPLICONS OF ALPHAPROTEOBACTERIA

Keywords: *repABC replicons, plasmid pAMI4, partitioning system, CRISPR-Cas system*

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BACKGROUND

Partitioning systems (par) enable the stable maintenance of bacterial low-copy-number replicons. These loci consist of two genes encoding partition proteins A and B, and a parS centromere-like sequence. The par system is often located in close vicinity to the replication system. An extreme example of this co-localization are alphaproteobacterial repABC replicons, where the partition (repAB) and replication (repC) genes form a single operon, with parS sequences usually positioned in close proximity to these genes.

OBJECTIVE

We found that in Paracoccus aminophilus plasmid pAMI4 (438 kb) has a more complex repABC composition. Besides the repABC operon (with parS located between repB and repC) this plasmid contains a 2-kb-long non-coding locus, carrying three additional parS repeats. The main objective of our study was to examine whether the 3parS locus is involved in pAMI4 maintenance.

METHODS

Common molecular techniques were used. RepBpAMI4-6His recombinant protein was purified of Ni-NTA resin and used together with (FAM)-labeled parS-containing DNA fragments in DNA binding assays. Fluorescence microscopy was applied to visualize in vivo distribution of GFP-RepBpAMI4 fusion protein in bacterial cells.

RESULTS

We demonstrated that 3parS is bound by partition protein RepB in vitro and is essential for proper pAMI4 partitioning in vivo. In search of similar loci, we conducted a comparative analysis of parS distribution in other repABC replicons. This revealed different patterns of parS localization in Rhodobacterales and Rhizobiales. Many of these additional parS repeats are located inside protein coding sequences, with an interesting example being the cas genes of CRISPR-Cas systems.

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1596 / MICROBIAL COMMUNITY DIVERSITY AND POTENTIAL FUNCTION IN SLOW SAND FILTERS USED FOR DRINKING WATER PRODUCTION IN THE NETHERLANDS

Keywords: *Slow sand filter, microbial community, drinking water, biomarker*

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Slow sand filters (SSF) are the last cleaning steps in drinking water production. Microorganisms are the main constituents of the bioactive layer on top of the filters which has a key role in filtering contaminants. The microbial diversity and function in such manipulated and dark-mixotrophic ecosystem has not been yet determined, limiting the predictability of the SSF efficiency. Here, two full-scale slow sand filters in the Netherlands were sampled several times within a year from 2020 to 2021. DNA extracted from different layers and locations of sediment within the filters and amplicon 16S and 18S rRNA gene sequencing was performed to determine both the bacterial/archaeal and eukaryotic diversity in the different samples. Stable isotope labeling showed high uptake of glucose in microbial lipids followed by label transfer to higher organisms. One of the main targets in this study are specific biomarkers for Archaea since their role in SSFs and in general in aquatic sediments is not fully understood. These results would help in the understanding of better and more predictable functioning of the slow sand filters with less down time. Targeting biomarkers of Bacteria and Archaea will provide insights about the contribution of these two domains of prokaryotes to the degradation of organic matter and eventually maturation of bioactive layer in SSFs.

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1619 / NHEJ ACTIVITY IN PSEUDOMONAS PUTIDA KT2440

Keywords: *putida, CRISPR-Cas9, NHEJ*

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Pseudomonas putida is an essential organism for biotechnology. Due to the high production of redox agents, it is well suited for producing complex organic molecules. For precise regulation of P. putida metabolism a bunch of instruments is used. CRISPR-Cas9 is such an instrument, and it is widely spread in the industry nowadays. Cas9 complex introduces double-strand breaks (DSB) in DNA, allowing to manipulate the sequence near the break. DSB can be repaired in an error-prone way, which can lead, for instance, to the silencing of the gene. But there are different ways to repair such breaks, so our objective was to characterize mutations arising after work of the error-prone pathway - non-homologous end joining (NHEJ). Unlike homologous recombination, this pathway does not need the DNA template to recover double-helix after DSB.

We produced strains of P. putida with overproduction and deletions of the main proteins of this pathway - Ku and LigD. We investigated changes in mutations in these different genetic backgrounds by high-throughput sequencing. We found that mutation rate depends on the life cycle phase: mutations occur lower during the log-phase. The specter of these mutations depends on the expression level of NHEJ genes, with their overexpression and deletion frequency of mutations varies. Moreover, the mutation rate depends on the site of DSB, which can be connected with varied GC content.

273 / STAPHYLOCOCCUS AUREUS BIOFILM INFLUENCED BY BONE MICROENVIRONMENT

Keywords: *Biofilm, Bone and joint infections, Staphylococcus aureus, nan*

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Bacteria colonize implant surface thanks to biofilm development during bone and prosthesis infection. However, therapeutic approaches are not adapted to the specific features of biofilm in bone context whereas the infectious environment is known to dramatically influence biofilm structure. In the present study, our first objective was to develop an in vitro model using different supports prepared to mimic the periprosthetic environment. We compared Staphylococcus aureus biofilm formation (adhesion by counting methods, matrix production thanks to confocal microscopy and biofilm-related gene regulation by q-PCR) of two methicillin-sensitive (MSSA) and one methicillin-resistant strain (MRSA) on a synthetic calcium phosphate coating, a mineral part of the bone or a bone explant (mineral and organic parts, no living host cells). We also studied the bacterial adhesion on titanium covered with fibronectin, mimicking an implanted prosthesis. We demonstrated the influence of three-dimensional structure, organic and mineral parts of bone on S. aureus biofilm development excluding the possibility of using an artificial bone-like surface. MRSA adhesion was more important on different supports and bone explant contrary to both MSSA strains. The mechanisms involved in bacterial adhesion in bone context were strain-dependent: production of a matrix for MSSA vs metabolism responses for MRSA. The simultaneous presence of bone explant and fibronectin coating titanium was also found to change the bacterial behavior. These results highlighted the need for new biofilm models, more representative of the infectious environment with adapted culture medium and presence of in situ supports in order to better evaluate therapeutic strategies on biofilm.

ACKNOWLEDGEMENTS/REFERENCES

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280 / LACK OF IMPACT OF POXB ON P. AERUGINOSA VIRULENCE AND CARBAPENEM RESISTANCE OF STRAINS WITHOUT TYPICAL UNDERLYING MECHANISMS

Keywords: *Pseudomonas aeruginosa, PoxB (OXA-50) intrinsic beta-lactamase, virulence, carbapenem resistance*

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BACKGROUND

Pseudomonas aeruginosa (PA) harbors an intrinsic β–lactamase, PoxB, displaying mild carbapenem hydrolytic capacity. The impact of this un-inducible enzyme over clinical resistance has been barely studied and thus, few cases of hyper-producing strains have been reported.

OBJECTIVES

i) ascertain whether a set of clinical PA isolates displaying carbapenem resistance but devoid of typical underlying mechanisms could base the phenotype on PoxB-related features;

ii) assess the impact of PoxB hyper-production on PA virulence, as a potential fitness-related explanation for the apparently low prevalence of PoxB-based resistance.

METHODS

we used 6 fully-sequenced carbanemem-resistant clinical strains (from a multicenter study) to determine if activity-increasing amino-acid changes and/or hyper-expression (RT-PCR) affecting poxB could explain their phenotypes. We cloned PAO1 strain's poxB (PA5514) in pUCP24 multicopy vector, transformed the construct into PAO1 and a peptidoglycan recycling-defective mutant, and analyzed β–lactam resistance (E-test). We determined the Lethal Dose 50 (LD50) of strains harboring the poxB plasmid over Galleria mellonella larvae as virulence indicator.

RESULTS

the 6 strains displayed wildtype poxB expression levels, whereas their sequences showed polymorphisms common to previously sequenced carbapenem-susceptible strains. Hyper-expression of pUCP24-cloned PoxB (≈600-fold compared to PAO1) significantly decreased meropenem/piperacillin susceptibility (MICs increased ≈3/4-fold), but had no impact on virulence (LD50s comparable to controls, ≈50 bacteria) in contrast with other cloned β–lactamases used as comparators. Therefore, PoxB does not seemingly support PA carbapenem resistance in strains without typical underlying mechanisms. The reason for this result is apparently not an unbearable biological cost of PoxB that could negatively select its hyper-production.

ACKNOWLEDGEMENTS/REFERENCES

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281 / NON-STEROIDAL ANTI-INFLAMMATORY DRUG CELECOXIB POTENTIATES THE IN VITRO ANTI-STAPHYLOCOCCAL EFFECT OF OXACILLIN

Keywords: *Staphylococcus aureus, anti-inflammatory, anti-inflammatory, celecoxib*

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BACKGROUND

Staphylococcus aureus (S. aureus) causes musculoskeletal infections (MI) such as osteomyelitis, which poses a serious global health challenge [1,2]. There is a current search for new antibiotics to combat S. aureus associated infections, however, no new antibiotics have been approved for clinical use in recent years [3]. Non-steroidal anti-inflammatory drugs (NSAIDs) such as celecoxib is used to treat inflammation caused by MI [4]. Since certain NSAIDs enhances the efficacy of some antibiotics through synergistic interaction [5], they are seen as a potential drug candidate to potentiate the efficacy of antibiotics.

OBJECTIVES

To investigate the in vitro synergistic interactions of celecoxib with oxacillin against S. aureus strains.

METHODS

The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method using 96-well microtiter plates [6]. The fractional inhibitory concentration (FICs) were assessed by the checkerboard assays [7]. The combinatory effects against five strains of S. aureus were subsequently determined, where $\Sigma FIC \leq 0.5$ = synergy., $\Sigma FIC > 0.5-4$ = no interaction., and $\Sigma FIC > 4$ = antagonism [7].

RESULTS

Celecoxib showed strong synergistic interaction with oxacillin against S. aureus strains at tested concentrations 16 to 0.5 µg/mL. The ∑FICs for these concentrations ranged from 0.087 to 0.471. Previous reports showed that celecoxib enhances the activities of certain antibiotics such as vancomycin and linezolid [5], however, to our best knowledge, this is the first study of the synergistic interaction of celecoxib with oxacillin against S. aureus.

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314 / ANTIMICROBIAL RESISTANCE PROFILES, VIRULENCE GENES, AND GENETIC DIVERSITY OF THERMOPHILIC CAMPYLOBACTER FROM LAYER CHICKEN

Keywords: *Campylobacter, Quinolones, Genotyping, Layer chicken*

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BACKGROUND

Campylobacter are among the major etiologies of gastroenteritis globally.

OBJECTIVE

Assessing the antimicrobial resistance (AMR) profiles, virulence genes, and genetic diversity of Campylobacter isolated from layer chicken farms.

METHODS

In total, 153 chicken feces were collected and Campylobacter were isolated by culture while PCR and sequencing were used for species confirmation. Antimicrobial susceptibility testing for six antimicrobials (ciprofloxacin, nalidixic acid, sitafloxacin, erythromycin, tetracycline, and gentamicin) was carried out by broth microdilution. Three AMR and nine virulence genes were screened by PCR. Genotyping was performed by flaA-restriction fragment length polymorphism (RFLP) and multilocus sequence typing (MLST).

RESULTS

Of the 153 samples, Campylobacter were detected in 55 (35.9%), with C. jejuni and C. coli being 49 (89.1%) and six (10.9%), respectively. High-level resistance was observed for ciprofloxacin (100%) and NAL (100%). The mutations (C257T) in gyrA and tet(O) genes were confirmed by sequencing. The rate of multidrug-resistance was 8.2%, and all were C. jejuni. All Campylobacter isolates possessed five virulence genes (cdtB, cstII, flaA, cadF, and dnaJ) while the rates for other genes (csrA, ciaB, and pldA) were 33.3-95.9%. We found 26 flaA types while the MLST showed 10 sequence types (STs) for C. jejuni and three STs for C. coli, with CC-607 (STs 3611) and CC-460 (ST-460) being predominant. Among the 10 STs of C. jejuni, three were newly assigned. The findings highlight increased resistance to quinolones and TET, the virulence potential, and diverse genotypes among Campylobacter strains isolated from the layer poultry farm.

355 / SEROLOGICAL IMMUNE RESPONSE TO ADENOVIRUS AND RESPIRATORY SYNCYTIAL VIRUS: DIFFERENCES BETWEEN CHILDREN AND ADULTS

Keywords: *respiratory infections, pneumoslide, nan, nan*

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BACKGROUND

Adenovirus and Respiratory syncytial virus (RSV) are the main agents among viruses responsible for causing upper and lower respiratory tract infections. Standard microbiologic techniques are very difficult for detection of these pathogens. Objective: to analyze most often detected specific IgM and IgG antibodies towards Adenovirus and RSV and to compare the results between children and adults.

MATERIAL AND METHODS

A total of 3050 patients were examined in period of 12 months (2021), by indirect immunofluorescent method, using the Pneumoslide test (Vircell). This test also detects antibodies to four bacteria and antibodies towards three other viruses.

RESULTS

From a total of 3050 sera, 17 were posive (0.55%) for Adenovirus IgM abs, 453 (14.8%) for IgG abs, for RSV 6 (0.19%) IgM were positive and for IgG abs 619 (20.29%).

From a total of 759 sera from children for Adenovirus 9 (1.18 %) IgM abs were positive and IgG abs 144 (18.97%). For RSV there were not IgM abs detected in this period and for IgG abs 130 (17.12%).

From a total of 2291 sera from adults 8 (0.34%) were positive for Adenovirus IgM abs and IgG abs 309 (13.48%). For RSV positive were 6 (0.26%) IgM abs and 489 (21.34%) for IgG abs.

336 / INFECTION COMPLICATIONS AND RESISTANCE PATTERNS OF COMBAT WOUNDS

Keywords: *wound, war, resistance, trauma*

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Up to one third of combat wounds get infectious complications. After hemorrhagic shock it is the second most common cause of mortality among battlefield injuries. Infection related morbidity and mortality depends on a severity of case as well as timing of primary medical help and initial antibiotic use after injury.

Current study explored the short term and long-term war wound infectious complications in one of the orthopedic hospitals in Armenia in 2020 during 44 days of war and one year after discharge. The soft tissue infections as well as osteomyelitis were included in the study.

138 patients injured during the war casualties and admitted to orthopedic hospital were included in the study. Study excluded abdominal, thoracic and head injuries. The study population included only upper and lower limb soft tissue and bone injuries. The retrospective analysis of patient medical records as well as bacteriological laboratory findings were collected and analyzed. The prospective follow up of the patients was done during 12 months after discharge.

Out of the 138 soldiers admitted to the hospital 13 (9.4%) had infectious complications. Overall, 29 microbes were isolated. The most common isolated microbes where Enterococcus Faecalis, Bacillus spp., Staph. Aureus, Staph. Saprophyticus. The highest resistance was observed against third and fourth generation of Cefalosporins: Ceftriaxone (n=13) and Cefepime (n=11) and to the Co-trimaxazole (n=11). 2 strains of isolated Acinetobacter baumanii were resistant to all antibiotics except from Meropenem. Medical charts review identified 41% non-compliance of de-escalation practices to the existing evidence.

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363 / COMPARATIVE ANALYSIS OF PVL-POSITIVE MRSA ISOLATED IN COMMUNITY AND HOSPITAL SETTINGS IN JAPAN

Keywords: MRSA, PVL

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BACKGROUND AND OBJECTIVES

Incidences of MRSA-producing Panton–Valentine leucocidin (PVL) have recently been increasing in community and hospital settings in Japan. PVL can cause serious infections even in healthy individuals. It is generally considered that PVL-positive MRSA was widely disseminated among healthy people and then spread into healthcare facilities; however, the difference between the strains isolated in each setting has not been clarified. In this study, the molecular epidemiological characteristics of PVL-positive MRSA strains isolated in community and hospital settings in recent years were investigated and compared.

METHODS

Approximately 300 and 4,300 MRSA strains isolated from 20 clinics and 9 hospitals, respectively, in Japan between 2016 and 2020 were used. The respective antibiotic resistance genes and virulence genes were detected via PCR. Molecular epidemiological analysis was conducted by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

RESULTS

The isolation rates of PVL-positive strains among MRSA were approximately 29 % in clinics and 10 % in hospitals. Molecular epidemiological analysis revealed that approximately 70 % of PVL-positive MRSA isolated in community settings were classified as ΨUSA300, a variant of the representative PVL-positive clone, USA300. In contrast, in hospital settings, approximately 10 % of PVL-positive MRSA were classified as ΨUSA300, and several other USA300 variants were detected. Among the PVL-positive strains, those belonging to clonal complex (CC) 1, 5, 30, and others were isolated only from hospitals and not from clinics. This study revealed that the PVL-positive MRSA strains in hospital settings are more diverse than in community settings in Japan.

368 / ANTIMICROBIAL EFFECT OF PYROPHYLLITE/SILVER AND PYROPHYLLITE/ZINC NANOCOMPOSITES AGAINST PLANKTONIC AND BIOFILM-EMBEDDED NONFERM

Keywords: *nanocomposites, biofilm, bacteria*

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BACKGROUND

Nanocomposites (NCs) are multiphase structures with at least one phase on the nanoscale dimension. Although antimicrobial properties of silver and zinc nanoparticles are well investigated, there is no data about antimicrobial capacity of nanocomposites containing pyrophyllite clay embedded with zinc (Pyro/Zn) and silver (Pyro/Ag) nanoparticles.

OBJECTIVES

Our aim was to explore possible antimicrobial effects of Pyro/Zn and Pyro/Ag NCs against planktonic and biofilm producing isolates of Pseudomonas aeruginosa and Acinetobacter baumanii.

METHODS

Experiments were performed in microtiter plates. Planktonic isolates (100 µl of bacterial culture in final concentration of 106 CFU/ml) were treated with 10 mg of Pyro/Ag and Pyro/Zn NCs with Zn and Ag concentration range of 5, 10, 15, 20, 25, 50, 75 and 100 mg/ml and incubated with 250 rpm agitation for 24h at 37°C. Antibiofilm activity assay was performed with 10 mg of NC in concentration of 20 mg/ml of Ag and Zn nanoparticles. Effect of NC on the process of biofilm formation was analyzed during overnight incubation of bacterial isolates with NC. NC activity against the mature biofilm was examined in a two-step process (first incubation without treatment, followed with incubation of mature biofilm with NC).

RESULTS

Pyro/Ag NC showed a bactericidal effect on planktonic isolates in concentration over 25 mg/ml, while Pyro/Zn showed only bacteriostatic activity with a slight reduction in bacterial number. When analyzing antibiofilm effects, only Pyro/Ag NC showed the ability to reduce BF production, while both NCs could not eradicate mature biofilm. Research was under project No. 19.032/961-78/19.

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380 / SEASONAL VARIATION OF PSEUDOMONAS SPECIES IN INTENSIVE UNIT

Keywords: *Pseudomonas, healthcare-acquired infection, seasonal variation, intensive care unit*

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Pseudomonas species strains are frequent intra-hospital pathogens in intensive care units and they are should be prioritized as important public health problem. Medical devices colonisation is often responsible for nosocomial infections in intensive care. Pseudomonas species colonization and its ability to become endemic through creating biofilm in medical environment highlight this organism as emergent pathogen. Also, Pseudomonas strains demonstrate seasonal variation. This study will reviewed Pseudomonas colonization on respiratory devices -endotracheal tubes and nasal cannulas. The study will determine its seasonality variation and it will indicate the critical seasons. This research included microbiological examined materials from medical respiratory devices of patients from Clinic for Anesthesiology, Resuscitation and Intensive Care – Skopje, sent to the Institute of Microbiology and Parasitology in Skopje for microbiological analysis for eight years period (2010 – 2017). The Pseudomonas isolation rate was observed and then compared with official climate data for average humidity of the air. Pseudomonas has an affinity to both respiratory medical devices but the percentage difference of its presence in the cannula, was significantly larger. The analysis of the total sample according to the values of the seasonal index over 100% (above the monthly amount), indicated that Psedomonas was the most present in November – 150,4% for the examined period. The highest value of the seasonal index for Pseudomonas strains from tubes were in November – 154,61% and analysis for cannula indicated the highest value in May – 152,44%. The months with high air humidity positively affect the development of these bacteria.

383 / INCREASED PREVALENCE OF DOXYCYCLINE LOW-SUSCEPTIBLE CUTIBACTERIUM ACNES ISOLATED FROM ACNE PATIENTS IN JAPAN

Keywords: *Cutibacterium acnes, Acne vulgaris, Antimicrobial treatment, Tetracyclines*

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The prevalence of antimicrobial-resistant Cutibacterium acnes is an important concern for the antimicrobial treatment of acne vulgaris. We hypothesized that antimicrobial treatment regimens for acne vulgaris would change following the revisions in the Japanese acne treatment guidelines, which added a statement regarding appropriate antimicrobial usage. Here, we studied the changes in antimicrobial use and antimicrobial-resistant C. acnes isolated from acne patients.

A total of 127 C. acnes isolates collected from 212 patients with acne between 2013 and 2018 were used. Roxithromycin and clindamycin resistance rates were approximately 50% and 40%, respectively. In contrast, the prevalence of low doxycycline-susceptible strains (minimum inhibitory concentration [MIC] ≥8 μg/ml) in 2018 (17.4%) was 5.6-fold higher than that in 2013 (3.1%). Although the number of patients with severe and moderate acne did not change, the number of patients with a history of oral tetracycline use increased. The incidence of low doxycycline-susceptible strains was high in patients with a history of oral tetracycline use. The prevalence of strains with a 16S rRNA mutation, which confers reduced susceptibility to tetracyclines, increased by 8.6-fold (12.1%) from 2016 to 2018 in comparison with the previously revised guidelines (1.4%). Furthermore, strains with tet(W) were found with approximately 10%.

Our data show that the antimicrobial resistance pattern in C. acnes changes according to the trend of antimicrobial usage for acne treatment. Therefore, we should pay heed to the rapid dissemination of tetracycline resistance in C. acnes owing to acquisition of 16S rRNA mutation and tet(W).

413 / NEOSARTORYA FISCHERI ANTIFUNGAL PROTEIN 2 – A POTENTIAL NEW THERAPEUTIC APPROACH FOR TREATMENT OF SUPERFICIAL CANDIDIASIS

Keywords: *antifungal protein, Candida spp., superficial candidiasis, nan*

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BACKGROUND

As a consequence of emerging number of fungal infections caused by (multi)drug-resistant Candida isolates, there is an urgent need to develop novel antifungal treatment strategies. The features of the Neosartorya (Aspergillus) fischeri antifungal protein 2 (NFAP2) render it to be a promising candidate.

OBJECTIVES

We investigated the potential applicability of NFAP2 (alone and in combination with licensed antifungal drugs) in the treatment of superficial Candida infections.

METHODS

The in vitro antifungal efficacy of NFAP2 and its combination with licensed antifungal drugs to inhibit the growth of different Candida spp.; and their ability to eradicate Candida biofilms was investigated according the recommendations of CLSI M27-A3 protocol. A toxicity test on primary human keratinocytes and dermal fibroblasts was applied to study the safe applicability of NFAP2 on human skin. The curative effect of NFAP2 alone and in combination with fluconazole was studied in a murine vulvovaginal candidiasis model.

RESULTS

NFAP2 was able to inhibit the growth of different Candida spp. and to eradicate their biofilms alone and in combination with licensed antifungals. NFAP2 exerted synergy with all tested antifungal drugs. NFAP2 was not toxic to human keratinocytes and dermal fibroblasts. The murine vulvovaginitis model experiments indicated that NFAP2 is able to significantly decrease the burden of fluconazole-resistant Candida albicans cells, and its combined application with fluconazole enhances the efficacy.

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02

450 / COMPREHENSIVE ANALYSIS OF BACTERIOCIN PRODUCTION POTENTIAL OF THE PROBIOTIC STRAIN STREPTOCOCCUS DENTISANI 7746

Keywords: *bacteriocins, antimicrobial peptide, quorum sensing, probiotics*

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BACKGROUND

Previous investigations from our group demonstrated the antimicrobial potential of the S. dentisani 7746's supernatants due to the activity of molecules of peptidic nature (1). Other studies reported the presence of up to 11 bacteriocin peptides coded within a bacteriocin coding region in S. dentisani 7746's genome (2, 3).

OBJECTIVES

In this study we aimed at i) identifying all the bacteriocin precursor genes encoded by S. dentisani 7746, and ii) identifying all their potential regulatory elements.

METHODS

We performed bacteriocin genome mining searches for core peptides with the web-based tool AntiSMASH. The online programs SMART and InterPro were used for protein structural and functional predictions.

RESULTS

The AntiSMASH analysis gave two areas of interest for the presence of core peptides: one corresponding to the region previously reported (2, 3), and a second area for bacteriocin production which had not been described before. Thorough investigation of these two regions shows that S. dentisani 7746 has complete sets of Com-like and Blp-like quorum sensing systems containing SunT-type transporters which are involved in the processing and export of antimicrobial peptides or peptide pheromones. We identified three additional bacteriocin precursor peptides increasing to 14 the number of putative bacteriocins encoded within the S. dentisani 7746 genome, making it one of the bacterial isolates with the largest bacteriocin repertoire known to date.

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ACKNOWLEDGEMENTS

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459 / PEPTIDOGLYCAN RECYCLING IMPAIRMENT INCREASES P. AERUGINOSA BIOFILM SUSCEPTIBILITY TO LYSOZYME AND/OR SUBINHIBITORY COLISTIN

Keywords: *Pseudomonas aeruginosa, biofilm, peptidoglycan recycling, treatment*

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BACKGROUND

Blocking peptidoglycan (PGN) recycling pathways poses an alternative to fight Pseudomonas aeruginosa (PA) infections (1). Increasing cell-wall permeability using sub-inhibitory concentrations of colistin boosts planktonic PA susceptibility to the immune protein lysozyme, phenomenon enhanced after PGN recycling blockade (2), especially when combined with AmpC intrinsic β–lactamase hyperproduction.

OBJECTIVES

To explore if the combination of lysozyme/colistin, together with the PGN recycling blockade/AmpC hyperproduction could increase the elimination of PA biofilms.

METHODS

Biofilm of studied strains (Figure 1B) were prepared by normalizing an overnight culture to an OD600nm=0.1 in 8-well slides. Forty-eight-hour biofilms were treated with 25 mg/L of human lysozyme (LZ), 0.05 mg/L of colistin (COL) or both combined (LZ_COL) for 2h, stained with 20µM propidium iodide and immediately imaged (confocal microscope). Dead cells were quantified (Biofilm Viability Checker). Statistical analysis was performed with GraphPad Prism 7, using paired one-way ANOVA, calculating the increase in biofilm mortality ratio (BMR) referred to the untreated control

RESULTS

A significant increase in BMR was observed for the 3 PGN recycling-deficient mutants when treated with LZ and for PA14ΔnZ, PA14ΔDDh2Dh3 and PA14ΔDDh2Dh3-pucpAD when treated with COL (Figure 1A). Interestingly, all strains showed a significantly increased BMR when treated with LZ_COL. PA14ΔDDh2Dh3 presented the maximum mortality, which was partially restored to wildtype levels after complementation with pUCPAD plasmid. These results, which correlate with our previous observations (1,2), suggest that the synergy LZ_COL together with the interplay between PGN recycling blockage and AmpC hyperproduction could be explored as a strategy to fight PA biofilms infections.

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AKNOWLEDGMENTS

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464 / EXTENDED-SPECTRUM ß-LACTAMASE ESCHERICHIA COLI AND KLEBSIELLA SPP. IN COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS

Keywords: *Escherichia coli, Klebsiella spp., ESBL*

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INTRODUCTION

Urinary tract infections (UTI) are common infections aquired in the community. Knowledge of local antibiotic susceptibility profile is important in decision making of the optimal choice for empirical antimicrobial therapy and its duration. Extended-spectrum β-lactamase (ESBL)-producing strains of Enterobacterales are considered to be emerging pathogens. Recurrent urinary tract infection (UTI) is one of the risk factors for infection with ESBL-producing bacteria.

OBJECTIVE

To assess the prevalence of ESBL-producing E. coli and Klebsiella spp. in community-acquired urinary tract infections in 2020 in Canton Sarajevo.

MATERIALS AND METHODS

In this retrospective study, we collected antibiotic susceptibility data from urine samples analysed in the Microbiology Laboratory of the Institute for Public Health of Canton Sarajevo from 1. January to 31. December 2020. Urine samples were inoculated on CUA III Liofilchem® medium and incubated at 37º C for 24-48 hours. A count of ≥ 100,000 colony forming units (CFU) per milliliter of urine was considered positive after incubation, and these isolates were identified. The antimicrobial susceptibility testing was performed using the disk diffusion method, according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines and results interpreted following EUCAST breakpoint tables v.10.

RESULT

There were total 34087 urine samples from outpatients analysed in 2020. Out of this number, 23,72% (n=8085) were positive. E.coli was identified in 70,27% (n=5682) samples and Klebsiella spp. in 21,41% (n=1731). ESBL E.coli was identified in 3,36% (n=191) of all E.coli positive samples and ESBL Klebsiella spp. in 7,33% (n=127) of all positive Klebsiella spp. samples.

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477 / ISOLATION, CHARACTERIZATION, AND COMPARATIVE INVESTIGATION OF NOVEL BACTERIOPHAGES AS PHAGE THERAPY CANDIDATES

Keywords: Bacteriophages, Phage therapy, Resistance

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BACKGROUND

Antibiotic resistance is becoming increasingly common in the world and represents a serious threat to public health. One of the major challenges in the world is the rise of pathogenic species resistant to antibiotics. So, new alternative treatment strategies are urgently needed. Bacteriophages (phages) are viruses that infect bacteria and they have become a promising alternative to conventional antibiotics.

OBJECTIVES

The aim of this study is to isolate and characterize new bacteriophages able to infect clinical strains as phage therapy candidates.

METHODS

Phages were isolated from water samples. After the enrichment of phages from water samples, the supernatant was filtered through 0.22mm syringe filters. The lytic spectrum of phages was determined using a spot test assay and obtained individual plaques using double-layer assays. They were tested against 50 clinical bacterial strains.

RESULTS

Three phages (6EC,18PA,19SA) were isolated from various natural water samples. Phage-6EC which was isolated from a lake produced big plaque with halos. Phage-18PA which was isolated from sewage water formed small clear plaques. Phage-19SA which was isolated from seawater formed tiny clear plaques. Phage-18PA was able to infect all Pseudomonas aeruginosa. However, it was able to lyse %31 of all Staphylococcus aureus and Escherichia coli strains. Phage-6EC was able to lyse %55 of all E.coli strains. However, it infected only three S.aureus strains. Phage-19SA was able to lyse %50 of S.aureus and E.coli strains and infected only one P.aeruginosa strain.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by a grant from the Research Fund of the University of Istanbul (Istanbul, Turkey). Project number: TDK-2021-37374

485 / METABOLIC CONSEQUENCES OF ANTIBIOTIC EXPOSURE IN ESCHERICHIA COLI AND PSEUDOMONAS AERUGINOSA

Keywords: *antibiotic resistance, metabolic modelling, metabolism*

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Besides genetic mutations, metabolic state of bacterial cells represents another driving factor in the emergence of antibiotic resistance. In this direction, studying how bacteria reprogram their metabolism when facing antibiotics exposure(1) is crucial to develop new efficient treatments, facilitating the discovery of new resistance mechanisms and thereby enhancing our ability to limit the development and spread of antibiotic resistance(2).

Here we have studied the metabolic consequences of antibiotic exposure in bacteria using an integrated approach that exploits transcriptomics and constraint-based metabolic modelling. Specifically, we ask whether common metabolic strategies emerge during the exposure to antibiotics, regardless of the kind of antibiotic used or, on the contrary, whether antibiotic-specific pathways exist. To this purpose, we have used very heterogeneous datasets from 9 studies (accounting for 13 experiments) that encompass two different microbes -E. coli and P. aeruginosa- grown on different media and exposed to different concentrations/types of antibiotics. We show that, despite antibiotic-specific metabolic signatures do exist, specific trends could be identified. These include, for example, the activation of glycerophospholipid metabolism in E. coli, being present in most of the treated models, i.e. metabolic models in which the presence of the antibiotic was simulated. For P. aeruginosa, carbon metabolism and amino acids biosynthesis pathways were the ones that significantly changed their activity upon antibiotic exposure. Our work might suggest general metabolic strategies to face the stress posed by antibiotic exposure and that, in turn, may represent a novel field of action in the fight against antibiotic resistance.

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492 / ANTIMICROBIAL ACTIVITY CHARACTERIZATION OF PEP20, A PLANTARICIN 149 DERIVATIVE PEPTIDE

Keywords: *multidrug-resistance, Plantaricin, Antimicrobial Peptide*

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The scarcity of therapeutic options for infections caused by multidrug-resistant bacteria increases the interest in antimicrobial peptides, such as Plantaricin149 (PLN-149). We synthesize several derivative peptides from PLN-149, testing their antimicrobial activities and hemolysis and obtained Pep20, a promising FMOC-undecapeptide. Pep20 had a fast bactericidal effect in a concentration-dependent manner, with no viable cells within one hour, and post-antibiotic effects of 5h and 2,5h for Staphylococcus aureus (Gram-positive), and Acinetobacter baumannii (Gram-negative), respectively. During 30 days of directed evolution, PEP20 minimal inhibitory concentration increased but less than the known control antibiotics. Finally, Pep20 showed a low selectivity index in the cytotoxicity assay. We also accessed PEP20 synergy with ciprofloxacin, tobramycin, daptomycin, polymyxin B, vancomycin, ampicillin and imipenem and observed synergism with vancomycin in Gram-negative strains, indicating a PEP20 action in the outer membrane. Pep20 causes cytoplasmatic depolarization in disproportionate increments to concentration, suggesting that Pep20 needs to reach a threshold for its action, such as observed for carpet mode of action. Finally, transmission electron microscopy showed damage to the membrane, in addition to mesosome-like membranous invaginations. In Gram-negatives, we also observed granules of high electron density, as already reported for cathelicidin. In conclusion, we demonstrated that PEP20 was an optimized peptide compared to the original due to its smaller size, greater and broad-spectrum antimicrobial activity, in addition to less hemolysis. Based on the characterization of PEP20, we hypothesized that its mode of action is on cytoplasmic and outer membranes in a carpet-like action.

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496 / IMPACT OF MASS BATHING EVENTS ON THE NATURAL ECOSYSTEM AND HUMAN HEALTH

Keywords: *Mass gatherings, microbiome, Human health, AMR*

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BACKGROUND

The Kumbh Mela is the world's largest periodic religious mass gathering event (MGE) hosted on the banks of 4 holy rivers in rotation every three years. The rise in the number of devotees, from 0.4 million in 1903 to 120 million in 2013 can dramatically impact the river ecosystem. A massive load of skin microbiota introduced during bathing events acts as a critical biotic strain, affecting the microbial community and the environmental factors. The assemblage of such contaminants in rivers during MGE increases the risk of water-borne infections and diseases that may cause serious health issues.

OBJECTIVES

In the present study, a spatiotemporal meta-analysis was performed to assess the bacterial communities from the samples collected before and during 2015 from the Godavari river. A comparative analysis was also performed with the samples collected during the lockdowns and travel restrictions, providing a unique opportunity to evaluate the impact of minimum anthropogenic activity on the river water ecosystem.

RESULTS

Targeted metagenomics revealed a 0.87-fold increase in the bacterial diversity during the restricted activity of lockdown. A significant increase in the resilient phyla viz. Pseudomonadota (70.6%), Bacteroidota (22.5%), Verrucomicrobia (1.8%), Actinomycetota (1.2%) and Cyanobacteria (1.1%) was observed. Minimal incorporation of allochthonous bacterial communities of human origin was observed. However, functional profiling using imputed metagenomics showed a reduction in infection and drug resistance genes by -0.71-fold and -0.64-fold, respectively.

CONCLUSION

These observations highlight the positive implications of COVID19 lockdown measures resulting in restoring autochthones microorganisms and minimizing the spread of pathogens.

ACKNOWLEDGEMENTS/REFERENCES

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509 / ANTIMICROBIAL RESISTANCE AND TRANSMISSION OF A MULTIDRUG-RESISTANT PLASMID IN CUTIBACTERIUM SPECIES

Keywords: *multidrug-resistance, opportunistic pathogen, Cutibacterium species*

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Cutibacterium species are skin inhabitants, while it may cause opportunistic infections. It is a concern that antimicrobial-resistant C. acnes strains are frequently isolated from patients with acne vulgaris. Thus, antimicrobial resistance may have been disseminated not only to C. acnes but also to other skin inhabitants. Here, we studied the molecular epidemiology of Cutibacterium species isolated from patients with acne vulgaris and those with other infections. Clinical C. acnes, C. avidum, and C. granulosum isolates were used. Antimicrobial susceptibility was evaluated by measuring minimum inhibitory concentration. Transconjugation of pTZC1 was performed using the filter mating method.

In all species, the rate of antimicrobial resistance from patients with acne vulgaris was higher than those from patients with other infections. The antimicrobial resistance rates of C. avidum and C. granulosum were higher than those of C. acnes, and several multidrug-resistant strains were found. In particullar, rates of macrolides-clindamycin resistance were high. The major resistance factors within those were the 23S rRNA mutation in C. acnes and C. granulosum, as well as erm(X) acquisition in C. avidum. Additionally, we found the multidrug-resistant plasmid pTZC1 in C. granulosum for the first time, which is a plasmid that carries erm(50) and tet(W), which have previously only been found in C. acnes. The pTZC1 was transferred with high frequency by transconjugation between C. acnes and C. granulosum.

This study showed that Cutibacteirum species exhibit antimicrobial resistance. Furthermore, the pTZC1 multidrug-resistant plasmid is capable of transfer among Cutibacterium species with high frequency.

519 / OREGANO ESSENTIAL OIL SENSITIVITY OF MULTIRESISTANT ACINETOBACTER BAUMANNII STRAINS ISOLATED FROM COVID-19 PATIENTS

02

Keywords: *Acinetobacter baumannii, COVID-19, ICU, Oregano essential oil*

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BACKGROUND

COVID-19 pandemic brought a burden to the health care system including a higher rate of patients admitted to the intensive care unit (ICU). Mechanical ventilation, needed in COVID-19 patients admitted to ICU, increases the risk of developing multidrug-resistant (MDR) Acinetobacter baumannii superinfection, which may cause major problems in antibiotic treatment.

OBJECTIVES

The aim of this research was to examine the susceptibility of A. baumannii isolates recovered from COVID-19 patients to antibiotics usually used as treatment options, and to Oregano essential oil (EO) as naturally derived compound.

METHODS

The 54 A. baumannii isolates were recovered from COVID-19 patients admitted to ICU, during period from December 2020 to February 2021. Biofilm formation ability, as the factor that contributes to bacterial resistance, was examined. Minimum inhibitory concentrations (MICs) were determined for meropenem, imipenem, colistin, gentamicin, tobramycin and levofloxacin. In addition, all 54 isolates of A. baumanii were tested for susceptibility to Oregano EO.

RESULTS

Results obtained in this study revealed a significant MDR profile of tested A. baumannii isolates. Even 74% (40/54) were strong biofilm producers. A. baumannii isolates were resistant to all tested antibiotics, except to colistin, leaving colistin as the only treatment option. On the other hand, MIC results obtained for Oregano EO were extremely promising: 0.279 mg/mL for all tested isolates. The obtained results emphasize a potentially new treatment approach to infections caused by MDR A. baumannii, using natural products such as EOs.

ACKNOWLEDGEMENTS/REFERENCES

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534 / CHARACTERIZATION AND VALIDATION OF AN ANTI-INFLUENZA PEPTIDE PRODUCED BY THE HUMAN INNATE IMMUNE SYSTEM

Keywords: *antimicrobial, peptide, influenza, immunity*

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BACKGROUND

Influenza virus is one of the major causes of respiratory infections worldwide, causing outbreaks with a severe impact on our society. Despite its high prevalence, there are individuals who remain resistant to develop flu symptoms, in a similar situation to asymptomatics in SARS-CoV2 infections. In a previous work, human DNA samples from these "flu-resistant" individuals were analyzed and a human antimicrobial peptide was identified with anti-influenza activity, as revealed by Hemagglutination Inhibition Assays.

OBJECTIVES

To detect this antimicrobial peptide in different human fluids. To compare its levels between symptomatic and asymptomatic individuals. To identify possible DNA sequence variations in the corresponding coding gene.

METHODS

An Enzyme-Linked ImmunoSorbent Assay test was set up to detect this peptide. Different human samples from hospitalized and asymptomatic individuals were analyzed, including saliva, nasopharynx swab, tear and oral mucosa. In addition, DNA from the oral mucosa samples were extracted, primers against its coding region were designed and amplicons were purified for PacBio sequencing and bioinformatic analysis.

RESULTS

This human antiviral peptide is produced in all routes of entry of the virus infection (saliva, nasopharynx and tears). In the absence of infection, flu-resistant individuals have significantly higher basal levels of the peptide compared to sensitive ones (p value < 0.0001). The amount of antiviral peptide increases significantly (p value = 0.009) during influenza virus infection when compared to basal levels.

CONCLUSIONS

The development of this human antiviral peptide could be a new tool in the prevention and treatment of influenza virus infections.

ACKNOWLEDGEMENTS/REFERENCES

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538 / QUORUM QUENCHING ACTIVITY OF HETEROLOGOUSLY EXPRESSED YTNP LACTONASE FROM CHRYSEOBACTERIUM AQUATICUM SRL1

Keywords: Quorum quenching, YtnP lactonase, Pseudomonas aeruginosa, nan

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BACKGROUND

Novel therapeutic strategies are needed to prevent the emergence and spread of antibiotic-resistant bacteria. In Pseudomonas aeruginosa, biofilm formation and the synthesis of virulence factors are regulated by quorum sensing (QS). Therefore, quorum quenching could be an efficient therapeutic approach against this bacterium, and lactonases that degrade N-acyl-homoserine lactones (AHLs), signalling molecules in two P. aeruginosa QS pathways, have emerged as a promising tool for pathogenesis inhibition.

OBJECTIVES

To characterize the quorum quenching activity of heterologously expressed YtnP lactonase from Cryseobacterium aquaticum SRL1.

METHODS

C. aquaticum SRL1 was isolated from a freshwater sponge on a minimal medium supplemented with N-hexanoyl-L-homoserine lactone as a sole carbon source. The ytnP gene identified from C. aquaticum SRL1 genome was cloned into the pETite vector and expressed in Escherichia coli BL21 (DE3). YtnP protein was purified using affinity chromatography and its AHL-degrading activity was analyzed by bioluminescence assays and HPLC. P. aeruginosa virulence factor production and biofilm formation, after 16 h of treatment with YtnP, were analyzed.

RESULTS

Lactonase encoding ytnP gene from C. aquaticum SRL1 was successfully cloned and overexpressed. Purified lactonase did not require cofactors, exhibited maximal activity at pH 6.5 and 30 °C, and retained a high level of activity after preincubation at 20 to 50 °C. YtnP degraded 70% of N-(3-oxododecanoyl)-L-homoserine lactone and 50% of N-butyryl-L-homoserine lactone. It also inhibited las and rhl systems in P. aeruginosa up to 70% and 35%, respectively. YtnP prevented Pseudomonas biofilm formation by 50% but did not affect pyocyanin production.

550 / CORRELATION OF K LOCI, SEQUENCE TYPES AND CARBAPENEMASE ENCODING GENES AMONG PANDRUG-RESISTANT ACINETOBACTER BAUMANNII

Keywords: *Acinetobacter baumannii, MLST, KL, blaNDM*

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BACKGROUND

Acinetobacter baumannii is among the most concerning multidrug-resistant organisms responsible for nosocomial infections and therefore constitutes a strong challenge when treating patients in hospital settings.

OBJECTIVES

Objective of this study was to explore the association between carbapenemase encoding genes, sequence types (STs), and capsular types among pandrug-resistant A. baumannii (PDRAB) isolates.

METHODS

This study included 30 randomly selected non-redundant PDRAB isolates recovered from hospitalized patients throughout Serbia from 2018-2021. All isolates were subjected to whole-genome sequencing (WGS) by the shotgun method on an Illumina platform. STs, K locus (KLs) for capsule synthesis and export, and resistance genes were derived from the genome assemblies using the pubMLST website, Geneious Prime program, and webtool Resfinder.

RESULTS

Multilocus sequence typing analysis revealed the presence of three and six STs by the Pasteur and Oxford MLST schemes, respectively (Fig. 1). Analysis of capsular loci revealed the presence of six types: KL9 (53.3%), KL27 (23.3%), KL2 (10%), KL30 (6.7%), KL4 (3.3%), and KL40 (3.3%). All isolates harbored blaOXA-66 and either blaOXA-23 (76.7%) or blaOXA-72 (23.3%), and three isolates (10%) were blaNDM-1-positive (blaOXA-66/blaOXA-72/blaNDM-1/STPas-492/STOxf-495/KL4 and blaOXA-66/blaOXA-72/blaNDM-1/STPas-492/STOxf-Novel/KL30). Obtained results showed that two disseminated clones (blaOXA-66/blaOXA-23/STPas-2/STOxf-195/KL9 and blaOXA-66/blaOXA-23/STPas-2/STOxf-1633/KL27) accounted for 76.7% of circulating PDRAB in Serbia (Fig. 1).

In conclusion, the results of this study showed the association between STs, KL, and carbapenemase genes and genetic relatedness among PDRAB all clustering into the international clonal complex II (ICII).

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562 / BACTERIAL ISOLATES FROM FRESH-WATER FISH GILLS AS A SOURCE OF BIOFILM MATRIX DEGRADING ENZYMES

Keywords: *Biofilm, Pseudomonas aeruginosa, Biofilm matrix degrading enzymes, nan*

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BACKGROUND

Antibiotic resistance is spreading and infectious diseases have become more difficult to treat. The common cause of antibiotic resistance is the formation of biofilm which is the important virulence characteristic of many pathogenic bacteria including Pseudomonas aeruginosa. The biofilm-embedded cells can be up to 1000-fold more resistant to antibiotics than planktonic forms, they are difficult to eradicate and often lead to chronic infection which can be life-threatening in immunocompromised individuals. The main structural and functional components of biofilm matrix are polysaccharides, lipids, proteins, and extracellular DNA. The promising strategy to increase the efficacy of antibiotics against biofilm-associated infections is to use them together with enzymes that disintegrate biofilm matrix.

OBJECTIVES

To identify new enzymes from environmental sources specifically targeting different biofilm matrix components.

METHODS

Substrate specific plate assays, 16S rRNA sequencing, whole-genome sequencing, genome mining, biofilm disruption assay.

RESULTS

Total of 67 morphologically different isolates collected from fish gills were screened for cellulase, alginate lyase, protease, DNase, and lipase activity. All enzymatically active isolates were identified using 16S rRNA sequencing. Based on the number of detected enzymatic activities and the level of each activity, five isolates identified as Aeromonas sp., Kocuria sp., Rhodococcus sp., Microbacterium sp., and Pedobacter sp. were selected for whole-genome sequencing. Supernatants and the cell lysates of selected bacteria were tested for the ability to disintegrate mature P. aeruginosa biofilms. Microbacterium sp. BG28 demonstrating cellulase and alginate lyase activity caused an 80% reduction of biofilm biomass. Genome annotation was used for identifying targeted enzymes.

563 / INFECTION-INDUCED MEMBRANE RUFFLING INITIATES DANGER AND IMMUNE SIGNALING VIA THE MECHANOSENSOR PIEZO1

Keywords: *Intestinal pathogens, Immune detection, Invasive infection, Danger signals*

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BACKGROUND

Tight regulation of the immune response is crucial to establish protective immunity against pathogens, while tolerating commensals and avoiding autoimmunity. The detection of microbes is the first step of the immune response to infection. Incoming microorganisms are generally sensed by Pattern Recognition Receptors (PRRs) binding characteristic microbial molecules, which evokes anti-microbial defences through changes in cellular activities and gene expression. Intestinal epithelial cells (IECs) are a barrier to gut microbes and important immune sentinels. Several Gram-negative intestinal pathogens induce secretion of the pro-inflammatory danger signal ATP as a rapid alert response of IECs, resulting in overt intestinal inflammation. However, what triggers ATP secretion during infection is unknown.

OBJECTIVES

We determined what triggers ATP secretion during infection with invasive pathogens.

METHODS

We collected pharmacological, genetic and live microscopy-based evidence during in vitro infections of IECs.

RESULTS

We show that the formation of plasma membrane ruffles accompanying the invasion of IECs by Gram-negative or Gram-positive bacteria is a physical immune signal of infection, which is detected by the mechanosensor PIEZO1. PIEZO1 activation is necessary and sufficient to trigger mechanically-induced ATP secretion via $Ca2+$ influx and also reprogrammes inflammatory gene expression in IECs. Thus, mechanosensation of invasion-induced plasma membrane distortion initiates immune signaling upon infection, independently of detection of microbial molecules. Given that formation of plasma membrane ruffles is a characteristic and unifying feature of invasive bacteria, our results indicate that, unlike PRRs which are driven by ligand binding, PIEZO1 acts as a mechanically driven immune sensor for invasive infection.

604 / PRECISION METAGENOMICS FOR DETECTION OF MARKERS ASSOCIATED WITH FLUOROQUINOLONE RESISTANCE IN UTIS

Keywords: *metagenomics, next generation sequencing, urinary tract infections, nan*

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BACKGROUND

150 million cases of urinary tract infections (UTIs) occur globally each year. Of woman effected by UTIs, 25-30% develop recurrent infections. In Europe, the rate of fluroquinolone (FQ) resistance in UTIs is reported at 25-40%. Urine cultures fail to identify the etiology in ~60% of patients. The Urinary Pathogen ID/AMR Panel (UPIP) uses Precision Metagenomics (PM) to detect and quantify >170 uropathogens and >2,000 antimicrobial resistance (AMR) markers.

OBJECTIVES

We compared AMR marker detection by UPIP to antimicrobial susceptibility testing (AST) for FQs, commonly used antimicrobials for UTIs.

METHODS

Culture was performed per routine protocols and AST determined on a BD Phoenix system. For UPIP, urines were spiked with internal control (bacteriophage T7, Microbiologics), DNA extracted (Quick-DNA Urine Kit, Zymo), sequencing libraries prepared, target-enriched in 3-plex with UPIP reagent (IDbyDNA), sequenced at 1x147bp to a depth of 1 million reads/sample (NextSeq550, Illumina) and automated data analysis was performed with Explify (IDbyDNA). UPIP is for research use only, and not for use in diagnostic procedures.

RESULTS

Culture and AST results were available for 290 urine samples that grew gram-negative pathogens. Top gram-negative uropathogens detected were Escherichia coli (90/290, 31%), Klebsiella pneumoniae (44/290, 15.2%) and Pseudomonas aeruginosa (19/290, 6.6%). Of these, 46 (15.9%) were intermediate or resistant to FQs. UPIP predicted phenotypic resistance to FQs in 38/46 (82.6%). The top FQ mechanisms of resistance were gryA (24/38, 63.2%), par (19/38, 50%) and oqx (16/38, 42.1%). UPIP's PM approach demonstrated high agreement between AMR marker detection and AST in gram-negative uropathogens.

ACKNOWLEDGEMENTS/REFERENCES

We are grateful to the entire Microbiology Unit at PathGroup Labs for coordination of sample selection, de-identification and shipment, and to Illumina for reagents for library preparation and enrichment in support of this study. We also thank the IDbyDNA Laboratory, R&D, MSA and Bioinformatics Teams.

611 / THE ORAL CAVITY AS A RELEVANT STAPHYLOCOCCUS AUREUS COLONIZATION SITE AND POTENTIAL SOURCE OF ANTIMICROBIAL RESISTANCE

Keywords: *Staphylococcus aureus, Oral carriage, Antibiotic resistance*

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BACKGROUND

Staphylococcus aureus is a pathogen that frequently causes serious nosocomial and community-acquired infections. Antibiotic resistance is common and detection remains a priority in clinical environments. Despite the nostrils being considered its preferred host habitat, the oral cavity has demonstrated to be an ideal starting point for S. aureus transmission, although its role is not fully understood.

OBJECTIVES

This study aimed to evaluate oral S. aureus prevalence and antibiotic susceptibility and compare it to the nasal cavity.

METHODS AND RESULTS

Oral and nasal swabs were collected from 101 healthy young adults and cultured in Mannitol-Salt Agar. Isolates were identified by MALDI-TOF MS. A total of 51 S. aureus isolates (oral=24 and nasal=27, from 36 participants) were tested for antibiotic susceptibility by disc diffusion for amoxicillin, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, quinupristin-dalfopristin, tetracycline, trimethoprim+-sulfamethoxazole, following EUCAST/CLSI guidelines.

Prevalence of exclusive oral and nasal S. aureus carriage was 12.0% and 13.9%, respectively, whereas simultaneous carriers represented 9.9% of the population. Regarding antibiotic susceptibility, among S. aureus carriers, 86% presented isolates resistant to at least one antibiotic and 28% presented Multi-Drug Resistant (MDR≥3 antibiotic classes) isolates. Similar percentages of resistance were observed in both oral and nasal isolates (to at least one antibiotic: 83% vs. 82%, $p>0.05$, and MDR: 21% vs. 30%, $p>0.05$).

In conclusion, this study demonstrates that the oral cavity is as relevant as the nasal cavity as a colonization site and a potential source of antimicrobial resistance to commonly prescribed antibiotics, which has been widely neglected so far.

ACKNOWLEDGEMENTS/REFERENCES

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617 / UNDERSTANDING THE THERAPEUTIC POTENTIAL OF HYDROGEN SULFIDE FOR TREATING MICROBIAL SKIN INFECTIONS

Keywords: *Dermatophytosis, Wound infection, Trichophyton, MRSA*

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BACKGROUND

Skin infections are very common, e.g. dermatophytosis which is caused by fungi, affecting 20-25% of the world population (Havlickova et al., 2018). In addition, bacteria e.g. Staphylococcus aureus commonly cause infections of skin and nails, in particular when the skin is damaged. The administration of antimicrobials is challenging due to the properties of skin and nails as protective tissues of the body. Topical treatments therefore can be lengthy and often fail due to poor penetration of antimicrobials. However, we hypothesise that hydrogen sulfide (H2S), which is small and has antimicrobial activity, provides an alternative treatment strategy as this may penetrate much better into skin or nails as compared to conventional antimicrobial agents.

OBJECTIVES

This study is to understand the effectiveness and mechanism of actions of H2S to pathogens that cause skin and nail infections, including fungal dermatophytes and S. aureus.

METHODS AND RESULTS

H2S generated using the H2S donor NaHS was shown to be fungicidal against Trichophyton rubrum and interdigitale. H2S was fungistatic against other fungi that cause nail infections, including Neoscytalidium dimidiatum, Fusarium oxysporum, and Candida albicans. Early confocal studies showed that H2S kills dermatophytes in a time- and fungal life cycle-dependent manner. It also has a strong bactericidal activity against S. aureus, even at high bacterial density levels. The mechanism of action is not clear yet, but one hypothesis is that H2S leads to the production of reactive oxygen species. We will investigate this by using redox-sensitive fluorescent probes through confocal analysis and the pre-treatment with antioxidants.

ACKNOWLEDGEMENTS/REFERENCES

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624 / WHOLE GENOME ANALYSIS OF CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIAE ST378 ISOLATES FROM COMPANION ANIMALS

Keywords: *Carbapenemase, Klebsiella, Hospital infection, Companion animal*

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BACKGROUND

The increased number and contact of companion animals with human poses threat of multidrug resistant (MDR) bacterial transmission to humans. In our previous study, 2 strains of metallo-β-Lactamase NDM-producing K. pneumoniae ST378 were isolated from clinical lesions of companion animals. However, the origin of these strains was unidentified and whether these strains are transmissible between humans and animals is unclear.

OBJECTIVES

This study was designed to investigate the genetic origin of these strains and identify the unknown transmission pattern of K. pneumoniae ST378.

METHODS

Complete genome sequence of the whole bacterial chromosome was read with short-read Illumina NovaSeq 6000 platforms following a paired-end 2×150-bp protocol. Sheared DNA was used as input for library preparation using the Illumina TruSeq DNA library preparation kit and variations were annotated using the SnpEff software (v5.0). To investigate the genetic phylogeny of these strains, phylogeny based on Multi locus sequence typing (MLST) was performed comparing with 109 K. pneumoniae strains accessed from South Korea, available from NCBI database.

RESULTS

The phylogenetic analysis showed that these strains are closely related to K. pneumoniae strain D16KP0008, which was isolated from human bloodstream infection in 2016, South Korea, Chungbuk. Our results suggested that CPKP can be a threat not only to companion animals but also to humans. This study was supported by National Research Foundation (NRF- 2020R1A2C2008794), BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Republic of Korea.

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This study was supported by National Research Foundation (NRF- 2020R1A2C2008794), BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Republic of Korea.

641 / INVESTIGATION OF ANTIMICROBIALS AND HEAVY METALS AS SELECTIVE PRESSURES ON HORIZONTAL GENE TRANSFER IN ENTEROBACTERIACEAE

Keywords: *antimicrobial resistance, bacterial conjugation*

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Antimicrobials and heavy metals are used in animal production for disease treatment, but also for prophylactic purposes and growth promotion in many parts of the world. However, recent evidence indicates that antimicrobial resistance (AMR) transmission can be affected by antimicrobials or heavy metals. Nonetheless, our understanding of factors influencing AMR transmission is still limited. The aim of this study was to investigate the effect of diverse agents as potential selective pressures, namely two antimicrobials (ampicillin and rifampicin) and one heavy metal (zinc nitrate) on bacterial conjugation. Sub-inhibitory concentrations of agents were used, as might occur in practical on-farm situations. One Escherichia coli donor strain was used, harbouring the beta-lactamase resistance gene blaTEM-1B on the plasmid IncI1_1_Alpha, and three rifampicin-resistant Salmonella enterica serovars as recipient strains. Donor and recipient strains were mixed together in the presence and absence of the additives and transconjugants were enumerated after 24 hours. To examine the temporal effect, conjugation frequencies were monitored hourly for one strain combination in the presence of zinc nitrate. Furthermore, whole-genome sequencing was used to reconstruct the transferred plasmid. For all three strain combinations different conjugation frequencies were obtained. While ampicillin significantly decreased conjugation frequencies, rifampicin slightly increased frequencies, but also reduced transfer at higher concentrations. Zinc nitrate showed less effects on frequencies of transfer. This study provides evidence that the presence of antimicrobials and heavy metals can differentially impact bacterial conjugation, but is concentration and strain dependent.

ACKNOWLEDGEMENTS/REFERENCES

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647 / EXPERIMENTAL EVOLUTION OF THE OPVAB OPERON IN SALMONELLA ENTERICA

Keywords: *Salmonella, opvAB*

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BACKGROUND

In the 1960s, Richard Levins proposed a game theory model predicting that phenotypic polymorphism is beneficial for populations living in changing environments. However, the cost of having more than one phenotype is too high to maintain polymorphism in stable environments. Because natural selection operates on phenotypes, it seemed reasonable to test whether the Levins' prediction can be extended to epigenetic polymorphism.

OBJECTIVES

We have performed an experimental analysis of the Levins' model using the opvAB operon of Salmonella enterica, which encodes proteins that alter the length of the O-antigen. Expression of the opvAB undergoes phase variation, producing two subpopulations: one subpopulation (OpvABOFF) is large and virulent but sensitive to phages that use the lipopolysaccharide O-antigen as receptor while the other subpopulation (OpvABON) is small and avirulent but phage-resistant. Because phage resistance can be also conferred by mutation, we compared the relative frequencies of phenotypic variation and mutation in fluctuating and nonfluctuating environments.

METHODS

The central experiments have consisted in infection of bacterial cultures with phage, phenotypic analysis to distinguish resistant mutants from OpvABON cells, electrophoretic analysis of lipopolysaccharide (LPS) profiles, intramacrophage proliferation assays, and analysis of resistance to guinea pig serum.

RESULTS

We have observed that mutants are predominant when the environmental conditions remain constant while OpvABON cells prevail in a fluctuating environment. Analysis of LPS profiles has revealed that the majority of mutants obtained in the presence of phages present LPS modifications. Only cells with normal LPS survive in the presence of serum and proliferate inside macrophages.

655 / BACTERIAL ANALYSIS BY MICROENCAPSULATION AND ITS APPLICATION TO STUDY PROLIFERATION HETEROGENEITY UNDER ANTIBIOTIC EXPOSURE

Keywords: *Single-cell analysis, antibiotic resistance, microencapsulation*

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BACKGROUND

Antimicrobial resistance remains a serious threat to public health around the world due to increased morbidity and mortality. The majority of antibiotic resistance mechanisms are genetically stable, where all cells in a population from a specific isolate show the same resistance phenotype. However, occasionally one observes isolates where different subpopulations within a particular isolate exhibit different susceptibilities to a particular antimicrobial agent.

OBJECTIVES AND METHODS

We have exploited new technologies based on single-cell encapsulation, which facilitates the isolation and analysis of microscopic colonies, to study the contribution of phenotypic heterogeneity to antibiotic resistance. We have developed a protocol to encapsulate individual cells of S. enterica in order to obtain one bacterium per microcapsule. This protocol, easily reproducible in laboratories, allows the monitoring of the growth of microorganisms to study the bacterial proliferation under antibiotic exposure.

RESULTS

The results show that not all cells have the same behaviour, observing diversity in colonies size within microcapsules in absence and presence of antibiotics, suggesting the existence of an enormous bacterial cell heterogeneity. This study opens us the possibility of using alginate microcapsules to investigate how the phenotypic heterogeneity and non-genetic mechanisms contribute to the antibiotic resistance.

657 / THE HEALTH-PROMOTING EFFECT OF EXTRACELLULAR VESICLES RELEASED BY VAGINAL LACTOBACILLI

Keywords: *Extracellular vesicles, Lactobacilli, Microbiota*

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Lactobacilli (LB) species dominate the vaginal microflora performing a first line defence against infections (1). Lactobacilli metabolites mediate the health-promoting effect acting as antimicrobials or are used for quorum-sensing and cross-talk communication (2). In this sense, extracellular vesicles (EVs) from LB demonstrate an antimicrobial activity (3,4), modulate host's nervous and immune system (5) and induce apoptosis in cancerous liver cells (6). Notably, EVs derived from vaginal LB isolates reduce HIV-1 infection (7). Here, we investigate the health-promoting activity of EVs produced by two strains of LB isolated from human vagina (L. gasseri BC12 and L. crispatus BC5) in terms of anti-adhesive effect on pathogens and pro-adhesive effect on LB. EVs were recovered from LB cultures by ultracentrifuge cycles and yield (1010 EVs/mL), size (90-150 nm diameter) and protein content were characterized. Adhesion assays, performed on HeLa cells monolayer, demonstrated that LB-EVs were able to reduce at least 25% the adhesion of all pathogens tested: Escherichia coli, Staphylococcus aureus, Enterococcus faecalis and Streptococcus agalactiae. Moreover, LB-EVs stimulated more than 26% the adhesion of all LB strains, including the same producer strains and two other vaginal isolates belonging to the same species (L. gasseri BC9, BC13 and L. crispatus BC1, BC3). L. gasseri BC12 EVs demonstrated the best health-promoting profile. These results highlighted that the barrier effect exerted by lactobacilli can be mediated by EVs released in the vaginal environment. This opens to the hypothesis of using postbiotics from human vaginal bacteria as a new strategy to prevent/treat human infections.

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668 / YERSINIA ENTEROCOLITICA BACTEREMIA ASSOCIATED WITH A RUPTURED ABDOMINAL AORTIC ANEURISMS: A CASE REPORT

Keywords: *abdominal aortic aneurism rupture, bacteremia, Yersinia enterocolitica, nan*

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BACKGROUND

Yersinia enterocolitica is a food-borne pathogen, mainly associated with infections involving the gastrointestinal tract including diarrhea, ileitis, and mesenteric lymphadenitis. Extraintestinal presentation is uncommon in healthy individuals but bacteremia is reported in immunocompromised hosts. With the current case report, we aimed to emphasize the association of Y. enterocolitica bacteremia with the complication of abdominal aortic aneurism.

CASE

A 74-year-old male with periodical abdominal pain was treated in several General surgery wards. The patient had a history of diarrhea and abdominal discomfort for nearly two months. During the last admission, an abdominal aortic aneurysm (AAA) was found, the patient was admitted to Vascular Surgery Clinic. Computed tomography angiography was conducted and demonstrated ruptured AAA with big retroperitoneal haematoma. Emergency AAA resection was undertaken – intraoperatively 2 ruptures of the sac were found – retroperitoneal and aortocaval fistula. A tube graft was implanted. Blood was collected for blood culture. Following a post-operative recovery period, the patient was discharged from the hospital with improved laboratory parameters and clinical condition. The blood cultures became positive after 2 days of incubation. The isolate was identified as Y. enterocolitica and was susceptible to all clinically relevant antibiotics.

CONCLUSION

Better surveillance could not only reduce morbidity and mortality but could also lead to a reduced incidence of severe life-threatening complications due to Y. enterocolitica in high-risk populations with vascular diseases, such as elderly men.

692 / PLANT DERIVED SURFACTANT FOR SUPPORT BACTERICIDAL COMPOUNDS

Keywords: *Antimicrobial resistance, Antibiotics, Plant derived surfactants*

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Antimicrobial resistance (AMR) is a growing worldwide problem caused by overuse of the antibiotic therapies and nowadays it constitutes an emergency threat to human health as there is still the lack of efficiently methods to inhibit the growth of the specific pathogenic bacterial strains.

The aim of the research was to study if there is a support action of plant surfactants containing saponins with bactericidal compounds. Therefore, a number of various concentrations of combinations of three antibiotics, differing in their mechanism of action (Ciprofloaxin, Polymyxin B, Tobramycin) along with plant surfactant extract (derived from Glycyrrhiza glabra) were tested.

The MTT (using a tetrazolium salt (3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide) assay [1] was applied to assess the biological activity of selected bacterial strains, such as: Escherichia coli, Pseudomonas aeruginosa and Psudomonas fluorescens. In addition, pictures of bacterial cells in the environment of the tested systems were recorded using SEM and TEM microscopy techniques.

The results showed that the lower concentrations of the plant-derived extract together with the higher concentration of the antibiotic, inhibited the metabolic processes of the examined strains. Hence, the results obtained may make a significant contribution to the development of further research on selected formulations for the creation of novel and efficient antimicrobial compounds for pharmacological and biotechnological industries' markets.

ACKNOWLEDGEMENTS/REFERENCES

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710 / STRUCTURE-FUNCTION ANALYSIS OF A RGG QUORUM SENSING SYSTEM IN STREPTOCOCCUS PNEUMONIAE

Keywords: *Streptococcus pneumoniae, transcriptional regulator, Rgg, structure*

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In Gram-positive bacteria, quorum sensing (QS) systems commonly utilise peptide pheromones for activation of cytoplasmic response regulators (RR). Knowledge of the interaction between the peptide and RR reveals vital information for the phenotypic manifestation of the system, and can potentially be used to design inhibitory antiinfective peptides. The Streptococcus pneumoniae Rgg144/SHP144 regulator-peptide QS system is critical for nutrient utilisation, oxidative stress response, and virulence. Here we characterised this system by assessing the importance of each residue within the active SHP by alanine-scanning mutagenesis and testing the resulting peptides for receptor binding and activation of the receptor. Interestingly, several of the mutations had little effect on binding to Rgg144 but reduced transcriptional activation appreciably. In particular, a proline substitution (P21A) reduced transcriptional activation by but bound with 3-fold higher affinity than the wild-type SHP. Consistent with the function of Rgg144, the mutant peptide led to decreased utilisation of mannose and increased susceptibility to superoxide generator paraquat. Pangenome comparison showed full conservation of P21 across SHP144 allelic variants. Crystalization of Rgg144 in the absence of peptide revealed a comparable structure to the DNA bound and free forms of its homologues suggesting similar mechanisms of activation. Together, these analyses identify key interactions in a critical pneumococcal QS system. Further manipulation of the SHP has the potential to facilitate the development of inhibitors that are functional across strains. The approach described here is likely to be effective across QS systems in multiple species.

ACKNOWLEDGEMENTS/REFERENCES

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716 / GENE-TAGETED SEQUENCING REVEALED CLONAL SPREAD OF ANTIBIOTIC RESISTANCE AMONG TUNISIAN MYCOPLASMA GALLISEPTICUM ISOLATES

Keywords: *GTS, Mycoplasma gallisepticum, Clonal complex, Antibioresistance*

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BACKGROUND

Mycoplasma gallisepticum is one of the most economically significant pathogen for poultry in Tunisia and worldwide. To our knowledge, very little is known about avian mycoplasma infections in Tunisia, as well as about the phylodistribution of drug resistance in Mycoplasma gallisepticum.

OBJECTIVES

This study was conducted to assess the antimicrobial susceptibility of Mycoplasma gallisepticum clinical strains in Tunisia, to identify the molecular basis of antibiotic resistance, and to investigate the phylogenetic relationships of resistant strains.

METHODS

Genetic typing and phylogenetic relationships of 40 Mycoplasma gallisepticum strains were investigated by gene-targeted sequencing (GTS) using 4 loci (pvpA, mgc2, vlhA and the InterGenic Spacer Region (IGSR) between the 16S and the 23S rRNA genes). The antimicrobial susceptibility was tested using the broth microdilution method. Minimum spanning tree was constructed to establish the phylogenetic relationships among resistant isolates.

RESULTS

GTS reveals 12 STs that were found to spread over 2 clonal complexes (CC) and 5 singletons. The emersion of antibiotic resistance to enrofloxacin and spiramycin has been identified as being related to a distinctive clonal complex formed by 4 different STs (ST2, ST3, ST4 and ST5) which would suggest that this phenotype was clonally disseminated.

ACKNOWLEDGEMENTS/REFERENCES

This study was funded by the Tunisian Ministry of Higher Education and Scientific Research

731 / ANTIBIOFILM EFFICACY OF RE(I)-TRICARBONYL COMPLEXES ON CYSTIC FIBROSIS-ASSOCIATED STAPHYLOCOCCUS AUREUS: THREE LINES OF EVIDENCE

Keywords: *anti-biofilm, Staphylococcus aureus, rhenium(I)- tricarbonyl complexes,cystic fibrosis*

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BACKGROUND

Chronic pulmonary infections represent the major hallmarks of cystic fibrosis (CF), an inherited disease caused by mutations in the CFTR gene. Staphylococcus aureus infections are prevalent in up to 70% of CF infants and have a critical role in the early disease development. The use of anti-staphylococcal antibiotic prophylaxis accelerates the emergence of antibiotic resistance as well as the onset of the further microbial lung infections. As a potential treatment solution, targeting virulence factors instead of growth is an attractive alternative to antibiotic therapy. Recently, Re(I)-tricarbonyl complexes have been recognized as potent agents against staphylococcal infections, both in vitro and in vivo.

OBJECTIVES

The main aim of this study is to reveal anti-biofilm activity of the rhenium(I)-tricarbonyl complexes library on CF-associated S. aureus isolates.

METHODS

Bacterial isolates from CF patients' sputum samples belonging S. aureus were examined for biofilm-forming potential. Re(I)-tricarbonyl complexes were tested for antibacterial activity according to CLSI standard and assessed for toxicity using the zebrafish (Danio rerio) model. The selected non-toxic complexes were evaluated for anti-biofilm activity in vitro in CF-sputum mimicking and BHI-glucose media, and in vivo using the zebrafish-wounded tail biofilm model of infection.

RESULTS

The majority of complexes that had no effect on S. aureus growth were capable of inhibiting biofilm formation in at least one of the tested conditions. Four most potent complexes inhibited biofilm formation up to 90% upon all tested stimuli, and did not provoke any toxic side effect in vivo at anti-biofilm doses.

765 / ANTIMICROBIAL ACTIVITY OF BIOGENIC SILVER NANOPARTICLES SYNTHESIZED USING SPHAGNUM MOSS

Keywords: *Silver nanoparticles, antimicrobial, Alcanivorax borkumensis, antibiotics*

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BACKGROUND

Currently, there is a significant problem of resistance of microorganisms to the applied antibiotics. [1, 2]. One of the possible solutions may be the production of an antimicrobial drug with new properties that make it difficult to develop bacterial resistance, for example, silver nanoparticles (AgNPs). A lower ability of microbes to develop resistance to AgNPs was found in comparison with antibiotics [3]. Biogenic AgNPs synthesized using extracts of plants with their own antimicrobial properties, such as sphagnum moss, may have a synergistic effect.

OBJECTIVES

The objectives of this study were the synthesis of biogenic silver nanoparticles using Sphagnum fallax moss extract and the evaluation of their antibacterial properties on the example of the bacterium Alcanivorax borkumensis (AB).

METHODS

AgNPs were obtained by a self-assembly method from the precursor $AgNO_3$ in the presence of an aqueous extract of sphagnum. The nanoparticles were subjected to ultrasonic treatment. AB bacteria were incubated in liquid broth in the presence of AgNPs for 48 hours, after which growth curves were constructed based on hourly optical density measurements. Further, using the disco-diffusion method, the effectiveness of the antibacterial activity of AgNPs was determined by the size of the suppression zone. Microscopic and spectrometric studies were carried out.

RESULTS

Biogenic AgNPs ranging in size from 10 to 60 nm with characteristic peaks in the absorption spectrum near the wavelength of 410 nm were synthesized and characterized. Antimicrobial activity against AB of a new type of biogenic AgNPs has been proven, in comparison with pure sphagnum extract.

ACKNOWLEDGEMENTS/REFERENCES

ACKNOWLEDGEMENTS

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784 / THE MULTIPLE ANTIBIOTIC RESISTANCE ACTIVATOR (MARA) INDUCES PERSISTER CELL FORMATION BY REGULATING METABOLIC GENES

Keywords: *MarA, Persister, metabolic*

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Multiple antibiotic resistance in bacteria can be driven by the transcriptional regulators in the AraC-XylS family. The Escherichia coli ‘mar’ regulon is considered a paradigm for such systems. The mar locus consists of 3 genes; marR, marA, and marB. A transcriptional activator encoded by marA enhances drug resistance by binding to “marbox” sequences at target promoters. Previously, we identified 33 MarA targets by ChIP-Seq analysis in a pathogen, Enterotoxigenic E. coli (ETEC) and reported two mechanisms of resistance to quinolones and tertacyclines. Further analysis of MarA targets has revealed that some metabolic genes that are involved in nucleotide and amino acid biosynthesis are regulated by MarA. Here, we show that MarA regulation of these genes inhibits growth and thereby induces persister cell formation.

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789 / COMBINED EFFECT OF SILVER NANOPARTICLES AND ELECTROPORATION PROVIDES NEW ANTIBACTERIAL TOOL IN DESTRUCTION OF BIOFILMS

Keywords: *Silver nanoparticles, Biofilms, Electroporation, nan*

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BACKGROUND

Increasing Pseudomonas aeruginosa resistance to the antibacterial drugs is the main problem that rise the research for the novel antibacterial tools. Moreover, the biofilm formation is a worrying issue, especially, among hospitalized patients. The antimicrobial properties of silver have long been known; therefore, the antimicrobial properties of silver nanoparticles (AgNPs) are currently of great interest. Regularly used techniques for producing AgNPs are physical and chemical synthesis, however, the biological synthesis of this nanomaterial is getting more interest as inexpensive and environmentally friendly alternative. In addition, the electroporation is a promising tool reducing the viability of pathogenic microorganisms and likely enhancing the effectiveness of AgNPs.

OBJECTIVES

This study reports the effect of AgNPs obtained by Geobacillus sp. strain 25 induced biosynthesis on P. aeruginosa planktonic cells and biofilms.

METHODS

thermophilic bacteria induced biosynthesis of AgNPs, microbiology, electroporation

RESULTS

The results show that 10 μg/mL concentration of Geobacillus strain 25 AgNPs effectively removes P. aeruginosa biofilms. Furthermore, the synergistic effect of AgNPs and electroporation was evaluated as well (concentration of Ag-NPs was 5 μg/mL, parameters of electroporation were eight 100 μs impulses, 15; 20; 25 kV/cm electric field). To sum up, the antibacterial properties of AgNPs and electroporation make them the alternative biocontrol tools against pathogenic bacteria and formation of biofilms.

791 / DISTRIBUTION OF CAPSULAR TYPES AND ANTIBIOTIC RESISTANCE IN GROUP B STREPTOCOCCI ISOLATED FROM COLONIZED PREGNANT WOMEN

Keywords: *Group B streptococci, antibacterial resistance, capsular types, colonization*

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BACKGROUND

Streptococcus agalactiae (group B streptococci, GBS) is the most common cause of neonatal sepsis in developed countries. The major risk factor for neonatal infections is maternal colonization. Therefore, the monitoring of maternal colonization, antibiotic resistance, and capsular polysaccharide (CPS) is needed for the prevention and treatment of GBS infections.

OBJECTIVES

This study aimed to determine the distribution of capsular types, the prevalence of antibiotic resistance, and hypervirulent clone ST17 among GBS isolates from colonized pregnant women in Serbia.

METHODS

A total of 800 GBS strains isolated from colonized pregnant women from January 2019 to December 2021 were enrolled in this study. Strains were isolated from five hospitals in Serbia and were collected and processed in the National Reference Laboratory for Streptococci. Antimicrobial susceptibility testing was done using the disk diffusion method. The detection of species-specific dltR gene and capsular types (Ia, Ib, II-IX) was done using multiplex PCR. The hypervirulent ST17 strain was detected by PCR amplification of the hvgA gene.

RESULTS

All tested strains were susceptible to penicillin and vancomycin. The erythromycin, clindamycin, tetracycline, and high-dose gentamicin resistance were 27.5%, 29.25%, 79.5%, and 9.75%, respectively. Chloramphenicol resistance was rare and found only in 1% of tested strains. Only two strains were resistant to fluoroquinolones. The prevalence of capsular types and distribution of antibiotic resistance are present in Figure 1. The hvgA gene was detected in 10.1% of tested strains. Most of the ST17-positive strains were among CPS type III (77/81), while only four isolates were CPS type IV.

02

795 / DIFFERENT EFFECT OF AMOXICILLIN CLAVULANIC ACID ON BIOFILM FORMATION OF ORAL STREPTOCOCCUS

Keywords: *amoxicillin / clavulanic acid, d-PTFE membranes, Streptococcus*

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BACKGROUND

Streptococcus are one of the early colonizers that binds to all pellicol-coated surface structures in the oral cavity, followed by subsequent colonization and biofilm formation. This applies to both natural and restorative or prosthetic materials. Knowledge of the dynamics of biofilm formation can help in the improvement of procedures performed in dental practice.

OBJECTIVES

Determine the effect of amoxicillin/clavulanic acid (AMC) on adhesion and biofilm formation of oral Streptococcus, on dense polytetrafluoroethylene membranes (d-PTFE) which is used as a barrier membrane in regenerative dental procedures was determined.

MATERIALS

Membranes were coated with artificial saliva and exposed to individual bacterial suspensions of S. mutans, S. oralis and S. salivarius at a concentration of 107 CFU/mL. To determine the effect of AMC on adhesion and biofilm formation, bacteria were exposed to the ½ and ¼ minimum inhibitory concentrations (MIC) of antibiotics, under the stated conditions. The antiadhesive effect was determined by counting the bacteria after 4h and the antibiofilm effect after 72h. For visualization of antiadhesive and antibiofilm effect Dead Live fluorescence staining and SEM were used.

RESULTS

AMC at a concentration of ½ MIC showed a antiadhesive effect in all tested streptococci, which did not lead to a decrease in the number of bacteria in the biofilm. Moreover, the formation of S. salivarius biofilm resulted in an increase in the number of bacteria compared to the biofilm created without the presence of antibiotics. ¼ MIC had an antiadhesive effect only on S. oralis, which, however, resulted in enhanced biofilm formation.

825 / POTENTIAL OF THE ESSENTIAL OIL NANOEMULSIFICATION TO BE USED IN ENDODONTICS: THE CASE OF CYMBOPOGON CITRATUS

Keywords: *oral biofilm, nanoemulsion, Enterococcus faecalis, root canal*

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BACKGROUND

The main culprit for the failure of the infected root canal treatment is Enterococcus faecalis biofilm which frequently cannot be eradicated by commonly used endodontic antimicrobial agents. In addition, numerous conventional antimicrobials are known to induce oxidative stress. Nanoemulsions containing essential oils (EOs) seem to possess a promising antioxidant, antibacterial and antibiofilm potential that may be utilized in infected teeth root canal therapy.

OBJECTIVES

The objective was to determine antioxidant and antibacterial potential of the C. citratus EO, as well as to formulate the C. citratus EO-based nanoemulsion (Cc-NE) and to investigate its antioxidant, antibacterial and antibiofilm potential, all toward E. faecalis.

METHODS

Characterization of the EO (GC/MS analysis) and Cc-NE (dynamic light scattering instrument), and determination of antibacterial (microdilution assay), antibiofilm (crystal violet assay) and antioxidant properties (lipid peroxidation TBA assay) were provided. In addition, CFU-counting assay was used to evaluate irrigative potential of Cc-NE against ex vivo formed E. faecalis biofilm.

RESULTS

Notable antibacterial activity, with MIC values ranging within 0.5-2 mg/mL, was determined. Significant inhibition of the biofilm formation (61.8-47.2%), as well as its disruption (22-36%) was observed. Ex vivo tooth root canal irrigation, involving Cc-NE, showed remarkable antibiofilm potential (Δlog CFU 3.4). EO induced some antioxidant activity (24% inhibition of lipid peroxidation) that was not decreased by the emulsification. Obtained results encourage further research with the aim of application of the Cc-NE in endodontics.

ACKNOWLEDGEMENTS/REFERENCES

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02

836 / PREVALENCE OF EXTENDED-SPECTRUM Β-LACTAMASE (ESBL) AND CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIA (KPC) IN CLINICAL SAMPLES

Keywords: *Klebsiella pneumoniae, ESBL, KPC, infection control*

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BACKGROUND

Klebsiella pneumoniae ESBL and KPC poses a significant threat to patients and healthcare system. Infections causes with these pathogens are associated with high mortality, primarily due to the limited treatment options.

OBJECTIVES

The aim of this study was to assess the prevalence of KPC and ESBL in overall isolates of K. pneumoniae in clinical samples.

METHODS

Retrospective study was conducted from November 2020 to November 2021. Identification and antibiotic susceptibility testing of K. pneumoniae was performed by standard laboratory methods according the EUCAST standards. Detection of carbapenemase and ESBL were performed by phenotypic methods such as E test, combined-disk test containing meropenem with various inhibitors (ROSCO Diagnostica A/S), chromogenic medium for detection ESBL/CPE isolates and VITEK 2 Compact system, BioMerieux.

RESULTS

In the study period a total of 944 isolates of Klebsiella pneumonia were detected in various clinical specimens, including blood and urine, wound swabs, lower respiratory tract samples, and screening ICU swabs. Among this isolates K. pneumoniae ESBL was detected in 349/944 (37%), while K. pneumoniae KPC in 188/944 (20%) of isolates, respectively. The rest of the isolates 407/944 (43%) belong to the wild type. Since the first outbreak of K. pneumoniae KPC in our hospital was in September 2017 with six isolates, our results show significant increase in resistance of these bacteria in last period. It makes a great challenge both in the treatment as well as in prevention and control of infection in hospital settings.

ACKNOWLEDGEMENTS/REFERENCES

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2. KPC-Producing Klebsiella pneumoniae Isolates in Croatia: A Nationwide Survey

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838 / MELALEUCA ERICIFOLIA ESSENTIAL OIL AS A PROSPEROUS AGENT AGAINST ENDODONTIC PATHOGENS

Keywords: *teeth intracanal isolates, facultative anaerobes, obligate anaerobes, antibacterial potential*

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BACKGROUND

Considering the shortcomings of the antibacterials commonly used in infected root canals therapy and the growing antibacterial resistance, the necessity for adjuvant/alternative antibacterial agents is evident. Since Melaleuca spp. essential oils have already been proved for promising antibacterial potential, but their impact on endodontic pathogens has not already been determined, Melaleuca ericifolia essential oil (MeEO) was selected as an object of this research.

OBJECTIVES

The aims were to isolate and identify numerous facultative and obligate anaerobes from the infected root canals, as well as to characterize chemical content and antibacterial potential of the MeEO.

METHODS

The clinical isolates were obtained from infected root canals with apical periodontitis of pediatric patients. Their identification was done using MALDI-TOF MS. MeEO was chemically characterized by GC-MS, while its antibacterial potential was determined by microdilution assay.

RESULTS

Out of all 35 examined infected root canals, 21 was with symptomatic and 14 with asymptomatic apical periodontitis, with total number of 148 isolates. Facultative anaerobes counted 102 isolates, with genera Streptococcus (58 isolates), Actinomyces (10) and Enterococcus (8) as the most dominant. Among obligate anaerobes (46) the most frequent were Veillonella isolates. MeEO the most dominant components were linalool (49.1%), 1,8-cineol (26%) and α-pinene (12%). Antibacterial potential, determined against selected strains, was moderate with MICs ranges 0.65-5.30 and 1.00-23.35 mg/mL, for obligate and facultative anaerobes, respectively. Obtained results encourage further analysis of biocompatibility of the MeEO, as well as its potential to be nanoemulsified, in order to be easily delivered within root canals.

863 / PHENOTYPIC CHANGES IN S. AUREUS CELLS CAUSED BY SUBINHIBITORY CONCENTRATIONS OF NEW LIPOGLYCOPEPTIDE ANTIBIOTIC 5812-A

Keywords: *Staphylococcus aureus, lipoglycopeptide antibiotic, tolerance*

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BACKGROUND

The emergence and rapid spread of multidrug-resistant pathogens observed in recent decades continues to be one of the important public health problems, which, to a certain extent, is being solved by the constant search for new antibiotic drugs.

One of these compounds is the recently described and characterized new lipoglycopeptide antibiotics complex produced by Streptomyces roseoflavus INA-Ac-5812 [1]. According to the mechanism of action, 5812-A resembles daptomycin [2]. In the present work, we are continuing the study of this perspective antibiotic.

OBJECTIVES

Evaluation of the dynamics of the resistance development and phenotypic changes occurring in the bacterial population of S. aureus when exposed to subinhibitory concentrations of new lipoglycopeptide antibiotic 5812-A.

METHODS AND RESULTS

In this work, the strain S. aureus 209P was used as a model object. After 20 cycles of passages, resistant phenotype was obtained. It was found that the restoration of sensitivity to the antibiotic does not occur during 15 passages through the medium without 5812-A. It was shown that the resistant phenotype also resistant to daptomycin, ramoplanin and vancomycin.

Comparison of the proteomic profiles of susceptible S. aureus strain and its isogenic resistant mutant was carried out using PAAG-electrophoresis. It was found differences in the protein abundance which corresponding to protein band with a molecular weight of 45 and 55 kDa. Comparative analysis of the fatty acid composition of cell membranes of sensitive and resistant strains using GC-MS showed an increase in anteiso-fatty acids in the mutant resistant to 5812-A.

ACKNOWLEDGEMENTS/REFERENCES

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885 / MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN THE ARCHANGELSK REGION OF NORTHERN RUSSIA: TWENTY YEARS AFTER

Keywords: *Mycobacterium tuberculosis, multidrug-resistant, Beijing genotype*

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BACKGROUND

In the Arkhangelsk region in northern Russia, the tuberculosis incidence rate decreased from 48.0 in 2000 to 20.8 in 2018 (per 100,000) but the proportion of primary multidrug resistance (MDR) is very high (33.1% in 2018).

OBJECTIVES

To analyze the current population structure of Mycobacterium tuberculosis in the Arkhangelsk region.

METHODS

A total of 89 M. tuberculosis isolates (2018) were characterized for drug susceptibility using the Bactec MGIT960. Mutations of the rpoB, katG and inhA were analyzed by Real-time PCR. The Beijing genotype and its main Russian clusters B0/W148 and Central-Asian/Russian were detected by PCR testing of the specific markers. Non-Beijing isolates were spoligotyped.

RESULTS

33.7% (30/89) of the isolates were MDR, rpoB Ser531Leu и katG Ser315Thr was identified in 90.0% of the MDR isolates. Beijing genotype was detected in 67.4% (60/89) of isolates and mostly represented by its subtypes Central-Asian/Russian (36 isolates) and B0/W148 (18 isolates). Spoligotyping of 29 non-Beijing isolates identified 17 spoligotypes of genetic families: T (14.6%; 13/89), LAM (2.2%), Haarlem (4.5%), Ural (4.5%), CAS1-Delhi (1.1%) and Unknown (5.6%). The majority of MDR isolates belonged to the Beijing family (93.3%; 28/30) of which 64.3% (18/28) and 21.4% (6/28) belonged to B0/W148 and Central-Asian/Russian clusters, respectively. Comparison with the published data on the Arkhangelsk region for 1998 (Toungoussova et al., 2002) showed that over 20 years the proportion of Beijing strains in the total M. tuberculosis population increased from 44.5% to 67.4%, and among MDR strains from 76.7% to 93.3%.

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02

922 / IN VITRO EVALUATION OF THE ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF ANTISEPTIC AGENTS AGAINST CLINICAL ACINETOBACTER BAUMANNII

Keywords: *Acinetobacter baumannii, biofilms, antiseptics*

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BACKGROUND

The ever-increasing number of multidrug-resistant Acinetobacter baumannii encourages researchers around the world to investigate and study new therapeutic strategies as an alternative to antibiotics.

OBJECTIVES

The aim of this study was to investigate the antimicrobial effect of antiseptics on reference and clinical antibiotic resistant strains of A. baumannii in planktonic form and their antibiofilm-forming ability.

METHODS

There were studied 42 clinical strains of A. baumannii, isolated from patients with infectious complications. The following quaternary ammonium antiseptics were tested: decamethoxine 0.02%, chlorhexidine digluconate 0.05%, octenidine dihydrochloride 0.1%, polyhexanide 0.1%, miramistin 0.01%. Minimum inhibitory and bactericidal concentrations (MIC and MBC) were evaluated by means of standard macromethod of serial double-fold dilutions. The biofilm-forming ability was determined by the microtiter-plate Christensen test (quantitative crystal violet assay). The effect of antiseptics on biofilm formation was assessed by reproducing biofilms with the addition of antiseptics at subbacteriostatic concentrations during 24 hours and subsequent spectrophotometric ODU assessment.

RESULTS

Clinical strains of A. baumannii remain susceptibility to modern antiseptics. With regard to MBC of antiseptics, decamethoxine (MBC 38.32 ± 6.34 µg/ml) and octenidine (MBC 36.82 ± 4.69 µg/ml) were shown to be the most effective agents. Bactericidal concentrations of miramistin (67.95 ± 5.03 µg/ml) and polyhexanide (72.37 ± 7.94 µg/ml) were similar in their efficacy. But chlorhexidine had twice higher MBC ($p > 0.05$). Subbacteriostatic concentrations of decamethoxin and chlorhexidine reliably ($p < 0.001$) inhibited the formation of biofilms by clinical strains of A. baumannii during the first 24 hours of cultivation.

924 / ONE YEAR ESBL IN GRAZ, AUSTRIA

Keywords: *ESBL,ß-lactamase, antibiotic resistance*

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Extended spectrum beta lactamases (ESBL) producing Enterobacteriaceae are wide spread in our days that they can be found almost everywhere. In this study we analyses all ESBL producing Enterobacteriaceae which were isolated at our Institute for Hygiene in 2017. All phenotypic ESBL Enterobacteriaceae first isolates t from one patient were treated as separated first isolates. PCR detection and gene identification were performed for different β-lactamase gene families, blaCTX-M-1-group, blaCTX-M-2 -group, blaCTX-M-9-group, blaGES, blaSHV, and blaTEM and blaVEB. PCR and sequencing procedures were performed and carried out for all isolates that showed an ESBL-positive phenotype. Additionally, the resistance data of all strains was collected. Of the 469 isolates included in the statistical analysis 83.7% were E. coli followed by 11.7% K. pneumoniae isolates.

All isolates of all species were resistant to AM and CTX according to the guidelines from 2017. CMEM with only the 5 isolates of E. cloacea and SXT with only the single isolate of C. koseri showing resistance are the two most effective drugs available in in vitro testing. The most abundant genes for E. coli were found in the CTX-M-1 group (73,5%), for K. pneumoniae the SHV group genes were most commonly found (98,2%). Double positive isolates were identified in 38% of the cases, threefold positive isolates in only 7,7%. Future studies will focus on the epidemiology of ESBL in the hospital as well as double or threefold positive isolates.

02

928 / EARLY-LIFE FUNGI VERTICAL TRANSMISSION AND ANTIFUNGAL SUSCEPTIBILTY

Keywords: *early-life, antifungal resistance, vertical transmission, yeast*

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BACKGROUND

Yeast acquisition seems to begin at birth, however, the precise contribution of the mother on the transmission of these pathogens to the offspring is yet to be determined. Currently there is an increase in antifungal resistance worldwide.

OBJECTIVES

To explore yeasts' vertical transmission by assessing the similarity of species between mother/child pairs, and to determine their antifungal susceptibility.

METHODS

Oral, fecal, and breastmilk samples were collected from 73 mother-child pairs one-month after birth, inoculated in Sabouraud-Dextrose Agar with chloramphenicol and isolates identified by MALDI-TOF MS. Antifungal susceptibility profiles were determined by broth microdilution assay for fluconazole/voriconazole/miconazole/anidulafungin /nystatin, according to CLSI guidelines.

RESULTS

A total of 133 isolates were identified in 53% of mother-child pairs, namely Candida albicans (48.1%), Candida parapsilosis (39.8%), Candida tropicalis (3%), Lodderomyces elongisporus (3%), Candida guilliermondii (2.3%), Candida dubliniensis (1.5%), Rhodotorula mucilaginosa (1.5%), and Geotrichum silvicola (0.8%).

Moreover, at least a yeast species was shared in 25.6% of the mother-child pairs and the presence of C. albicans was significantly associated between the oral cavities of the mother-child dyads ($p=0.001$). In children, it was observed an association in C. parapsilosis colonization between the oral cavity and the gut ($p<0.001$). Regarding the antifungal susceptibility, all isolates were susceptible to all antifungals, except for three C. albicans isolates from a single mother-child pair (breastmilk n=1 and child-gut n=2), which presented resistance to voriconazole and fluconazole. In conclusion, vertical transmission of yeasts is likely, but results must be confirmed by genotyping. Antifungal resistance appears to be low in early-life, but its monitoring should be continued.

ACKNOWLEDGEMENTS/REFERENCES

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937 / THE IMPACT OF OXIDATIVE STRESS AND THE SOS RESPONSE ON THE DEVELOPMENT OF DE NOVO ANTIMICROBIAL RESISTANCE IN ESCHERICHIA COLI

Keywords: *Antimicrobial resistance (AMR), Reactive oxygen species (ROS), Adaptive mutagenesis, SOS response*

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BACKGROUND

The molecular mechanisms involved in de novo development of antimicrobial resistance (AMR) are poorly understood. Exposure to antimicrobial molecules is believed to trigger the SOS response and the generation of reactive oxygen species (ROS) (Beaber et al., 2004; Kohanski et al., 2007). These two processes may initiate resistance by accelerating mutagenesis and increasing genetic variability.

OBJECTIVES

The aim of this study was to investigate the roles of oxidative stress and the SOS response in adaptation mechanisms of Escherichia coli (E. coli) towards different antimicrobial molecules and to find a common mechanism for the acquisition of de novo resistance.

METHODS

Four different E. coli strains (MG1655, ΔdinB, ΔkatE, and ΔoxyR) with deficient oxidative stress or SOS response were exposed to stepwise increasing sub-lethal concentrations of different antibiotic classes (beta-lactams, fluoroquinolones, aminoglycosides and tetracyclines). The resulting strains were analysed by whole genome sequencing.

RESULTS

A common response could not be identified based on the outcome of this study. The results indicate that development of AMR upon sub-lethal antimicrobial exposure is dependent on the antimicrobial class and the molecular interactions of the drug on a cellular level. The different adaptive mutations observed suggest that different classes may activate distinct stress responses. Although these results may shed some light on the development of de novo AMR, further research is needed to fully elucidate the molecular mechanisms involved.

ACKNOWLEDGEMENTS/REFERENCES

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We thank the Netherlands Food and Consumer Product Safety Authority for research funds.

948 / PREVALENCE OF COLISTIN RESISTANCE IN ESCHERICHIA COLI: IS THERE A BIOLOGICAL COST ASSOCIATED TO MCR-BORNE PLASMIDS?

Keywords: *Food-producing animals, Colistin resistance, Fitness, nan*

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BACKGROUND

Dissemination of plasmid-mediated colistin resistance genes (mcr) poses a critical threat to multidrug human infections. Colistin is still used in animal production.

OBJECTIVES

A better understanding of the biological cost imposed by mcr-plasmids may facilitate decision on the ban of colistin in livestock. Our study aims to assess the prevalence of ESBL and mcr-borne plasmids in Escherichia coli isolated from food-animals and the impact on bacterial fitness of mcr-1 acquisition.

METHODS

Phenotypic screening of ESBLs and mcr producers was performed in 98 E. coli. Plasmid incompatibility groups, ESBL and mcr-1 to -10 genes were screened by PCR and sequencing. E.coli J53 was the recipient in conjugation assays. Growth kinetics of recipient and mcr-1-transconjugants were conducted in antibiotic-free LB-broth and with 4 mg/L colistin. Plasmid stability was investigated in six transconjugants and donors by serial transfer during 10 days and plated on antibiotic-free and 4 mg/L colistin MH-agar. Plasmid retention was determined. Statistics were done with paired, two-tailed t-test and ANOVA test.

RESULTS

mcr-1 gene was found in 15,3% of E. coli, mostly from pigs, blaTEM-1, blaCTX-M-1, and blaCTX-M-15 co-existed with mcr-1 in four strains. Transconjugants have a mean growth rate of 1.66 and 1.32 in absence and in presence of colistin ($p=0.0003$). In pairwise assays with recipient and transconjugants with different plasmids (IncHI2, IncP), significant growth rates differences were observed ($p<0.05$). Plasmid retention was diverse, ranging from complete loss to full retention, suggesting that other factors beyond colistin pressure regulate the stability of the plasmid maintenance in bacterial population.

ACKNOWLEDGEMENTS/REFERENCES

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952 / EFFECT OF VAPOR-PHASE ESSENTIAL OIL ON RESISTANT CANDIDA SPECIES: MECHANISMS OF ACTION

Keywords: *Resistant candida species, Essential oil, Vapor-phase, Biofilm*

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BACKGROUND

Vulvovaginal candidiasis VVC is one of the most prevalent vaginal infectious diseases. Indeed, the growing need to apply antifungal intensive therapies have contributed to an emergence of drug-resistant Candida strains. Thus, the increasing incidence of drug-resistant vaginal strains, the high difficulty to treat VVC, the extremely limited and toxic therapeutic options make it crucial the development of effective and non-toxic alternative treatments. In this sense, essential oils (EOs) have been suggested as a promising alternative due to their low toxicity and high antimicrobial activity. Interestingly, the vapour phase of EOs (VP-EOs) was shown to present higher activity towards Candida growth than its liquid phase.

OBJECTIVES

This study aims to evaluate the VP-EO effect on biofilms of antifungal-resistant vaginal isolates of Candida species (C. albicans and C. glabrata) and determine its mode of action.

METHODS

The VP-EO effect on C. glabrata and C. albicans biofilms were evaluated by the time-dependent killing assay over 24h on pre-formed biofilms and in biofilm formation. Then, membrane integrity and metabolic activity of this biofilms were evaluated by flow cytometry, using the fluorescent probes propidium iodide (PI) and FUN1, respectively.

RESULTS

The results revealed high VP-EO antifungal activity against two drug-resistant isolates. In fact, at 12-14 h with VP-EOs, there was a significant reduction (> 4 Log CFU) in biofilm formation and reduction in pre-formed biofilm in both Candida strains. Furthermore, flow cytometry results show that the mechanisms of action of VP-EO are related to membrane integrity (PI internalization) and metabolic activity.

955 / METABOLIC FUNCTIONS OF THE AMINOGLYCOSIDE RESISTANCE GENE 'APH' OF PSEUDOMONAS AERUGINOSA

Keywords: *Antibiotic resistance, Pseudomonas aeruginosa, nan, nan*

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Pseudomonas aeruginosa possesses sophisticated antibiotic resistance mechanisms encoded by its large genome, making rather challenging to eradicate. These mechanisms must be well understood to develop new antimicrobial strategies. In P. aeruginosa, an intrinsic aph gene confers general insensitivity to kanamycin-class antibiotics by encoding an aminoglycoside phosphotransferase enzyme. Interestingly, aph has previously been reported as being regulated by 4-hydroxyphenylacetic acid interacting with the HpaA transcriptional regulator.
Yet, the background of this interaction is still a mystery.

This study investigates whether this association occurred by chance and the aph gene is involved in different bacterial metabolisms, as it seems to be regulated by environmental factors. First, in-frame Δaph and ΔlasIRΔaph deletion mutants were constructed, and changes in phenotypes were observed. Then, regulatory associations of the aph gene with las and pqs quorum-sensing systems were examined using bioluminescence-based gene reporters. The results showed that the production of pyocyanin, one of the virulence factors of P. aeruginosa, decreased in the mutant. However, ΔlasIRΔaph mutant showed an elevated fluorescence level reflecting increased siderophore production. Further analyses revealed that there is no change in the activity of the promoter of the pqsABCDE operon in the aph mutant. In contrast, the promoter of the aph gene is significantly derepressed in the ΔlasIR mutant.

Altogether, these results suggest that the biological function of aph may be broader than assumed. Elucidating unknown metabolic functions may enable developing specific inhibitors for this gene, thus enabling the use of kanamycin-class antibiotics in the treatment of P. aeruginosa infections.

975 / ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC SYNERGISM OF SOME ESSENTIAL OILS IN MULTIRESISTANT PSEUDOMONAS AERUGINOSA STRAINS

Keywords: *multiresistant bacteria,, essential oils,, Pseudomonas aeruginosa,, antibiotic synergism*

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BACKGROUND

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen with increased resistance, commonly found in clinical and natural reservoirs. Essential oils (EOs) contain biologically active substances and are resourceful candidates for the design of antimicrobial ecologic agents, while limiting the selection of resistant mutants.

OBJECTIVES

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen with increased resistance, commonly found in clinical and natural reservoirs. Essential oils (EOs) contain biologically active substances and are resourceful candidates for the design of antimicrobial ecologic agents, while limiting the selection of resistant mutants. In this work, we aimed to characterize patterns and mechanisms of global epistasis in microbial metabolism.

METHODS

Minimun inhibitory concentrations (MICs) and minimum biofilm eradication concentrations (MBECs) of the EOs were determined by microdilution assay, while biofilm inhibition was established by crystal violet method. Synergistic interaction of the EOs with antibiotics was analyzed by adapted disk diffusion method.

RESULTS

At least 60% of the analyzed strains (n=36) were antibiotic resistant and multi resistant. Average MICs values varied among the analyzed EOs, with the lowest index obtained for Thyme (0,34%) and Tea tree (0,96%) EOs, as compared to Sage (4,6%) and Eucalyptus (2,7%) EOs. All analyzed EOs inhibited the production of monospecific biofilm in a dose and strain dependent manner. The analysis of MBEC values revealed that Thyme give the lowest average concentration (0.028%), being very sucesfull in biofilm inhibition in most of the P. aeruginosa isolates. The evaluation of EOs-antibiotics synergistic interaction showed that Tea tree and Thyme singnificantly enhance the activity of TZP, CAZ, MEM and DOR antibiotics, followed by Eucaliptus and Sage EOs.

976 / NOVEL SOLUTION FOR EFFECTIVE ORAL CANDIDIASIS TREATMENT

Keywords: *Oral candidiasis*

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BACKGROUND

Oral candidiasis represents an emerging health problem worldwide. This fungal infection, predominantly caused by Candida albicans, is common in individuals with diabetes, HIV, oncological patients, and long-term denture wearers. Current oral antifungal therapies that rely on polyenes (nystatin) and azoles (miconazole) are insufficiently effective and lead to incomplete infection elimination and infection relapse. As filamentation presents a key virulence trait responsible for limited efficacy of antifungal treatments, the applicationof filamentation-inhibiting agents together with antifungals presents a promising therapeutic strategy.

OBJECTIVES

To evaluate antifungal and anti-filamentation efficacy of delphinidin-rich Aristotelia chilensis (maqui berry) extract and lozenge formulated based on this extract. .

METHODS

A set of preclinical in vitro and in vivo assays on zebrafish (Danio rerio) model was employed to address antifungal activity, inhibition of filamentation, synergy, and toxicity with clinically used antifungals nystatin and miconazole. A clinical study involving individuals with diabetes and denture wearers with oral candidiasis was performed, aiming to evaluate the antifungal efficacy of combination therapy based on miconazole and lozenge containing maqui berry extract

RESULTS

Sole extract and lozenge formulation showed no effect on the growth of C. albicans clinical strains, while successfully inhibited fungal filamentation and potentiated antifungal activity of nystatin and miconazole in vitro and in vivo. No toxic effects of combination treatments have been detected in vivo. Results of the clinical study showed much higher efficacy of combination therapy against oral candidiasis compared to clinical therapy alone, with a higher proportion of cured patients and lower frequency of infection relapse.

1001 / ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES FROM WATER SOURCES EFFECTIVE AGAINST KLEBSIELLA PNEUMONIAE

Keywords: *bacteriophage, Klebsiella pneumoniae, carbapenem resistant, ESBL*

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BACKGROUND

Klebsiella pneumoniae is an opportunistic pathogen that causes various serious human infection. ESBL and carbapenem-resistant(CR) K.pneumoniae have increasingly been reported worldwide, making bacteriophage therapy a promising alternative to multidrug-resistant K.pneumoniae strains.

OBJECTIVES

This research was conducted to isolate and identify biological characteristics of novel phages effective against such isolates from environmental water sources in Turkey.

METHODS

ESBL and carbapenemase encoding genes CTX-M, SHV, TEM, OXA-48, KPC, and NDM were detected by PCR. Phages were isolated and purified from water sources and their activity spectrums were tested against 62 CR, 30 ESBL-positive and 34 susceptible K.pneumoniae by spot test. The one-step growth curve, optimum multiplicity of infection (MOI), pH and temperature stabilities were calculated. Their morphologies were determined by TEM.

RESULTS

ive phages were isolated and one of them, KP-74, was able to infect a wide spectrum of K. pneumoniae strains with 80.6% of CR, 50% of ESBL-positive and 67.6% of ESBL-negative activity. The one-step growth curve demonstrated that the KP-74 phage had a significantly short latent period of 5 minutes, and the burst size was approximately 8000 pfu/infected cells (figure 1). The optimum MOI was calculated as 0.001 PFU/CFU. KP-74 phage was thermally stable and tolerant to a wide pH range (figure2,3).

The significant number of virtually complete lysis zones of CR and ESBL-positive isolates indicated that KP-74 phage can be a potential therapeutic agent for controlling infections caused by drug-resistant K. pneumoniae. Isolated novel phage can be a promising candidate for further in vivo studies.

ACKNOWLEDGEMENTS/REFERENCES

This study was approved by Baskent University Institutional Review Board (Project No.KA22/25) and supported by Baskent University Research Fund.

1056 / ANTIBIOFILM ACTIVITIES OF CERAGENINS FOR URINARY TRACT INFECTIONS DUE TO MYROIDES SPP.

Keywords: *Ceragenins, Myroides, antibiofilm activity, nan*

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BACKGROUND AND OBJECTIVES

Myroides spp. Gram-negative, formerly known as Flavobacterium spp. are opportunistic pathogens. It can cause urinary tract infections to endocarditis, especially in immunocompromised patients. Myroides has several extracellular and cell-associated virulence factors. One of the most important virulence factor is biofilm formation on host tissues and on abiotic surfaces such as glass, plastics and medical devices. Antibiotic options for the treatment of Myroides infections are very limited due to drug resistance mechanisms and virulence factors, thus causing treatment failures and high mortality rates. Therefore, there is an urgent need to develop alternative compounds and approaches to combat biofilm-based infections.Ceragenins(Cationic Steroid Antibiotic-CSA) designed to mimic the activities of antimicrobial peptides, are a new class of antimicrobial agent.

MATERIAL AND METHOD

Biofilm eradication assays were performed in 96-well plates. After adjustment of thirty-seven strains to 106 cfu/ml, prepared inoculums were added to the wells and incubated at 37 °C for 24 h to form biofilms. Next day, medium and planktonic cells were removed and the wells were washed with PBS twice. Ceragenins were added into wells and the plates were incubated at 37 °C for additional 24 h. Then, wells were washed again with PBS and sonicated. Dilutions were collected, diluted and dropped to TSA. Colonies were counted the next day.

RESULTS

Using this model, we were able to reproduce known patterns of global epistasis and illustrate their mechanistic basis. We show that metonary simulations. Our results have implications for our understanding of global epistasis and, more generally, the predictability of evolution.

CONCLUSION

The results demonstrated that CSA-131 and CSA-138 could be alternative approach against Myroides biofilm infections treatment.

1071 / ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCUS ISOLATED FROM FROZEN WHITING (MERLANGIUS MERLANGUS)

Keywords: *antimicrobial resistance, fish, North Sea, Staphylococcus*

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Antimicrobial resistance (AMR) is an important global public health. So far, antimicrobial resistance bacteria (ARBs) in terrestrial animal received much attention while ARBs in marine environment has been overlooked. In this study, we evaluated the occurrence of ARBs in fish caught in a sea exposed to anthropization. We isolated and identified bacterial communities associated with several whiting (Merlangius merlangus) from the North Sea. AMR was detected by disk diffusion, following the CLSI standards (CLSI, 2015). In addition, we collected individuals life history traits, under the assumption that human (i.e. integration of riverine inputs, potentially carrying ARBs) may drive the composition of fish-associated bacterial community or ARBs occurrence

We detected and identified 4 major bacterial genus in fish samples (Staphyloccus, Bacillus, Psychrobacter and Aerococcus). We determined the AMR of several strains belonging to the genus of Staphylococcus isolated in diverse areas. Interestingly, 7 strains were found to be multi-resistant (i.e. remaining to at least 3 classes of antibiotics). We identified several AMR genes (like tet59, sul2...) in these strains by whole genome sequencing.

We demonstrated that rivers had local influence, especially on the English coast, on the occurrence of multi-resistant Staphylococcus. Moreover, the measurement of several marine environmental factors showed that depth, temperature or salinity is not involved in the occurrence of antimicrobial resistant Staphylococcus. These results highlighted the importance of considering multi-parameters along with bacterial occurrence to investigate AMR from marine environment, and open the way for further researches prior to develop monitoring plans.

1089 / DETECTION OF COLISTIN RESISTANCE FROM THE SALMONELLA STRAINS ISOLATED FROM THE ENVIRONMENTAL SAMPLES IN POULTRY FARMS

Keywords: *Antimicrobial resistance, Colistin, Salmonella, mcr*

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In this study, the Salmonella strains (n= 300) isolated and serologically identified from the environmental swab samples from the poultry farms in Turkey, have been tested to investigate if these are resistant to colistin. The study also aimed to reveal the presence of the phenotypic and genotypic resistance to colistin for the first time in Turkey from the Salmonella strains isolated from the environmental swab samples from the poultry housing environment.

Of all the Salmonella isolates tested (n=300), 72 isolates were considered to be phenotypically resistant and the remaining 228 were susceptible according to the EUCAST criteria with the specified MIC value of the colistin (1,2). These 72 resistant Salmonella isolates were then examined by the Multiplex PCR protocol published by the European Union Antibiotic Resistance Reference Laboratory (3).

Some of the isolates identified as phenotypically resistant to colistin, were also resistant to pefloxacin (34.72%), ampicillin (5.55%), sulphamethoxasol/trimethoprim (4.16%), gentamycin (2.77%) and cefotaxime (1.38%)

It was determined that none of the strains to carry the mcr genes by using the current PCR protocol mentioned above.

Although mcr genes were not detected in the tested isolates, it is important to understand the detection of Salmonella strains that is even phenotypically resistant may pose a potential risk to food safety and public health. Conducting further studies by using the whole genome sequencing analysis and any other new techniques might contribute to a better understanding of the colistin resistance and its transmission in the environment, humans and animals.

ACKNOWLEDGEMENTS/REFERENCES

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1102 / MOLECULAR DOCKING OF COLISTIN WITH THE PMRA PROTEIN MODEL USING COLISTIN-RESISTANT ACINETOBACTER BAUMANNII

Keywords: *Acinetobacter baumannii, Protein Conformation, Protein Modeling, Molecular docking*

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BACKGROUND

The aim of this study was to investigate the molecular docking of PmrA with colistin.

METHODS

the primary model of PmrA was obtained using the I-TASSER platform. The functional domains of PmrA were determined using the ROSETTA prediction tool. FUpred (Folding Unit predictor) was used to detect the domain's boundaries from protein sequences based on contact map prediction. The favorable interactions between the selected colistin and modeled receptor were identified using the extra precision (XP) feature of Grid-based Ligand Docking with Energetics (GLIDE, a Schrödinger module) [25]. Ligands were energy minimized using the Universal Force Field and converted to the pdbqt format in PyRx0.8 for virtual screening [1, 2].

RESULTS

The secondary structure and solvent accessibility of PmrA by the I-TASSER platform is shown in Fig. 1. The PmrA contains two domains (phosphate receiver domain (REC) and the C-terminal DNA binding domain DBD). Each activated REC contains a five-stranded parallel β-sheet (β 1–5) surrounded by five α-helices (α1–5). The DBD contains 3 β-sheet (β 6–8) and three α-helixα (6–8). Several residues spanning the C-terminal form H-bonds with a DNA phosphate backbone through Arg198 and Asp 188 on the helix α8 are shown in Fig. 2. The docking of the PmrA protein with the colistin molecule is shown in Fig. 3. Graphical representations of the PmrA indicated that the docking of colistin was with the amino acid residues ASP 4, GLY 49, LYS 96, and ALA 75 of the PmrA.

ACKNOWLEDGEMENTS/REFERENCES

The above research is a collaboration between the Department of Microbiology and virology, Kerman University of Medical Sciences, Kerman and Department of Biotechnology, Tips college of arts and science, Coimbatore, Tamil Nadu, India

1114 / MONITORING OF INCREASING TREND OF COLISTIN RESISTANCE IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

Keywords: *Acinetobacter baumannii, colistin , MIC, broth microdilution*

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BACKGROUND

Once out of use due to numerous side effects, polymyxins now serve as the last therapeutic option for life-threatening infections caused by Acinetobacter baumannii. For antimicrobial susceptibility testing of colistin, EUCAST recommends the broth microdilution method (BMD).

OBJECTIVES

This study aimed to show the increasing trend of MIC value for colistin in clinical isolates of A. baumannii and to indicate the problem of developing resistance to this antibiotic.

METHODS

This retrospective study was conducted in two periods of time: June 2019 to October 2020 and April-December 2021. The study included 457 isolates of Acinetobacter baumannii detected by VITEK® 2 COMPACT, BioMerieux. MIC values for Colistin were determined by broth microdilution according to the EUCAST recommendations (MIC-Strip Colistin, Merlin A Bruker Company, Germany).

RESULTS

A.baumannii was most commonly isolated from the ICU, with 334 (73%), mostly from lower respiratory tract specimens, wounds, and drain swabs. The MIC value for colistin ranged from 0.25–2 µg/ml in the first and 0.25–4 µg/ml in the second period of time. The highest number of isolates in both periods had a MIC value of 0.5 µg/ml, 248 (54.26%), followed by 1µg/ml, 90/19.69%, and 24/10.96% with 0.25 µg/ml, respectively. In the second period, the MIC value increased to 2 µg/ml and was detected in 9 (1.96%) of the isolates. The first resistant isolate with an MIC value of 4 µg/ml was detected in 2021 and effective control and prevention measures stopped its further spread.

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1123 / EVALUATION OF MICROORGANISMS ISOLATED FROM BLOOD CULTURES OF PEDIATRIC PATIENS

Keywords: *Bacteremia-fungemia, Pediatric blood culture, COVID-19, Antimicrobial resistance*

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BACKGROUND

Bacteremia/fungemia is an important cause of morbidity and mortality in children.

OBJECTIVES

In this retrospective study, it was aimed to analyze the microorganisms isolated from blood cultures of pediatric patients, their resistance patterns and also SARS-CoV-2 test results.

METHODS

Between November 2020-December 2021, 5213 blood cultures were examined and microorganisms accepted as pathogen in 257 cultures of 214 patients were included.

Cultures were incubated at BD Bactec system for identification conventional methods and VITEK-2 Compact system were used. Susceptibility tests were performed according to EUCAST/CLSI recommendations. Intermediate susceptible strains were considered as sensitive.SARS-CoV-2 tests were performed with RT-PCR.

RESULTS

There were 44 outpatients, 170 inpatients; 184 of them tested for SARS-CoV-2, 8 were positive. Patients' genders were 109 female/105 male and the mean age was 3.23 years.

The isolated bacteria and fungi with their susceptibilities are shown in Table-1,2 and Table-3.

The rate of carbapenem resistant isolates among K.pneumoniae (n:31) strains was 32.2%. P.aeruginosa strains were found resistant to piperacillin-tazobactam (6/11), ceftazidime (7/11), imipenem (6/11), ciprofloxacin (5/9), cefepime (4/9), levofloxacin (4/5), seven of the 11 strains examined were resistant to meropenem. Resistance rates of Acinetobacter baumannii strains were found for ciprofloxacin (4/10) gentamicin (3/11), imipenem (4/11) and meropenem (4/11). No erythromycin,clindamycin,linezolid (0/7) and rifampicin (0/3) resistant strains were found in MRSA; on the other hand, in MRCoNS, erythromycin (42/45) and clindamycin (36/47) resistance were found at higher rate and similar to MRSA no linezolid-resistant strains were found. These results confirm the importance monitoring the increase of antimicrobial resistance.

1133 / PREVALENCE OF DIARRHEAGENIC E. COLI AMONG HOSPITALIZED CHILDREN IN A CLINICAL CENTRE

Keywords: *E.coli, diarrhea, enterocolitis, pediatric*

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BACKGROUND

Escherichia coli is a common cause of acute diarrhea mainly in young children and, less frequently, in elderly or immunosuppressed patients. Many types of E. coli are part of the normal enteric fora, but can cause urinary tract or nervous system infections. Objective. To study the prevalence of the main types and serogroups of diarrheagenic E. coli among hospitalized children with enteric infections.

MATERIAL AND RESULTS

Over a period of 5 years, 1,160 hospitalized children with acute diarrhea syndrome were studied. Fecal samples underwent culturing, biochemical and phenotypic identification. Among the studied patients, 112/1,160 children (9.7%) had diarrhea caused by E. coli, and only 4 of the isolates were lactose-negative. The most common was diarrhea caused by ETEC – 65/112 (58.0%), followed by EPEC – 38/112 (33.9%), and in third place – EHES 9/112 (8.0%). We did not isolate EIEC types. Depending on the group of E. coli, we observed some differences in the clinical presentation and specifics in the distribution of patients by age. The study shows that this causative agent is common among Bulgarian children with diarrhea. Unfortunately, the microbiological network is still not able to adequately respond to the challenges of the extended serodiagnosis for detection of diarrheagenic E. coli.

ACKNOWLEDGEMENTS/REFERENCES

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1135 / IS CEFTAZIDIME/AVIBACTAM A VIABLE TREATMENT OPTION FOR CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE IN SEVERE COVID-19?

Keywords: *Klebsiella pneumoniae, carbapenemase, ceftazidime/avibactam*

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BACKGROUND

Patients with severe COVID-19 infections treated in intensive care units often require prolonged antimicrobial therapy. Hospital settings are considered to be reservoairs of multidrug-resistant organisms such as carbapenemase-producing Klebsiella pneumoniae (CPK). The emergence of CPK with growing number of colistin-resistant isolates is observed in the most severe COVID-19 patients. Ceftazidime/avibactam (CZA) inactivates class A and C beta-lactamases as well as OXA-48 from class D, commonly found in CPK. CZA has been in clinical use in Serbia since late 2020.

OBJECTIVES

Objective of the study was to determine the susceptibility of CZA of CPK isolated from patients with severe COVID-19 infection.

METHODS

CPK were isolated from 57 patients with severe COVID-19 infection during 2021. Identification and susceptibility testing was done using Vitek 2 system. Susceptibility to colistin was done using ComASP Colistin (Liofilchem). Carbapenemase-production was detected using lateral flow assay (Resist-4 O.K.N.V., Coris BioConcept).

RESULTS

Total of 84 isolates of CPK was obtained from blood, urine, lower respiratory samples and wounds (37%, 34%, 27%, and 2% respectively). OXA-48 was detected in 83% and NDM in 17% of isolates. CPK were resistant to all beta-lactams except imipenem and meropenem (susceptibility was 39%, 16% respectively), fluoroquinolones and cotrimoxazole. Susceptibility to amikacin was 51%, gentamicin 28% and colistin 20%. All OXA-48 producing isolates were susceptible to CZA (MIC range: 0.38-2µg/ml). In vitro synergy of CZA and aztreonam was observed in NDM-producing isolates (Image). Combination of aztreonam and CZA may be a promising treatment option for severe COVID-19 infection with NDM-producing Klebsiella pneumoniae.

1153 / NATURAL PEPTIDES AS SCAFFOLD FOR SYNTHETIC ANTIBIOFILM AND ANTIMICROBIAL PEPTIDES (SAAMPS) USEFUL TO CONTROL ANTIMICROBIAL RESIS

Keywords: *Synthetic antibiofilm antimicrobial peptides (SAAMPs), Biofilm associated infections, antibiotic-resistance*

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Novel antimicrobials agents against multidrug-resistant pathogens are necessary considering the big threat for public health of the continuous spread of AMR resistance worldwide. We looked at different organisms (freshwater and marine invertebrates, terrestrial and marine plants1, truffles) as unconventional sources of antibiotics focusing in particular on antimicrobial activities against relevant pathogens (Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes) of polypeptide-enriched extracts from defensive cells of crustaceans or from plant and fungal cells extracts. The antimicrobial activity was tested not only in planktonic bacterial cells but also in biofilm structured bacteria, considering the relevant role of bacterial communities in antibiotic resistance. Starting from biological activities, polypeptide-enriched extracts were further investigated by a high-resolution mass spectrometry (RP-HPLC/nESI-MS/MS) and database search, to detect the presence of novel natural peptides responsible for the observed activity “in vitro”.

However, to design new and more effective synthetic antibiofilm and antimicrobial peptides (SAAMPs), bioinformatic tools and online servers were employed with the aim to improve natural peptides features and to select derivative molecules on the basis of favorable chemical-physical and structural properties. Biological activities and allergic, hemolytc and toxic potentials were predicted too.

In conclusion, bioinformatic analysis has revealed that identified natural peptides may represent chemical platforms for the development of effective SAAMPs useful to tackle infections caused by antibiotic resistant pathogens.

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Identification of New Antimicrobial Peptides from Mediterranean Medical Plant Charybdis pancration (Steinh.) Speta. Antibiotics (Basel, Switzerland), 9(11), 747. https://doi.org/10.3390/antibiotics9110747

1171 / METHYL EUGENOL: A NATURAL PROPANOID WITH PROMISING ANTI-FUNGAL POTENTIAL TARGETING MEMBRANE DISRUPTION AND VIRULENCE

Keywords: *Candida albicans, methyl eugenol, antifungal, virulence traits*

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BACKGROUND

The growth in immunocompromised patients and concomitant Candida albicans resistance to currently existing therapies necessitates the classification of innovative medications with novel targets. The current study looks at the antifungal activity of methyl eugenol (ME), a key component of clove and basil extract that has powerful antibacterial and antifungal effects.

MATERIAL AND METHODS

The broth microdilution technique was used to test drug susceptibilities. Effects of ME on the plasma membrane were assessed by fluorescent microscopy, PM-ATPase activity, flow cytometry, and ergosterol quantitation. The MTT method was used to measure biofilm inhibition in ME-containing treatments. The suppression of hyphal development in the presence of ME was tested using hyphal producing cells. The suppression of hydrolytic enzyme secretion (Proteinase and Phospholipase) was studied in cells cultured in a bovine serum albumin and egg yolk emulsion medium.

RESULTS

ME was shown to have antifungal activity against Candida albicans at 750 µg/ml. Insight studies into the mechanisms suggested that the plasma membrane was a potential target, which could be attributed to compromised cell membrane integrity as evidenced by hypersensitivity to the membrane perturbing agent SDS, depleted ergosterol levels, and decreased plasma membrane ATPase activity. ME also influences virulence properties such as yeast to hypal transition, hydrolytic enzyme production, biofilm formation, and adherence to human buccal epithelial cells.

CONCLUSION

This study's results clearly show ME's antifungal nature, which may be used to improve treatment techniques; more research into the molecular processes, in vivo antifungal action, and synergistic activity with recognised antifungal medicines is necessary.

ACKNOWLEDGEMENTS/REFERENCES

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1182 / NEW SMALL MOLECULE INHIBIT PYOVERDIN BIOSYNTHESIS IN PSEUDOMONAS AERUGINOSA

Keywords: *Pyoverdine, Pseudomonas aeruginosa, antivirulence*

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BACKGROUND

Due to the it's innate insensitivity to a wide range of antimicrobials and its quick acquisition of new resistance mechanisms, antimicrobial resistance is a significant challenge for clinically important pathogen Pseudomonas aeruginosa (1).

The use of anti-virulent chemicals to disrupt pathogenesis without directly impacting bacterial growth is one way to get around this challenge. Inhibition of the pyoverdine biosynthesis is a promising strategy for the development of new treatments. Here we report identification of a small molecule which inhibits pyoverdine secretion and its potential target proteins.

OBJECTIVES

To develop High Throughput Screening (HTS) method for functional pyoverdine secretion which is key virulence determinants involved in acute as well as chronic infections of P. aeruginosa and identify small compounds block pyoverdine secretion.

METHODS AND RESULTS

We developed a HTS method to identify small molecule inhibitor for pyoverdine biosynthesis. Using this method we identifed a piperidinecarboxyamide derivative inhibitor which inhibit pyoverdine secretion at low concentrations (IC50=2 µM). To identify potential protein targets we used Drug Affinity Responsive Target Stability (DARTS) technique (2). According to Mass spectrometry (MS) results we identified 7 putative protein targets. Aspartate aminotransferase was found to be the most likely target of the small molecule.

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1513 / CHARACTERIZATION OF TWO NEW PHAGES ACTIVE ON MULTIDRUG-RESISTANT PSEUDOMONAS AERUGINOSA

Keywords: *bacteriophages, mdr, pseudomonas aeruginosa, nan*

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BACKGROUND

Multidrug-resistant bacteria are emerging at an alarming rate. Among them, carbapenem-resistant P. aeruginosa is a leading cause of healthcare-associated infections worldwide. Various intrinsic and acquired mechanisms of antibiotic resistance make it a serious therapeutic challenge. One of the promising alternative treatments employs the use of bacteriophages, natural enemies of bacteria.

OBJECTIVES

The key objective was to isolate, purify and characterize natural lytic bacteriophage(s) that could serve as potential therapeutic(s) for combating multidrug-resistant P. aeruginosa.

METHODS

2 lytic phages, PSJM and PLST, were isolated from Belgrade wastewaters, purified and concentrated using CsCl gradient ultracentrifugation. The phages host range were screened using spot assay against 5 P. aeruginosa stains from ATCC collection, and 28 clinical isolates resistant to carbapenems, obtained from different hospitals in Serbia. Genomic DNA was isolated from both phages, sequenced and genomes were analysed in detail. PSJM phage was analysed using electron microscopy for morphology assessment.

RESULTS

PSJM genome analysis revealed more than 97% identity with lytic phage "Delta", isolated 13 years ago from Danube around Novi Sad, Serbia. Morphological assessment placed it inside the Podoviridae family. It produced small plaques on more than 90% of the strains, surrounded with large halos in portion of strains, indicating depolymerase production. Phage PLST is also characterized by wide host range, lysing 78% of analysed strains, while its genome analysis showed high (92%) identity to lytic phage "PA26" that belongs to Myoviridae family.

Characterization of parameters of lytic cycles, as well as bactericidal kinetics is in progress.

02

1516 / CONSTRUCTION AND ACTIVITY TESTING OF A CHIMERIC MULTIMODULAR ENZYBIOTIC AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Keywords: *Methicillin Resistant Staphylococcus aureus, ENZYBIOTICS, BACTERIOPHAGE*

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BACKGROUND

Antibiotic resistance in bacteria is a serious concern worldwide. There is an upsurge of methicillin resistant Staphylococcus aureus strain in Saudi Arabia, with a prevalence rate of 38%. Novel strategies to counter these antibiotic resistant strains are of significant research interest around the world.

OBJECTIVES

The objective of the study was to create a chimeric multimodular enzybiotic against clinical MRSA isolate.

METHODS AND RESULTS

The novel endolysin was constructed by replacing the cell wall binding domain of the endolysin of phage φ11 with a truncated cell wall binding domain encompassing 100 amino acids from a minor tail protein, P17 from Staphylococcus aureus phage φ44AHJD. The chimeric protein CA100 was tested for its antibacterial activity against S. aureus 8325-4, clinical MRSA isolate, a veterinary isolate of S. aureus and against a clinical E. coli isolate. Following denaturation, refolding of CA100 was done with rapid dilution technique. The CA100 was found to be active against S. aureus 8325-4 and MRSA, with 52 % and 48 % activity after 60 min following the treatment. Interestingly, no significant activity was observed against the veterinary isolate of S. aureus and E. coli. Spot analysis of CA100 was also done on S. aureus 8325-4 and MRSA strain. The CA100 was also found to be stable in comparison with CA with specific binding observed towards S. aureus cells. To best of our knowledge, this work is the first instance wherein domain replacement of protein from two unrelated phages has been successfully tested.

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1562 / ISOLATION AND CHARACTERIZATION OF TWO NEW LYTIC BACTERIOPHAGES, SJM3 AND LASTA, ACTIVE AGAINST KLEBSIELLA PNEUMONIAE

Keywords: *Klebsiella , bacteriophage*

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BACKGROUND

Acquisition of genetic elements for antibiotic resistance and the emergence of hypervirulent strains escalates the severity of K. pneumoniae infections, not only in immunocompromised but also in healthy individuals. Bacteriophages are being evaluated as a potential way to combat multidrug resistant pathogens. Phages are suitable to precisely target the infection-causing bacteria whilst not disturbing the beneficial microorganisms present in the body.

OBJECTIVES

Isolation of bacteriophages of Klebsiella pneumoniae, their detailed characterization and bioinformatic analysis, as well as lytic and antibiofilm activity testing.

METHODS

Two lytic phages (SJM3 and LASTA) active against nosocomial carbapenem- and colistin- resistant K. pneumoniae strains, were isolated from wastewater, purified and concentrated using CsCl gradient ultracentrifugation. Their genomes were sequenced and analyzed. Adsorption rate, parameters of replication cycle, as well as virion morphology were determined, too. Bactericidal efficiency of the two phages, applied individually or as a cocktail, was tested on planktonic and biofilm cells of K. pneumoniae Ni9 strain, and surviving colonies were tested for resistance.

RESULTS

Bacteriophages SJM3 and LASTA belong to Podoviridae family and have a narrow host range. Within 5 minutes of incubation, about 90% of LASTA and SJM3 particles adsorb to host cells. Both phages have circular genomes the size of 62.4 kb and differ by only 13 nucleotide substitutions. SJM3 and LASTA have similar lytic effect when applied individually or as cocktail. However, no combination could eradicate K. pneumoniae biofilm. Phage resistance in Ni9 strain develops as soon as 3 hours post infection and is of transient nature.

ACKNOWLEDGEMENTS/REFERENCES

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1569 / BLOOD-CULTURES ETIOLOGY SPECTRUM AND ANTIMICROBIAL RESISTANCE IN 2021: LESSONS TO BE LEARNED FROM COVID-19 MANAGEMENT

Keywords: *etiology of bacteremia, multidrug resistant organisms, Antimicrobial stewardship*

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BACKGROUND

COVID-19 pandemic influenced substantially the spectrum of infections viewed from the Microbiology laboratory and especially the rate of highly antibiotic resistant microorganisms

OBJECTIVES

2021 – surveillance and analysis of positive blood-cultures from a university hospital with predominant COVID-19-infected patients.

METHODS AND RESULTS

Microbiology methods included blood-cultures investigated by Bactec, BD, USA; Gram stain, culture method, MALDI-tof identification and antimicrobial susceptibility testing by EUCAST. From a total 1430 blood-culture sets submitted, 141 blood cultures were positive. The etiological spectrum consisted of: 23 Enterococcus spp (20 E. faecium and 3 E. faecalis); 5 Staphylococcus aureus, 2 confirmed Staphylococcus epidermidis cases; 3 Escherichia coli; 3 Enterocobacter cloacae; 2 Proteus mirabilis; 8 Klebsiella pneumoniae; 16 Acinetobacter spp, (incl. 15 A. baumannii); 6 Stenotrophomonas maltophilia; 2 Pseudomonas aeruginosa; 2 Cupriavidus mettallidurans; 27 Candida spp (C. albicans – 8, C. glabrata – 5, C. parapsilosis – 11, c. guilliermondi – 2, C. tropicalis – 1), the other 42 presented single CoNS. Isolation of problematic resistant organisms was high – incl. to carbapenems: 14 A. baumannii were CRAB; 7 K. pneumoniae were CRE, 1 E. cloacae and 1 P. mirabilis were CRE; and 3 of E. faecium were VRE. Such results are disturbing and obviously a consequence of breaches in the principles of Antimicrobial stewardship and Infection control. The conclusion is that the care for patients with COVID-19 should not be prescribing unnecessary antibiotics (that in fact contribute to the selection of resistant microorganisms and provoke system mycoses), but applying the classical principles of Antimicrobial stewardship and Infection control.

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1573 / BDELLOVIBRIO BACTERIOVORUS, A 'LIVING ANTIBIOTIC' TO FIGHT GRAM-NEGATIVE BACTERIAL PATHOGENS

Keywords: *Bdellovibrio bacteriovorus, antibiotic*

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BACKGROUND

B. bacteriovorus is a small deltaproteobacterium that possesses the peculiar capability to prey upon other Gram-negative bacteria. The predator is capable of preying on various species of bacteria regardless of their antibiotic resistance profile. Antibiotic resistance is an ever-growing global problem that is projected to have severe repercussion on the capability of healthcare systems to treat infectious diseases. The lipopolysaccharide (LPS) of the Gram-negative bacterial outer membrane constitutes the first barrier that B. bacteriovorus encounters when attacking a potential prey. The capability to anchor and penetrate the LPS layer of the outer membrane is thus a crucial step for B. bacteriovorus predation.

OBJECTIVES

Investigation of the roles of the core LPS genes of Escherichia coli in relation to susceptibility to B. bacteriovorus predation with particular focus on genes involved in biogenesis of the inner and outer core LPS constituents.

METHODS

Utilization of a novel polyclonal antibody specific for B. bacteriovorus. Colony forming unit measurements and flow cytometry-based assays were used to follow the interactions between the antibody-labeled predator and green fluorescent protein (GFP)-labeled prey. Furthermore, transmission electron microscopy (TEM) was used to visualize the predation process.

RESULTS AND CONCLUSION

Identification of different predation kinetics among E. coli mutants with the help of a novel polyclonal antibody against B. bacteriovorus highlights a major role of the outer core constituents of LPS in resistance to B. bacteriovorus predation.

1597 / ANTIMICROBIAL POTENTIAL OF LACTOCOCCUS LACTIS SUBSP. LACTIS BV. DIACETYLACTIS BGBU1-4

Keywords: *bacteriocin, Lactococcus, antimicrobial*

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BACKGROUND

Lactococcus lactis BGBU1-4 strain was isolated based on its antimicrobial potential against many Gram-positive bacteria including food spoilage and food-borne pathogens. The first analysis revealed that strain BGBU1-4 produces at least two bacteriocins of 3,642.62 Da and 5,160.94 Da. (1)

OBJECTIVES

Analyze antimicrobial potential of strain BGBU1-4.

METHODS AND RESULTS

To determine the overall antimicrobial potential of strain BGBU1-4, we applied genome analysis, and repeated isolation and purification of the two antimicrobials by strain BGBU1-4 and transformant MG7284 carrying plasmid pBU6. Genome mining indicated that strain BGBU1-4 has the potential to produces additional eight antimicrobial peptides.

Among those eight peptides, which were expressed in pMAL-c5X system, only peptide 6 had weak antimicrobial effect against lactococcal strains. Strains BGBU1-4 and transformant MG7284, carrying plasmid pBU6, synthesize only lactolisterin BU, while the 3,642.62 Da antimicrobial peptide is a degradation product of lactolisterin BU, which retains antimicrobial activity. Variants in lactolisterin BU length, BU1-24 and BU1-31, showed very high antimicrobial activity against all tested indicator strains, indicating that domains I and II are crucial for the antimicrobial effect.

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1615 / PHYLOGENETIC AND RESISTOME ANALYSIS OF THE EPIDEMIC MULTIDRUG-RESISTANT ST25 ACINETOBACTER BAUMANNII CLONE.

Keywords: *blaOXA-23, Animals, Spread, OneHealth*

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BACKGROUND

Acinetobacter baumannii ST25 clone includes multidrug-resistant isolates responsible for severe infections. In France, OXA-23-producing ST25 isolates have been collected from humans and animals during 2010-2019.

OBJECTIVES

To analyze genomic relationships and resistome of ST25 French isolates from humans (n=11) and animals (n=34) together with all ST25 genomes available in public genomic repository (NCBI) for gathering knowledge on the evolution of this multidrug-resistant clone.

METHODS

The genome of the 45 French isolates was sequenced generating short reads (Illumina). Among the genomes of the NCBI database (n=5837 on the 18/01/2022), 103 ST25 non-redundant genomes, including two from animals, were selected for further phylogenetic analysis together with the French isolates (MrBayes). The resistome was analyzed using ResFinder4.1 (https://cge.cbs.dtu.dk/services/ResFinder). The genetic elements harboring antimicrobial resistance genes were investigated by long reads sequencing (Nanopore Oxford Technology).

RESULTS

The 148 ST25 genomes grouped in four clades (CI-CIV). Clades II and IV included genomes from South America, whereas geographical origin of the genomes in the other clades was more diverse. The genomes of the French isolates grouped with others of the NCBI database in clades CI and CIII, highlighting similarities between isolates from humans and animals. Fifty-two genes conferring antibiotic resistance were found. In clade CIII, >50% of isolates harbored a blaOXA-23 gene. In the isolates sequenced for this study, blaOXA-23 was mainly located on an AbaR4 resistance island.

The ST25 A. baumannii clone is globally expanded but presents genomic divergence among isolates. ST25 multidrug-resistant isolates are able to colonize humans and animals overcoming host-related barrier.

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1760 / PRECISION METAGENOMICS FOR DETECTION OF MARKERS ASSOCIATED WITH TRIMETHOPRIM-SULFAMETHOXAZOLE RESISTANCE IN UTIS

Keywords: *metagenomics, next generation sequencing, urinary tract infections*

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BACKGROUND

150 million cases of urinary tract infections (UTIs) occur globally each year. Of woman effected by UTIs, 25-30% develop recurrent infections. In Europe, the rate of trimethoprim-sulfamethoxazole (TMP-SMX) resistance in UTIs is 15-60%. Urine cultures fail to identify the etiology in ~60% of patients. The Urinary Pathogen ID/AMR Panel (UPIP) uses Precision Metagenomics (PM) to detect and quantify >170 uropathogens and >2,000 antimicrobial resistance (AMR) markers.

OBJECTIVES

We compared genotypic AMR marker detection by UPIP to phenotypic antimicrobial susceptibility testing (AST) for TMP-SMX, an antibiotic commonly used for UTIs.

METHODS

Culture was performed per routine protocols and AST determined on a BD Phoenix system. For UPIP, urines were spiked with internal control (bacteriophage T7, Microbiologics), DNA extracted (Quick-DNA Urine Kit, Zymo), sequencing libraries prepared, target-enriched in 3-plex with UPIP reagent (IDbyDNA), sequenced at 1x147bp to a depth of 1 million reads/sample (NextSeq550, Illumina) and automated data analysis was performed with Explify (IDbyDNA). UPIP is for research use only, and not for use in diagnostic procedures.

RESULTS

Culture and AST results were available for 290 urine samples that grew gram-negative uropathogens. Top gram-negative uropathogens detected were Escherichia coli (90/290, 31%), Klebsiella pneumoniae (44/290, 15.2%) and Pseudomonas aeruginosa (19/290, 6.6%). Of these, 47 (16.2%) were intermediate or resistant to TMP-SMX. UPIP predicted resistance to TMP-SMX in 45/47 (95.7%). The mechanisms of resistance for TMP-SMX were dfr (43/45, 95.6%), sul (42/45, 93.3%) and oqx (22/45, 48.9%). UPIP's PM approach demonstrated high agreement between AMR marker detection and AST in gram-negative uropathogens.

ACKNOWLEDGEMENTS/REFERENCES

We are grateful to the entire Microbiology Unit at PathGroup Labs for coordination of sample selection, de- identification and shipment, and to Illumina for reagents for library preparation and enrichment in support of this study. We also thank the IDbyDNA Laboratory, R&D, MSA and Bioinformatics Teams.

295 / UTILIZATION OF PRODUCED WATER AS A GROWTH MEDIUM FOR ALGAE CULTIVATION AND BIOFUEL PRODUCTION

Keywords: *Algae, Biofuel, Bioremediation, Biotechnology*

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BACKGROUND

Oil fields face adverse environmental conditions owing to the outflow of enormous volumes of produced water (PW) that is contaminated with hydrocarbons, heavy metals, and high salt concentrations.

OBJECTIVES

To use PW as an appropriate algal cultivation medium for the selective growth of native oil-resistant and hypersaline algal species.

METHODS

Algae were isolated on PW after supplementation with nutrients, oil bioremediation was assessed using GC-MS, and lipid accumulation and biodiesel production were assessed using solvent extraction and GC analysis.

RESULTS

35 algal strains were isolated and screened for various biotechnological applications including bioremediation, and biofuel production. Most strains showed capability to grow in PW. Three strains were selected, namely Asterarcys sp. RA100 and Dunaliella sp. NM2, and NU3. Growth of these strains showed their need for supplementation with phosphorus more than nitrogen and their tolerance to high salinities, temperatures, and light intensities. Their growth was successfully scaled up to 240 L in an experimental raceway under greenhouse conditions, and under direct sun (max. temperature of 50oC). Bioremediation experiments exhibited the capability of Dunaliella sp. NM2 and NU3 to degrade >95% of TPH in water. While Asterarcys sp. RA100 and Dunaliella sp. NM2 possessed high lipid content of 26 and 36%, respectively, their stress under high salinities and nutrient deprivation enhanced their lipid content. Utilization of oil produced water as a cultivation medium increases the feasibility of a large-scale biodiesel production in non-arable desert sites besides their role in produced water bioremediation.

313 / BACILLUS STRAINS ISOLATED FROM MARINE SPONGES EXHIBITS ANTIMICROBIAL AND BIOSURFACTANT ACTIVITIES

Keywords: *surfactant, bacteria, genome*

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Sponge-associated bacteria stand out as sources of biotechnologically-relevant substances. Two strains previously isolated from marine sponges in our recent study, Bacillus pumilus 64-1 and Bacillus subtilis 84-5, showed antibacterial and biosurfactant activities, respectively. Therefore, this study aimed to deepen our understanding on both activities of these two strains by a coupled strategy using guided-assays and genome mining.

For that, chemical analyses, thin-layer chromatography and bioassays were performed of B. pumilus 64-1. The methanolic extract of strain 64-1 exhibited bacteriostatic activity on Staphylococcus aureus cells at a minimum inhibitory concentration of 29.6 µg/mL. At the genomic level, B. pumilus 64-1 (3.6 Mpb) showed 3,705 coding sequences (CDS), 12 biosynthetic gene clusters (BGCs), including types I and III polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS), bacteriocins, betalactones, sactipeptides, terpenes, and siderophores. In particular, an NRPS potential product showed 85% similarity with bacilysin, with high homology with the cluster present in other Bacillus strains. The investigation of the potential biosurfactant activity of B. subtilis strain 84-5 revealed a surface tension of 26.91 ± 0.29 mN/m, with emulsifying activity on both mineral oil and n-hexadecane. In addition, 84-5's genome (4.1 Mbp) showed 4,088 CDS. Eleven BGCs were predicted, including types I and III PKS, bacteriocins, terpenes, thiopeptides, and NRPS. Particularly, one of these BGC sequences showed 82% similarity with a surfactin from Bacillus velezensis FZB42. These results are promising for the employment of sponge-associated Bacillus for the search for new treatments against antimicrobial-resistant bacteria and green alternatives for bioremediation processes and other biomedical applications.

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341 / BEAUVERIA BASSIANA SAN01: A POTENTIAL SOURCE FOR INDUSTRIAL BIOCATALYSTS

Keywords: *Beauveria bassiana , industrial biocatalysts*

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The quest for a better sustainable environment has necessitated the replacement of chemically driven industrial processes with enzyme-catalyzed reactions. A novel strain of an endophytic entomopathogen, Beauveria bassiana SAN01, with remarkable enzyme secreting potential, was isolated from onion leaves in its endophytic state.

Aside from being a novel enzyme source, B. bassiana has been demonstrated in previous studies to be safe for animal and human use. The enzyme profiling of the fungal strain was carried out both qualitatively and quantitatively using wheat bran as the growth medium. The total RNA from the fungus was sequenced using a NextSeq 550 sequencer for qualitative profiling, while the HotPep tool was employed in the functional prediction of the CAZymes in its transcriptome. Results showed that the fungus secretes various important biomass-degrading enzymes in significant quantities. These enzymes have been identified to include amylase, β-glucosidase, β-xylosidase, cellulase (exoglucanase and endoglucanase), polygalacturonase and laccase. Furthermore, several other enzymes, including arabinofuranosidase, cellobiose dehydrogenase, chitosanase and esterases, were also identified at the transcriptomic level.

Subsequently, significant production levels for the biocatalysts were recorded under unoptimized submerged fermentation conditions, which ranged from 2.88 U/ml (laccase) to 16.17 U/ml (endoglucanase) to 304.48 U/ml (xylanase); corroborating the transcriptomic analyses. The significant activities of Beauveria enzymes recorded in this study and its previously highlighted safety thus open a new vista for its use as a source of important industrial enzymes and, by extension, other biotechnological applications.

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356 / BIOSURFACTANT FROM THERMOHALOPHILIC STRAIN OF BACILLUS ALVEAYUENSIS: CHEMICAL CHARACTERIZATION AND ANTIFUNGAL ACTIVITY

Keywords: *Lipopeptide, Petroleum, Antifungal*

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Biosurfactants (BS) are microbial secondary metabolites capable of reducing the surface and interfacial tensions and form stable emulsions, characteristics that make them highly applicable in different industrial sectors. Some BS produced by Bacillus strains have shown antifungal activity, especially lipopeptides belonging to iturin, fengycin and surfactin families. The goal of this work was to characterize the BS produced by a thermohalophilic Bacillus alveayuensis (strain Ar70C7-2) isolated from a Brazilian offshore oil reservoir, determining its chemical structure, and its antifungal activity. Crude biosurfactant obtained by acid precipitation was extracted with chloroform followed by semi-purification on a silica gel 60 column and purification on a C18 column. The amino acid profile was determined by HPLC, the quantification of fatty acids by gas chromatography and the chemical structure by mass spectroscopy. Antifungal activity was determined by the agar-well diffusion method against eleven different phytopathogenic fungal strains using BS semi-purified. Fungal mycelium and spore suspensions were cultured on Potato-Dextrose-Agar at 28 ºC for 15 days, with and without rifampicin. As for the BS composition, results indicated the presence of 7% of protein, fatty acid chains varying from C14 to C18 atoms, with C16 homolog being the most abundant. Mass spectra showed the presence of surfactin, iturin and fengycin lipopetides families. Complete inhibition of hyphal growth was observed in cultures of Moniliophthora perniciosa, Phytophthora sojae and Lasidiplodia euphorbicola, and mycelia growth was considerably reduced in five fungal strains (Ceratocystis paradoxa, Sclerotinia sclerotiorum, Rhizopus microsporus, Aspergillus flavus and Aspergillus niger).

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365 / HETEROLOGOUS EXPRESSION OF BACTERIOCINS IN SACCHAROMYCES CEREVISIAE

Keywords: *Antimicrobial peptides, Bacteriocins, Heterologous expression, Saccharomyces cerevisiae*

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BACKGROUND

The emergence of multidrug drug-resistant bacteria is of great concern and there is a growing need for alternative strategies to combat antibiotic resistance. Antimicrobial peptides, such as bacteriocins, are excellent candidates; however, their high manufacturing costs limit their application as alternative antimicrobials. Recombinant gene expression offers the potential to produce these peptides more cost-effectively at a larger scale.

OBJECTIVES

Bactericidal yeast strains were constructed to express two class IIa bacteriocins, namely AX and BX, that explored codon optimisation and secretion signals.

METHODS

The native and codon-optimised variants of the ax and bx genes were cloned into episomal expression vectors containing either the Saccharomyces cerevisiae alpha mating factor (MFα1) or the Trichoderma reesei XYN2's (XYNSEC) secretion signals. Antimicrobial activity of the recombinant strains was evaluated against Listeria monocytogenes using agar overlay and well-diffusion assays, as well as Tricine-SDS-PAGE analysis and Scanning Electron Microscopy.

RESULTS

The bacteriocins were successfully expressed in S. cerevisiae as the recombinant peptides retained their activity and stability, with the MFα1 secretion signal being superior to the XYNSEC secretion signal for the secretion of the bacteriocins. Antimicrobial activity assays indicated that codon optimisation of the bx gene resulted in an 8-fold increase in activity of the recombinant peptide, but had no impact in the AX-producing strains. Scanning electron microscopy revealed that L. monocytogenes cells treated with the peptides were shrivelled and intracellular components leaked out of the cells. The development of an optimised yeast expression system for antimicrobial peptides could improve commercial bio-manufacturing of antimicrobial peptides.

ACKNOWLEDGEMENTS/REFERENCES

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370 / DIFFERENT PROMOTER-INTRON COMBINATIONS FOR ENHANCED AMYLASE EXPRESSION IN SACCHAROMYCES CEREVISIAE

Keywords: *Consolidated bioprocessing, Introns, Amylase, Saccharomyces cerevisiae*

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BACKGROUND

The world is striving towards a circular bioeconomy in which biorefineries will play a pivotal role in the conversion of cheap and renewable biomass to value-added products. Starchy material is one of the most abundant renewable sources of biomass and its conversion by engineered microbial hosts has been vital to the growth of the bioethanol industry. Consolidated bioprocessing (CBP) of starchy materials requires recombinant strains of Saccharomyces cerevisiae to produce raw starch degrading enzymes (RSDEs) and ferment the resulting sugars in a single step. Ideally, the recombinant yeasts should produce maximal amounts of RSDEs to ensure faster hydrolysis and more efficient ethanol production. One strategy to improve RSDE production levels is to increase heterologous gene transcription by including promoter-proximal introns in the expression cassette. However, few studies have looked at the use of different introns in combination with native yeast promoters for enhanced RSDE expression.

OBJECTIVES

This study investigated the effect of two native S. cerevisiae introns on the heterologous expression of α-amylase and glucoamylase genes when placed proximal to four different native yeast promoters.

METHODS

All strains were constructed using a yeast-mediated ligation strategy. Introns were inserted directly downstream of the different promoters. Two alpha-amylase and one glucoamylase genes were evaluated for enhanced protein production.

RESULTS

Our results showed significantly enhanced protein production and extracellular enzyme activity for several recombinant strains due to the presence of promoter-proximal introns. Results also eluded that the effect of the promoter-intron combination is dependent on the gene it is expressing.

ACKNOWLEDGEMENTS/REFERENCES

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374 / PROTEOME-WIDE ANALYSIS OF PAENARTHROBACTER NICOTINOVORANS ATCC 49919 NICOTINE- METABOLISM

Keywords: *Paenarthrobacter nicotinovorans, proteome, nicotine metabolism*

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BACKGROUND

Paenarthrobacter nicotinovorans is a soil Gram-positive nicotine-degrading microorganism (NDM) that harbors a 165 kb pAO1 catabolic megaplasmid. Both the chromosome and the pAO1 have recently been sequenced, but details on the regulation and interplay of the nicotine-degrading pathway with the general metabolism of the cell are scarce. The objective of this work was to provide the nicotine-induced proteome of the strain by using a time-based shotgun proteomics study.

METHODS

P. nicotinovorans was grown in the presence or absence of nicotine and the cells harvested at 3 different time intervals: 7-, 10- and 24-hours after inoculation. The cells were lysed, separated on SDS-PAGE, digested in-gel digestion using trypsin, and the resulting peptides mixture was analyzed using nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS).

RESULTS

An extensive number of proteins that are both plasmidal- and chromosomal-encoded and were shown to work together via Krebs cycle and nicotine pathway. This data provides insights into bacterial cell's adaptation to the nicotine metabolic intermediates and could serve as a basis for future attempts to genetically engineer the pAO1 encoded catabolic pathway for increased bioremediation efficiency or for the production of valuable chemicals. The mass spectrometry-based proteomics data have been deposited to PRIDE with the dataset identifier PXD012577.

ACKNOWLEDGEMENTS/REFERENCES

MM was supported by a grant from the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI PN-III-P4-ID-PCE-2020-0656.

377 / BIOTECHNOLOGICAL APPLICATIONS OF PIGMENTS FROM EPIPHYTIC BACTERIA FROM LORANTHUS SP.

Keywords: *Epiphytic bacteria, Pigments, Textile dye, Nanoparticles*

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BACKGROUND

Epiphytic bacteria produce a variety of pigments for Ultra-Violet tolerance, leaf colonization, and epiphytic survival. Microbial pigments are environmentally and ecologically safer than synthetic colorants and are gaining attention from researchers. Mass production and extraction of microbial pigments is easy and cheap as compared to plant sources.

OBJECTIVES

The current study investigates the applications of pigments from epiphytic bacteria for use as mainly ecofriendly colorants.

METHODS

Epiphytic bacteria were isolated from the leaves of Loranthus sp. from mangrove plants. Two strains LF-O and LF-Y producing orange pigment and yellow pigment were chosen for further studies. Pigment extracts from the strains were assessed for their potential for dyeing cotton textiles and gel candles. The pigments were also assessed for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and production of silver nanoparticles.

RESULTS

LF-O and LF-Y produced bright orange pigment with absorption maxima of 475 nm and yellow pigment with absorption maxima of 436.5 nm respectively. LF-O and LF-Y were identified as Corynebacterium sp. and Micrococcus sp. respectively. LF-O and LF-Y exhibited maximum growth and pigment production at 28oC. The pigments efficiently dyed cotton cloth whereas the stained gel candles retained the color until 1.5 years after preparation. The orange pigment from LF-O exhibited strong antioxidant activity. Within 30 min of incubation, silver nanoparticles were formed from both the pigment extracts. The average diameter of spherical silver nanoparticles synthesized from LF-O was 117.2 nm and that synthesized by LF-Y was 168.5 nm.

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405 / CATCH ME IF YOU CAN: TRACKING FUNGAL CONTAMINATION IN METALWORKING FLUIDS

Keywords: *MWFs, flow cytometer, spore detection*

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Water-miscible metalworking fluids (MWFs) are submitted to multiple microbial contaminations through non-sterile environment and tap water (1). Biofilm development and fungal sporulation may threaten product stability (1) and workers' health (2). However, microbial monitoring through liquid sampling is limited as it detects only planktonic microorganisms.

Although detection of fungal contaminations are infrequent, they are likely ubiquitous in MWFs. These obligate aerobes grow mainly on machine surfaces at junctions, sidewalls, pipes and tanks in close proximity of the fluid, but not in direct contact. During fungal development, spores are likely to be released in MWFs, which could lead to cross-contamination of machines placed in the same area. Moreover, many of these opportunistic fungi are thought to be pathogenic (4). This makes fungal detection important for MWF monitoring. However, fungal spores are only present at low concentration in MWFs, making their detection with routine monitoring technics challenging.

We hypothesized that traditional culture-based methods, like dip-slide, underestimate fungal concentration in used MWFs. To improve detection resolution, we investigated different spore enrichment methods, including gradient concentration and used flow cytometry combined with SYTO 9 and propidium iodide staining to segregate fungal spores from bacteria (5). All experiments are performed with Fusarium oxysporum, a fungus frequently found in MWFs (6).

This technic enables a better detection and monitoring of fungi in MWFs and is an experimental tool to generally investigate fungal establishment in MWFs.

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03

414 / COMPLETE GENOME SEQUENCE OF THE WELL-STUDIED NICOTINE-DEGRADING MICROORGANISM PAENARTHROBACTER NICOTINOVORANS STRAIN ATCC 49919

Keywords: *Paenarthrobacter nicotinovorans, nicotine metabolism, genome, nan*

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BACKGROUND

Paenarthrobacter nicotinovorans ATCC 49919 is a nicotine-degrading microorganism with potential applications for the conversion of nicotine-containing waste into precursors for the synthesis of drugs and insecticides.

OBJECTIVES

The research objective is to provide the complete genome of the strain, which is the first step towards the envisioned complete omics characterization of nicotine metabolism in Paenarthrobacter nicotinovorans ATCC 49919.

METHODS

gDNA extracted from Paenarthrobacter nicotinovorans ATCC 49919 cultures was sequenced using two technologies: short-read sequencing was performed with Illumina NovaSeq 6000, while the Oxford Nanopore Technology (ONT) MinION provided the long-read sequences.

RESULTS

Following hybrid assembly using Unicycler, the complete genome comprised of two circular replicons was obtained. The largest replicon is the 4 316 184 bp chromosome with an overall GC content of 63.2%. Present in the chromosome were a total of 3953 coding sequences, 54 tRNAs, 2 ncRNAs, 1 tmRNA and 6 identical ribosomal operons. The second replicon, a 165 141 bp circular megaplasmid with an overall GC content of 59.7%, has a level of 99.99% identity with the previously described sequence of the pAO1 megaplasmid, GenBank entry AJ507836.131. The genome opens the way for engineering the strain with the aim of improving the conversion of nicotine-containing waste.

ACKNOWLEDGEMENTS/REFERENCES

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419 / MICROALGAE MICROBIOMES – A NATURAL SOURCE FOR THE PREVENTION AND TREATMENT OF DISEASES IN AQUACULTURE

Keywords: *Microalgae Microbiomes , Anti-biofilm, omics, aquaculture*

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Aquaculture is one of the fastest growing food sectors in the world with land-based aquaculture gaining increasing interest. Diseases caused by bacteria like Pseudomonas sp., Flavobacterium sp., Aeromonas sp. or Edwardsiella sp. are an obvious challenge to the aquaculture industry exacerbated by many pathogen's biofilm building abilities. Antibiotics are still in use in many regions of the world, contributing to increased antimicrobial resistance. Our work builds on the hypothesis that a novel approach to disease treatment in aquaculture can be achieved by exploiting the healthy properties of microalgae and their associated microbiomes.

To advance this field, we performed both functional and sequence-based screening of microalgae and microbial consortia for anti-biofilm activities. Five promising candidates of microalgae which showed significant anti-biofilm traits were chosen for further investigation. Ongoing metagenomes-, metaproteomes- and metatranscription analyses gave first insights into the main enzymes involved in these antibiofilm effects and potential candidates for industrial applications.

ACKNOWLEDGEMENTS/REFERENCES

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We declare no conflict of interest.

429 / PROSPECTS FOR THE USE OF FICUS CARICA L. LEAVE EXTRACT AS BIO-PRESERVATIVE AGENT

Keywords: *Ficus carica L, Saccharomyces cerevisiae, antiradical activity, mitochondrial ATPase*

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BACKGROUND

Fruits, roots and leaves of F. carica have been used in traditional medicine to treat various ailments and have anti-inflammatory, antispasmodic and antimicrobial activity[1].

OBJECTIVES

The aim of our research was to study the influence of F. carica leave extracts on Saccharomyces cerevisiae ATCC 9804 (wine yeast strain) in order to evaluate its potential as bio-preservative agent for "reduced alcohol" drinks.

METHODS

Antimicrobial activity determination was done using disk-diffusion method. Specific growth rate (SGR, μ), generation succeeding factor (GSF, G), the mitochondrial FoF1-ATPase activity was evaluated by Taussky and Shorr method[2]. Antiradical activity was studied by DPPH-assay[3]. The concentration of total phenolics and flavonoids were investigated using Folin-Ciocalteu reagent and $AlCl_3$ colorimetric assay[4].

RESULTS

Obtained results show that F. carica was rich in phenolic substances (149.32±4.01mg (GAE)/g dry weight) and flavonoids (21.1±0.19 mg (QE)/g dry weight). IC50 value in DPPH-assay was 120.34 µg/ml. The plant extract suppressed the SGR of yeast by 1.5 times (µ=0.139). Significant growth of GSF has been observed (G=5 instead of the same parameter in control version (G=3.27)). Plant extract suppressed ATPase activity by 29.8 % (1.873 µg P/ml min mg protein).

On the basis of these results it is possible to suppose that investigated plant extract antimicrobial influence conditioned with the changes in membrane-associated properties. Obtained data show that F. carica is perspective as a bio-conservative agent in wine industry.

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462 / ANTI-MELANOGENIC EFFECT OF RECOMBINANT LACCASE FROM STREPTOMYCES SP. BV286

Keywords: *Streptomyces, laccase, anti-melanogenesis, zebrafish*

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BACKGROUND

Melanin is a part of heterogeneous group of molecules that provide skin pigmentation and photoprotection. Hormonal changes, skin injuries and inflammation can trigger the overproduction of melanin – hyperpigmentation. Most of commercially available anti-melanogenic agents have adverse side effects. Although recalcitrant, melanin can be decolourized by microbial oxidative enzymes – laccases and peroxidases, with the use of redox mediators. Eco-friendly and biodegradable microbial enzymes emerged as a promising alternative to toxic and carcinogenic anti-melanogenic compounds.

OBJECTIVES

To investigate the potential of recombinantly expressed laccase from Streptomyces sp. BV286 for anti-melanogenesis in vivo.

METHODS

In order to analyze anti-melanogenesis in vivo, wild type zebrafish (Danio rerio) embryos were treated at 6 h post fertilization (hpf) with 0.5 % cell free extract (cfe) of E. coli BL21 (DE3), containing overexpressed laccase from Streptomyces sp. BV286. Cfe of E. coli BL21 (DE3) was used as a control. Anti-melanogenic effect was evaluated at 24 and 48 hpf. Cardiotoxicity, hepatotoxicity and teratogenicity were monitored up to 120 hpf.

RESULTS

Melanogenesis was successfully inhibited in embryos treated with cfe containing overexpressed laccase from BV286. No toxic effects have been observed up to 120 hpf, contrary to the embryos treated with 5 µg/ml of commercially available hydroquinone. These results imply that recombinant laccase from BV286 is effective and safe for the enzymatic skin whitening.

ACKNOWLEDGEMENTS/REFERENCES

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03

463 / MICROBIAL COMMUNITIES OF ACTIVATED SLUDGE OF LARGE-SCALE WASTEWATER TREATMENT PLANTS OF MOSCOW CITY ON A GLOBAL SCALE

Keywords: *wastewater treatment, activated sludge, microbiome*

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Microbial communities in wastewater treatment plants (WWTPs) play a key role in water treatment. Microbial communities of activated sludge vary extensively based on plant operating technology, influent characteristics and WWTP capacity. In this study, we performed 16S rRNA gene profiling of activated sludgeat nine large-scale WWTPs responsible for the treatment of municipal sewage from the city of Moscow, Russia. Two plants employed conventional aerobic process, one plant - nitrification/denitrification technology, and six plants were operated with the University of Cape Town (UCT) anaerobic/anoxic/oxic process. Microbial communities were impacted by the treatment technology and dominated by the Proteobacteria, mostly of classes gamma and alpha. Other major groups were the Bacteroidota, Actinobacteriota, Chloroflexi, Myxococcota, Firmicutes, Patescibacteria, Verrucomicrobiota, Bdellovibrionota, Nitrospirota, and Planctomycetota. WWTPs employing the UCT process enabled efficient removal of not only organic matter, but also nitrogen and phosphorus, consistently with the high content of ammonia-oxidizing Nitrosomonas, nitrite-oxidizing Nitrospira and Candidatus Nitrotoga, and phosphate-accumulating bacteria. The latter group was represented by Candidatus Accumulibacter, Tetrasphaera sp. and denitrifying species. Co-occurrence network analysis provided information on key hub microorganisms in activated sludge, which may be targeted for manipulating the activated sludge stability and performance. Comparison of activated sludge communities from Moscow WWTPs and ones analyzed worldwide by a Global Water Microbiome Consortium revealed that Moscow samples clustered together indicating that influent characteristics, related to socio-cultural and environmental factors, could be more important than a plant operating technology.

ACKNOWLEDGEMENTS/REFERENCES

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466 / CULTIVATION OF KERATINOLYTIC MICROMYCETE ASPERGILLUS CLAVATUS ON POULTRY WASTE

Keywords: *Aspergillus clavatus, keratinases, biodegradation*

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Keratinases are enzymes in demand in industry and agriculture. The search for a highly active keratinase producers and the development of sustainable and profitable methods of their cultivation are an important goal of modern biotechnology. It was shown that Aspergillus clavatus synthesizes extracellular keratinases in submerged conditions on a medium with milled chicken feathers as the main source of carbon and nitrogen. The next task was to study the growth of the micromycete during solid-state fermentation (SSF).

SSF was carried out on 0.7 g chicken feathers with 10 ml liquid medium under static conditions at 28 °C. Keratinolytic and caseinolytic activity was measured spectrophotometrically in the eluate (0.05 M Tris-HCl buffer, pH 8.2) with wool keratin suspension and Hammerstein casein solution. One unit (Uk/Uc) of activity was defined as an increase of A280 for keratinolysis and A275 for caseinolysis by 0.01 under the assay conditions (37 °C, pH 8.2, 600 rpm). The decomposition of feathers was studied by scanning electron microscopy (SEM).

Visible hydrolysis of feathers was observed on the 3rd day of cultivation, which is consistent with the SEM data. All feathers lost their integrity and changed their microscopic structure by 6-7 days. The maximum proteolytic activity occurred on the 4th day (keratinolysis – 50.9 Uk; caseinolysis – 64.6 Uc). The data obtained indicate the promise of Aspergillus clavatus as a source of keratinases due to the high target enzymatic activity and the ability to grow under conditions of low costs for substrates and cultivation waste disposal.

468 / STUDY OF PATHOLOGICAL MUTATIONS IN HUMAN CDKL5 PROTEIN THROUGH A CO-EXPRESSION KINASE ASSAY IN A MARINE POLAR BACTERIUM

Keywords: *CDKL5, Psychrophiles, Kinase assay, Co-expression*

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BACKGROUND

CDKL5 is a serine/threonine protein kinase whose activity is crucial for the development of the brain. Dozens of CDKL5 pathological variants have been associated with CDKL5 Deficiency Disorder (CDD) but clear genotype-phenotype relationships have not been defined yet [1]. Most of the difficulties in the characterization of CDKL5 stem from the failed attempts in its recombinant soluble expression. On the other hand, the polar bacterium Pseudoalteromonas haloplanktis TAC125 (PhTAC125) can produce CDKL5 in soluble form, differently from Escherichia coli [2,3].

OBJECTIVES

We propose the use of the bacterium PhTAC125 as an orthogonal system for studying CDKL5 functions and disease-related alterations. To prove this point, we aimed to co-express 12 CDKL5 variants with their substrate, EB2, and measure their intracellular activity. In a bacterium, no confounding effects due to phosphorylation/dephosphorylation cascades would be present.

METHODS

To co-express CDKL5 variants with EB2 in PhTAC125, multiple polycistronic plasmids have been developed. The efficacy of these systems has been monitored and iteratively improved by using a fluorescent reporter. The optimized plasmid was then used for the synthesis of the two human proteins. The catalytic activity of each CDKL5 variant was measured by analyzing EB2 phosphorylation levels with a specific antibody.

RESULTS

Our data prove that, during co-expression, CDKL5 can phosphorylate EB2 in PhTAC125 and, hence, is soluble and active in this bacterium. Furthermore, CDKL5 pathological variants show various degrees of hypoactivity, demonstrating that this tool can shed light on genotype-phenotype links in CDD and quantitatively measure the effect of each mutation.

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483 / IDENTIFICATION OF NEW CARBOXYLIC ACID ESTER HYDROLASES DEGRADING POLYESTERS IN THE GENUS ASPERGILLUS

03

Keywords: *carboxilic acid ester hydrolases, polyethylene terephthalate, biodegradation*

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Carboxylic acid ester hydrolases (CEH) (EC 3.1.1. X) catalyzes the hydrolysis and synthesis of carboxylic acid esters and comprises 117 subdivisions, including lipases, carboxylesterases and cutinases (1). Cutinases (EC. 3.1.1.74) are enzymes belonging to the group of those whose primary substrate is the biopolymer cutin, which is naturally found in plants as a protective barrier against pathogens (2). Due to the reactions that catalyze, it gives it various applications in the industry; among the most important are food, oils, fat modification, obtaining aromas, biodiesel production, enzymatic degradation of toxic substances and synthetic polymers (3). Therefore, the objective of this work was to identify carboxylic acid ester (CEH) enzymes in the Aspergillus genus with the ability to degrade polyesters expressed in the presence of different inducers, such as olive oil, apple cuticle and synthetic polyesters such as polyethylene terephthalate (PET). The kinetics of CEH production in the presence of these inducers were performed, and the enzymes with activity were identified. The results of hydrolytic activity "in situ" in zymogram in the two Aspergillus strains showed the presence of 2 bands with esterase activity, with molecular weights of 34 kDa and 54 kDa. The 34 kDa protein from Aspergillus terreus was expressed in the presence of PET and was identified as a hypothetical α/β hydrolase protein of 35 kDa. A novel CEH enzyme with the ability to hydrolyze PET was detected, which is of great interest for further studies.

ACKNOWLEDGEMENTS/REFERENCES

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511 / IN DEPTH COMPARATIVE MICROBIOME ANALYSIS OF MEDITERRANEAN FISH HATCHERIES

Keywords: *metagenomics, marine biotechnology, aquacultures, live feeds*

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BACKGROUND

The importance of the fish hatcheries' microbial communities to fish health and welfare has been long recognized and several studies have been conducted to map these communities during healthy rearing conditions but also during disease outbreaks. Most studies focus on the intestinal and epidermal microbiome of the developing fish, which is mainly shaped by the live feeds (microalgae, rotifers, and copepods) used at the rearing process. The microbiome of live feeds, however, has not been investigated thoroughly so far.

OBJECTIVES

We have analyzed the microbiome of one research and two commercial fish hatcheries producing Mediterranean fish species with emphasis on their live feed sectors. The aim was to compare the microbiomes of hatcheries with different characteristics (water source, production intensity, and practices) and provide baseline information about their structure with a focus on putative pathogenic bacteria .

METHODS

The hatcheries were sampled seasonally over one year. Sampling involved 5 stations: inlet, microalgae, rotifers, artemia, and outlet. Water samples were filtered using a vacuum filtration system and DNA was extracted from filters. 16s amplicon Novaseq sequencing was conducted. Bioinformatics included amplicon sequence variant analysis.

RESULTS

Alpha diversity analysis revealed a relatively stable microbiome between seasonal samplings for every hatchery. Nevertheless, a diverse microbiome between sampling stations was discovered. A core microbiome for every hatchery was cataloged, which included important putative fish pathogens belonging to Vibrio and Tenacibaculum genera. Mapping of the microbiome in the live feeds through metagenomics is the first step towards efficient microbial control in fish hatcheries.

ACKNOWLEDGEMENTS/REFERENCES

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517 / MICROBIAL COMMUNITY DIVERSITY OF BIOFILMS ON CARBONIZED POLYANILINE-MODIFIED ANODES OF MFCS USING MUNICIPAL WASTEWATER

Keywords: *microbial fuel cell, metabarcoding, wastewater, microbial community*

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MFCs (microbial fuel cells) have garnered attention in recent years as a potential green energy source employing select microbial strains' extracellular electron transfer (EET) abilities. Wastewater is both a source of exoelectrogenic biofilm-forming microorganisms, and the organic matter necessary for their metabolic processes. Both power generation and bioremediation can be obtained through the use of MFC systems. Our aim was to use a carbonized polyaniline-derived carbon nanostructure (NCN) as an anode modifier for a MFC system supplied with municipal wastewater, analyze the microbial community enriched on its surface, and identify the main taxa capable of power generation.

Modified carbon cloth with NCN obtained from polyaniline pyrolysis was used as an anode of dual chamber MFCs, operated in anaerobic conditions, at 30°C. The anode chamber was supplied with wastewater enriched with acetate. Total DNA was extracted from the initial wastewater and the MFC anodes through an optimized process involving a DNA concentration step. The 16S rRNA gene V3-V4 was sequenced, and resulting data was analyzed using an ASV-based sorting method (QIIME2) and the Silva database. The microbial diversity observed from the anodes was greatly reduced when compared to the initial wastewater, determining the significant enrichment of EET-capable bacterial taxa. Proteobacteria became the dominant phylum in the anode samples. The Geobacter, Arcobacter, Acinetobacter, Comamonas and Shewanella genera increased in relative abundance on the anodes, when compared to the initial wastewater.

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526 / CLONING AND CHARACTERIZATION OF N-CARBAMOYL ß-ALANINE AMIDOHYDROLASES FROM RHIZOBIUM RADIOBACTER MDC 8606

Keywords: *N-Carbamoyl-ß-Alanine Amidohydrolases , Rhizobium radiobacter MDC 8606, crystal structure*

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Optically pure L-amino acids find many uses in industry, as feed and food additives; and as intermediates for pharmaceuticals, cosmetics, and pesticides [1].

We describe the cloning, expression, purification and structure determination of the N-Carbamoyl-β-alanine amidohydrolase (RrCβAA), from Rhizobium radiobacter MDC 8606 strain of Microbial Depository Center of SPC "Armbiotechnology" NAS RA.

Specific activity of RrCβAA was determined using an assay able to detect β-alanine concentration, upon conversion into an isoindole derivative by reaction with ortho-phthalaldehyde [2].

RrCβAA is a member of the amidohydrolase family, with significant sequence identity to the Agrobacterium tumifaciens C58 enzyme.

A Hexa-histidine-tagged RrCβAA protein construct was purified to homogeneity using nickel affinity and size exclusion chromatography. We also present a high-resolution crystal structure of the RrCβAA in complex with 3-ureidopropionic acid and demonstrate ligand binding and activity is metal dependent. The purified RrCβAA showed high-levels of activity against N-carbamoyl β-alanine, with an optimum activity at 55 °C.

The substrate spectrum of RrCβAA was investigated toward various N-carbamoyl-DL- L- and D-amino acids. It was found that RrCβAA showed stereo specificity toward N-carbamoyl-L-amino acids with greatest catalytic efficiency for N-carbamoyl-L-β-alanine. Thus, RrCβAA can be used to obtain L-α, L-β-, L-γ, L-δ- amino acids.

ACKNOWLEDGEMENTS/REFERENCES

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This work was supported by State Committee Science MES RA, Research Project 21SCG-2I017

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570 / EVALUATION OF YEAST SURFACE DISPLAY SYSTEM FOR SYNTHESIS OF ANTHOCYANIN-DECORATING ENZYMES

Keywords: *Yeast, Anthocyanin*

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Plants produce anthocyanins to incite the pollination and seed dispersion performed by pigment-attracted animals. These natural blue-to-red coloured pigments are also of industrial interest, especially as a blue food colorants. Stability and the bathochromic effect of the anthocyanin colour are enhanced by various sugar and other moieties introduced by transferases. Exposing these anthocyanin decorating enzymes on the yeast cell surface would potentially allow creating the biotransformation systems that emit flow limiting precursor transfer across cellular membranes.

In this study, Aga2-fused Vaccinium corymbosum and Lupinus angustifolius anthocyanidin 3-O-glucoside-5-O-glucosyltransferase (Vc5OGT, Lang5OGT), L. angustifolius anthocyanidin 3-O-glucoside-6’’-O-coumaroyl-transferase (Lang6OCT) and L. albus anthocyanidin 3-O-glucoside-6’’-

O-malonyltransferase (Lalb6OMT) proteins were successfully synthesised in yeast Saccharomyces cerevisiae EBY100 strain and were shown to be exposed to the cell surface. In comparison, the synthesis of only Vc5OGT and Lang6OCT were successful as soluble N’-polyhistidine tagged proteins in S. cerevisiae. Preliminary tests of whole-cell incubation with anthocyanin extract show no substantial effect on anthocyanin colour transformation, plausibly due to high degree of glycosylation of exposed enzymes, N’terminal fusion, and limited supply of modifying precursors to the medium. Further studies will aim to determine species-specific activity of these enzymes and factors that would increase their activity in the surface display system.

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03

580 / BIOSYNTHESIS OF (2R,3S)-ISOCITRIC ACID BY YEAST YARROWIA LIPOLYTICA, GROWN ON WASTE OF ETHANOL PRODUCTION INDUSTRY

Keywords: *Isocitric acid (ICA)*

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There is ever increasing evidence that isocitric acid (ICA) can be used as a promising compound with powerful antioxidant activity to combat oxidative stress. ICA is commonly produced by chemical synthesis with the formation of a mixture of stereoisomers extremely persistent. Alternatively, ICA can be produced using the yeast Yarrowia lipolytica as producer and pure substrates as sources of carbon and energy.

The aim of this work was to study the possibility of using ester-aldehyde fraction (EAF) (the waste of ethanol production industry) for ICA production.

The potential producer of ICA, Yarrowia lipolytica VKM Y-2373, was selected by screening of 35 wild strains, as well the mutant and recombinant strains of Y. lipolytica. The selected strain showed sufficient growth and good acid formation in media with growth-limiting concentrations of nitrogen, sulfur, phosphorus, and magnesium. A shortage of Fe2+ and Ca2+ ions suppressed both Y. lipolytica growth and formation of ICA. The preferential synthesis of ICA can be regulated by changing the nature and concentration of nitrogen source, pH of cultivation medium, and concentration of EAF. Experiments in this direction allowed us to obtain 65 g/L ICA with a product yield of 0.65 g/g.

618 / BIODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF DEEP-SEA ACTINOBACTERIA FROM THE MADEIRA AND AZORES ARCHIPELAGOS

Keywords: *Marine Actinobacteria, Deep-sea, Natural products*

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Actinobacteria are a chemically prolific source of biotechnologically relevant secondary metabolites. The genetic and metabolic diversity of marine Actinobacteria reflect their adaptation to extreme marine conditions, such as the deep-sea. However, the difficulty of accessing these environments makes their exploration as a source of natural products quite challenging. In this study, we used 72 deep-sea samples of sediments, corals and sponges collected (150–3200 m depth) in poorly surveyed areas of Madeira and Azores archipelagos for the bioprospection of Actinobacteria capable of producing new natural products. Actinobacteria were isolated using two pre-treatments and five selective culture media supplemented with different antibiotics. A total of 298 actinobacterial strains distributed by 20 genera, including potential novel taxa, were isolated from the analysed deep-sea samples. Antimicrobial assays revealed 17 extracts active against one or more tested microorganisms. The corresponding minimum inhibitory concentration (MIC) values were in the range of 7.81–1000 µg mL-1. For cytotoxic activity, extracts from 33 of these strains had the ability to reduce the cellular viability of the cancer cell lines T-47D (breast ductal carcinoma) and HepG2 (liver cancer), after 48h of exposure.

Anti-inflammatory activity was observed in 45 extracts that significantly reduced nitric oxide (NO) production by the inflamed cell line RAW264.7. Metabolic analysis of actinobacterial extracts showed the presence of several metabolites related to known natural products, but also potentially new ones. The next steps will include guided mass isolation of the new natural products identified in the metabolic profiling of deep-sea Actinobacteria.

ACKNOWLEDGEMENTS/REFERENCES

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620 / IRON-ASSOCIATED ELECTROGENICITY IN A CLINICAL STRAIN OF KLEBSIELLA PNEUMONIAE AND CORRELATION WITH VIRULENCE POTENTIALS

Keywords: *Electrogenicity, Virulence, Iron, Klebsiella pneumoniae*

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Klebsiella pneumoniae is a pathogenic bacterium implicated in a variety of infections. The pathogenicity of various Klebsiella strains still holds a lot of unclear descriptions with respect to mechanisms of infection. Klebsiella pneumoniae is a good iron metabolizer and has been described as an electrogenic organism. Although some evidence has linked its electrogenicity with virulence, a correlative effect of iron as an electron carrier on Klebsiella pneumoniae electrogenicity in line with its virulence is still largely under-explored.

This work aimed at determining iron-association in electrogenicity of a clinical strain of Klebsiella pneumoniae and its correlation with virulence.

Influence of iron concentrations (10 - 50µm) on electrogenicity and virulence was studied on a Klebsiella pneumoniae strain which was isolated from a clinical sample (urine). Electrogenic potential of the isolate was determined by observing the open circuit voltage (OPV) in a microbial fuel cell while the virulence potential of the isolate was determined by assessing the quantities of urease and protease enzymes produced in urea and casein media respectively using spectrophotometry.

Between 10 and 50 µm iron concentrations, OPV of urea medium increased from 581 to 596 mV while that of Casein medium increased from 575 to 592 mV. There was also increase in both urease and protease production as the absorbance (420nm) increased from 1.203 to 1.403 and 1.087 to 1.287 respectively. The results show that increase in the iron concentration from 10 to 50 µm led to increase in electroactivity and hence increase in virulence potential of the isolate.

628 / MOLECULAR DIVERSITY AND BIOCATALYTIC POTENTIAL OF THE HALOALKALIPHILIC ACTINOMYCETES OF SEA ORIGIN

Keywords: *Alkaline Proteases, Haloalkaliphilic actinomycetes, 16S rDNA-based Phylogeny, Canonical Correlation Analysis (CCA)*

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BACKGROUND

Haloalkaliphilic actinomycetes of sea origin have recently focused attention with particular reference to their distribution, diversity and biocatalytic potential. The aspects include cultural and morphological features, cell wall constituents, biochemical properties, extracellular enzymes, antibiotic resistance, 16S rDNA-based phylogeny, ARDRA and DGGE.

OBJECTIVES

Investigations on the Molecular diversity and Enzymatic potential of Actinomycetes of seawater in different seasons.

METHODS

The molecular diversity was judged by the amplification of the 16S rRNA genes using different universal and species-specific primers. The most probable secondary structure of the 16S rDNA gene was predicted for the early cycle PCR inhibition. The group-specific patterns were also generated based on the 16S rRNA gene fragments analyzed by the DGGE. The Jaccard's Similarly Coefficient and Phenograms by PAST Algorithms were significant approaches. The amplified restriction patterns of 16S rRNA genes generated by the tetra-cutter restriction endonucleases revealed distinct ARDRA patterns and clusters. While the CCA established the correlation of the physico-chemical factors and the variation of the actinomycetes in sea water in different seasons, with the dominance of the Nocardiopsis genus.

Proteases reflecting unique features of salt dependent temperature profile, resistance against urea denaturation and catalysis under various extreme conditions were prevalent. The recombinant proteases expressed in E. coli were compared with their natives. The co-production of amylases and proteases in marine actinomycetes highlighted the significance of biocatalysis in extremophiles.

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638 / NUTRITIONAL INTER-DEPENDENCIES AND A CARBAZOLE-DIOXYGENASE ARE KEY ELEMENTS OF A BACTERIAL CONSORTIUM RELYING ON A SPHINGOMONAS

03

Keywords: *biodegradation, bacterial consortium, multi-omics, B12 auxotrophy*

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Thiabendazole (TBZ), is a persistent fungicide/anthelminthic and a serious environmental threat. We previously enriched a TBZ-degrading bacterial consortium, described its composition and provided first evidence for Sphingomonas involvement in TBZ transformation. Here, SIP and amplicon sequencing analysis of the heavy and light DNA fraction of cultures grown on 13C-labelled vs unlabelled 12C-TBZ showed that 66% of the 13C-labelled phenyl moiety of TBZ was assimilated by Sphingomonas, with a relative abundance ratio of heavy-13C to light-13C DNA fraction of 36.8 (unlike with other members with ratios < 1.6). Non-target mass spectrometry (MS) showed thiazole-4-carboxamidine being a single dead-end transformation product, in agreement with the carbon assimilation of the 13C-labelled TBZ phenyl by Sphingomonas.

Metagenomic analysis resulted in 16 metagenome-assembled genomes (MAGs) with 96.2-100% estimated completeness with the most abundant being Sphingomonas, Sinobacteriaceae, Bradyrhizobium, Filimonas and Hydrogenophaga. Meta-transcriptomics/proteomics suggested TBZ transformation by Sphingomonas via initial cleavage to thiazole-4-carboxamidine and catechol by carbazole dioxygenase (car), and subsequent catechol transformation by enzymes encoded in a catechol ortho-cleavage (cat) operon.

Docking analysis of carbazole dioxygenase CarAa, showed high affinity for TBZ, further supporting its involvement in the transformation of TBZ. All the above point to a Sphingomonas-mediated TBZ transformation. Co-occurrence and gene expression networks showed strong interactions between Sphingomonas and a Hydrogenophaga, with Hydrogenophaga activating cobalamin biosynthesis and Sphingomonas cobalamin salvage, suggesting potential B12 auxotrophy by Sphingomonas. Our findings indicate microbial interactions aligning with the "black queen hypothesis" where Sphingomonas (detoxifier, B12 recipient) and Hydrogenophaga (B12 producer, enjoying detoxification) act as both benefactors and beneficiaries.

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639 / SUPPRESSION OF BICARBONATE TRANSPORT INCREASES CYANOBACTERIAL GROWTH AT HIGH CO2

Keywords: *cyanobacteria, bicarbonate transporter, carbon dioxide (CO2)*

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Phototrophic cyanobacteria use CO2 concentrating mechanism (CCM) to enhance their photosynthetic performance. At ambient concentration of inorganic carbon (Ci: CO2 + HCO3-), CCM activity raises intracellular Ci concentration and CO2 availability to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). However, above certain strain-specific concentrations, CO2 can suppress cyanobacterial growth, whereas the suppression mechanism is not well understood. In Synechocystis sp. PCC 6803 (S. 6803), CCM comprises a permanently-expressed, low-affinity, high-flux sodium/bicarbonate symporter BicA. Overexpression of BicA mutated to retain its activity at high CO2 concentrations decreased the CO2 concentration threshold, which limits growth of the extra-BicA mutant. Suppression of BicA activity in the mutant and wild type S. 6803 strains increased tolerance of both to higher CO2 concentrations. These results corroborate our hypothesis that at high Ci concentration BicA activity adversely effects cyanobacterial growth.

644 / LIGNOCELLULOSE-DEGRADING POTENTIAL OF NOVEL ACIDOPHILIC FUNGI

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Switching to a sustainable bio-based economy encompasses harnessing the power of microorganisms and turning waste streams into value-added products and energy. Residual lignocellulosic biomass from agricultural or forestry by-products can serve as an abundant feedstock for this purpose. However, for it to be feasible, a pretreatment step to overcome the recalcitrant nature of biomass is necessary. This step typically either uses acids as catalysts or produces acids as by-products. The resulting low pH inhibits the downstream steps of biomass valorization. Utilizing acidophilic biocatalysts for the breakdown of pretreated lignocellulosic biomass may prove an efficient way to overcome this hurdle and skip the washing steps that make the process less sustainable.

In the present project the wide tropical fungal biodiversity of Vietnam has been assessed by isolating and molecularly characterizing hundreds new strains from various sources. Among them, there were acidophilic strains with lignocellulose-degrading potential. These strains are thus perfect candidates for industrially relevant applications. Our goal now is to study the enzymatic mechanisms by which novel acidophilic fungi can degrade lignocellulose, and thereby discover new efficient biocatalysts.

656 / LACTOBACILLUS VAGINALIS BC17 DERIVATIVES EXERT BIFIDOGENIC ACTIVITY ON-DEMAND POSTER - BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY

Keywords: *vaginal lactobacilli, bifidogenic activity, postbiotics , paraprobiotics*

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Bifidobacteria colonize the intestine of the newborn immediately after birth. This occurs especially in babies born vaginally, while in infant born by c-section the presence of bifidobacteria drops dramatically, increasing the risk of developing gastrointestinal disorders [1]. Considering that newborns naturally come in contact with maternal lactobacilli as they pass through the birth canal, the aim of this work is to exploit for the first time the bifidogenic activity exerted by a Lactobacillus of vaginal origin (L. vaginalis BC17) [2]. Here, we focused on cell-free supernatants (CFS) and heat-killed cells (HKC) as postbiotics and paraprobiotics, respectively [3, 4].

CFS was recovered by filtering the supernatant of BC17 incubated for 7h, 13h or 24h [2], whilst 24 h-cultured BC17 cells were suspended in sterile saline (10^7 CFU/mL) and heat-treated (70°C for 2h for 3 times) to obtain HKC [5]. CFS and HKC were sought for the ability to stimulate the planktonic growth and the biofilm formation of 11 Bifidobacterium strains through turbidimetric method and crystal violet staining [6], respectively.

We found that the proliferation of bifidobacteria was strongly improved by both CFS, especially by 7h-CFS (324-523%), and HKC (122-443%). Moreover, the incubation of bifidobacteria with CFS and HKC also favored the biofilm formation (232-396% and 144-311%, respectively). Importantly, no stimulating effects were observed on intestinal Escherichia coli strains, indicating that BC17 derivatives can represent a valid alternative to selectively promote the growth of beneficial bacteria.

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661 / CHARACTERIZATION OF PROPITIOUS MICROBIAL ISOLATES FROM VARIOUS ENVIRONMENTS FOR THEIR BENEFICIAL PLANT GROWTH PROMOTING TRAITS

Keywords: *plant growth promoting bacteria, abiotic stress, soil, microorganisms*

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BACKGROUND

Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms. The variety of conditions may influence the evolution of microorganisms and shape their phenotype. Especially the soil, which is the main niche of PGPB is very heterogeneous in terms of nutrients, pH and particle size. Bacteria origin deferred due to various environmental traits such as drought or salinity stress.

OBJECTIVES

The aim of the research was to obtain PGPB candidates able to survive different abiotic stresses.

METHODS

Different bacterial strains were isolated from: bulk soil and vegetables samples collected from agricultural lands in north-eastern Poland; sand from Pustynia Błędowska and Dead Sea mud from Israel.

The analysis of selected PGPB properties was carried out among 33 bacterial isolates based on:

(i) qualitative estimationof phosphate (P) (ii) potassium (K) and (iii) zinc (Zn) solubilization activity using plate tests; (iv) quantitative colorimetric estimation of auxin production and (v) qualitative estimation of siderophores production.

Moreover, bacterial strains have been identified based on the 16S rRNA gene sequencing, and then a phylogenetic analysis was performed.

RESULTS

81.8%, 75.8% and 61% of tested 33 bacterial isolates were capable of P, K and Zn solubilization, respectively. Klebsiella spp. AF3II1 showed the highest P and K solubilization, while Serratia plymuthica EDC15 and Pseudomonas putida AF1I1 showed the highest auxin and siderophores production ability, respectively. The obtained data indicates that the isolates from different origin differ in PGP properties.

ACKNOWLEDGEMENTS/REFERENCES

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685 / EFFECT OF CARBON SOURCES AND TEMPERATURE ON MICROBIAL POPULATIONS IN BIOLEACHING REACTORS

Keywords: *bioleaching, molecular analysis*

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We studied the effect of additional carbon sources on the microbial community during the continuous biooxidation of gold-bearing pyrite-arsenopyrite flotation concentrate. Biooxidation was carried out in laboratory reactors in a continuous mode at 40, 45, and 50°C, the residence time was 6 days. Molasses or CO2 were added as a carbon source in two bioreactors while the third reactor was used as control. Results of 16S rRNA gene profiling revealed that at 40°C the addition of carbon sources had almost no effect on the composition of microbial communities. At 45°C, the relative abundancies of Acidithiobacillus, Ferroplasma, and Acidiplasma increased in all three reactors. Addition of CO2 also increased the shares of Cuniculiplasma and A-plasma archaea. At 50°C Acidithiobacillus, Sulfobacillus and Acidiplasma dominated in the communities. Notably at 45°C and 50°C the total concentration of microbial cells in CO2-supplemented reactor was 2-3 times higher than in other reactors. Metagenome-assembled genomes were obtained for Acidithiobacillus caldus and five archaea of the phylum Thermoplasmatota. The analysis of the metagenome-assembled genomes enabled to reconstruct their central metabolism. In the microbial communities, biooxidation of sulfide minerals was enabled by the activity of Acidiplasma sp., Sulfobacillus, Ferroplasma and Acidithiobacillus caldus. The obtained data on the influence of various carbon sources and temperature on the composition of the microbial community performing biooxidation of sulfide concentrates is the basis for increasing the efficiency of the bioleaching process by targeting the community.

ACKNOWLEDGEMENTS/REFERENCES

The work was supported by the Russian Science Foundation grant No. 21-64-00019.

694 / CHARACTERIZATION OF A NOVEL MARINE POLYHYDROXYALKANOATE - PRODUCING STRAIN OF THE LEISINGERA GENUS

Keywords: *bioplastic, marine bacteria, crude glycerol, draft genome*

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Due to its ubiquitous use, increasing amounts of plastic waste are accumulating everywhere in the environment. Replacement of petroleum-based plastic with biopolymers, such as polyhydroxyalkanoates (PHA), which are completely biodegradable, could be a viable solution. For this reason, efficient production of PHA, through using higher-yield strains and cheap biomass as a carbon source, need to be improved. In this aim, a marine bacterial strain, C2.10, previously screened positive for PHA granule accumulation, was phenotypically and genotypically characterized for bioplastic production.

Using Biolog Phenotypic Microarray plates, the strain was tested for the ability to assimilate various carbon and nitrogen sources, and its pH tolerance range. After optimizing genomic DNA isolation, the genome was sequenced using the Illumina platform. For quantification of PHA production, shake flask cultivations were carried out at 30°C on 2% pure or biodiesel-derived (crude) glycerol.

Biolog profiling showed highest preference for glucose and disaccharides, organic acids, and glycerol assimilation. Genome annotation revealed all genes involved in class I PHA synthesis (phaABC) and metabolism (phaP, phaR, and phaZ), and all genes necessary for fatty acid catabolism. 16S rRNA gene sequence analysis places strain C2.10 in the Leisingera genus. TYGS in silico hybridization results indicated that C2.10 was significantly different from existing Leisingera strain genomes. Shake flask cultivation yielded a four-fold higher cell dry weight (CDW) using crude glycerol in comparison to pure glycerol. PHA accumulation in the cell reached more than 67% of the CDW, indicating the strain's high potential for bioplastic production from cheap biomass.

ACKNOWLEDGEMENTS/REFERENCES

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695 / BIOGENIC GOLD NANOPARTICLES FROM STREPTOMYCES SP. M137-2 AS VACCINE ADJUVANT

Keywords: *Gold nanoparticles, Streptomyces, Green synthesis, Adjuvanticity*

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Biosynthesis of gold nanoparticles (AuNPs), called biogenic AuNPs that provides the synthesis of more stable and monodisperse AuNPs, non-toxic chemical, environmentally friendly and cost-effective instead of conventional synthesis methods has been attaining a surge of interest in recent years. Herein we describe its adjuvanticity by comparing microbiologically synthesized AuNPs with alum, a widely used commercial adjuvant.

In our previous study, the synthesis of AuNPs, which are spheres around 60 nm was performed using cell-free fermentation broth of Streptomyces sp. M137-2. In present study, bovine serum albumin (BSA) as the model protein antigen was conjugated onto AuNPs, and BSA-conjugated AuNPs were characterized by UV-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Moreover, the cytotoxicity, TNF-α and IL-6 cytokine levels and cellular uptake of BSA-conjugated gold nanoparticles were compared with BSA absorbed onto alum, in vitro studies used human monocytic cell line (U937 cells) differentiated into macrophages.

In the cytotoxicity assay, BSA-conjugated AuNPs and alum showed high viability (over 70%) in the macrophage cells. TNF-α and IL-6 cytokine levels of the macrophage cells induced with BSA-conjugated AuNPs were 3-fold ($p<0.05$) and 2-fold ($p<0.05$) higher than that induced by BSA absorbed onto alum, respectively. The adjuvanticity of the biogenic AuNPs was likely due to induce strong inflammatory response and increase the uptake of the antigen by the macrophage cells. This study is the first report that biogenic AuNPs synthesis by microorganism has the potential to serve as vaccine adjuvant.

ACKNOWLEDGEMENTS/REFERENCES

This study was financially supported by Aliye Üster Foundation

712 / SECRETOME ANALYSIS OF ABORTIPORUS BIENNIS GROWN ON CORN STOVER REVEALED TWO NOVEL LACCASES WITH SUPERIOR PROPERTIES.

Keywords: *Abortiporus biennis, laccase, secretomics, plastics degradation*

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BACKGROUND

White-rot basidiomycetes are the only microorganisms with the ability to produce both hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) enzymes for degrading all the constituents of lignocellulosic biomass. Their rich enzymatic machinery makes them ideal for the targeted discovery of novel enzymes with desirable properties.

OBJECTIVES

In the present work, Abortiporus biennis, a white-rot fungus isolated from a Greek habitat, was studied in regard to its lignocellulolytic enzymatic potential.

METHODS

The strain was grown in corn stover, a common agroindustrial residue, and the production of lignocellulose-acting enzymes was studied with standard biochemical methods, as well as secretome analyses. Two novel laccases, AbiLac1 and AbiLac2, were isolated from the culture supernatant of this fungus with ion-exchange chromatographic methods.

RESULTS

The results revealed the presence of all the necessary enzymatic activities for complete breakdown of the lignocellulosic substrate, while the prominent role of the oxidative enzymes in the lignocellulolytic strategy of white rot fungi became apparent. Biochemical characterization of purified laccases revealed that they are able to oxidize a wide variety of phenolic and non-phenolic substrates. AbiLac1 was found to oxidize polystyrene powder and the degradation yield was evaluated by variation in molecular weight. The enzyme showed high depolymerization potential, so current efforts primarily focus on expanding the scope of polymers treated. Overall, the results of the present study demonstrate the prominent biotechnological potential of the unexplored enzymatic machinery of white-rot Basidiomycetes from Greek habitats.

ACKNOWLEDGEMENTS/REFERENCES

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733 / ISOLATION AND CHARACTERIZATION OF CARBAZOLE DIOXYGENASE DRIVING THE TRANSFORMATION OF THIABENDAZOLE

Keywords: *thiabendazole, carbazole dioxygenase*

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Thiabendazole (TBZ) is a benzimidazole fungicide used for the control of postharvest fungal infestation of fruits. Monitoring programs in Europe showed that thiabendazole is commonly detected in fruits at levels entailing an unacceptable risk for consumers, and it constitutes one of the most common pollutants of surface water systems in the Mediterranean region. Therefore, there is a need for immediate action to limit its dispersal. Previous work in our lab demonstrated the efficient degradation of the fungicide TBZ by a bacterial consortium and showed the key role of Sphingomonas in its degradation. Meta-transcriptomic and meta-proteomic analysis suggested that Sphingomonas activate a carbazole dioxygenase operon during the initial cleavage of TBZ. Carbazole is an N-heterocyclic aromatic hydrocarbon, structurally similar to TBZ. The objective of this study is to confirm the role of carbazole dioxygenase, a multicomponent enzyme consisting of terminal oxygenase (CarAa), a ferredoxin (CarAc), and a ferredoxin reductase (CarAd) unit, on the transformation of TBZ. Each enzymatic component was overexpressed in Escherichia coli strain BL21 (DE3) with histidine tag and was purified. The functionality of the purified proteins in the degradation of thiabendazole is currently tested at in vitro assays and the results will be reported in the conference. This multicomponent enzyme will be the first one reported able to detoxify TBZ. The application of this enzyme in appropriate formulations would be an innovative approach for the removal of thiabendazole from agro-food produce and the environment.

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735 / A NOVEL APPROACH FOR IN SILICO DESIGN OF DNA VACCINE CANDIDATE AGAINST LATENT TOXOPLASMOSIS

Keywords: *Toxoplasma gondii, latent toxoplasmosis, DNA vaccine, SRS13*

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Latent toxoplasmosis caused by Toxoplasma gondii (T. gondii), which infects one third of the world's population, is characterized by tissue cysts in brain resulting in headache, confusion, seizures and other neurological symptoms. There has been attaining a surge of interest in targeting tissue cysts in order to develop new therapeutics for toxoplasmosis recently. In our group's previously study, the cyst wall protein SRS13 was found to be highly immunogenic by protein microarray screening. Herein, we describe a design of DNA vaccine encoding SRS13, which has high stability and immunogenicity using in silico methods. Using this novel approach T and B cell epitopes of SRS13 extracellular domain were evaluated by in silico methods for antigenicity, allergenicity, toxicity, stability and solubility. The three-dimensional structure was predicted and validated with bioinformatics tools, and molecular docking analysis for MHCI and MHCII receptors was performed. Based on in silico analysis, we have constructed the DNA vaccine candidate with seven rich-epitopes domain including highly scored T and B-cell epitopes. Docking studies demonstrated that the vaccine has persistent interactions with MHC Receptors. Finally, the predicted SRS13 construct was incorporated into the pVAX1 vector to generate a DNA vaccine. This highly immunogenic DNA vaccine can be considered as a promising vaccine candidate against latent toxoplasmosis for further in vitro and in vivo studies.

747 / COMPARISON OF TWO CLONING SYSTEMS FOR OENOCOCCUS OENI 229M GENES IN E. COLI AND THEIR IMPACT ON PROTEIN YIELD AND LACCASE ACTIV.

Keywords: *Bacterial laccases, Cloning vectors, Cloning systems*

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Bacterial laccases are multicopper oxidase enzymes with potential in wine industry as biological tools for degrading toxic and undesirable compounds. E. coli DH5α has been widely used for cloning different genes, including bacterial laccases inserts, due to its fast-growing, easy manipulation and its large number of plasmids used as cloning vectors, among them pET28a and pETNKI [1], compared in this work. Both plasmids express a His-Tag tail with Ni-affinity in the laccase protein, helpful for its purification by column chromatography. PET28a has been successfully employed for cloning laccase genes [2], while pETNKI is more versatile given that many genes can be cloned without needing specific restriction enzymes or ligases. In addition, pETNKI has a hydrolysis site for a lab-easy-to-produce protease called PreScission to remove the His-Tag tail in the attached protein.

The objective of this work was to compare two cloning systems regarding the clones with insert percentage and enzymatic activity using either pET28a (5369 bp) [2] or pETNKI (5343 bp) [3] vectors for cloning the laccase gene of Oenococcus oeni 229M (ENOLAB 4002) in E. coli DH5α.

Transformation of the vector+insert was performed by electroporation at 1.7 V, obtaining 12.5% of clones with insert with pETNKI and 61.1% with pET28a. 3.7 mg of the purified protein was obtained from 2 L of cell culture with the pET28a cloning method, and its specific activity was 12.12 U/mg over ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) substrate, while the result for pETNKI is about to be obtained in order to compare widely both methods.

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753 / BIOCATALYTIC PROPERTIES OF TWO STREPTOMYCES SPP. CO-ISOLATED FROM RHIZOSPHERE SOIL OF PAPAVER RHOEAS

Keywords: *Streptomyces, biocatalysis, co-culture, heterologous expression*

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Although Streptomycetes are widely known as producers of antibiotics and other secondary metabolites, their biocatalytic potential should not be overlooked. A variety of industrially important enzymes originate from this genus, which are very significant in the era of biotechnological development. Considering that certain petrochemical polymers require a combination of enzymes for their degradation, utilization of Streptomycetes with enhanced biocatalytic properties could present an adequate solution for their biodegradation.

The aim of this work was to explore biocatalytic potential of two co-isolated Streptomyces spp., grown as individual strains or in co-culture. Additionally, these strains were used as hosts for heterologous expression of additional enzymes in order to improve their biocatalytic potential.

A screening for biocatalytic activities was performed on agar plates supplemented with specific substrates. Strains were evaluated for several enzymatic activities including different hydrolases and oxidoreductases. Additionally, strains were tested for their plastic-degrading potential on several monomers and polymers and post-consumer textiles. After incubation period activities were noted either as pigmentation or clear or depigmented halo around colonies. Genes for additional enzymes (esterases and laccases) were introduced into these strains in-trans, on expression plasmids, in order to boost their biocatalytic properties.

Obtained results confirmed high hydrolytic activity and presence of laccases, and for some enzymes the notable difference in efficiency was observed when two Streptomyces spp. were grown in co-culture. Also, these strains were recognized as potential suitable hosts for heterologous expression of enzymes that could be used for biodegradation of polymeric materials including textiles.

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760 / ISOLATION OF SPONGE-ASSOCIATED ACTINOBACTERIA FOR BIOPROSPECTION OF NOVEL NATURAL COMPOUNDS

03

Keywords: *Natural Products, Actinobacteria, Bioprospection, Bioactive Metabolites*

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Actinobacteria are commonly found as part of the rich microbiome of marine sponges (Phylum Porifera). Various species of these microorganisms have been shown to be prolific sources of metabolites valuable for drug development. This work aimed to isolate and identify sponge-associated Actinobacteria for future bioprospection of novel natural bioactive compounds. Nine marine sponges were collected in two underexplored regions: Maputo, Mozambique and off-coast Ovar, Portugal. The sponges from Mozambique were identified as Haliclona sp. (n=2), Psammocinia sp. (n=3) and Callyspongia perforata (n=1), whereas those from Portugal were identified as Cliona cf. celata (n=1), Axinella sp. (n=1) and Desmacidon fruticosum (n=1). In total, over 240 bacterial strains were recovered from these sponges. Several genera of Actinobacteria were identified, including Micromonospora and Streptomyces, which are characterized by their high ability to produce a great variety of bioactive compounds [1]. Species of rare and generally unexplored Actinobacteria such as Micrococcus, Brachybacterium, Kocuria, Dietzia, Mycolicibacterium, Williamsia, Rothia, Nesterenkonia and Kytococcus were also recovered. A potential new species of Mycolicibacterium has been isolated as well. In conclusion, a high number of isolates and a great variety of Actinobacteria were isolated from the analyzed marine sponges, including a potential new species. These isolates constitute a promising source for the bioprospection of novel bioactive secondary metabolites.

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767 / INCREASING THE EFFICIENCY OF PIR2-BASED YEAST SURFACE DISPLAY SYSTEM

Keywords: *recombinant proteins, surface display, Pir2*

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BACKGROUND

Saccharomyces cerevisiae is frequently used as a host for the production of recombinant proteins. S. cerevisiae also provides the immobilization of recombinant proteins on the cell surface as an alternative to conventional immobilization methods. Yeast surface display systems are based on genetic fusion of a gene coding for a protein of interest and a gene coding for one of yeast cell wall proteins. Since immobilization of the protein through its N- or C-terminal end can affect its activity, different surface display systems were developed. Proteins of the Pir family are standardly used for N-terminal immobilization of recombinant proteins.

OBJECTIVES

Pir-based display systems developed so far involve fusion of the whole Pir protein with the desired protein, resulting in a large recombinant protein whose expression cause problems in its secretion and cell wall binding. To address this problem, we introduced changes in the Pir2 sequence and the protein secretion mechanisms to increase the efficiency of the Pir2-based surface display system.

METHODS

Using bacterial β-lactamase as a reporter enzyme, we assessed the efficiency of surface display of non-modified and modified recombinant protein Pir2bla in different yeast mutants. Amount of protein incorporated in cell wall was assessed by measurement of β-lactamase activity and by western blot.

RESULTS

Results show higher β-lactamase activity in strains expressing recombinant proteins containing the modified Pir2 binding region, lacking multiple internal repeats, and an increase of activity in most of the tested strains lacking activity of certain genes involved in endoplasmic reticulum-associated degradation (ERAD) pathways, or endocytosis.

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771 / THE PROSPECTS OF ALCOHOL PRODUCTION WASTE UTILIZATION USING GREEN ALGAE

Keywords: *Chlorella vulgaris, waste utilization, H2 production*

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BACKGROUND

Application of green algae in large scale industry has a potential of becoming the future of green biotechnology. Opportunities include, but not limited to industrial waste utilization with possible use of produced algal biomass for bulk chemical production, as biofertilizer or for production of green energy, which is specifically of interest considering the current global environmental challenges. Industrial wastes like brewery spent grains (BSG) and cereal distiller's grains with solubles (DGS) contain various carbon and nitrogen sources and trace elements, which are essential for microbial growth and can be used as great sources for H2 generation by green algae.

OBJECTIVES

To analyze the potential of green algae as agents for alcohol production waste utilization, biomass and H2 production by green algae Chlorella vulgaris Pa-023 was performed using DGS and BSG as substrates.

METHODS

The growth of microorganisms was monitored by changes in the optical density and the H2 yield was recorded by the decrease of redox potential to low negative values during microbial growth.

RESULTS

Though the algae used showed varying efficacy utilizing different types of alcohol production wastes, both biomass and H2 yield in waste containing media were enhanced compared to control Tamiya medium in some cases reaching ~2-3 times increase. The substrates used required pre-treatment for effective utilization by algae, including appropriate dilutions. The technology of the process may be in early stage of research, but the results obtained suggest a potential for future application.

813 / GROWTH INHIBITION BY MALTOSE IN XYLOSE-FERMENTING SACCHAROMYCES CEREVISIAE

Keywords: *Xylose, Maltose inhibition, Osmotic stress, Saccharomyces cerevisiae*

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Lignocellulosic biomass widely existing on the earth is a pivotal feedstock used to produce sustainable energy and platform chemicals. Fermentable sugar mixtures produced by pretreatment of lignocellulose make xylose or glucose-xylose (co-)consumption an urgent problem. Maltose has been found to inhibit xylose-metabolism in the specialist strain IMX730 lacking the genes encoding for hexokinase [1], but inhibition also occurs in its ancestor CEN.PK 2-1D, albeit with weaker growth defects. The effect cannot be attributed to a transport deficiency as xylose uptake is insensitive to maltose inhibition. However, the inhibitory effect is augmented in a IMX730 strain devoid of maltase (dMal), while deletion of all maltose transporter genes protects cells from maltose inhibition. These data suggest that intracellular maltose exerts an inhibitory effect on xylose metabolism.

The effect is not unique for xylose metabolism, as it occurs also for other sugars to different degrees. Maltose uptake assays indicate the accumulation of high intracellular maltose levels in the dMal strain. Comparative transcriptome analysis of dMal under maltose shock indicates the upregulation genes involved in calcium signaling and cell wall stress. Additionally, the expression of genes participating in cell cycle and DNA replication stress is affected consistent with the observation of growth arrest. It is hypothesized that the high levels of intracellular maltose exert a general inhibitory effect of metabolism possibility related to high intracellular osmolarity.

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835 / HYDROGEN PHOTOPRODUCTION BY NITROGEN-DEPRIVED MICROALGAE PARACHLORELLA KESSLERI AND CHLORELLA KESSLERI

Keywords: *Green microalgae, Hydrogen photoproduction, Nitrogen deprivation*

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Microalgae are promising objects of biotechnology due to their diversity, high growth rates and low production costs. They are widely used as food supplements, plant growth stimulators, and as removers of pollutants from industrial wastewater. Microalgae are able to produce biologically active compounds and to generate hydrogen (H2), which is of interest in biofuels technology. In this work the comparative analysis of H2 photoproduction by Parachlorella kessleri and Chlorella kessleri under nitrogen deprivation has been done. P. kessleri RA-002 (Microbial Depository Center, NAS, Yerevan, Armenia) and C. kessleri IBCE C-3 (Algae Collection, Institute of Biophysics and Cellular Engineering, NAS, Minsk, Belarus) were cultivated in standard Tamiya medium upon continuous illumination. Nitrogen-deprived media were prepared by omitting KNO3 from Tamiya medium. The H2 production was observed in both algae under nitrogen-deprived conditions. The highest H2 yield was detected in P. kessleri, which was 5 fold higher in comparison with control cells, cultivated without nitrogen deprivation. Moreover, H2 production by P. kessleri was ~1.7 fold higher than by C. kessleri. The use of photosystem (PS) II inhibitor, diuron demonstrated that H2 production by these algae under nitrogen-deprivation relies on the PS II activity. Photochemical activity of PS II decreased under nitrogen deprivation leading to anaerobic conditions formation. The data indicate that nitrogen deprivation triggers H2 generation via creation of anaerobic conditions stimulating H2-producing enzymes, such as Fe-hydrogenase. Thus, nitrogen deprivation is an effective way to enhance H2 generation and can provide the potential applications of green algae in biofuel technology.

ACKNOWLEDGEMENTS/REFERENCES

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845 / THE EFFECT OF GREEN EXTRACTION METHOD, SUBCRITICAL, WATER EXTRACTION, ON SELECTED BIOLOGICAL ACTIVITIES OF CHAGA MUSHROOM

Keywords: *biological activities*

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Inonotus obliquus, or Chaga, is a medicinal mushroom with a long history of folk medicinal use and growing popularity worldwide. It cannot be cultivated so novel, greener extraction methods are needed to satisfy the market and obtain high-quality supplements. In this study, we evaluated the biological activity (antimicrobial and antioxidant) of Mongolian (IM) and Serbian (IS) Chaga extracts obtained by subcritical water extraction. They were also screened for general chemical composition. Both IM and IS, have high phenolic content. HPLC analysis revealed that extracts are especially rich in chlorogenic acid, followed by catechins, p-coumaric and cinnamic acids.

ABTS assay confirmed the remarkable scavenging ability of extracts, reaching 98% when a concentration of 2 mg/mL was tested. The antimicrobial potential was examined by microdilution method using Gram-positive and (Enterococcus faecalis and Staphylococcus aureus), Gram-negative bacteria (Escherichia coli and Acti-netobacteria baumannii). According to the obtained minimal inhibitory concentration (MIC) for E. faecalis S. aureus (MIC -1.25 mg / mL), E. coli, and A. baumannii (MIC -2.5 mg / mL) both extracts were shown to have the same effect on the tested bacteria. Hereby, we demonstrated the high biological potential of extracts obtained by subcritical water extraction.

ACKNOWLEDGEMENTS/REFERENCES

The Science Fund of the Republic of Serbia (project IDEAS-AniNutBioMedClays) supported this work

907 / UNLOCKING THE DIVERSITY AND BIOACTIVE POTENTIAL OF SEAWEED-ASSOCIATED ACTINOBACTERIA

Keywords: *Marine Actinobacteria, Natural Products, Seaweed, Bioactivity*

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Microbial secondary metabolism from untapped marine sources represents a valuable route to uncover chemical novelty. Actinobacteria, elite class drug producers, often live in association with marine organisms, as seaweeds, and such promising – yet poorly explored – symbiosis might translate into the synthesis of exclusive natural products. A collection of over 470 seaweed-associated Actinobacteria, recovered from three macroalgae species collected from Portuguese shores, was screened for the production of antimicrobial and/or anticancer compounds. Although dominated by Streptomyces, our collection covers 26 other actinobacterial genera, including some rare and new taxa. About 34% of the crude extracts obtained from these microorganisms showed positive results in the bioactivity assays: 119 extracts were able to inhibit the growth of Candida albicans, Bacillus subtilis and/or Staphylococcus aureus; 92 extracts revealed cytotoxicity towards the human cancer cell lines breast carcinoma T-47D, colorectal carcinoma HCT116 or neuroblastoma SH-SY5Y, with several of them causing no deleterious effect on the non-cancerogenic cell line hCMEC/D3, translating into a possible anticancer asset. The extracts are currently being screened for anti-obesity activity using zebrafish larvae. Although dereplication data indicated the presence of well-known metabolites in most of the active extracts, it also revealed the likely presence in some extracts of novel bioactive compounds that are being isolated following a bioactivity-guided pipeline. Results obtained so far highlight not only the rich diversity of seaweed-associated Actinobacteria but also their value as a potential source of biotechnologically-relevant molecules to address urgent clinical problems, such as infections caused by antibiotic multi-resistant bacteria or cancer.

ACKNOWLEDGEMENTS/REFERENCES

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926 / THE INFLUENCE OF CHROMATE ON THE RESPIRATION ACTIVITY OF BAKER'S AND BREWER'S YEAST

Keywords: *Saccharomyces cerevisiae, Respiration*

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Metals are widespread in nature and organisms are constantly exposed to them through a variety of sources, therefore, there is a need for microorganisms to have the ability to respond to potentially toxic metals in their environment.One of such metals is chromium (Cr), and the toxicity of Cr is dependent on oxidation state and solubility. Chromate (Cr(VI)) is a powerful oxidizing agent, and is more toxic then Cr (III).

The aim of this work was to determine the influence of chromate on the respiration and enzymatic activity of baker's and brewer's yeast, Saccharomyces cerevisiae.

The baker's and brewer's yeast were grown overnight in a liquid medium containing 400 µM and 600 µM Cr(VI). The effects of chromate on respiration were analyzed using a Micro-Oxymax respirometer (Columbus Instruments, USA). Additionally, the specific activity of superoxide dismutase (SOD) was measured. All the results obtained were compared to the control samples which were grown in the liquid medium without Cr.

The results suggest that the concentrations of chromate used were not sufficient to affect the respiration activity in both baker's and brewer's yeast; however, some effects are visible on the enzymatic level. The specific activity of SOD in brewer's yeast decreases with the increase of Cr(VI), whereas in baker's yeast the enzyme remains active. In baker's yeast there is also an increase in SOD activity in the presence of 400 µM Cr(VI), while the activity in the presence of 600 µM Cr(VI) is similar to the control sample.

930 / WINE GRAPE WASTE APPLICATION FOR ESCHERICHIA COLI BIOMASS AND H2 PRODUCTION

Keywords: *Escherichia coli, biohydrogen, wine grape waste*

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Wine grape waste (WGW), composed of lignocellulose, proteins, organic acids, etc., is a promising source for microbial cultivation and biohydrogen production. Escherichia coli can utilize different organic compounds and produce molecular hydrogen (H2) by the operation of hydrogenase enzymes.

Growth parameters, H2 production, oxidation-reduction potential (ORP) kinetics of E. coli BW25113 wild type were investigated upon utilization of WGW with different dilutions.

ORP and H2 production of bacterial cultures were studied with the platinum (Pt) and titanium-sili¬cate (Ti-Si) redox electrodes. Physicochemical pretreatment of WGW was done using 0.4 % H2SO4, at 121 °C. K2HPO4 and KOH were used for the pH adjustment of the mediums.

Bacteria were grown anaerobically on 0-4-fold diluted WGW mediums, pH 7.5. The significant decrease of readings of Pt electrode from positive up to low negative (-440 to -560 mV) values has been shown after 24 h, which indicates H2 production during bacterial growth in both 2- and 4-fold diluted WGW mediums. Compared to the non-diluted WGW medium, 1.2-fold higher (2.14±0.07 mmol L-1) H2 yield was observed in a 2-fold diluted medium. Less acidification, prolonged H2 production, and high biomass formation (OD600,1.5) were observed in a 2-fold diluted medium after 48 h when instead of KOH pH was controlled using K2HPO4. The obtained results showed the importance of the high buffering capacity of the medium for H2 and biomass formation. Consequently, the possibility of using inexpensive side-streams of the dairy industry is a perspective for economical biomass and biohydrogen production.

ACKNOWLEDGEMENTS/REFERENCES

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933 / SURFACTIN PRODUCTION BY BACILLUS VELEZENSIS IN THE CONTEXT OF CIRCULAR ECONOMY

Keywords: *surfactin, biocontrol, bioprocess, circular economy*

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The antibiosis and the systemic induction of plant resistance are recognized as key elements of the Bacillus species' plant-beneficial activity. Surfactin as a Bacillus-derived lipopeptide has been extensively studied due to its strong tensioactive and antimicrobial activity, as well as the role in plant colonization, as an important aspect in biological control. Industrial production of biocontrol agents could benefit from the circular economy approach in terms of industrial waste utilization as a substrate, especially considering production cost affecting the final product price and market competitiveness. The aim of this study was to investigate the potential of industrial waste as a medium for the cultivation of Bacillus velezensis IP22, previously designated as an effective biocontrol agent. The cultivation medium was based on raw glycerol from biodiesel production performed using two different raw materials: waste frying oil and cold-pressed rapeseed oil. The overall cultivation broths were used for in vitro antimicrobial activity testing against Xanthomonas spp. isolated from diseased cabbage and pepper. The HPLC-MS (High-Pressure Liquid Chromatography-Mass Spectrometry) analysis confirmed the presence of three lipopeptides from the surfactin family in the cultivation broth supernatant, while the obtained inhibition diameters indicated the synergistic effect of bacterial biomass and produced extracellular metabolites against the tested phytopathogens. The results of this study pointed out the multi-mechanism biocontrol potential of the investigated strain, indicating the necessity for further bioprocess optimization to maximize surfactin and bacterial biomass yield, and techno-economic evaluation of the potential industrial level bioprocess scale-up.

ACKNOWLEDGEMENTS/REFERENCES

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935 / APPLICATION OF MICROBIAL INOCULANTS UPSTREAM OF THE COMPOSTING PROCESS TO ACTIVATE THE BIODEGRADABILITY OF PLANT RESIDUES

Keywords: *Composting, Lignocellulose, Biodegradability*

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Composting is considered an effective strategy to transform plant residues into a sustainable product of high agronomic value. Microorganisms play a crucial role in this process since they drive the transformation and degradation of organic matter thanks to their enzymatic versatility. However, the structure of lignocellulose involves a great challenge to the microbiota inhabiting a compost pile. Considering the above, the main objective of this work was the application of lignocellulolytic microorganisms to increase the biodegradability of lignocellulosic wastes in a step prior to the composting process.

For this purpose, a collection of bacteria and fungi was characterized in terms of their in vitro capacity to degrade cellulose, hemicellulose and lignin. The strains that showed the best capabilities were identified using molecular techniques. Then, a protocol for the application of the selected strains on plant wastes was designed to optimize the dose and incubation time. Finally, the effect of the microbial inoculation was tested by fiber analysis (cellulose, hemicellulose and lignin) and quantification of oxygen consumption (DRI24 and AT4).

Among the best biotechnological tools, two strains of Bacillus spp. and other one of Fusarium oxysporum were identified. The results related to fiber composition and respirometric parameters showed a clear influence of the applied doses and incubation time, although this effect was variable according to the strain applied. Therefore, this work would represent an advance in relation to the optimization of the composting process, providing new solutions for the effective treatment of lignocellulosic waste.

ACKNOWLEDGEMENTS/REFERENCES

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939 / A NOVEL CE16 ACETYL ESTERASE FROM THERMOTHELOMYCES THERMOPHILUS

Keywords: *acetyl esterase, hemicellulose degradation xylanase-esterase synergy*

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BACKGROUND

The biotechnological utilization of hemicellulose, though appealing, is usually obstructed by its complicate structure, necessitating a variety of enzymes towards decomposition. Acetyl substitutions are common decorations of the xylan backbone, esterifying up to 70% of the xylopyranose residues (xylp). Thus, the use of acetyl xylan esterases (AXEs) has already been established. Esterases of the CE16 family are of particular interest due to their specificity against 3-O-acetylation of non-reducing end xylps O-2-substituted by 4-O-methyl-glucopyranosyluronic acid, which is one of the most recalcitrant units of the polymeric xylan.

OBJECTIVES

The mode of action of CE16 esterases is not yet fully elucidated. The unique functional characteristics of TtCE16 as novel biocatalyst could provide new insights on enzymatic bioconversion of hemicellulose towards circular bioeconomy.

METHODS

TtCE16 acetyl esterase from Thermothelomyces thermophilus was heterologously expressed in Pichia pastoris and was biochemically and physicochemically characterized. A comparative study between TtCE16 and AXEs of known specificities, was performed. Moreover, the synergistic relationships between the esterases and different families of xylanases was investigated upon hydrolysis of pretreated lignocellulosic substrates. Structural studies have also been implemented. TtCE16 has been crystallized in space group P3121 and X-ray diffraction data have been collected to 1.9 Å resolution. The structure of TtCE16 is currently under determination, applying the molecular replacement technique.

RESULTS

The TtCE16 esterase exhibits activity against acetylated xylo-oligosaccharides. The specificity of the enzyme is complementary to a CE6 AXE. The xylanase-esterase synergy with TtCE16 was pronounced, especially when the esterase was combined with other AXEs.

ACKNOWLEDGEMENTS/REFERENCES

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965 / RUSTICYANIN AND SULFOCYANIN HOMOLOGS IN ALICYCLOBACILLUS SP. S09 AND OTHER FIRMICUTES

Keywords: *iron oxidation, rusticyanin, Firmicutes, acidophiles*

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Rusticyanin is a blue copper protein known to be involved in iron oxidation by extremely acidophilic Proteobacteria such as Acidithiobacillus ferrooxidans, At. ferridurans and Acidihalobacter prosperus. The related sulfocyanin has been reported to be involved in sulfur oxidation by the archaeon Sulfolobus acidocaldarius.

Based on interests in bioleaching of sulfidic minerals in presence of chloride, we have recently isolated the acidophilic and moderately halophilic Firmicutes strain S09. It obviously represents a new species of the genus Alicyclobacillus. In order to identify possible genes related to iron and sulfur oxidation on the genome of Alicyclobacillus sp. S09 and on genomes of other Firmicutes, the genes for rusticyanin A and B of At. ferrooxidans and for sulfocyanin of Sl. acidocaldarius and Sulfobacillus thermosulfidooxidans served as queries.

Most of the sequences found to share high similarity to the queries originated from various species of Alicyclobacillus (especially Al. ferrooxydans), of Acidibacillus (especially Ab. ferrooxidans) and of Sulfobacillus. By far most of the sequences had been annotated as hypothetical proteins, some as plastocyanin, and some as multicopper oxidase domain. Two clades were identified of predicted proteins that carry a lipoprotein signal peptide, and of which the genes are neighbored by genes of other proteins related to respiratory processes such as cytochrome C oxidase subunits. These clades (one with an S09 sequence) were more closely related to sulfocyanins of Sl. acidocaldarius and Sb. thermosulfidooxidans than to rusticyanins of Acidithiobacillus. Other sequences of Alicyclobacillus sp. S09 formed separate branches in the sulfocyanin and in the rusticyanin clades.

ACKNOWLEDGEMENTS/REFERENCES

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967 / OPTIMIZATION OF ALKALINE SUGARCANE BAGASSE PRE-TREATMENT FOR SUSTAINABLE BIOETHANOL PRODUCTION BY CANDIDA TROPICALIS Y5

Keywords: *Bioethanol, Candida tropicalis, pretreatment, agrowaste*

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BACKGROUND

Environmental pollution associated with combustion of fossil fuels calls for development of renewable energy sources like biofuels. Sugarcane Bagasse (SB) is a major waste of the sugar industry and which is known to contain cellulose and hemicellulose that can be converted to bioethanol. However, the recalcitrant nature of plant biomass demands optimal pretreatment method to make sugar components available for enzymatic depolymerisation.

OBJECTIVES

In the present work, we optimized SB alkaline pre-treatment using potassium hydroxide (KOH) and evaluated the capacity of Candida tropicalis Y5 (Accession number MG321586) to produce ethanol from the pre-treated SB enzymatic hydrolysate.

METHODS

SB alkaline pre-treatment was optimized with respect to KOH load, treatment time, and temperature, using response surface methodology (RSM). The objective function was the sugar yield after each pre-treatment condition, following enzymatic hydrolysis with a commercial cellulase cocktail. Fermentation of the optimally pre-treated material was carried out through separate hydrolysis and fermentation (SHF) with C. tropicalis Y5.

RESULTS

Optimum pretreatment conditions were found to be KOH load at 150mg/g bagasse, 86oC and 120 minutes of pre-treatment time. Maximum total reducing sugar of 600mg/g bagasse was observed. Ethanol yield was 19g/L with fermentation efficiency of 98% (calculated on hexose content). Our results show that pre-treatment of SB using low concentrations of KOH enhanced enzymatic hydrolysis without production of inhibitors (acetic acid and furfural). Candida tropicalisY5 successfully converted both hexose and pentose sugars present in pretreated hydrolysate to ethanol.

1021 / SULFATE-REDUCING AND FERMENTATIVE BACTERIA FROM PETROLEUM RESERVOIRS

Keywords: *oil fields, fermentative bacteria, sulfate-reducers*

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Sulfate-reducing and fermentative prokaryotes are dominant components of the microbial community of petroleum reservoirs with sulfate-containing formation water. Production of hydrogen sulfide causes environmental and economic problems associated with corrosion of steel equipment, a decrease in oil quality, reservoir clogging by sulfides. Thus, the study of oil reservoir microorganisms is essential for the development of methods of sulfide producers growth suppression and for development of biotechnologies for the enhancement of oil recovery.

The aim of the work was to study the functional and phylogenetic diversity of microorganisms in formation waters of oil fields in Kazakhstan and Russia, to isolate microbial strains forming oil-displacing metabolites and to describe new taxa of sulfate-reducing and fermentative bacteria.

METHODS

High-throughput sequencing of V3–V4 region of the 16S rRNA gene, cultural, analytical and biochemical methods, whole-genome sequencing was performed using HiSeq 2500 platform.

RESULTS

Sulfate-reducing bacteria of the genera Thermodesulfobacterium, Defluviitoga, Desulfovirgula, and Desulfonauticus predominated in water samples from Kazakhstan.

Fermentative bacteria of the genera Anaerobaculum, Coprothermobacter, and Soehngenia, were also presented in these samples. New strains of fermentative bacteria producing oil-displacing metabolites were isolated. A number of unique bacteria including both the known species of the genera Desulfovibrio, Pseudodesulfovibrio, and Acetobacterium, and new species Sphaerochaeta halotolerans, Soehngenia longivitae, and Actinotalea subterranea has been phenotypically and phylogenetically characterized.

ACKNOWLEDGEMENTS/REFERENCES

This study was supported by the Russian Science Foundation (grant 21-64-00019).

1025 / EFFECT OF MORPHOLOGY OF CARBONIZED POLYPYRROLE-MODIFIED ANODES ON THE BACTERIAL DIVERSITY OF MICROBIAL FUEL CELLS

03

Keywords: *microbial fuel cell, MFC, wastewater, microbial community*

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Microbial fuel cells (MFC) are an emerging green and sustainable technology for single-step wastewater treatment and bioenergy production. Their performance is largely dependent on the materials used for their construction, affecting both bacterial biofilm formation and the ease of electron flow. Our objective was to determine the influence of different morphologies of carbonized polypyrrole (CPPY), used as an anode modifier in a MFC system, on the anodic microbial community enriched from municipal wastewater.

CPPY was obtained through polypyrrole pyrolysis with round (CPPY-R) and tubular (CPPY-T) morphologies under a nitrogen atmosphere. The morphology of the samples was highlighted by Scanning Electron Microscopy (SEM). Dual chamber MFC systems were set up with anodes consisting of a carbon cloth modified with CPPY-R or CPPY-T, 40% Pt/C ink-coated cathodes, and 1 kohm external loading. Municipal wastewater supplemented with acetate and phosphate buffer were used as anolytes and catholytes, respectively. The microbial communities of the initial wastewater and, following operation, of the anode biofilm were analyzed through 16S rRNA gene V3-V4 metabarcoding. The maximum power density of 43.2 mW/m2 was obtained in the case of the MFC anode modified with CPPY-T, compared with 29.3 mW/m2 for CPPY-R. Polarization measurements show that the tubular morphology determines an increase in MFC power generation. MFC operation led to the enrichment of known exoelectrogens at anode level, and the use of CPPY-T determined reduced alpha diversities with little variation between biological replicates.

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1034 / EVALUATION OF GENETICALLY MODIFIED YEAST STRAINS FOR THEIR ABILITY TO ETHANOL PRODUCTION

Keywords: *yeast mutants, alcoholic fermentation, Msn2/4, transcription factor*

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BACKGROUND

Alcohol fermentation is the central process in wine, beer, alcoholic beverage production, bioethanol production employing carbohydrate sources, and food industry byproducts. Many studies have indicated that the genetic engineering of yeast cells can be a useful approach in increasing the alcoholic fermentation rate as well as their ethanol tolerance. These modifications could be the overexpression of a key protein using a strong promoter or the modification of a specific domain or amino acid which can also lead to the desired outcome.

OBJECTIVES

In this study, we attempt to study the influence of alteration in two proteins of a specific Saccharomyces cerevisiae strain for the improvement of alcoholic fermentation characteristics.

METHODS

We used Reversed Flow Gas Chromatography for kinetic and physicochemical studies and HPLC for measuring ethanol production.

RESULTS

Four different mutants in which serine residues have been replaced by alanine are studied. Our results indicate that substitution at position 533 of Msn4 protein (W_M4_533) significantly increases the fermentation rate even at low temperatures (12 °C), by lowering the fermentation's activation energy. Similar results but to a lesser extent were obtained by the S582A substitution in Msn2 protein. In addition, W_M4_533 seems to have a more balanced utilization of must hexoses. From the present work it is concluded that genetic modification of Msn2/4 proteins represents a promising procedure for shortening the fermentation time, even at low temperatures, which in many cases constitutes an important technological requirement.

ACKNOWLEDGEMENTS/REFERENCES

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call SUPPORT FOR REGIONAL EXCELLENCE (MIS 5047289)

1042 / KINETIC STUDY OF FIG SYRUP FERMENTATION BY GENETICALLY MODIFIED S. CEREVISIAE YEAST STAINS

Keywords: *Figs syrup, Reversed Flow Gas Chromatography, Yeast strains life cycle, Alcoholic fermentation*

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BACKGROUND

Figs are fruit of high glycemic index because of high glucose and fructose content. Souma is a traditional beverage of Chios Island. It is the distillate of the fermentation product of dried figs. Transcription factors Msn4/Msn2 have been reported to regulate cell's stress response.

OBJECTIVES

In this study the fermentation process was monitored using RF-GC technique from which the ability of the different genetically yeast strains to ferment the fig's syrup can be investigated.

METHODS

Reversed Flow Gas Chromatography used for kinetic and physicochemical studies and for estimating the duration of each yeast strains life cycle phase.

RESULTS

The kinetic analysis of each fermentation phase showed that W_M4_533 strain was the quickest during lag-phase and especially log-phase at low temperatures. As the temperature increased this advantage was abolished, which is a characteristic of a low temperature fermenting strain. In contrast W_M4_558 strain was proved to ferment more efficiently during the stationary phase also at low temperatures and the increase of the temperature led to the abolishment of this advantage. The main characteristic of stationary phase is the increased levels of ethanol. Considering this, the observed behavior of W_M4_558 strain implies the assumption that the replacement of this serine residue by alanine confers to the cell an ethanol resistant character at low temperatures. Gathering all these together, it can be concluded that the Ser533Ala substitution on the transcription factor msn4, resulted in a psychrophilic phenotype, whilst the Ser558Ala resulted in a possible ethanol resistant phenotype in relatively low temperatures.

ACKNOWLEDGEMENTS/REFERENCES

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call SUPPORT FOR REGIONAL EXCELLENCE (MIS 5047289)

1055 / THE BIAS PROJECT: INNOVATIVE BIOFERTILIZERS FOR SUSTAINABLE AGRICULTURE TO PROTECT HUMAN HEALTH AND THE ENVIRONMENT

03

Keywords: *Biofertilizers, Plant Growth-Promoting Bacteria , bioactive molecules*

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BACKGROUND

Chemical fertilizers affect the chemical-physical properties of the soils, causing the accumulation of heavy metals, the eutrophication of water and the loss of nutrients (Savci, 2012). Biofertilizers, based on mix of plant growth-promoting bacteria (PGPBs), have emerged as an economically attractive and ecologically sustainable alternative to provide nourishment to the plants (Mahanty et al., 2017). PGPBs control plant growth by diverse mechanisms, including plant growth hormones biosynthesis - such as indole-3-acetic acid (IAA), gibberellic acid and cytokinins - production of siderophores causing the bioavailability of iron and of antibiotics that strengthen the plant biological control against biotic stresses (Nehra and Choudhary, 2015).

OBJECTIVE

The BIAS project, funded by Sicily Region through the European Regional Development Fund (PO-FESR Sicilia 2014-2020), aimed to develop innovative biofertilizers based on microbial activity.

METHODS

Different bacteria, including streptomycetes and bacilli, were assayed for PGP activity. In particular, the strains were assayed for the capability of i) producing IAA by spectrophotometric assay, ii) solubilizing organic and inorganic phosphate, iii) growing under saline and hydric stresses, iv) fixing nitrogen. The most interesting strains, namely Streptomyces coelicolor, Streptomyces violaceoruber, Bacillus thuringensis and Kocuria rhizophila, were cultured i) to analyse the bacterial secreted and cellular metabolome by HPLC-MS and ii) to evaluate the PGP properties on tomato (Solanum lycopersicum) plants.

RESULTS

The results revealed that the selected strains, producing different bioactive compounds, promotes the growth of tester plant and, therefore, they can be considered good candidates to develop novel biofertilizers for a low-input and sustainable agriculture.

ACKNOWLEDGEMENTS/REFERENCES

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Nehra, V., & Choudhary, M. (2015). A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. Journal of Applied and Natural Science, 7(1), 540-556.

1067 / DEVELOPMENT OF BACTERIAL FORMULATION TO PREVENT TENACIBACULUM MARITIMUM OUTBREAKS IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

Keywords: *Tenacibaculum maritimum, Microorganisms, Recirculating Aquaculture Systems (RAS), nan*

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Tenacibaculum genus is highly abundant in marine environments. Among this genus, the species Tenacibaculum maritimum has been reported as pathogenic for fish and associated with a high mortality and economic losses in several fish species production.

This work aimed to explore the culturable bacterial community from a recirculating aquaculture system (RAS), to develop a bacterial formulation to prevent outbreaks of T. maritimum. For that, water and biofilter carriers were used as inoculum for the direct isolation of culturable bacterial community. Previously, correlation and network analysis (obtained from next generation sequencing (NGS) data from the RAS), was performed to assess potential bacterial genera that can be negatively associated with T. maritimum. Then, different culture media, rich in different carbon sources and micronutrients were used, seeking the isolation of the bacterial genera with negative correlation with T. maritimum. Inhibition assays were developed based on disc diffusion and method and striking method.

So far, 259 bacterial isolates were retrieved from water and biofilter carriers. Among the 259 bacterial isolates, 52 were tested against T. maritimum in which 9 showed inhibitory potential. Bacterial isolates with inhibitory potential will be taxonomically identified. Besides, these isolates will be tested alone and in a bacterial consortium in microcosms experiments. Additionally, the community from water and biofilter carriers will be characterized trough NGS.

The results from this work can contribute for the development of new formulations to improve RAS biofilters. Furthermore, with NGS techniques, it will be possible to understand their biological context and presence in the system.

ACKNOWLEDGEMENTS/REFERENCES

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1075 / THE EFFECT OF ALUMINIUM NANOPARTICLES AND ALUMINIUM IONS ON THE GROWTH OF S. AUREUS AND E. COLI

Keywords: *aluminium, nanoparticles, antimicrobials, antibiotics*

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BACKGROUND

The development of new antibiotics is too slow and complicated to sufficiently stabilize the growth of resistant bacterial strains. We therefore consider it appropriate to focus on an alternative direction of the antimicrobial effect of heavy metals and metal nanoparticles (NPs).

OBJECTIVES

Our research investigates the inhibitory effect of aluminum nanoparticles (AlNPs) synthesized by the green method from Azadirachta indica. Bacterial growth curves (S. aureus (MSSA, MRSA) and E. coli) were measured at 450 nm/540 nm for 24 hours at 25°C, 300 rpm at 0.01 OD in 2YT medium, repeated in four cycles. AlNPs were added to the bacteria at concentrations of 300–1600 µg/ml and aluminium ions (Al3+) at concentrations of 3–25 µg/ml. The results were evaluated as AUC growth curves.

METHODS AND RESULTS

Bacterial cultures were grown reproducibly in pure medium (AUCs were considered baseline). Following the addition of AlNPs, the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) were observed to be lower for E. coli (MIC = 310 µg/ml, MBC = 630 µg/ml) than for S. aureus (MSSA (MIC = 470 µg/ml, MIB = 1380 µg/ml), MRSA (MIC = 480 µg/ml, MBC = 1560 µg/ml)). Al3+ alone were more effective for E. coli (MIC = 6 µg/ml, MBC = 22 ug/ml) than for S. aureus (MSSA (MIC = 9 µg/ml, MBC = 25 µg/ml), MRSA (MIC = 7 µg/ml, MBC = 23 µg/ml)).

CONCLUSION

The study provided initial information on the effect of AlNPs and Al3+ on S. aureus and E. coli.

ACKNOWLEDGEMENTS/REFERENCES

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1174 / EFFECTS OF CONSORTIUM OF INDIGENOUS BACTERIA IN THE TREATMENT OF METABOLIC SYNDROME

Keywords: *Autoprobiotics, Metabolic Syndrome, Microbiome biochemical paprameters, nan*

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BACKGROUND

Currently used biotechnological probiotics are heterogenous to humans and may be rejected due to incompatibility. The use of indigenous beneficial bacteria (autoprobiotics) can become a promising direction in the personalized treatment of metabolic syndrome (MS).

Objectives was to investigate the effectiveness of autoprobiotics in the share of indigenous consortium of fecal bacteria (IC) in treatment of patients with MS.

METHODS

The study group consisted of 38 patients with obesity, carbohydrate and lipid metabolism disorders. All patients in addition to the basic therapy received a personified functional food product based on IC prepared by anaerobic cultivation of own fecal samples of the patients for 10 days. Samples of IC and feces before and after the introduction of autoprobiotic were study bacteriologicaly, using qPCR and metagenome analysis (16 S rRNA).

RESULTS

The representatives of lactobacilli, bifidobacteria and enterococci significantly increased in IC. The decrease in the population of the of opportunistic Enterobacteriaceae family members and the greater representation of Bacteroides thetaiotamicron after taking the IC were shown. A positive clinical effect was manifested in the disappearance of dyspeptic symptoms, a tendency to normalize the lipidogram, liver enzymes and decrease of concentration of glucose and C reactive protein in blood serum. The decrease in the manifestation of “low grade inflammation”, improved clinical and laboratory parameters, allow to consider, that IC could be useful component of the complex therapy of MS.

ACKNOWLEDGEMENTS/REFERENCES

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1179 / AUTOPROBIOTIC AND PROBIOTIC ENTEROCOCCI IN TREATMENT OF COLORECTAL CANCER

Keywords: *Autoprobiotic , Microbiome, Colorectal cancer*

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Significant changes in intestinal microbiota of patients with colorectal cancer (CRC) were noted, causing worsening of clinical course and leading to a need of correction after surgical intervention.

Objective was to study the effectiveness of autoprobiotics (AP, indigenous beneficial bacteria) and probiotics in the treatment of CRC.

The study group consisted of 41 patients with CRC. All patients received 1 day before surgery and 19 days after AP (personified functional food product based on indigenous non-pathogenic Enterococcus faecium) or probiotc E.faecium L-3 (P). Samples of feces before and after the introduction of AP or P were studied using qPCR and metagenome analysis (16 S rRNA).

In patients with negative growth of non-pathogenic enterococci (18 people) changes in intestinal microbiota were revealed more often. In all patients there was a decrease (or elimination in the case of AP usage) of Bacteroides fragilis, Fusobacterium nucleatum, Parvimonas micra, Clostridium perfringens, Clostridioides difficile, Citobacter sp. and Candida spp.. The presence of Enterobacter sp. was noted in all patients, but after using AP, the representation of these bacteria decreased to a greater extent.

Microbiota changes was accompanied by the disappearance of dyspeptic symptoms, decrease in the concentration of C reactive protein and liver enzymes in the blood serum . Thus, the possibility of using AP is one of the new promising areas of supplementary therapy of CRC. P is the mean of its replacement in case of impossibility of creating autoprobiotics.

ACKNOWLEDGEMENTS/REFERENCES

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, №075-15-2020-902 (16.11.2020) FSBSI «IEM».

1386 / PRODUCTION OF THE RIBOFLAVIN (VITAMIN B2) ON WHEY BY THE FLAVINOGENIC YEAST CANDIDA FAMATA

Keywords: *riboflavin, milk whey, genetic engineering, bioreactor*

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Riboflavin serves as biosynthetic precursor of flavin nucleotides FMN and FAD and is important biotechnological product with annual market around 1 billion US dollars. It is mostly used as component of feed premixes for animals (80%), in food industry as food colorant, in medicine and component of polivitamin mixtures and as drug for treatment of some diseases.

Apart from proteins, fat, microelements and vitamins, whey contains lactose which can be source of carbon for specific C. famata strain to produce riboflavin (with addition of ammonium sulfate only). It's a promising discovery to utilize byproduct and receive valuable product. The aim of the current work was to improve the available riboflavin-overproducing strains C. famata regarding growth and utilization of lactose.

Results showed that C. famata effectively utilizes lactose as sole carbon source and overproduces riboflavin. The synthesis of vitamin B2 on whey was significantly elevated by expression of homolog of mammal riboflavin efflux protein BCRP and expressing SEF1 transcription activator under control of LAC4 promoter induced by lactose. Outcomes so far are very promising. Maximally, 1.2 g of riboflavin per liter was achieved during C. famata bioreactor cultivation on whey with ammonium sulfate. Further studies on optimization of dissolved oxygen supply will be done to get even the higher titers of riboflavin.

ACKNOWLEDGEMENTS/REFERENCES

This study was supported by Polish National Science Center, grant Opus UMO-2018/29/B/NZ1/01-497

1507 / ADAPTIVE SEASONAL DYNAMICS OF THE GUT MICROBIOME OF THE WILD IBEX FROM THE STELVIO NATIONAL PARK, ITALY

Keywords: *microbiome, circular economy, side streams valorization, nan*

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BACKGROUND

Modern agricultural production still relies on unsustainable practices while crop-derived recalcitrant lignocellulose (LC) accumulates in large quantities as unused material, making the circular valorization of this waste biopolymer a top priority research area. Natural microbiomes - as ultracomplex microbial communities populating every ecosystem on Earth - represent a still untapped source of functionalities for the valorization of recalcitrant plant biopolymers, thanks to a vast array of hydrolytic and oxidative enzymes allowing their biotransformation into platform chemicals (e.g., volatile fatty acids), with the concomitant production of several bioactive compounds.

OBJECTIVES

We aimed to identify the natural microbiomes (or hubs or strains) for the biotransformation of LC side streams by characterizing the fecal microbiome of Alpine ibex Capra ibex, to emphasize adaptive shifts in LC-degrading microbiome properties alongside the increasing amount of fibers that animals feed on during cold periods.

METHODS

We collected fresh fecal samples from 85 adult ibex across 3 different seasons (winter, spring, and summer) in Stelvio National Park. Samples were analyzed by multiomics (shotgun metagenomics and metabolomics), and the microbiome structural and functional dynamics were explored at the network level.

RESULTS

In the ibex gut microbiome, we identified specific modules and hubs with strategic functionalities involved in LC metabolism (e.g., cellulases, hemicellulases, lignin-modifying enzymes), and showed a well-recognizable temporal dynamic matching seasonal changes in forage quality. While still preliminary, these findings may pave the way for engineering sustainable microbiome-based applications for efficient bioconversion and valorization of LC side streams.

521 / ADVANCES IN MICROBIOME-DERIVED SOLUTIONS AND METHODOLOGIES ARE FOUNDING A NEW ERA IN SKIN HEALTH AND CARE

Keywords: *Microbiome , Dermocosmetic, postbiotics, microbiome metabolites*

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BACKGROUND

The microbiome, as a community of microorganisms and their structural elements, genomes, metabolites/signal molecules, has been shown to play an important role in human health, with significant beneficial applications for gut health. Skin microbiome has emerged as a new field with high potential to develop disruptive solutions to manage skin health and disease. Despite an incomplete toolbox for skin microbiome analyses, much progress has been made towards functional dissection of microbiomes and host-microbiome interactions. A standardized and robust investigation of the skin microbiome is necessary to provide accurate microbial information and set the base for a successful translation of innovations in the dermo-cosmetic field.

OBJECTIVE AND RESULTS

This review provides an overview of how the landscape of skin microbiome research has evolved from method development (multi-omics/data-based analytical approaches) to the discovery and development of novel microbiome-derived ingredients. Moreover, it provides a summary of the latest findings on interactions between the microbiomes (gut and skin) and skin health/disease. Solutions derived from these two paths are used to develop novel microbiome-based ingredients or solutions acting on skin homeostasis are proposed.

The most promising skin and gut-derived microbiome interventional strategies are presented, along with regulatory, safety, industrial, and technical challenges related to a successful translation of these microbiome-based concepts/technologies in the dermo-cosmetic industry.

1588 / BIOCONVERSION OF INDUSTRIAL WASTE INTO NEUTRAL INTRACELLULAR LIPIDS USING OLEAGINOUS YEAST STRAINS

Keywords: *industrial waste, biofuels, yeasts, intracellular lipids*

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BACKGROUND

Neutral intracellular lipids produced by yeasts are a class of compounds of high biotechnological interest, used to obtain biofuels. The use of oil-based yeasts on industrial scale is still limited by the high production costs. A solution might consist in using cheap alternatives such as lignocellulosic substrates high in xylose or molasses, for biomass accumulation.

OBJECTIVES

The study aims to select oleaginous yeast strains able to assimilate various residues produced in large quantities in Romania, and the preliminary optimization of cultivation conditions aimed to increase the yield of intracellular lipid accumulation.

METHODS AND RESULTS

Six yeast strains isolated from different plants, spontaneously fermented products and industrial biomass from Romania, were characterized using PCR-RFLP / sequencing of ITS1-5.8S-ITS2 region, and identified as belonging to: Pichia, Lachancea, Metschinikowia and Candida genera. In a first step, the yeast strains able to assimilate D-xylose and molasses in presence of organic/ inorganic nitrogen source were selected.

Subsequently, various substrate concentrations were tested. All the strains assimilated the substrates, but Lachancea thermotolerans CMGB-ST12, Metschinikowia reukaufii CMGB-ST10 and Pichia membraniefaciens CMGB-ST53 showed the best yields. Nile Red staining and quantitative quantification expressed as RFU showed that L. thermotolerans CMGB-ST12 and P. membraniefaciens CMGB-ST53 have a good yield of neutral intracellular lipids accumulation and can be further used as microbial resources in biotechnological processes for production of biofuels.

The study allowed the preliminary characterization of new oleaginous yeast strains that can be used to convert industrial wastes into precursors of biofuels.

1584 / EVOLUTION OF PSEUDOMONAS SP. M1 TOWARDS ENHANCED ß-MYRCENE BIOTRANSFORMATION

Keywords: *Evoution, Genomics, Biotechnology, Terpenes*

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BACKGROUND

Pseudomonas sp. M1 can mineralize a variety of hydrophobic monoterpenes (namely β-myrcene) due to a specialized set of genes present in a 28-kb genomic island [1]. Previous GC-MS analysis of the produced metabolites evidenced the effective production and accumulation of industrially relevant intermediates (e.g. the myrcen-8-ol used in scent formulations) [1].

OBJECTIVES

Enhance the monoterpene-associated biotransformation performance of M1 cells.

METHODS AND RESULTS

A strategy resorting to laboratorial evolution has been set and employed. M1 cells were submitted to long-term evolution, during 600 generations, using either β-myrcene (5 cultures) or lactate (5 cultures) as sole carbon and energy sources. A comparative genomics analysis was conducted both at culture population and lineage level, resorting to Illumina next-seq high-throughput sequencing and Breseq-driven bioinformatics. Overall, the number of mutations detected in lactate growing populations was higher than for β-myrcene growing populations in a ratio of 1:3 and different frequencies, suggesting that lactate populations were more homogeneous (closer to clonal) than β-myrcene ones.

We retrieved 150 isolates from β-myrcene-grown populations to select M1 lineages evidencing enhanced β-myrcene-catabolism performance. Among these, 6 evidenced over a 2-fold increase of β-myrcene-catabolism associated promoter activity, in comparison to the ancestor. Significantly, for all (6) isolates evidencing enhanced β-myrcene catabolic activity, sequencing data revealed mutations in the gene coding for the regulator FleQ. This regulator together with an ATPase (FleN) regulates the expression of genes responsible for flagella biosynthesis and exopolysaccharides, suggesting that alterations in mobility-associated apparatus may contribute for an enhanced β-myrcene biotransformation performance.

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1613 / THE ROLE OF THE HOST NUCLEASES IN PRESPASER GENERATION DURING PRIMED CRISPR ADAPTATION IN THE TYPE I-E CRISPR-CAS SYSTEM

Keywords: *CRISPR-Cas, CRISPR adaptation, prespacers, bacterial nucleases*

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CRISPR-Cas adaptive immunity allows bacteria and archaea to fight against phages and mobile genetic elements by a specific recognition mechanism. CRISPR-Cas immunological memory in Escherichia coli is acquired by the integration of 33-bp spacers, corresponding to foreign sequences, into the CRISPR arrays. Spacers are then transcribed to crRNAs and guide CRISPR interference machinery, which destroys foreign DNA containing complementary regions, protospacers. In parallel, CRISPR-mediated DNA degradation products are used by CRISPR adaptation machinery as sources of new spacers in a process called primed CRISPR adaptation.

The precursors of such spacers were demonstrated to be ~33bp dsDNA fragments with a ~4nt 3’ overhang. Yet, the mechanism of their generation is unknown. In this work we demonstrate the functions of several genome maintenance proteins during prespacer generation. Specifically, we show that the RecJ exonuclease is responsible for the trimming of prespacer 5’ ends, while the RecBCD complex unwinds double-stranded regions, enabling access for RecJ. The data suggest that the previously reported reduction of CRISPR adaptation efficiency in the ΔrecB ΔrecJ strain may be explained by the poor prespacer generation. Additionally, we show that ExoVII nuclease may partially substitute the RecJ function.

1758 / CHARACTERIZATION OF THE BACTERIAL DISTRIBUTION IN PRODUCTION PROCESSES OF PACKAGING MATERIALS WITH DIFFERENT TEMPERTURE PROFILES IN THE PROCESS WATER

Keywords: *nan, nan, nan, nan*

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Microbial growth and biofilm formation are common issues in production processes using water circulation systems, as it is the case in fibre based packaging manufactories. The entry of microorganisms can occur in several processes during production. Including a large volume of water, which is recycled during the production these days. In addition, microorganisms enter the production by used raw materials. This creates suitable conditions for microbial growth along the production process. Consequently, these factors can impair the production efficiency and the quality of the final product.

In this study, the microbial composition in biofilm, process water and packaging material of three production machines with varying temperature profiles (75 °C, 44-50 °C and 30 °C) were determined at three different time points using Illumina next generation sequencing.

At a temperature of 75 °C in the whole water cycle, five abundant taxa were identified in all samples, including Thermus (12 %), Tepidimonas (6 %), Meiothermus (5 %), Pseudomonas (3 %) and Bacillus (< 1 %). In the lower temperature production machine (44-50 °C) Tepidimonas (9 %) and Cloacibacter (7 %) were detected more frequently. At 30 °C a low relative abundance <1 %to 2 % of the top twenty genera could be determined but a higher bacterial diversity. In all samples taken from the different mills the genera composition differed from each other and was slightly but not significantly variable over time. The lower the temperature in the process water, the higher the bacterial diversity in the samples.

337 / GLUTAMATE DEHYDROGENASE (GDHA) OF STREPTOCOCCUS PNEUMONIAE IS REQUIRED FOR HIGH TEMPERATURE ADAPTATION

Keywords: *Streptococcus pneumoniae, GdhA, temperature , Galleria mellonella*

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During its progression from the nasopharynx to other niches of its human host, Streptococcus pneumoniae must cope with changes in temperature(1). We hypothesized that the temperature adaptation is an important facet of pneumococcus and glutamate dehydrogenase (GdhA) plays a role on it. Studies on GDHs demonstrated that it has roles in response to various signals(2,3), however, its involvement into temperature adaptation has not been studied well.

The objectives are to investigate the effect of temperature on pneumococcal metabolism and study the role of GdhA in thermal adaptation associated with virulence and survival.

To increase our understanding of pneumococcal thermal adaptation, we obtained transcriptional profiles of the pneumococcus at different temperatures. One of the identified genes differentially expressed at 40°C encoded glutamate dehydrogenase (GdhA). GdhA was deleted using SOEing PCR and the mutant was analysed by growth studies, cell size determination, biochemical assays, and biofilm formation. We also investigated GdhA's role in pneumococcal virulence in vivo.

The transcriptome data showed that gdhA was downregulated at 34°C and upregulated at 40°C. Deletion of gdhA attenuated the growth, cell size, biofilm formation, and biosynthesis of proteins associated with virulence in a temperature-dependent manner. Finally, ΔgdhA grown at 40°C was less virulent than other temperatures or the wild type at the same temperature in a Galleria mellonella infection model, suggesting that GdhA is required for pneumococcal virulence at elevated temperature.

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391 / STUDIES ON THE INTERACTION OF T4-LIKE PHAGES AND DIARRHEAGENIC ESCHERICHIA COLI STRAINS

Keywords: *ETEC, STEC, swine, T4-like phage*

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BACKGROUND

Bacteriophages are being reported as efficient in controlling E. coli infections in animals. T4-like phages are particularly known to exhibit wide host ranges and use their long tail fibers (LTFs) to recognize its hosts.

OBJECTIVES

To evaluate the potential of T4-like bacteriophages to tackle diarrheagenic E. coli strains isolated from pigs.

METHODS

The lytic spectrum of three bacteriophages, vB_EcoM_FJ1, vB_EcoM_FN and vB_EcoM_SP1 was assessed against 92 Enterotoxigenic and Shiga toxin-producing Escherichia coli isolates. We analysed the phage genomes, identified their host receptors and evaluated their ability to select insensitive bacterial variants escaping the complement system.

RESULTS

The T4-like E. coli phages FJ1, FN and SP1 belong to genus Tequatroviruses, order Caudovirales. Overall, they presented a narrow lytic spectrum among the tested strains (up to 3.3%). At the genome level, phages share >80% overall nucleotide identity with E. coli T4 phage, but a particular look at the distal part of the LTF (gp38) revealed a much smaller similarity with T4. All three phages recognize lipopolysaccharides as receptors, and additionally, FN binds to an outer membrane protein (OmpA). Both phages FJ1 and FN seem to produce insensitive bacterial variants that are generally more susceptible to porcine serum inactivation comparatively to the originating strain.

CONCLUSIONS

Unlike typical T4-like, these phages showed a very narrow host range. These results may be explained by the acquisition of novel adhesins (encoded by gp38) known to be primary determinants of host range in the T4-like phages.

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403 / ANTIBIOFILM ACTIVITY OF OZONE GAS AGAINST OXA-48 CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIAE

Keywords: *biofilm, hospital acquired infections, Klebsiella pneumoniae, ozone*

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BACKGROUND

OXA-48 carbapenemase producing Klebsiella pneumoniae has great clinical significance in epidemiology of hospital acquired infections. It can produce biofilm on hospital surfaces which is resistant to standard disinfectants, so there is a need for novel methods of disinfection in hospitals. Ozone gas has antimicrobial properties but there is lack of data on ozone action against K. pneumoniae biofilm.

OBJECTIVES

Aim of this study was to investigate efficiency of gaseous ozone on biofilm of OXA-48 carbapenemase producing Klebsiella pneumoniae.

METHODS

24 hour old biofilm of clinical strains of K. pneumoniae OXA-48 was formed on ceramic tiles and expose to 25 ppm of gaseous ozone for 1 hour using a model of experimental chamber. Afterwards, total bacteria count, total biomass, oxidative stress levels before and after ozone treatment have been measured. Morphological changes and topology of biofilm were observed with atomic force microscopy and scanning electron microscopy.

RESULTS

Reduction of total bacteria count for 2 log10 CFU/cm2 was determined after the ozone treatment, followed by total biomass reduction for 22.14% to 88.15%, depending on used method. Levels of reactive oxygen species after the ozone treatment were higher for 159% for K. pneumoniae ATCC 70063 and 182% for K. pneumoniae NCTC 13442 strain. Morphological changes in structure of biofilm were observed, as well as damage to the cell wall of bacteria in biofilm.

CONCLUSION

Ozone gas shows antimicrobial properties but lacks efficacy in given concentration to completely eradicate early K. pneumoniae biofilm. Further investigation on ozone mechanism of action is needed.

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411 / THE RELEVANCE OF ANTIFUNGALS AND ANDROGRAPHOLIDE ON CANDIDA SPP. BIOFILM FORMATION AND ERADICATION USING IMPEDANCE-BASED MEASURE

Keywords: *xCELLigence, fungal biofilms, impedance-based mearurements, andrographolide*

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BACKGROUND AND OBJECTIVES

Candida species cause life-threatening infections with high morbidity and mortality rates and their resistance to conventional therapy is closely linked to biofilm formation. Thus, the development of new approaches to study Candida biofilms and novel therapeutic strategies could facilitate better clinical outcomes.

METHODS

We have set up an impedance-based in vitro system to evaluate the effect of two different groups of conventional antifungals used in clinical practice, azoles and echinocandins, and compared these results to those obtained by classical E-test and microdilution.

RESULTS

Both fluconazole and voriconazole were not able to halt biofilm formation, while echinocandins showed biofilm inhibitory capacity at relatively low concentrations starting from 0.625 mg/L. However, analyses performed on 24h C. albicans and C. glabrata biofilms revealed that micafungin and caspofungin failed to eradicate mature biofilms at all tested concentrations ranging from 128 to 0.0625 mg/L and suggesting that once formed, Candida spp. biofilms are extremely difficult to eradicate. For this reason, we also evaluated whether the natural compound andrographolide, isolated from the plant Andrographis paniculata, had antifungal and/or anti-biofilm properties when added alone and in combination with micafungin. Our results showed an antagonistic effect between these two compounds, but highlighted the ability of andrographolide alone to prevent fungal biofilm formation in all tested strains at concentrations higher than 315 mg/L. In addition, this compound was capable of completely eliminating 24h biofilms, suggesting that it could be potentially used as a new approach to treat multi-resistant Candida spp. related infections.

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435 / FLIW2 PROTEIN MODULATES THE POST-TRANSCRIPTIONAL REGULATOR CSRA AND AFFECTS FLAGELLAR MOTILITY IN HELICOBACTER PYLORI

Keywords: *Helicobacter pylori, Motility, Carbon storage regulator A, FliW2*

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BACKGROUND

Helicobacter pylori infections are closely related to human gastric diseases. Owing to its acidic niche, H. pylori has evolved chemotactic and flagellar motility directed towards gastric epithelia. The expression of flagellar motility is multilaterally controlled by sigma factors and a global carbon storage regulator (CsrA). Despite the well-known role of CsrA in modulating virulence factors, no studies have yet determined the factors that modulate H. pylori CsrA activity. Based on comparative genomic analysis, we have identified fliW homologs (fliW1 and fliW2) and postulate that fliW2 modulates CsrA by sequestration and abolishing its capacity to block translation of target transcripts.

OBJECTIVES

To make the connection between how CsrA is regulated and how the CsrA regulon contributes to H. pylori pathogenesis, we aimed to investigate the role of FliW2 in modulating CsrA activity and examine the molecular interactions between CsrA and FliW2.

METHODS AND RESULT

Impaired motility was demonstrated in both isogenic ΔfliW2 and ΔcsrA mutants but only the ΔfliW2 mutant was observed with short flagella by electron microscopy. A reduction of major flagellin FlaA in the ΔfliW2 mutant implies that FliW2 affects FlaA via inhibition of CsrA activity. A FliW2-CsrA interaction was demonstrated by pull-down assay and in vivo bacterial two-hybrid analysis. The latter method revealed that the C-terminal region of CsrA was critical for interaction. The evolutionary role of fliW2 in differentiating H. pylori from other clinically important pathogens was also examined. Taken together, FliW2 sequestrates CsrA activity thus antagonizing CsrA's blocking on flaA translation and affecting flagellar motility.

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438 / GUT MICROBIOTA COMPOSITION AND DISEASE SEVERITY IN PATIENTS WITH COVID-19: FAMILIAL MEDITERRANEAN FEVER DISEASE

Keywords: *COVID-19, FMF, gut microbiota, disease severity*

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BACKGROUND

Previously, we have identified the association between the composition of the gut microbiota of healthy Armenian men and the severity of COVID-19 disease after they were infected with SARS-CoV-2. In addition, we showed that the percentage of male FMF patients with mild COVID-19 among all patients with familial Mediterranean fever (FMF) was approximately four times higher than that of "healthy" male COVID-19 patients after their infection. In women with FMF, this indicator was lower than in "healthy" women (67% versus 87%).

OBJECTIVES

The aim of this study was to characterize the severity of COVID-19 disease in FMF patients in Armenia in relation to their baseline gut microbiota composition.

METHODS

We used data from our previous study on gut microbiota composition in FMF disease (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111835). Associations between the composition of the gut microbiota of forty-five healthy individuals without MEFV mutations and forty-five FMF volunteers and the severity of COVID-19 disease after the people were infected with SARS-CoV-2, was performed by a third-generation, culture-independent, high-density DNA microarray analysis.

RESULTS

In total, 18,700 bacterial operational taxonomic units were described for the studied healthy and FMF populations. Both the effects of colchicine and gut microbiota composition through the gut Prevotella, Coprobacillus, Clostridium ramosum and Clostridium hathewayi as well as Faecalibacterium prausnitzii, Eubacterium rectale and bifidobacteria on the severity of COVID-19 disease are discussed.

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482 / A TWO-STEP PROTOCOL FOR THE DETECTION AND ISOLATION OF VIBRIO VULNIFICUS DANGEROUS IN PUBLIC AND/OR ANIMAL HEALTH

Keywords: *Vibrio vulnificus, detection, zoonosis, nan*

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Vibrio vulnificus is a zoonotic pathogen linked to fish farms that is spreading due to climate change. In this work, we combined a pre-enrichment step with a new multiplex PCR to detect and/or isolate V. vulnificus from seafood or feed for human or animal consumption. The advantage of this new protocol over previous methods is that it allows the identification of samples potentially hazardous to human health, nimal health, or both. The specificity and detection limit of the new protocol was tested in vitro with strains belonging to the main lineages of the species and other Vibrio spp and resulted to be 100% and 10 cfu/mL, respectively. Likewise, the efficacy of the protocol was demonstrated in vivo with healthy and artificially infected shrimps. Therefore, this two-step protocol could be implemented in health risk studies associated with seafood/feed consumption, as well as in routine identification and subtyping of V. vulnificus derived from environmental or clinical samples.

ACKNOWLEDGEMENTS/REFERENCES

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525 / MOLECULAR CHARACTERIZATION OF MYCOBACTERIUM TUBERCULOSIS STRAINS FROM PATIENTS WITH HIV-ASSOCIATED TUBERCULOSIS IN RUSSIA

Keywords: *Mycobacterium tuberculosis, HIV, Russia, Genotyping*

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BACKGROUND

Adverse trends of tuberculosis (TB) and HIV co-infection in Russia require in-depth analysis of underlying reasons including molecular study of the Mycobacterium tuberculosis strains.

OBJECTIVES

To study medically significant M. tuberculosis Beijing genotype and its major genetic variants in TB/HIV and TB/HIV-negative patients in Russia.

METHODS

In total, 508 isolates were studied: 105 from TB/HIV patients and 403 from TB patients. The Beijing genotype, its phylogenetic sublineages, and major subtypes Central Asian/Russian and Russian epidemic cluster B0/W148 were determined using real-time PCR of specific markers. The MIRU-VNTR profiles were compared to the MIRU-VNTRplus database.

RESULTS

88 (83.8%) of 105 strains from HIV/TB patients belonged to the Beijing genotype; 47 (44.8%) to the Central Asian/Russian cluster, 33 (31.4%) - to B0/W148, and 8 (8.5%) - to the ancient Beijing sublineage. In the TB/HIV-negative group, 221 strains (54.8%) belonged to Beijing genotype; 164 (40.8%) – to the Central Asian/Russian cluster, 51 (12.7%) – to B0/W148, and 5 (1.2%) – to the ancient Beijing sublineage. To conclude, M. tuberculosis strains from patients with HIV-TB were dominated by the Beijing genotype and its Russian epidemic cluster B0/W148. At the same time, the proportion of ancient Beijing strains in HIV/TB patients also significantly exceeded that in the group of TB patients without HIV (P=0.0004). This indicates the possible and underestimated clinical significance of these strains in HIV/TB coinfection. In view of the increasing circulation of MDR strains of the ancient Beijing sublineage in parts of Russia, in-depth studies of their pathogenetics are required.

527 / A RARE CASE OF RECURRENT CENTRAL NERVOUS INFECTION

Keywords: *RECURRENT CENTRAL NERVOUS INFECTION, MOLLARET, nan, nan*

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BACKGROUND

HSV is the most common cause of sporadic severe CNS infection and early treatment is of great importance. Although HSV-1 is more frequent and is characterized radiologically (MRI, CT) by clear temporal lesions, HSV-2 presents a vaguer radiographic picture and causes more frequent recurrent episodes.

OBJECTIVES

To present a case of a patient with a second episode of CNS herpes infection, without known immune deficiency.

CASE

A 28-year-old, female patient with a history of CNS and genital infection by HSV-2 five years ago, presented to the Emergency Department with acute bilateral frontotemporal headache, fever and neck pain, not responding to paracetamol. Laboratory and radiological tests (CT, MRI and chest x-ray) were normal. Lumbar puncture was performed and examination of the cerebrospinal fluid (CSF) revealed the following: 115 leukocytes/μl (lymphocyte type), normal glucose (61 mg/dl) and slightly elevated albumin (81 mg/dL), elements compatible with CNS viral infection. The multiplex PCR (FilmArray, Biofire) detected HSV-2 and the patient was treated directly with acyclovir intravenously. During her hospitalization, a quantitative measurement of immunoglobulins and serum supplementation was performed with normal results. On the third day, the symptoms completely resolved. After completing ten days of intravenous treatment, the patient was discharged, with instructions for a new immunological and gynecological examination.

RESULTS-CONCLUSIONS

The rare syndrome of recurrent herpetic meningoencephalitis (Mollaret) is characterized by severe neurological damage. Although characterized by three or more episodes, this patient is likely to be at increased risk. Close monitoring, thorough immunological testing and possibly prophylaxis are required.

532 / INCIDENCE OF ENDEMIC RESPIRATORY VIRUSES DURING SARS-COV-2 PANDEMIC

Keywords: *SARS-CoV-2, endemic respiratory viruses, nan, nan*

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BACKGROUND

The incidence of endemic respiratory viruses is seasonal, it is highest during winter months. During the severe respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, decrease in incidence rates of seasonal respiratory viruses was observed.

OBJECTIVES

Aim of this study was to compare the incidence of endemic respiratory viruses before and during SARS-CoV-2 pandemic and to determine the most probable cause for the differences.

METHODS

Nasopharyngeal swabs were collected from patients admitted to both intensive care units and medical wards during seasons of respiratory viruses (October – February; last period only to January 2022) for five consecutive years (2017-2022). Filmarray Respiratory Panel (BioFire, USA) assay was performed on 4774 samples and data for each year were compared to each other.

RESULTS

Pre-pandemic seasons saw a steady rates of respiratory viral infections. Adenovirus was detected in 0,42%, seasonal Coronavirus in 7,3%, Human Metapneumovirus in 1,1%, Human Rhinovirus/Enterovirus in 14,8%, Influenza A in 14,0%, Influenza B in 2,4%, Parainfluenza in 2,8% and RSV in 5,7% of nasopharyngeal swabs. During season 2020-2021, there was a drastic reduction of positivity rate for seasonal respiratory viruses. Apart from SARS-CoV-2, the only viruses detected at all were Coronaviruses (detected in 3,3% of samples) and Rhinovirus/Enterovirus (9,6%). In the last observed season (October 2021 – January 2022) positivity rates for seasonal respiratory viruses were again similar to those before pandemic started.

During winter 2020-2021, there were in force unprecedented COVID-related measures. They were loosened last season despite ongoing SARS-CoV-2 pandemic and again allowed spread of other respiratory viruses.

593 / THE FIRST CASES OF CANDIDA AURIS ISOLATION IN A TERTIARY HOSPITAL IN ATHENS, GREECE, AND THE PREVENTION OF AN OUTBREAK

Keywords: *Candida auris, infection control, nan, nan*

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BACKGROUND

C. auris is an emerging risk in healthcare facilities due to its antifungal resistance and its potential to cause outbreaks.

OBJECTIVES

We present the first cases of C. auris isolation during a three-month period and the outbreak prevention.

CASES

The first case was an 87-year-old male patient, hospitalized due to COVID-19 on September 2021, who developed fungemia. Positive blood cultures were immediately examined by multiplex PCR (FilmArray, BioFire Diagnostics, USA) and inoculated on Sabouraud Dextrose Agar (bioMerieux, France). FilmArray detected C. auris and fungal colonies were identified by MALDI-TOF (Bruker, USA). VITEK 2 System (bioMerieux, France) misidentified the strain as C. duobushaemulonii.

On November 2021, another C. auris isolate was cultured from the surgical tissue of an 82-year-old female, non-COVID-19 patient, hospitalized due to lower limb cellulitis.

In both cases, C. auris infection control and surveillance measures were immediately implemented, thus, extensive carriage screening was performed in patients' contacts. Nasal, axilla, groin swabs and urine samples were collected. Potential environmental sources of transmission were sampled as well. Only one positive nasal swab culture, collected from a patient who shared the same room with the first one, was detected and no outbreak occurred. Patients' common risk factor was the long-term treatment with dexamethasone and broad-spectrum antibiotics.

RESULTS-CONCLUSIONS

These cases highlight the importance of accurate species identification and immediate implementation of infection control measures. Moreover, during COVID- 19 pandemic all the hygiene measures have been improved and this may have also contributed to the prevention of C. auris outbreak in our hospital.

601 / DNA METABARCODING FOR DETECTION OF PATHOGEN TRICHODERMA SPECIES IN COMPOST AND CASING SOIL USED IN CHAMPIGNON CULTIVATION

Keywords: *metagenomics, green mold, mushrooms, pathogen detection*

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BACKGROUND

Champignon mushroom production is strongly influenced by the occurrence of pathogenic bacteria and fungi causing different kinds of diseases which can significantly lower the yield. Green mold disease is one of the most aggressive diseases of champignon mushrooms. In champignon production, green mold disease is connected to fungal species belonging to genus Trichoderma. The most aggressive are T. aggressivum f. europaeum (mainly present in Europe) and T. aggressivum f. aggressivum (mainly present in North America), but also T. harzianum.

OBJECTIVES

In this research we aimed to explore the efficiency of DNA metabarcoding approach to detect Trichoderma species present in compost and casing soil during champignon cultivation.

METHODS

Genomic DNA extracted from compost and casing soil was used as a template for amplification and sequencing of an ITS2 fragment. High-throughput sequencing was performed on the Illumina NovaSeq 6000 platform. Raw sequence data was processed using next-generation microbiome bioinformatics platform QIIME2. In total, 332 operational taxonomic units were defined and the trend of succession in fungal communities of compost and casing soil were detected between different time dependent sample series.

RESULTS

We identified eight Trichoderma species among which T. harzianum and T. aggressivum. The first was detected in all sample series, while the second was detected only in casing soil samples. Using DNA metabarcoding it was possible to detect aggressive Trichoderma species even before the appearance of visible green mold. However, there was no clear pattern in Trichoderma spp. detection among time dependent sample series.

ACKNOWLEDGEMENTS/REFERENCES

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605 / ANALYTICAL STUDY OF INFECTION OUTBURST UNDER STRONG MITIGATION MEASURES

Keywords: *quantitative biology, epidemiology, Infection outburst and dynamics (COVID-19), social distancing measures*

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While comprehensive studies on interventions such as vaccination or quarantine are conducted within epidemiological compartmental models, the effects of social distancing on infection outbursts are yet to be understood.

We developed a realistic and analytically solvable model for the COVID-19 dynamic under social mitigation measures, which presents a generalization of the SEIR (Susceptible-Exposed-Infected-Recovered) model. We derive a closed-form mathematical expressions for infected, detected cases, and fatalities, as a function of time. Joint analytical and numerical analysis reproduces these main infection progression data, generating empirically observed COVID-19 growth signatures of detected cases, i.e., its three distinct dynamical regimes (exponential, superlinear, and sublinear). Analytical guidance facilitates the extraction of key infection progression parameters, e.g., infected fatality rates and attack rate. It distinguishes the regions where equations are most effective to infer simple relations between model parameters and epidemiological observables. The main parameter quantifying the effect of social mitigation (α) is thus highly constrained and connected to the time-duration of the superlinear regime. Combined with the time of social measures introduction, it establishes a new parameter – protection time, relevant for public policy decision making, etc.

Our analytically tractable model can shed light on cause-effect connections that underlie the epidemiological dynamics, explain universal dynamical features of a system, and set tight constraints on infection parameters inference. Due to its generality, it applies to the outburst of any new pandemics when pharmaceutical interventions are unavailable, and timely and appropriately chosen social mitigation measures are crucial.

622 / COMPARISON OF DIAGNOSIS METHODS BASED ON PCR WITH RIFT VALLEY FEVER VIRUS (RVFV) RNA

Keywords: *Rift valley fever virus (RVFV), Reverse transcription-quantitative polymerase chain reaction (RT-qPCR), Reverse transcription droplet digital PCR (RT-ddPCR), In vitro transcription (IVT)*

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BACKGROUND

Rift valley fever (RVF) is an important zoonotic disease caused by the Rift valley fever virus (RVFV) which can affect ruminants and humans. RVFV is a single-stranded RNA virus and consists of three segments: large (L), medium (M), and small (S). The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) has been a gold standard method of diagnostics for viruses. In contrast to the relative quantification of RT-qPCR, reverse transcription-droplet digital PCR (RT-ddPCR) is a powerful method for absolute quantification

OBJECTIVES

A comparison was performed of the RT-qPCR and RT-ddPCR with primer-probe sets for RVFV

METHODS

In this study, the synthesized RNA of three RVFV strains (L, M, and S) were made using In vitro transcription. The negative reference virus RNA such as Zika, Dengue, Japanese encephalitis, CHIKV, and SFTS were used for screening and validation of RVFV primer-probe sets. All experiments were performed RT-qPCR and RT-ddPCR

RESULTS

Both the RT-qPCR and RT-ddPCR assays for RVFV do not react with any negative reference viral genomes, indicating that both the RT-qPCR and RT-ddPCR assays are specific to RVFV. The comparison of both the RT-qPCR and RT-ddPCR assays with serially diluted templates showed that the LoD of both assays are similar, and the concordant of the results was observed. The LoD of both assays reached the practical measurable minimum concentration. Combining these results, the sensitivity of the RT-qPCR and RT-ddPCR was similar, and the material measured by RT-ddPCR can be used as a reference material for RT-qPCR.

629 / A SYNERGISTIC ANTIADHESION ACTIVITY OF TEA TREE AND LEMON EUCALYPTUS TREE ESSENTIAL OILS ON LEGIONELLA PNEUMOPHILA

Keywords: *Essential oils, Antibacterial effect, chackerboard assay , nan*

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BACKGROUND

Biofilm protects L. pneumophila from different environmental factors and makes it more resistant to harsh environmental conditions and disinfectants. Therefore, it is necessary to examine alternative ways of preventing the formation of biofilm, and natural compounds show great potential. Essential oils (EO´s) obtained from a variety of plant materials are known for their antimicrobial activities. Objectives: In this study, we have investigated the antibacterial properties of tea tree (Melaleuca alternifolia (Maiden and Betche) Cheel oil) and lemon eucalyptus tree (Eucalyptus citridora Hook.) EO´s and their synergistic, and anti-adhesive effect against L. pneumophila biofilm on polystyrene.

METHODS

Tea tree and lemon eucalyptus tree essential oils from Australian native plants was tested individually and in combination against Legionella pneumophila 130b. To test different combinations EO´s a chackerboard assay was used.

RESULTS

Minimal effective concentration (MEC) for tea tree is 12.8 mg/mL and for lemon eucalyptus tree EO is 6.4 mg/mL. A different combination of these two EO oils have a synergistic and additive antimicrobial and antiadhesive effect on L. pneumophila which is also shown in the isobologram.

Tea tree and Lemon Eucalyptus tree EO´s show great potential in growth inhibition and antiadhesion activity to L. pneumophila and should be further investigated.

ACKNOWLEDGEMENTS/REFERENCES

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633 / NITRATE AS A PREBIOTIC AND NITRATE-REDUCING SPECIES AS POTENTIAL PROBIOTICS TO IMPROVE ORAL AND SYSTEMIC HEALTH

Keywords: *Oral Microbiome, Prebiotic, Probiotics, Caries*

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The salivary glands actively concentrate plasma nitrate, leading to high salivary nitrate concentrations (5–8 mM) after a nitrate-rich vegetable meal. Nitrate is an ecological factor that can induce rapid changes in the function of polymicrobial communities, but the effects on the human oral microbiome have not been clarified. To test this, saliva of 12 donors was collected to grow in vitro biofilms with and without 6.5 mM nitrate. Samples were taken at 5 h (most nitrate reduced) and 9 h (all nitrate reduced) of biofilm formation for ammonium, lactate, and pH measurements, as well as 16S rRNA gene Illumina sequencing. Nitrate did not affect biofilm growth significantly, but reduced lactate production, while increasing the observed ammonium production and pH (all $p < 0.01$). Significantly higher levels of the oral health-associated nitrate-reducing genera Neisseria (3.1 ×) and Rothia (2.9 ×) were detected in the nitrate condition already after 5 h (both $p < 0.01$), while several caries-, halitosis- and periodontitis-associated genera were significantly reduced ($p < 0.05$ at 5 h and/or 9 h). In a second in vitro study, we observed that adding Rothia isolates to oral communities can increase their nitrate reduction and lactate consumption capacities. Preliminary clinical work indicates that nitrate-rich supplements buffer salivary pH when sugar is consumed, resulting from lactate usage in the presence of nitrate. We propose that nitrate could be used as a prebiotic and nitrate reducing isolates as potential probiotics to reduce or prevent dysbiosis, improve general oral health and promote systemic health by increasing nitric oxide availability.

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665 / SAPONINS AS A TOOL TO INCREASE AN EFFECTIVENESS OF ANTIBIOTICS

Keywords: *saponins, antibiotics, biomembrane, nan*

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The large-scale use of antibiotics is one of the reasons for the increase in antimicrobial resistance, which is one of the greatest challenges of the 21st century. The use of surfactants, which can modify the permeability of the cell membranes, may increase the bioavailability of antibiotics to pathogenic bacteria. Hence, the co-use of surfactants and pharmaceutics may involve a synergistic effect, which contributes to the therapeutic effect at a reduced dose of the pharmaceutical.

In this study, the bacteria Pseudomonas aeruginosa were treated with nitrofuran antibiotics in combination with the saponin-rich extract of Sapindus mukorossi. Moreover, the toxicity of the extract on human colon epithelium cells was analyzed, to evaluate the non-toxic level of the used bioactive compounds.

Moreover, the permeability and adsorption capacity, as well as the enzymatic activity of cells treated with drugs and the extract were assessed. To confirm the modification bacterial cells morphology, size, and zeta potential of cells were measured along with their microscopic study.

The obtained results indicate an increase in the permeability of the cell membrane after the co-action of antibiotics and saponins, which leads to increased toxicity against bacteria. Moreover, it was confirmed, that at effective extract concentration, i.e. 10 mg/L, saponins have no toxic effect on the cells of the colon epithelium. The obtained results allow us to hope for an effective tool to intensify the action of antibiotics. This will help to maintain the therapeutic effect while reducing the dose of the antibiotics.

ACKNOWLEDGEMENTS/REFERENCES

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690 / THE COMPARISON STUDY BETWEEN THE INTERFERON-GAMMA RELEASE ASSAY AND TUBERCULIN SKIN TEST IN HEALTHY CHILDREN AND ADOLESCENTS VAC

Keywords: *BCG, interferon-gamma release assay, tuberculin skin test, nan*

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Bacille Calmette-Guérin (BCG) vaccine is the most widely used vaccine in the world. It is considered a major confounding factor in the interpretation of the results of tuberculin skin test (TST), the gold standard for the diagnosis of Mycobacterium tuberculosis (M.tb) infection. The interferon-gamma release assay (IGRA) can be used as an alternative to the TST.

The aim of the study was to compare the diagnostic parameters of TST and IGRA in relation to the diagnosis of latent M.tb infection (LTBI) among BCG-vaccinated Polish children and adolescents with contact with tuberculosis. The correlation between TST size and IFN-γ levels was also assessed.

Of the 216 cases, 47% had TST size <5 mm, 15% from 5 to 9 mm, and 38% ≥10 mm. The mean size of the TST was 6.5 mm. No indeterminate results were observed in IGRA testing; 33% cases were IGRA-positive and 67% IGRA-negative. There was a substantial agreement between the TST and IGRA results ([κ]=0,651). Sensitivity, specificity, positive and negative predictive values were higher for IGRA than for TST. ROC analysis confirmed higher AUC values for IGRA (0.980) compared to TST (0.883). A positive correlation was found between the TST size and age ($p < 0.001$), but there was no association between the age and IFN-γ levels and the TST size and IFN-γ concentration.

In conclusion, IGRA may be the most appropriate tool for the rapid screening of LTBI in the BCG-vaccinated population.

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707 / STUDY OF DISEASE - ASSOCIATED ORAL BACTERIA IN NASOPHARYNGEAL ASPIRATES OF CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE

Keywords: *IPD, NPA, Oral bacteria, Porphyromonas*

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BACKGROUND

Pneumonia, caused by Streptococcus pneumoniae infection, is one of the leading causes of mortality in children under age of 5(1) especially when presents as invasive pneumococcal disease (IPD)(2). Albeit the success of pneumococcal conjugate vaccines (PCVs) in reducing infant mortality, incidence worldwide remains high(3). Previous work shown higher bacterial diversity and richness in respiratory samples from children with IPD compared to healthy controls and an over-representation of Porphyromonas, among other oral-associated bacteria genus, in IPD patients(4). The keystone periodontal pathogen Porphyromonas gingivalis is capable of subverting the immune response and inducing dysbiosis with effects beyond the oral cavity(5). Thus, identification of periodontal-associated pathogens such as P. gingivalis in IPD patients might give an insight on disease onset.

OBJECTIVES

Isolation and identification of periodontal disease-associated bacteria in nasopharyngeal aspirates (NPA) of children with IPD.

METHODS

We analyzed NPA of children with IPD from Catalonia (Spain). Clinical variables and use of antibiotics were registered. Samples were cultured under anaerobic conditions for Porphyromonas isolation. 16S rRNA gene sequencing was used to analyze the microbiota composition of NPA and identification of bacteria isolates.

RESULTS

66 children with IPD (median age 42 months) were included in the study. The genus Porphyromonas could be identified in 32 samples by 16S sequencing, of which 7 presented Porphyromonas relative abundance greater than 1%. Colonies with black pigmentation were observed after 15 days and harvested for identification by Sanger sequencing of the 16S rRNA gene, confirming the presence of viable oral bacteria in the respiratory tract.

ACKNOWLEDGEMENTS/REFERENCES

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725 / PERFORMANCE OF RT-PCR DETECTION OF VIRAL RNA IN SALIVA WITHOUT EXTRACTION

Keywords: *RT-PCR, SARS-CoV-2, saliva, COVID-19*

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BACKGROUND

The use of saliva has proven to be an interesting diagnostic specimen for respiratory virus, including SARS-CoV-2, and its use in alternative to nasopharyngeal swabs (NPS) has many advantages.

OBJECTIVES

In this study a comparison between the results of SARS-CoV-2 in NPS and saliva is performed and the influence of the matrix on the RT-PCR is analyzed.

METHODS

Dual samples (saliva and NPS) of patients suspected of having COVID-19 were subjected to RT-PCR amplification of three viral targets (genes ORF1ab, E and N) using RNA extracted from NPS and also saliva directly without RNA extraction. Different amounts of saliva from different donors were tested in the amplification of the same amount of viral RNA.

RESULTS

An overall agreement of 94% was obtained between NPS and saliva results. Sensitivity of detection of viral genes ORF1ab, E and N in saliva is affected differently and detection of these genes in saliva samples presents great variability when NPS samples present Ct-values above approximately 20, with sensitivities ranging from 76.3% to 86.3%. An increase of the Ct-values (7.3 ± 4.8) is observed in saliva samples when comparedto NPS. The magnitude of the positive shift of the Ct-values in the presence of saliva presents a very high between -person variability.

843 / GENOMIC CHARACTERIZATION OF SARS-COV-2 ISOLATED FROM FAECES

Keywords: *SARS-CoV-2, Faeces, Genome characterization*

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BACKGROUND

SARS-CoV-2 transmission occurs mainly through aerosols and respiratory secretions1, but it has also been described that SARS-CoV-2 replicates at the intestinal epithelium, being excreted in faeces2. Although the evidence to date indicates that the faecal-oral route of transmission is highly unlikely, this route of transmission and thus the possible role of water and food in its transmission cannot yet be ruled out. Therefore, it is necessary to study whether the new variants have greater environmental stability that could favor better transmission of the virus through these sources.

OBJECTIVES

The aim of the study was the analysis of the SARS-CoV-2 genomes present in stool samples from COVID-19 patients and the comparison and characterization of the obtained genomes.

METHODS

Positive faecal samples were evaluated by RT-qPCR of the nucleocapside gene3. Genomes were sequenced using MinION technology with the primer scheme artic V4 and Midnight panel4. Obtained reads were aligned against the reference genome of SARS-CoV-2 isolate Wuhan-Hu-1 (MN908947) and genomic variations and consensus sequences were obtained with ivar software5. Molecular fitness of the spike protein was calculated with SpikePro software6.

RESULTS

SARS-CoV-2 concentrations in faeces ranged from 3.47 to 7.08 log (cg/g). Obtained genomes showed differences in spike protein fitness and were classified in different linages carrying specific mutations.

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854 / SERUM ZINC LEVELS IN PATIENTS WITH SARS-COV-2 INFECTION

Keywords: *Metallomics, Heavy metals, AAS analysis, Coronaviruses*

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BACKGROUND

Coronaviruses such as SARS, MERS and SARS-CoV-2 are considered respiratory viruses, but their effect is systemic. In addition to the respiratory system, the infection mainly affects the liver and kidneys. The severity of the course of the disease is therefore associated with a number of other pathophysiological changes, including oxidative status and associated elemental levels. Changes in zinc levels in patients with a severe disease course have been described in several studies.

OBJECTIVES

The aim of this work was to make an initial overview of zinc and copper levels in patients with SARS-CoV-2 virus infection.

METHODS AND RESULTS

Experimental monitoring began at the beginning of the autumn pandemic wave (September 2021), when the delta virus variant appeared and continued until January 2022, when the omicron variant appeared. A total of 450 serum samples were collected and analyzed for Zn and Cu by AAS. Zn levels were as follows: in October – MEAN: 10.02, MIN: 4.97 and MAX: 18.42, MEDIAN: 9.66 µM; in November – MEAN: 11.02, MIN: 4.12 and MAX: 26.46, MEDIAN: 10.30 µM; in December – 10.18, MIN 2.21 and MAX 20.76, MED 10.04 µM. A mean value of 10.4 was determined in patients with SARS and a value of 11.42 µM in other individuals, which was 8.9% lower.

CONCLUSION

The results of the change in Zn level can provide important information for a better understanding of the course of SARS-CoV-2 virus.

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927 / REMDESIVIR-MODIFIED AGNPS – THE FIRST STEP TO STUDY ITS ANTIVIRAL POTENTIAL

Keywords: *remdesivir, SARS-CoV-2, nanoparticles, antiviral*

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BACKGROUND

Remdesivir (RDV) is an antiviral drug originally targeted against the Ebola virus (1). However, it has also been used against the SARS-CoV-2 virus, where it shortens the recovery time in patients (2). Silver nanoparticles (AgNPs) show antiviral and antioxidative effects (3) and can be made from Salvia officinalis using green synthesis method.

OBJECTIVES

1. To bind RDV (50, 200, 500 nM) to AgNPs and release it from AgNPs in water and buffers with different pH.

2. To determine the kinetics of RDV release from AgNPs in water/buffers using LC-MS/MS and electrochemistry.

3. To study protein corona effect on RDV recovery.

METHODS AND RESULTS

Detection of RDV was done by LC/MS-MS method – mobile phase A (100% water, 0.1% HCOOH), mobile phase B (95% ACN + 5% water, 0.1% HCOOH), the column Zorbax C18, RT 1.88 min, quantification 200 m/z, qualification 261 m/z, LOD 0.3 ng/ml, LOQ 5 ng/ml, calibration 0–1000 ng/ml. For comparison, an electrochemical method was developed for measuring RDV concentration (DPV; 0.1 M phosphate buffer pH 6.98; start -0.9 V, end -1.5 V, step 5 mV, scan rate 10 mV/s, RDV signal -1.3 V). AgNPs were prepared by filtering S. officinalis extract through AgNO3 coated filter and dispersing in water by US. RDV-AgNPs complex was prepared by dispersion in water by US. RDV was successfully released from AgNPs. The protein corona effect on RDV recovery ranged from 30 to 60%.

CONCLUSION

RDV can bind to AgNPs by weak molecular interactions that allow its release under specific conditions.

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961 / LYMPHOGRANULOMA VENEREUM AMONG CHLAMYDIA TRACHOMATIS-POSITIVE MEN WHO HAVE SEX WITH MEN IN BULGARIA

Keywords: *LGV, MSM, Bulgaria*

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BACKGROUND

Lymphogranuloma venereum (LGV) is an STI caused by Chlamydia trachomatis serovars L1-L3 and is endemic among European men who have sex with men (MSM) since 2003, but diagnostic laboratories in Bulgaria largely do not test for these serovars.

OBJECTIVES

Our aim was to introduce a genotyping approach suitable for a diagnostic laboratory using nucleic acid amplification tests (NAATs) for detection of C. trachomatis in order to be able to detect serovars L1-L3 circulation among MSM in Bulgaria.

METHODS

Thirty-one consecutive clinical samples found positive for C. trachomatis during diagnostic testing from 2020 to 2021 were retrospectively analyzed for the presence of C. trachomatis serovars L1-L3. A commercially available NAAT was used to detect C. trachomatis in first void urine (FVU), pharyngeal and rectal swabs from suspected MSM. In C. trachomatis-positive samples, LGV genovars-specific C. trachomatis DNA was detected by pmpH PCR from the same specimen. LGV genovars were confirmed with partial ompA gene sequencing.

RESULTS

Altogether 31 C. trachomatis-positive samples (22 FVU, 1 pharyngeal and 8 rectal swabs) were successfully typed by pmpH PCR. Twenty-nine samples contained non-LGV and two samples LGV C. trachomatis types. All the C.trachomatis LGV types were found in rectal samples. Detected L types were confirmed to be serovar L2 with ompA sequencing. The data show that LGV infections circulate also among Bulgarian MSM, which underscores the importance of expanding LGV testing capacity in order to better inform patient management. Enhanced surveillance and genotyping programs could help measure and monitor LGV prevalence in Bulgaria.

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985 / COMPARATIVE IN-VITRO ACTIVITIES OF VARIOUS ANTIBIOTICS AGAINST ACHROMOBACTER SPP. ISOLATED FROM PATIENTS WITH CYSTIC FIBROSIS

Keywords: *Emerging bacteria, Cyctic fibrosis, Antibiotic therapy*

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OBJECTIVES

Achromobacter species is an emerging pathogen causing chronic bacterial infections in patients with certain conditions, such as cystic fibrosis (CF), hematologic and solid organ malignancies, and certain immune deficiencies (1). In the present study, the in-vitro antibacterial activities of meropenem, trimethoprim/sulfamethoxazole, colistin, tigecycline, or ceftazidime were comparatively assessed using 50 Achromotobacter spp. strains isolated from CF patients.

METHODS

Fifty non-epidemiological clinical isolates of Achromobacter spp were isolated from CF patients in the Department of Clinical Microbiology of a University Hospital. Identification, nrdA allele, and PFGE profile of clinical strains have been reported. The Pseudomonas aeruginosa reference strain ATTC 27853 was used to verify that MIC values were within the correct range stated by The Clinical and Laboratory Standards Institute guidelines (CLSI) (2). Minimal inhibitory concentrations (MIC)'s were determined by the microbroth dilution technique described by the CLSI guidelines.

RESULTS

The 'types-distributions' of the Achromobacter strains used in the study: A. xylosoxidans-21, A. insuavis-10, A. ruhlandii-9, A. aegrifaciens-2, A. genogroup-3, A. mucicolens-1, A. spirithus-1, A. dolens-1, A. piechaudii-1, A. denitrificans-1. The MIC's of meropenem, trimethoprim/sulfamethoxazole, colistin, tigecycline, and ceftazidime, for the 50 strains of Achromobacter spp. were comparatively investigated. According to MIC50/90 values the tested antibiotics were ranked as follows: meropeneme (MIC50/90 = 0.25/1µg/ml); trimethoprim-sulphame-taksazole (MIC50/90 = 0.5-9.5/4-76 µg/ml); colistin (MIC50/90 = 2/4µg/ml); tigecycline (MIC50/90 = 2/8µg/ml); ceftazidime (MIC50/90 = 2/8 µg/ml).

On an MIC50/90 basis, meropenem was found the most active antimicrobial agent against all tested strains After meropenem, trimethoprim-sulphamethoxazole was the second active antibiotic.

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1005 / THE FIRST STUDY OF CAMPYLOBACTERIOSIS IN HOSPITALIZED CHILDREN WITH ACUTE INFLAMMATORY DIARRHEA IN GEORGIA

Keywords: *campylobacteriosis, children, diarrheal illness, epidemiological pyramid*

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BACKGROUND

Campylobacter is the leading food-borne bacterial pathogen, causing gastroenterititis worldwide.

In Georgia clinical laboratories do not routinely analyze Campylobacter spp. while performing stool cultures for other enteric pathogens. Therefore, neither incidence nor burden of campylobaceriosis is known. Meanwhile, the official statistics of gastroenteritis remains alarmingly high, particularly in children population.

OBJECTIVES

The objectives of the study were: 1. to deliver the first systematic prevalence data of campylobacteriosis and the recent epidemiologic trends; 2. to reveal age distribution of three main studied pathogens; 3. to estimate "true incidence" of campylobacteriosis among city children population.

METHODS

382 stool samples were collected during an year at the main infection clinic of the city.

Culture-based bacteriological methods, followed by PCR tests for bacterial isolation, identification and confirmation for three main bacterial pathogens.

The Mann – Whitney test was used for comparing the age distribution of the patients.

An epidemiological pyramid was applied to extrapolate the results of the study to the city children population.

RESULTS

Among enteropathogenic bacteria Shigella sonnei, was the most common (19.1%), followed by thermophilic Campylobacter spp. (12.3%) and Salmonella spp. (4.9%).

The distribution of the patients ages with Campylobacter spp. infection (median 40) was significantly different compared to patients infected with Shigella sonnei (median 92 months), however, no significantly difference was found with salmonellosis (median age 57), and salmonellosis compared to shigellosis.

The 'true incidence' of campylobacteriosis among the city children population was estimated to be around 1800 cases annually an incidence of around 6 per 1000.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Tbilisi Children's Infec-tious Diseases Clinical Hospital (protocol code 1 and 15.06.2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

1011 / IMPACT OF COVID-19 PANDEMIC ON BACTERIAL RESPIRATORY TRACT INFECTIONS

Keywords: *COVID-19, coinfection*

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BACKGROUND

The coronavirus disease 2019 (COVID-19) is associated with high morbidity and bacterial co-infections in affected patients are an additional risk factor for patients' outcome. Although important, microbiological data on bacterial coinfections are sparse.

OBJECTIVES

Aim of this study was to evaluate the impact of COVID-19 pandemic on bacterial respiratory tract infections and to detect differences in composition of bacterial pathogens in lower respiratory tract (LRT) samples of COVID-19 and non-COVID-19 patients.

METHODS

Samples from LRT were collected from patients admitted to both intensive care units and medical wards during season with most frequent respiratory infections (October – February) for five consecutive years (2017-2021). Samples were cultivated and isolates were identified using MALDI-TOS MS (Brucker, USA). Data between pre-pandemic and pandemic periods were compared to each other.

RESULTS

In total, 2449 LRT samples were cultivated. Bacterial pathogens were detected in 75% from samples in pre-pandemic period and 66% from samples collected in pandemic period. There was a significant decrease of samples containing Haemophilus spp. (11,3% vs 4,2%) and Moraxella spp. (3,0% vs 0,2%) in COVID-19 patients. The most frequently detected pathogens in COVID-19 patients were Enterobacterales and the positivity rate was significantly higher (27,4%) than in samples from pre-pandemic period (13,0%). Significantly higher (4,8% vs. 0,7%) was also positivity rate for Acinetobacter spp. in samples from COVID-19 patients. There are significant differences in species composition of bacterial pathogens in samples from non-COVID-19 and COVID-19 patients. Microbial patterns should be taken into consideration when deciding for appropriate empiric antimicrobial therapy.

1028 / EMERGING BACTERIAL PATHOGENS USE OXYLIPINS TO 'JUMP' BETWEEN PLANTS AND HUMANS

Keywords: *emerging pathogens, oxylipins, lipoxygenase, cross-kingdom parhogens*

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BACKGROUND

In our recent paper [1], we have obtained bioinfiormatic evidence for association of lipoxygenases (and, probably, oxylipin synthesizing capacity) with multicellularity and opportunistic pathogenicity in some bacteria. The second association is of particular interest because of its clinical significance: some bacteria in our dataset appeared to be emerging pathogens. Moreover, the mechanism of lipoxygenase pathogenicity was earlier experimentally characterized for Pseudomonas aeruginosa [2]. Here, I follow up our previous research to better characterize the potential of oxylipin signalling for emerging pathogens.

OBJECTIVES

I used bioinformatic methods: network text analysis, phylogenetic analysis, conservation analysis and molecular modelling.

RESULTS

I have found that lipoxygenase phylogeny correlates with ecological traits in pathogenic and symbiotic bacteria. The most evident association was observed between lipoxygenase phylogeny and host category – vertebrate/human, plant, or marine invertebrate. Phylogenetic analysis shows multiple 'jumps' of lipoxygenase between plant symbionts, plant pathogens and vertebrate/human pathogen. Some of pathogens in respective phylogenetic clusters were versatile (capable of affect both plants and vertebrates). It allows to conclude that lipoxygenases provide emerging pathogens with cross-kingdom host jump capacity. Conservation analysis and molecular modelling show that this trait is associated with (n-6)-lipoxygenases, and the mechanism of pathogenicity could be the same as in Pseudomonas aeruginosa: immune response suppression.

CONCLUSIONS

This research scales up the problem of oxylipin signalling as a pathogenicity factor in emerging pathogens and shows the need for molecular epidemiological surveillance of bacterial lipoxygenases.

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The abstract image was created with BioRender.com

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1044 / EPIDEMIOLOGY OF YEAST PATHOGENS IN BULGARIAN POPULATION

Keywords: *pathogens, antifungal drug resistance, The principal component analysis*

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BACKGROUND

In Bulgaria, the epidemiology of yeast pathogens using molecular tools has not been studied so far.

OBJECTIVES

To conduct an epidemiological survey of yeast pathogens circulating in the Bulgarian population. To analyze the correlation between parameters characterizing the studied population (gender, age, diagnosis) and the resistance of the pathogens to antifungal drugs

METHODS

The strains were isolated from clinical samples collected in the Plovdiv region and were identified using the analysis of ITS1+2 region sequences (1).The principal components analysis was used to obtain a dimensional reduction of parameter space (gender, age, diagnosis, and antifungal resistance) to a smaller number of components (with the largest variance in the original data) and to reveal the correlation between parameters.

RESULTS

The majority of yeasts (out of total 113 strains) belonged to the following species Candida glabrata (36%), C. albicans (27%), C. parapsilosis (10%), and C. tropicalis(7%). The remaining yeast strains belonged to 17 yeast species (21%). Strains of C. albicans were associated with males and persons aged over 65 years and showed resistance to a single antifungal drug and did not show a pronounced correlation with a specific disease. Candida glabrata strains were associated with women, children aged up to 11 years, teens 12-17 years old, and adults 46-65 years old and showed resistance up to two antifungal drugs and most frequently were associated with women that hadpregnancy complications. The strains of C.parapsilosis were associated with children,demonstrated multidrug resistance, and were associated with the blood and blood cultures.

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1049 / ZINC TRANSPORTER SYSTEMS IN THE VIRULENCE OF BRUCELLA OVIS

Keywords: *Zinc, Brucella ovis, Virulence*

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BACKGROUND

Brucella ovis is an intracellular bacterial pathogen of worldwide distribution that causes a type of ovine brucellosis. Deciphering the virulence mechanisms of B. ovis is relevant not only to increase knowledge about the similarities and differences among the Brucella species, but also to identify attenuated strains useful as specific vaccines.

Zinc homeostasis is essential in intracellular pathogens to obtain amounts required for cellular processes but avoiding toxic concentrations. The three-protein ZnuABC import system for Zn2+ (transcriptionally regulated by Zur) and the ZntA Zn2+ exporter (transcriptionally regulated by ZntR) have been linked to virulence in B. abortus 2308 but have not been studied in other brucellae.

OBJECTIVES

To construct and characterize mutant strains for the ZnuABC-Zur and ZntA-ZntR Zn2+ import-export systems in the virulent strain B. ovis PA .

METHODS

The ΔznuA, ΔznuBCzur and ΔzntR mutants of B. ovis PA were obtained replacing each wild-type locus by a locus previously inactivated by overlapping PCR. Growth characteristics and virulence in mice of the mutant strains were evaluated in comparison with the parental strain.

RESULTS

Growth of the mutant strains in rich solid or liquid medium was indistinguishable from that of the parental strain. When the Zn2+ specific chelator TPEN was included in the liquid medium, the ΔznuA and ΔznuBCzur mutants, but not the ΔzntR mutant, showed a delayed growth. However, these in vitro defects did not influence virulence in the mouse model since the parental and mutant strains showed similar splenic colonization at week 3 and 7 post-infection in mice intraperitoneally infected.

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1054 / SARS-COV-2 AMINO ACID MUTATIONS DETECTION IN GREEK PATIENTS INFECTED AT THE FIRST WAVE OF THE PANDEMIC

Keywords: *pandemic , genome, mutations, first wave*

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BACKGROUND

SARS-CoV-2, a novel virus that belongs to the Coronoviridae family, emerged in December 2019 causing the COVID-19 pandemic in March 2020. Unlike previous SARS and MERS outbreaks, this virus has a higher transmissibility rate, albeit a lower mortality rate, that could result in accumulation of a significant number of mutations and a faster evolution rate. Pathogenicity of the virus is greatly affected by these mutations as it has been shown by the variety in disease severity caused by several variants that have emerged worldwide and the several studies focusing on the effect of distinct mutations on the virus.

OBJECTIVES

In this study, we aim to record and characterize mutations on SARS-CoV-2 viral genome and their effect on the proteins they are located on in Greek patients infected at the first wave of the pandemic.

METHODS

We performed SARS-CoV-2 Amplicon sequencing and NGS sequencing on Greek patients' samples and bioinformatic analysis of the results.

RESULTS

Although, it has been claimed that SARS-CoV-2 is genetically stable, we have discovered a variety of mutations on the viral genome. In detail, we detected 14 mutations in total on ten SARS-CoV-2 isolates. Mutations were detected several of the viral proteins and specifically on ORF1ab, S protein, M protein, ORF3a and ORF 7a. Most of them result in amino acid substitutions. Changes in the amino acid sequence of the proteins result in minor or major impact on the proteins structural stability and, potentially, on viral transmissibility and viability.

1066 / SARS-COV2 VARIANTS OF CONCERN ASSESSMENT IN A NEW SARI EPIDEMIOLOGICAL SURVEILLANCE NETWORK

Keywords: *SARS-CoV2, covid19, VOC, SARI*

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BACKGROUND

The adaptation of the epidemiological surveillance networks has been necessary to allow finding information about the impact of SARS-CoV2 in several countries. Syndromic surveillance systems offer advantages for covering emerging infectious agents.

OBJECTIVES

To assess the contribution of a sentinel hospital in the characterization of SARS-CoV2 variants of concern (VOC) at the beginning of a new national epidemiological surveillance network based on severe acute respiratory infections (SARI).

METHODS AND RESULT

The performance of SARS-CoV2 VOC determinations by RT-PCR detection (of its most characteristic mutations and deletions) with Molbiol® kits, was protocolized in a sentinel hospital. It was made in a representative sample of the reference population, considering SARI clinical surveillance. In the period from 2021 March 28th to 2022 January 19th, 223 samples of covid patients aged 0 to 100 years (59.9 +/- 21) were selected for VOC study. 88,3% of them came from Specialized Health Care Centres (the rest from Primary Health Care), and 52.9% belonged to men. Regarding VOC study, 44 of them were alpha (19.7%, from April to July), 45 were delta (20.2%, from July to January), and 18 were omicron (8.1%, from December to January), whilst no VOC was detected in another 45 samples (20.2%). Somehow, VOC study could not be performed in several samples because of insufficient swab quantity (27, 12.1%), or quality (19.7%). All these data were consistent with those from the new national SARI surveillance system.

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1068 / BACTERIAL-DERIVED EXOPOLYSACCHARIDES FAVORS BIOFILM RE-COLONIZATION ON BIOTIC AND ABIOTIC SURFACES

Keywords: *biofilm, biofilm matrix, titanium, enamel*

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BACKGROUND

Biomaterial surface properties modulate protein adsorption, microbial accumulation and exopolysaccharides (EPS) production, affecting biofilm susceptibility against antimicrobial treatments.

OBJECTIVES

To understand if EPS-enriched biofilms present advantages on allowing better bacterial re-colonization after antimicrobial treatments, when comparing biotic (dental surfaces) and abiotic (titanium) surfaces with regards to their protein adsorption profile and biofilm EPS-production.

METHODS

Protein adsorption on both surfaces was formed using saliva or blood plasma and evaluated using Liquid chromatography with tandem mass spectrometry. Next, Streptococcus mutans biofilms were formed in vitro for 48-h or polymicrobial biofilms were formed in situ for 3-days allowing EPS-enrichment by exogenous 1% [wt/vol] sucrose supplementation (for EPS synthesis), (negative control was 0.5%-glucose + 0.5%-fructose). Chlorhexidine 0.2% [vol/vol] was used as antimicrobial (negative control was 0.9% NaCl). After antimicrobial treatment the remaining biofilms were exposed to new bacterial inoculum of S. mutans for 2 hours. Finally, re-colonized biofilms were collected and analyzed for: viable bacteria (colony forming units), biofilm pH, EPS content, 3D-structure (confocal laser scanning microscopy) and % of S. mutans on in situ biofilms.

RESULT

Surface and protein adsorption significantly modulated the microbial profile. Enamel dental surface had higher EPS content ($p<0.05$), and higher bacterial biomass compared to titanium. EPS-enriched biofilms presented reduced antimicrobial susceptibility and favored re-colonization, regardless the surfaces, for in vitro and in situ ($p<0.05$). We showed that surface properties modulates the protein profile and the amount of EPS synthesized and that EPS content not only reduces antimicrobial susceptibility, but facilitates bacterial recolonization on biotic and abiotic surfaces.

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1082 / EFFECT OF DOUBLE COMBINATIONS AGAINST COXSACKIEVIRUS B3 AND POLIOVIRUS

Keywords: *double combinations*

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The human enteroviruses (EV) comprise one group of the picornavirus family. These diverse EVs cause a diverse array of clinical features, including aseptic meningitis; hand, foot, and mouth disease; neonatal sepsis-like disease; pancreatitis; encephalitis; myocarditis and pericarditis; paralysis and respiratory diseases. The best known members are the polioviruses (PV) and coxsackieviruses. There are no EV-specific drugs available for clinical use. One of the reasons is fast development of drug-resistant mutants. Synergistic combinations of two agents can overcome toxicity and restrict the emergence of resistance to the partners in the combination.

We studied the combined effects in cell culture of inhibitors with different mode of action against cardiotropic Coxsackievirus B3 (Woodruff strain) (CV-B3) and Poliovirus-1 (LSc–2ab strain) (PV-1). Theoretical additive interactions of expected effects for drug–drug interactions was calculated by using MacSynergy II. Interpretation of significance of the observed volumes of synergy or antagonism, depicted in the differential surface plots, was based upon the program guidelines.

The combinations of pocapavir with oxoglaucine, 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril agains CV-B3 demonstrated additive effect. The combination of pocapavir and oxoglaucine indicate synergistic antiviral activity on PV-1 replication in HEp-2 cells. An additive effect was exhibited when pocapavir was combined with 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril against PV-1. Tested combinations had no cytotoxicity effects on uninfected HEp-2 cells.

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1117 / CLINICAL CHARACTERIZATION OF HOSPITALIZED COVID19 AND SARI PATIENTS

Keywords: *Covid19, SARI*

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BACKGROUND

Assessing the differences between the clinical characteristics of patients with covid19 and other Several Acute Respiratory Infections (SARI) could facilitate their prognosis and treatment. These profiles can vary in each population, so both international and regional studies are important.

OBJECTIVES

To assess the differences between the clinical profile of patients with covid versus other SARIs.

METHODS AND RESULTS

A sample of SARI hospitalized patients was studied in the frame of a European epidemiological surveillance study, but for this work at a one-hospital level. Data were collected since the first case of covid19 in 2020 and until the end of 2021. Patients were tested for SARS-Cov2 for the diagnosis as cases (positives) or controls (other SARIs). Underlying chronic diseases, symptoms upon admission and other severity variables were compared by Chi2 and Student's T tests. Data from 2523 patients were analyzed. Among the patients with other SARIs, it was more likely a) to refer low oxygen saturation, coryza, dyspnoea, sudden onset, b) to have chronic disease such as asthma, cancer, dementia, heart disease, lung disease, neuromuscular disease, kidney disease, ictus, tuberculosis. Otherwise, among patients with covid-19, it was more frequent: a) to be woman and independent for activities of daily living; b) to refer ageusia, anosmia, cough, diarrhea, dizzy, dysgeusia, fever, general deterioration, headache, myalgia, nausea; c) to have chronic disease such as obesity and a higher body mass index ; to be hospitalized for more days, sent to icu, and die.

1124 / SENSITIVE AND SPECIFIC DIAGNOSIS OF FELINE LEUKEMIA VIRUS (FELV) BY A NOVEL POLYMERASE CHAIN REACTION (PCR) IN FELIDS

Keywords: *PCR, Feline Leukemia*

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BACKGROUND

Feline leukemia virus (FeLV) is a gammaretrovirus associated with multiple fatal disease syndromes in cats, oftenly causing death. Diagnosis is generally hampered by the lack of reliable commercial PCR kits, as the virus shares homology with sequences integrated by ancient FeLV (enFeLV) infections, ending in false positives results.

OBJECTIVES

To develop a novel polymerase chain reaction (PCR) for rapid, sensitive and specific molecular diagnosis of FeLV.

METHODS

A pair of primers was designed to amplify a 204 bp region within the env gene of FeLV provirus, using the GeneRunner program, and checking the homology with BLAST database (NCBI). Sixty-eight blood samples from cats with suspected FeLV infection were tested. The PCR was validated using the amplicon of a positive sample, previously cloned into a pGem-T vector, and verified by sequencing. The samples that tested positive were sequenced by the Genomics Sequencing Service of the University of La Laguna. The sequences obtained were analyzed using BLAST database (NCBI) and MEGA-X (Molecular Evolutionary GeneticsAnalysis) program.

RESULTS

Sixty out of the 68 samples showed DNA bands of the expected size. The analysis of the sequences showed a 100% homology with the FeLV virus, without any crossmatching with the endogenous sequence (enFeLV). An efficient detection technique for Feline Leukemia Virus has been developed, which does not amplify the endogenous sequences, ensuring the specificity needed for such a complex disease diagnosis.

1138 / ORAL IMMUNOGENICITY OF ENTEROTOXIGENIC ESCHERICHIA COLI OUTER MEMBRANE VESICLES ENCAPSULATED INTO ZEIN NANOPARTICLES COATED WITH

Keywords: *Enterotoxigenic Escherichia coli (ETEC), Outer membrane vesicles, Oral vaccine, Nanoparticles*

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BACKGROUND

Enterotoxigenic Escherichia coli (ETEC) infections have been identified as a major cause of acute diarrhoea in children in low-middle income countries, associated with substantial morbidity and mortality rates. However, there is no licensed vaccine against this pathogen. ETEC vaccine development is challenging since this pathotype expresses a wide variety of antigenically diverse virulence factors.

OBJECTIVES

We propose the use of outer membrane vesicles (OMVs) isolated from two representative ETEC clinical strains previously selected attending to the presence of common virulence factors and the oral route was selected for administration.

METHODS

Proteomic studies were carried out in order to determine the presence of important ETEC virulence factors in the OMVs. Moreover, it was investigated their antigenicity using sera from patients infected with ETEC. Finally, it was studied their immunogenicity. For this purpose, BALB/c mice were orally immunized with the vesicles, either free or encapsulated in zein nanoparticles coated with a Gantrez®–mannosamine conjugate. Groups of mice immunized through subcutaneous route and non-immunized mice were used as controls.

RESULTS

Proteomic studies revealed the presence of important immunogens, such as heat-labile toxin, critical colonization factors and adhesins. Furthermore, these vesicles proved immunogenic after subcutaneous administration in mice. Moreover, mice immunized with encapsulated OMVs orally showed higher levels of specific antibodies IgG2a comparing to those immunized with free OMVs. These results were supported with biodistribution studies which indicated that the encapsulation of OMVs delayed the transit through the gut. Further studies are needed to evaluate the protection efficacy of this vaccine approach.

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1169 / COMPARISON OF CANINE T CELL EPITOPE REPERTOIRE PREDICTED FOR DIFFERENT SARS-COV-2 OMICRON S-PROTEIN SEQUENCES

Keywords: *SARS-CoV-2 Omicron S-protein, MHC I-restricted epitopes, dogs, in silico*

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BACKGROUND

Omicron, the predominated SARS-CoV-2 variant worldwide, is still evolving, generating new mutations within its immunodominant spike protein (S-protein). Being extremely transmissible in humans, the Omicron variant may likely be highly infective in other susceptible species, especially, in pets.

OBJECTIVES

To analyze the effect of nonsynonymous mutations in the Omicron S-protein sequence on the repertoire of in silico predicted MHC I-restricted T cell epitopes and their potential immunogenicity in domestic dogs.

METHODS

Amino acid sequences of S-protein derived from six Omicron variants isolated in different parts of the world as well as reference sequence of the hCoV-19/Wuhan/WIV04/2019 strain were extracted from GisAid database. Canine MHC class I-restricted T cell epitopes and immunogenic properties of peptides were predicted using NetMHCPan 4.1 and Class I Immunogenicity tools, respectively (https://www.iedb.org).

RESULTS

While only 197 epitopes were discovered within the reference S-protein sequence under standard settings (strong binder % rank ≤1), 203 - 207 MHC I ligands were predicted for the Omicron variants with 151-154 peptides shared between reference and Omicron ones. Of 56 unique epitopes exclusively presented in Omicron sequences, only 17 (30%) were highly conservative and found in all six Omicron variants. Notably, the average immunogenicity score of distinct S-protein epitopes predicted for Omicron strains was higher than for those of reference Wuhan. Thus, our results suggest that mutations may lead to the emergence of more immunogenic spike protein variants possessing high potency to induce CD8+ T-cell immune response in dogs.
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1191 / IDENTIFICATION OF TYPE VI SECRETION SYSTEMS EFFECTOR PROTEINS THAT CONTRIBUTE TO INTERBACTERIAL COMPETITION IN SALMONELLA DUBLIN

Keywords: *Salmonella, T6SS, effector, interbacterial competition*

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The Type VI Secretion System (T6SS) is a multiprotein device that has emerged as an important fitness and virulence factor for many Gram-negative bacteria through the injection of effector proteins into prokaryotic and/or eukaryotic cells via a contractile mechanism. In Salmonella, five T6SS gene clusters have been identified within pathogenicity islands SPI-6, SPI-19, SPI-20, SPI-21 and SPI-22, which are differentially distributed among serotypes. Salmonella enterica serotype Dublin (S. Dublin) is a cattle-adapted pathogen that harbors the T6SS encoded in pathogenicity islands SPI-6 and SPI-19 (T6SS-6 and T6SS-19, respectively). Interestingly, while both systems have been linked to virulence and host colonization in S. Dublin, an antibacterial activity has not been detected for T6SS-6 in this serotype. In addition, there is limited information regarding the repertoire of effector proteins encoded within T6SS-6 and T6SS-19 gene clusters in S. Dublin. In this study, we demonstrate that T6SS-6 and T6SS-19 of S. Dublin CT_02021853 contribute to interbacterial competition. Bioinformatic and comparative genomic analyses allowed us to identify genes encoding 3 candidate antibacterial effectors located within SPI-6 and 2 candidate effectors located within SPI-19. Each antibacterial effector gene is positioned upstream of a gene encoding a hypothetic immunity protein, thus conforming an effector/immunity module. Of note, the genes encoding these effectors and immunity proteins are widely distributed in Salmonella genomes, suggesting a relevant role in interbacterial competition and virulence. Finally, we demonstrate that E/I modules SED_RS01930/SED_RS01935 (encoded in SPI-6), SED_RS06235/SED_RS06230 and SED_RS06335/SED_RS06340 (both encoded in SPI-19) contribute to interbacterial competition in S. Dublin CT_02021853.

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1192 / T3SS EFFECTORS GTGE AND SOPD2 CONTRIBUTE TO INTRACELLULAR SURVIVAL OF SALMONELLA TYPHIMURIUM IN DICTYOSTELIUM DISCOIDEUM

Keywords: *Type III Secretion Systems, Bacterial Effectors, Salmonella, Dictyostelium*

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Salmonella survives and replicates intracellularly in a specialized membranous compartment known as the Salmonella-containing vacuole (SCV). To establish the SCV, Salmonella translocates effector proteins into host cells through two “type III secretion systems” (T3SS). Many effectors subvert the function of small GTPases of the Rab family, as Rab32. Among other functions, Rab32 contributes to restricting the intracellular growth of bacterial pathogens. Effectors GtgE (serine protease) and SopD2 (Rab32 GTPase-activating protein) of Salmonella Typhimurium act cooperatively to cleave Rab32, preventing its recruitment to the SCV and allowing the intracellular survival of this pathogen in macrophages. The ability of Salmonella to survive within amoebae, including Dictyostelium discoideum, has been reported, but the molecular mechanisms involved have not been fully characterized. We have reported that S. Typhimurium requires both T3SS to survive intracellularly in D. discoideum residing in a SCV-like compartment. Here, we studied the role played by effectors GtgE and SopD2 during D. discoideum infection by S. Typhimurium. To this end, we generated ΔgtgE, ΔsopD2 and ΔgtgE ΔsopD2 mutants of S. Typhimurium and evaluated their intracellular survival in D. discoideum by competitive infection assays. All mutants displayed increased internalization and reduced intracellular survival in D. discoideum when compared to the wild type. The genome of D. discoideum encodes four putative Rab32 proteins. Our sequence-based analyses confirmed that all four Rab32 proteins of D. discoideum share a high sequence similarity with mammalian Rab32 proteins. Furthermore, the Rab32 proteins of D. discoideum retain the sequence motif experimentally defined as the GtgE cleavage site.

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1198 / AN UPGRADED SELECTIVE CULTURE MEDIUM FOR PRIMARY ISOLATION OF BRUCELLA ATTENUATED STRAINS FROM VETERINARY SAMPLES

Keywords: *Brucella melitensis, selective culture medium, antibiotics, veterinary samples*

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BACKGROUND

Animal brucellosis is characterized by abortion and bacterial shedding in livestock. Brucella isolation is the only definite confirmation of infections. For this, the use of adequate selective culture media able to inhibit the growth of overcoming contaminants is an essential tool. Currently, simultaneous culture in Farrell's (FM) and CITA (CM) media is the recommended method. However, attenuated mutants susceptible to polymyxins can be inhibited in these media, providing misled safety results in the natural host.

OBJECTIVES

To develop a selective culture medium for isolation of Brucella attenuated strains from veterinary field samples.

METHODS

The growth B. melitensis 16M, Rev1 and two polymyxin B susceptible mutants was analysed in FM and CM vs. plain agar. For this, we cultured serial ten-fold dilutions from 109 to 103 CFU/mL in each culture medium. To define the Brucella selective medium (BSM), we analysed the individual and combined activities of the CM's antibiotics (as less inhibitory than FM). Moreover, sensitivity and specificity of BSM were assessed, first, in laboratory conditions (by culturing Brucella and field contaminant strains) and, thereafter, with a total of 1,759 sheep field samples.

RESULTS

The growth of the four B. melitensis, more markedly Rev1Δwzm, were highly inhibited in FM and, with the exception of Rev1, also in CM (Fig. 1A). The inhibition in CM was related to a synergistic action between colistin and vancomycin (Fig. 1B). The redefined BSM (halving vancomycin and colistin) allowed the growth of all B. melitensis strains as in agar (Fig. 2A), and inhibited field contaminants as in CM (Fig. 2B). Overall, BSM improved the sensitivity vs. CM for primary isolation of both B. melitensis attenuated mutants (Fig. 3).

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1207 / IDENTIFICATION OF CANDIDA AND GEOTRICHUM COLONIZED IN THE GASTRO-INTESTINAL TRACT OF PATIENTS WITH CHRONIC URTICARIA

Keywords: *Chronic hives, Candida, Molecular typing, nan*

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INTRODUCTION

Urticaria is a reaction pattern that represents cutaneous mast cell degranulation, with the condition being defined as chronic if lesions recur for longer than 6 weeks.

OBJECTIVES

Considering the high incidence of chronic urticaria among the patients with colonization of yeasts in gastro-intestinal tract, we investigated all fungal colonization and invasion in the gastro-intestinal tract of cases involved hives. Our aim was identification of all isolates in the level of species.

METHODS

Our subjects included 200 cases with long time superficial lesions as Urticaria. Fresh stool sample from the cases with clinical symptoms were collected. A direct microscopic investigation performed for the detection of fungal growth in gastro-intestinal tract. The basic culture on sabouraud glucose agar used for confirming of the fungal detection. The Molecular methods and proteomic based MALDI-TOF system used for the identification of all fungal isolates.

RESULTS

The highest age range of our cases was 40-50 and included 25% of all. Women and men similarly involved (12 cases each). Our findings of microscopic investigation included budding cells in 13(54.2%) cases, blastospores 6(25%), arthrospores 3(12.5%) and pseudohypha 2(8.3%). Total of 24 fungal isolates, 7 (29.2%) cases of Geotrichum silvicola, 7 (29.2%) Candida albicans and 6(25%) Candida glabrata were the most frequent identified by MALDI-TOF system. Other yeast included C. Africana, C. tropicalis and C. glabrata one each. Only one un-known case by MALDI-TOF system recorded.

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1469/ VIRAL IMMUNITY AND THERAPEUTIC INTERVENTION FOR VIRAL INFECTIONS.

Keywords: *viral, immunity, therapeutic interventions, infections*

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Viral immunity is specifically the immune response to viral infections. The antibodies serve as cr barriers to viral infections. Viruses constitute the simplest life forms and causes of diseases and also a reservoir of enormous genetic diversity. Hormonal immunity to a virus is achieved through immune system in communicating the presence of invading pathogens At the infected site or cells to effectors' cell which will also interfere with the essential process for viral cycle. Individuals that successfully control infective virus, elicited antibodies can provide lifelong protection from future re-infection. One approach to understand the nature of successful immune response has been to utilize structural biology that will help to uncover the molecular details of anti-bodies derived from vaccines or natural infections and how they interact with their cognate microbial antigens. The ability to isolate antigen specific B-cells and rapidly solve structures or functional monoclonal antibodies in complex with viral glycoprotein surface antigen has greatly expanded our knowledge of the sites of vulnerability on viruses Therefore this review will compare the adaptive humoral immune response to Human Immuno-deficiency Virus Influenza and Filoviruses with a particular focus on neutralizing antibodies the pathogenesis of each of these viruses is quite different providing an opportunity for comparison of immune responses. Viruses use cell machinery to replicate their genome and produce viral proteins. Several intra-cellular factors including the redox state might directly or indirectly affect the progression of viral infection .Treatment with antivirals Immunodulators and Immunoprophylaxis by vaccination are currently licensed as therapeutic measures.

04

1511 / MOLECULAR PREVALENCE AND IDENTIFICATION OF BARTONELLA SPECIES IN DOGS AND ARTHROPOD VECTORS IN HAMEDAN AND KERMANSHAH, IRAN

Keywords: *Arthropods, Bartonella, Dogs, Zoonotic*

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BACKGROUND

Bartonella species are lesser-known pathogenic bacteria that infect a wide range of domestic and wild animals as well as humans. Currently, out of 40 Bartonella species/subspecies, at least 17 are associated with clinical signs in humans and animals. However, despite the zoonotic importance of bartonellosis there is limited information about prevalence and species infecting dogs and cats in Iran.

OBJECTIVES

The aim of this study was determination of molecular prevalence and identification of Bartonella species in dogs and arthropods infesting them in Hamedan and Kermanshah cities in west of Iran.

METHODS

Blood genomic DNA (gDNA) was extracted from 100 dogs (45 from Hamedan and 55 from Kermanshah) and, of 25 Ctenocphalides canis fleas, six Pulex irritans fleas and 12 Rhipecephalus sanguineus ticks collected from nine infested dogs were examined for the presence of Bartonella species. Conventional PCR targeting fragments of ITS and rpoB genes was performed, and PCR-positive samples were sequenced bidirectionally and analyzed phylogenetically.

RESULTS

Out of 100 dogs, 14 dogs (14%, ten from Hamedan and four from Kermanshah) were found infected with Bartonella species. Nucleotide sequencing confirmed the presence of four Bartonella species in the examined population i.e. Bartonella vinsonii subsp. berkhoffii and Candidatus B. merieuxii in dogs. None of the examined fleas scored positive but one Rh. sanguineus tick from a blood-negative dog was infected with Bartonella DNA.

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1553 / EFFICACY OF RAPID LAMP ASSAY AND QUANTITATIVE RT-PCR ASSAY IN DETECTING SARS-COV-2 VIRUS IN SPUTUM AND NASAL SAMPLES

Keywords: *LAMP, RT-PCR, SARS-CoV-2, nan*

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BACKGROUND

Since the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019, quantitative reverse transcription-PCR (RT-qPCR) has remained the diagnostic gold standard for COVID-19. However, methods such as reverse transcription-loop mediated isothermal amplification (RT-LAMP) may offer more rapid and accessible means of diagnosis (1, 2).

OBJECTIVES

This study evaluated the efficacy of using LAMP assay in detecting SARS-CoV-2 compared to RT-qPCR.

METHODS

We collected 74 sputum samples and 74 corresponding nasal swabs from patients in a large acute care setting. We obtained IRB approval for this study. The New England Biolabs SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit was utilized for viral detection in both sample types. Samples that turned yellow were interpreted as positive, while samples that remained pink were considered negative. RT-qPCR Mean Cq-FAM values below 35 were deemed to be significant.

RESULT

Of the 154 samples, RT-qPCR detected mean Cq-FAM values for 71% of samples, while the RT-LAMP assay detected 29%. Of the samples that displayed values for RT-qPCR, 52% were considered significant. Of these, RT-LAMP detected viral load in 42%. Nasal samples were 3.4 times more likely to have meaningful detection of spike protein using the RT-qPCR technique and 1.2 times more likely to detect viral burden using RT-LAMP than sputum samples. Nasal swabs were 2.4 times more likely to detect spike proteins using RT-qPCR and positive RT-LAMP results than sputum samples.

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04

1554 / IMMUNOGENIC DOMAINS OF VIRULENCE RELATED CELL WALL HYDROLASES OF STAPHYLOCOCCUS AUREUS RECOGNIZED BY HUMANS AND ANIMALS

Keywords: *staphylococcus aureus, surface proteins, vaccines, mastitis*

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Bovine mastitis is an infection of the mammary gland that is commonly associated with S.aureus. Within dairy industry approximately 11% of milk production is affected due to mastitis. Mastitis is a risk for human health due to possible ingestion of bacterial toxins and antibiotic residues upon treatment. The exposure of cattle to antibiotics contributes to development of resistance/virulence acquired by horizontal gene-transfer; In addition, some strains shown interspecies transmission that can increase their pathogenicity. Bacterial surface proteins represent a major reservoir of virulence factors involved in host colonization and immune evasion. Some of these proteins have already been tested for their protective function, being the perfect target of vaccine research. Vaccination against S.aureus antigens is a promising approach to prevent mastitis, but effective vaccines are not available or still in clinical trials. Therefore, achievement of effective anti-staphylococcal vaccines requires a better understanding of the surface exposed proteins.

Reversed vaccinology using S.aureus isolates and mastitic cow sera was applied to pinpoint conserved exposed epitopes of selected bacterial surface proteins to identify highly immunogenic candidate antigens.

Bioinformatic and immunological approaches were used to identify conserved immunogenic determinants of S.aureus mastitis isolates, focusing on cell surface-exposed proteins. Conserved potential immunogenic epitopes were selected using antigenicity-plots from CLC-workbench. Further, the B-cell epitope prediction tool Bepipred was used to compare conserved predicted epitopes with those that showed conserved antigenicity. Afterwards, proteins and their domains were purified to challenge their antigenicity in a serological-proteome assay with mastitic-cow sera to evaluate their immunogenic potential.

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1561 / CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE - AN ACTUAL PROBLEM IN CLINICAL PRACTICE

Keywords: *Carbapenem-resistance, Klebsiella pneumoniae, Antibiotics*

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The aim of the study was to determine the prevalence, susceptibility, and the type of carbapenemases of carbapenem-resistant K.pneumoniae (CRKp). During a four-year-period (2017-2020) all samples (urine, wound, blood, tubus/canila, sputum, tracheal aspirates) obtained from the hospitalized patients were analyzed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje. Determination of the susceptibility was done by disk diffusion method, Vitek 2 and E-tests. CIM method and carbapenemase set (Mast Diagnostic) were performed for detection of carbapenemase production to all strains that showed smaller inhibition zones to carbapenems according EUCAST. Easyplex SuperBug CRE (Amplex Biosystems, Germany) was performed for detection of beta-lactamase genes. Out of the total number of Klebsiella pneumoniae - Kp 215/394/363/277, carbapenem resistant were 21%/18%/19%/21%, respectively. The percentage of CRKp, according to the origin, was as follows: 25/13/11/13 from urine, 18/30/43/28 from wound, 29/30/36/17 blood culture, 20/25/41/35 tubus/canilla and 13/19/27/27 was from sputum/tracheal aspirate. In 76 randomly selected isolates with positive phenotypic tests for carbapenemase detection, metallo-beta lactamases encoding genes were detected, such as: NDM (89.5%), OXA-48 (9.2%), VIM (1.3%). Between 90-100% of Kp strains were resistant to 9 tested beta-lactams and ciprofloxacin. The resistance to meropenem and to cotrimoxazole was between 80-89%, to imipenem 50-80%, to gentamicin 40-80% and to amikacin 30-50%. Increased MICs to colistin showed 17.8%, 4.5%, 12% and 12.3% of Kp isolates in 2017, 2018, 2019 and 2020, respectively. The percentage of CRKp isolates is increasing and it is the right time to perform all strategies to control their spread.

1588 / CONNECTIONS BETWEEN EXOPROTEOME HETEROGENEITY AND VIRULENCE IN THE ORAL PATHOGEN AGGREGATIBACTER ACTINOMYCETEMCOMITANS

04

Keywords: *Aggregatibacter actinomycetemcomitans, exoproteome, serotype, virulence*

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BACKGROUND

Aggregatibacter actinomycetemcomitans (Aa) is a Gram-negative bacterial pathogen associated with severe periodontitis and non-oral diseases. Clinical isolates of Aa display a rough (R) colony phenotype with strong adherent properties. Upon prolonged culturing, non-adherent strains with a smooth (S) colony phenotype emerge. To date, most virulence studies on Aa have been performed with S strains of Aa, whereas the virulence of clinical R isolates received relatively little attention.

OBJECTIVES

Since the extracellular proteome is the main bacterial reservoir of virulence factors, the present study was aimed at a comparative analysis of this sub-proteome fraction for a collection of R isolates and derivative S strains, in order to link particular proteins to the virulence of Aa with serotype b.

METHODS

Extracellular proteins were identified by mass spectrometry. Bacterial virulence was assessed using infection models based on larvae of the greater wax moth Galleria mellonella, a human salivary gland-derived epithelial cell line, and freshly isolated neutrophils from healthy volunteers.

RESULTS

351 extracellular Aa proteins were identified by mass spectrometry, with the S strains consistently showing more extracellular proteins than their parental R isolates. A total of 50 known extracellular virulence factors was identified, of which 15 were expressed by all investigated bacteria. Importantly, the comparison of differences in exoproteome composition and virulence highlights critical roles of 10 extracellular proteins in the different infection models. Altogether, our present study provides novel cues for understanding the virulence of Aa, and for development of potential preventive or therapeutic avenues to neutralize this important oral pathogen.

ACKNOWLEDGEMENTS/REFERENCES

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1658 / COMPARATIVE GENOMIC ANALYSIS OF BOVINE STEC AND AEPEC STRAINS ISOLATED IN HUNGARY

Keywords: *STEC, EPEC, genomics, mobile genetic elements*

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BACKGROUND

Escherichia coli belonging to the enterohemorrhagic (EHEC), Shiga toxin-producing (STEC) and atypical enteropathogenic (aEPEC) pathotypes are significant foodborne zoonotic pathogens posing serious health risk with healthy cattle as their main reservoir.

OBJECTIVES

We aimed to perform a comparative genomic analysis of STEC and EPEC strains isolated during a representative sampling of Hungarian cattle farms in 2017-18, as well as those of earlier isolation and those of human sources.

METHODS

Twenty-seven draft genome sequences were determined using Illumina NextSeq platform. Assembly was performed with Spades 3.13.0, gene searches were conducted with VirulenceFinder, VFDB, ResFinder, SerotypeFinder 2.0, PHASTER and BLAST. Phylogenetic analysis was done using Interactive Tree of Life v5, HarvestSuite, and Mega-X.

RESULTS

Additive virulence gene arrays and genomic integration sites of key virulence genes showed correlation to the origin of strains, the most frequent site for the Stx1 prophage was yehV, for Stx2 it was wrbA and sbcB. In the case of O157:H7 strains, the locus of enterocyte effacement (LEE) pathogenicity island integrated into the selC site, in other serotypes into pheV, and in the case of O26:H11 strains, the pheU site.

Phylogenetic analysis showed no clear separation of the bovine and human lineages among the strains. The similar virulence arrays and close phylogenetic relations of strains from bovine and human sources underline the zoonotic potential of bovine aEPEC and STEC. The high number of prophages (9 regions on average per strain) and the various integrations patterns point to the ongoing evolution of these pathogens in cattle.

ACKNOWLEDGEMENTS/REFERENCES

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1660 / PRODUCTION AND PURIFICATION OF RECOMBINANT SARS-COV-2 SPIKE GLYCOPROTEIN-S2 SUBUNIT

Keywords: *Covid-19 Antigen, Purification, Recombinant S2 Subunit, SARS-CoV-2*

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BACKGROUND

S2 subunit mediating fusion between viral and host membranes. The high amino acid sequence identity of more than 90% in the S2 subunit suggests that the fusion mechanism during virus infection is well-conserved. With these properties, S2 is a better target for the development of a comprehensive therapeutic agent.

OBJECTIVES

The spike protein of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a major determinant of viral pathogenesis. Many researchers started to develop detection kits and anti-COVID-19 vaccines. It is aimed that production of target S2 subunit is required to facilitate the development of these tools.

METHODS AND RESULTS

To enhance expression of the S2 subunit of SARS-CoV-2, S2 gene fragment (1788 base pair) was cloned in pET30a expression vector added a His tag at the C terminus. S2 subunit was expressed in Escherichia coli BL21 and Rosetta 2 pLysS cells induced by IPTG. The expression of S2 was optimized under different conditions including time, media, temperature etc. to obtain the proteins in soluble form. The bacterial cell pellet containing the recombinant proteins was lysed. IMAC purification was carried out over the S2 containing bacterial supernatant using the Akta Avant 25 chromatography system. The purified S2 protein (65.5 kDa) was confirmed by Western Blot using anti-polyhis antibodies. The resultant purified S2 subunit would be useful tools for the development of immunodetection kits, vaccines, and immunoglobulin production.

ACKNOWLEDGEMENTS/REFERENCES

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1781 / DEVELOPMENT OF A MONOCYTE ACTIVATION TEST FOR MONO- AND MULTI- COMPONENT SHIGELLA GMMA-BASED VACCINES

Keywords: *Shigella, Vaccines*

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Shigellosis contributes to substantial childhood mortality and morbidity worldwide and levels of multi-drug resistance are increasing. Despite this, there are currently no licensed vaccines against Shigella. Generalised modules for membrane antigens (GMMA) are a promising approach for Shigella vaccines. GMMAs are produced via a high yield production method to purify outer membrane vesicles from genetically modified Gram-negative bacteria. The presence of lipopolysaccharides and lipoproteins in GMMA-based vaccines makes them intrinsically pyrogenic (capable of causing febrile response). The rabbit pyrogen test (RPT) and the bacterial endotoxin test are the most commonly employed assays to monitor the pyrogenic content and thereby the safety of vaccines and biological medicines. However, both tests have limitations when measuring inherently pyrogenic medicinal products. Here, we adapt the monocyte activation test (MAT) as an alternative to the RPT for monitoring the pyrogenicity of mono- and multi- component Shigella GMMA-based vaccines. Three methodologies (A-C) for the MAT are endorsed by the European Pharmacopoeia.

Our data identified method C, the reference lot comparison test, as the most suitable. A comparison of different reference materials demonstrated the highest levels of parallelism and stability for the drug substance when analysing the matched drug product. The MAT overcomes limitations of the RPT, including ethical considerations. Our results support the implementation of the MAT as a suitable alternative to the RPT. The assay parameters established here can also be extended to other GMMA-based vaccines currently in development further aiding vaccine batch release.

ACKNOWLEDGEMENTS/REFERENCES

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292 / RHODOCOCCI AS A MEANS OF PHARMACEUTICAL POLLUTION CONTROL

Keywords: *Rhodococcus, pharmaceutical pollutants, NSAIDs, biodegradation*

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BACKGROUND

The problem of pharmaceutical pollution is currently taking on alarming dimensions: The consumption of medicines and personal care products has increased dramatically during the COVID-19 pandemic. Administration of over-the-counter drugs, in particular analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), for the symptomatic treatment of acute respiratory infections is growing. These substantially contribute to the pressure on and risks for ecosystems. Among the microorganisms that promote natural attenuation from xenobiotics are Rhodococcus spp., which can degrade numerous pollutants and have multiple adaptive capabilities.

OBJECTIVES

To study the features and mechanisms of metabolization of pharmaceuticals by rhodococci.

METHODS

Combined atomic force and confocal laser scanning system was used to reveal changes in morphometric and physicochemical parameters of rhodococci exposed to pharmaceuticals. Respirometry was applied to investigate physiology of biodegraders. Comparative bioinformatic analysis of complete genomes was applied to study biodegradation genes and the role of antioxidant enzymes in Rhodococcus adaptation to drug-induced stress. Using chromatographic and spectral methods, the pathways of pharmaceutical biodegradation were predicted. Biological activities and ecotoxicities of metabolites formed were determined in silico.

RESULTS

The potential of rhodococci to decompose diclofenac, ibuprofen, ketoprofen, meloxicam, and naproxen, NSAIDs most often found in aquatic ecosystems, was shown. Novel strains capable of complete degradation of the above NSAIDs were selected. Optimal conditions of the NSAID bioconversion were revealed, and the most typical responses of rhodococci exposed to these toxicants were identified. Biocatalysts with high functional stability were obtained. The developed biocatalysts are promising for pharmaceutical wastewater post-treatment and pharmaceutical waste disposal.

ACKNOWLEDGEMENTS/REFERENCES

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325 / FUNGAL AGRICULTURE UP TO DATE: WHAT ELSE CAN WE LEARN FROM HOW INSECTS DO FARMING?

Keywords: *Fungiculture, Microbial Ecology, Biomimesis*

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The invention of agriculture is a revolutionary moment in the history of humankind that allowed a transition to sedentarism, the creation of urban settlements, and a titanic population explosion. We owe such technology the complexity of our society, but humans are not the only farmers. Groups of highly social insects were growing fungal crops tens of millions before us. The most studied group of fungus-growing insects are Attini ants, but termites of the Macrotermitinae subfamily and ambrosia beetles have also selected nutritional mutualisms with fungi. Insects' systems are described as multipartite symbiosis and many associated microbes show ecologically and technologically interesting traits.

Traits such as the production of pesticide-like substances or antibiotics that prevent antagonists to attack the cultivar, the fertilization of crops by enrichment with nutrients, or the degradation of the highly recalcitrant substrate of the cultivar, have shown us that nature has developed the answer to the problems humans fight too and that we can learn a lot about how insects do farming. Although there exist important reviews about this topic, some of these publications are decades old and here we update the advances in the study of the microbiology of fungus-farming insects' systems. We critically comment on gaps related to the functional necessity of some described associations and expose new described traits that can also be found in human agriculture. Finally, we highlight the unbalance between the discoveries in Attini ants and other groups of fungus-growing insects, and how it can be used to develop analogous studies.

333 / GROWTH OF TRICHODERMA ASPERELLUM AT DIFFERENT CONCENTRATIONS OF TWEEN SURFACTANT

Keywords: *Trichoderma, Tween*

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The present study determined changes in the morphological structure and dry biomass of T. asperellum MSCL 309 grown in different concentrations of Tween 20, Tween 40 and Tween 80, as well as the antifungal activity of the cultivation liquid against the phytopathogenic fungus Cladosporium herbarum MSCL 276. During the experiment, T. asperellum suspension was introduced into six standing flasks with sugar-yeast extract medium. Culture medium with T. asperellum biomass was drained from each flask every day since the start of cultivation (3 days) to detect changes in T. asperellum biomass. Changes in biomass weight were recorded. In addition, microscopy of T. asperellum biomass was performed. According to the obtained experimental data, it could be concluded that chlamydospores began to form within 48 hours, but no morphological differences were observed between samples with different concentrations of Tween 20, 40 and 80, and none of the samples formed inhibition zones against phytopathogenic fungi, but after 72 hours of culture T. asperellum supernatant formed growth inhibition zones against the phytopathogen at low concentrations of Tween 20 and 80. The highest yields of T. asperellum dry biomass were achieved at 1.5% and 2% Tween 20, 40 and 80 concentrations.

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361 / THE BIOLOGICAL ACTIVITY OF MICROBIAL VOLATILE ORGANIC COMPOUNDS ON DIFFERENT ORGANISMS

Keywords: *volatile organic compounds, quorum sensing, phytopathogens, Arabidopsis thaliana*

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Microorganisms emit a huge variety of volatile organic compounds (VOCs). These substances can affect the activity of enzymes, the expression of genes of other organisms, act as chemical signals, and affect the bacterial Quorum Sensing (QS) system.

Our research aimed to investigate individual pure VOCs, synthesized by bacteria and fungi, with different chemical structures (alcohols, ketones, terpenes, etc.) and their effects on various biological objects.

We showed that studied VOCs have an inhibitory effect on the phytopathogenic Agrobacterium tumefaciens strains. Also, they suppressed the formation of agrobacteria biofilms, their swarming motility, and killing bacterial cells in mature biofilms.

We investigated the ability of VOCs to modulate bacterial communication by influencing LuxR and LuxI type QS systems of different bacteria using specific lux-biosensors. The amounts of VOCs that did not have a significant bactericidal effect on the cell growth of biosensors were used. Moreover, some VOCs demonstrated a specific effect on only one of the studied QS systems.

It was found that VOCs affected the Arabidopsis thaliana growth and the germination of its seeds. In the presence of some VOCs at certain amounts, the total plant biomass increased.

The fruit flies Drosophila melanogaster died most quickly under the action of terpenes, although these substances did not show such a significant effect on other biological objects under this study. Other VOCs also strongly inhibited the vital activity of flies. It was noted that the suppression of the vital activity of flies increased with the elongation of the hydrocarbon chain of ketones.

ACKNOWLEDGEMENTS/REFERENCES

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409 / ANALYSIS OF PUTATIVE BIOSYNTHETIC GENE CLUSTERS AND ANTIMICROBIAL PEPTIDES IN THE GENOME OF BACILLUS SUBTILIS RC 25

Keywords: *Bacillus subtilis, Illumina, Biosynthetic gene clusters , AMPs*

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BACKGROUND

Several bacteria produce a wide range of bioactive compounds with important applications in abiotic stress amelioration, agriculture and plant growth promotion. In this study we aim to analyze the genome of potential Bacillus subtilis RC 25 (NCBI Accession- MZZI01000000) for putative biosynthetic gene clusters, bacteriocins and antimicrobial peptides.

OBJECTIVES

The present study is focused on the analysis of putative biosynthetic gene clusters and antimicrobial peptides in the genome of Bacillus subtilis RC25

METHODS AND RESULTS

Genome of a potential Bacillus subtilis RC25 (3.98 Mb) isolated from chickpea rhizospheric soil was sequenced using illumia Hiseq 2500 and annotation was performed using Rapid annotation by SEED Subsystem (RAST). antiSMASH version 5.1.2, and the integrated ClusterFinder algorithm, an hidden Markov model based probabilistic algorithm was used to identify biosynthetic gene clusters (BGCs) like regions and antimicrobial peptides in genome. A total 4195 genes distributed in 7 scaffolds, 4115 protein coding genes, 80 non protein coding genes, 885 putative proteins, 3 rRNA (5s, 16s and 23s) and 73 tRNAs were identified in the genome. A total 23 regions of BGCs and 468 bactriocins were identified, out of which the AMPs (Bacteriocins) viz: Fengicin, Bacilliobactin, Teichuronic acid, subtilosin A, Bacilysins and surfactin gives the lead that the strain could be prospected for control of minor pathogens in addition to nutrient mobilization and growth promotion under abiotic stress conditions. Presence of large number of Bacteriocins in the genome might contribute to the environmental suitability of the strain and make them suitable organism for technology development.

415 / ISOLATION OF DNA AND PROTEINS FROM MARINE MICROBIAL EPIPHYTES

Keywords: *DNA and protein isolation, marine mcrophytes, Cymodocea nodosa, Caulerpa cylindracea*

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BACKGROUND

Marine macrophyte surfaces are inhabited by diverse microbial communities. When culture-independent methods such as 16S rRNA sequencing, metagenomics, and metaproteomics are used in the study of these communities, it is advisable to perform selective isolation of DNA and proteins prior to analysis. Methods for separating microbial cells from host tissue, such as shaking, scraping, and ultrasonication, do not result in complete cell detachment and, in the case of ultrasonication, may result in host tissue disruption.

OBJECTIVES

Our objective was to adapt protocols for selective DNA and protein isolation from microbial communities inhabiting two marine macrophyte species, the seagrass Cymodocea nodosa and the macroalga Caulerpa cylindracea, and to evaluate the suitability of the recovered material for culture-independent molecular methods..

METHODS

The developed protocols use universally available laboratory chemicals and are based on direct cell lysis on surfaces of marine macrophytes. The removal efficiency of the procedures was evaluated using confocal microscopy, while the recovered DNA and proteins were tested by 16S rRNA sequencing, metagenomics and metaproteomics.

RESULTS

The adapted protocols allowed almost complete removal of epiphytic microbial cells, regardless of host species. The obtained DNA was suitable for metagenomic and 16S rRNA sequencing, while isolated proteins were identified by mass spectrometry. The low presence of host DNA and proteins observed in the isolated samples indicates a high specificity of the protocols for epiphytic communities.

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418 / ANTIBIOGRAM AND MOLECULAR IDENTIFICATION OF PATHOGENIC ESCHERICHIA COLI GENE IN DRINKING WATER SOURCES FROM JOS, NIGERIA

Keywords: *Antibiogram, Escherichia coli, Gene, Multiplex Polymerase Chain Reaction and Water*

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Quality water is essential for the well-being of all people and one of the fundamental global advancement strategies of public health is accessibility to potable water. Escherichia coli (E. coli) have been known to cause diseases in the guts of human. This study was aimed at evaluating the antibiogram and identification of pathogenic E. coli strains gene in drinking water sources from Jos, Nigeria. Water samples were collected from sixty (60) sites in Jos metropolis. The coliform count was determined using the most probable number (MPN) technique. Water samples were subjected to microbiological assessment using spread plate method.

The multiplex polymerase chain reaction (MPCR) technique was used to identify the pathogenic E. coli strains gene. Results revealed that borehole water had coliform counts ranging from 2 to 150 coliforms/100 mL, while well water had coliform counts ranging from 2 to 1700 coliforms/100 mL. The virulent gene fliC (flagellin E.coli strain k12) encountered from two sample sites (Dangara and Dahwak) was identified as pathogenic E.coli flagellin K12. The results of susceptibility study revealed difference among E. coil isolates in their susceptibility and resistance patterns to antibiotics. The high susceptibility of most E. coil was observed with gentamycin, ciprofloxacin, sulfamethoxazole, oxfloxacin and enrofloxacin. This study revealed the presence of virulent gene fliC, contamination of water sources with pathogenic E. coli and their antibiogram. Water sources from these sites should be properly treated before consumption to avoid risk of infection by these organisms.

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426 / EVALUATION OF BACTERIAL DIVERSITY AND COMMUNITY-LEVEL PHYSIOLOGICAL PROFILES ASSOCIATED TO SEDIMENTS AND WATER FROM A KARST CAVE

Keywords: *: caves, bacterial diversity, metabolic fingerprinting, amplicon-based sequencing*

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Cave ecosystems feature constant temperature, lack of light, high humidity, and limited energy sources for trophic webs. The variety, interactions and ecological roles of microorganisms living in cave microhabitats are largely unknown. Therefore, detailed studies of these habitats may provide a wealth of information on geomicrobial interactions microbial-driven biogeochemical cycles

Here we aimed at evaluating the diversity, abundance, and organic substrate preferences of microbial communities associated with sediments and water from a pristine karst cave located in Romania.

Culture-independent techniques were employed to evaluate bacterial diversity, for which total environmental DNA (eDNA) was extracted using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Germany) from sediment and water aseptically collected samples. After aligning all reads against the SILVA ribosomal RNA reference database, community-level taxonomic profiles were generated and analyzed using the DADA2 package in R. Community-Level Physiological Profiling (CLPP) based on BIOLOG EcoPlates™ estimated the ability of communities to metabolize different carbon substrates and was assessed using derived particular parameters of the carbon-source utilization pattern.

Proteobacteria were the most prevalent among the 15 identified phyla in all samples. The abundances of the remaining detected phyla varied depending on the sampling site and sample type. Carbon utilization indicates a more diverse pattern in relation to the sampling site. Our findings indicate that metabolic fingerprinting and amplicon-based high-throughput sequencing provide important information about active communities and substrate preferences. This information, which is accompanied by physicochemical analysis, serves in elucidating the links between taxonomic and functional diversity of subterranean microbial communities.

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436 / RSMA REGULATES PYOCYANIN PRODUCTION THROUGH MVAU AND RPOS IN PSEUDOMONAS AERUGINOSA ID4365, AN OVERPRODUCER STRAIN

Keywords: *Rsm system, RpoS, pyocyanin, Pseudomonas aeruginosa*

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Pyocyanin is a phenazine with redox activity produced by Pseudomonas aeruginosa. This metabolite serves a virulence factor but also, due to its characteristics, can be used in different biotechnological fields. Its synthesis and regulation have been addressed widely in the type strains PAO1 and PA14 and in the environmental strain M18. It has been found that the three quorum sensing systems, the regulators RsaL, MvaU and RpoS and the post-transcriptional Rsm system controls its synthesis. In this work, we determine the regulation by RsmA on the pyocyanin synthesis in P. aeruginosa ID4365, an overproducer strain. By using a set of mutants, transcriptional and translational fusions and western blot assays, we found that rsmA inactivation increase pyocyanin production compared with the wild type strain, when are growing in the limited-phosphate PPGAS medium. This overproduction surpasses to that done by PAO1 and PA14 strains. Also, we found that RsmA regulates the expression of both phz operons, involved in pyocyanin synthesis; the phz1 operon is negatively regulated at translational level but the phz2 operon is positively regulated at transcriptional level in an indirect way through MvaU. Besides, we determined that the phz1 operon mainly contributes to pyocyanin production. Finally, we found that expression of the alternative sigma factor RpoS, which is a negative regulator of pyocyanin production, is positively regulated by RsmA. These results indicate that RsmA regulate not only the biosynthetic operons for pyocyanin production but also their regulators.

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442 / DEGRADATION OF SULPFONATED POLYETHYLENE BY BIO-PHOTO FENTON APPROACH

Keywords: *Biocatalysis, Hyrogen peroxide, Hydroxyl radicals, Photo-catalysis*

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Polyethylene (PE) plastics are highly recalcitrant and resistant to photo-oxidative degradation due to its chemically inert backbone structure. We applied two novel reactions such as, Bio-Fenton reaction using-glucose oxidase (GOx) enzyme alone and Bio-Photo-Fenton reaction using GOx immobilized on TiO2 nanoparticles (TiO2- GOx) under UV radiation, for (bio)degradation of pre-activated PE with sulfonation (SPE). From both the reactions, GC-MS analyses identified small organic acids such as, acetic acid and butanoic acid as a major metabolites released from SPE. In the presence of UV radiation, 21 fold and 17 fold higher amounts of acetic acid (4.78 mM) and butanoic acid (0.17 mM) were released from SPE after 6 h of reaction using TiO2-GOx than free GOx, which released 0.22 mM and 0.01 mM of acetic acid and butanoic acid, respectively. Our results suggest that (bio)degradation and valorization of naturally weathered and oxidized PE using combined reactions of biochemistry, photochemistry and Fenton chemistry could be possible.

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449 / BISMUTH OXYHALIDE (BIOX) NANOPARTICLES FOR DRINKING WATER DISINFECTION

Keywords: *Photocatalysis, Bismuth oxyhalide, Water disinfection*

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BACKGROUND

The pathogens in drinking water distribution systems pose a health risk to humans, therefore drinking water disinfection is the most important treatment step to keep the consumers away from waterborne diseases such as diarrhoea, dysentery, cholera, typhoid and polio [1]. Chlorination is the most applied technology in the developed countries. However, the formation of harmful disinfection by-products (DBPs) makes the applicability of this method limited [2]. Thus, it is necessary to find alternative non-toxic methods.

Recently, nanomaterials with photocatalytic properties, such as bismuth oxyhalide (BiOX, X = Cl, Br, I) materials have attracted attention due to their structural characteristics, excellent electronic and optical properties. Photocatalysis by BiOX has been recognized as promising method to inactivate microorganisms in water while producing CO2 and H2O as the end products [3]. Thus, the objective of this study was to demonstrate the antimicrobial activity of BiOX nanoparticles on the survival of microorganisms in drinking water under visible light irradiation.

METHODS

Escherichia coli ATCC®25922 was used as an indicator microorganism for faecal contamination to evaluate the disinfection efficiency. Antibacterial activity of bismuth oxychloride (Bi12O17Cl2) nanoparticles against E. coli was evaluated with plate count technique and calculated as reduction in the number of CFU.

RESULTS

The results showed that after 2 hours of incubation in aqueous solution, with 1 mg/mL of Bi12O17Cl2 nanoparticles, cultivable E. coli decreased to 1.3-log (95.2% inactivation). Bi12O17Cl2 nanoparticles were able to inhibit the growth of cultivable E. coli and demonstrated their potential in drinking water disinfection.

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455 / OPTIMIZATION OF DNA AND ENDOTOXIN EXTRACTION FROM SETTLED AIRBORNE DUST

Keywords: *DNA, Endtoxin, Dust*

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BACKGROUND

Settled airborne dust is considered an important proxy for human exposures to airborne microorganisms. The introduction of the electrostatic dust collector (EDC) has allowed scientists to collect bacterial endotoxin and DNA in settled dust to study the airborne bacterial communities. However, low DNA yield on exposed EDC´s has been a key challenge.

OBJECTIVES

The aim of the study was to increase DNA yield from EDC to allow reliable description of indoor airborne bacterial communities. We also aimed to optimise the extraction procedure by performing dual DNA and endotoxin extraction from a single EDC.

METHODS

We tested five different DNA extraction methods. The abundance, diversity, and bacterial communities' structure were assessed using qPCR and 16S rRNA amplicon sequencing with Illumina MiSeq. To test the feasibility of dual extraction from a single EDC, we compared the dual EDC extraction to a traditional method for endotoxin extraction.

RESULTS

Among the five DNA extraction methods, Lever et al. (2015) and DNeasy PowerSoil Pro Kit ® DNA extraction methods showed the highest DNA yield. The Lever-method yielded higher bacterial diversity and more consistent DNA yield than the DNeasy PowerSoil Pro Kit ®, while the latter one was faster and had higher throughput, supporting analysis of large number of samples (> 1000). The traditional and dual extraction methods had similar endotoxin concentrations (P = 0.21). Our results suggest that deploying a single EDC is sufficient to efficiently sample DNA and endotoxin in studies on airborne microbial exposure.

460 / GENOME-WIDE SEQUENCING TO STUDY GENOMIC STABILITY AND POLYMORPHISM IN INDEPENDENT CYANOBACTERIAL TRICHORMUS VARIABILIS STRAINS

Keywords: *cyanobacteria, sequencing, genomics, polymorphism*

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BACKGROUND

Studies of the last few decades have shown that the strains of heterocyst-forming cyanobacteria, Trichormus variabilis, are characterized by widespread distribution from polar to tropical climatic zones and several laboratories have the collections of independent isolates of this cyanobacteria.

OBJECTIVES

At our Department was assembled a collection of 16 T.variabilis strains from different world regions, identical in 16S-rRNA genes. So genome-wide sequencing technology provides a unique opportunity for a detailed comparative analysis of the level of genomic polymorphism in these independent T.variabilis strains.

METHODS

Whole genome sequencing (Illumina, Nanopore) of 7 strains including the model strain T.variabilis ATCC29413, two original strains: BAC9610 (Lake Baikal) and 0441 (hot spring, Buryatia, GCF_009856605.1), and strains isolated in Vietnam, USA and Israel, was carried out.

RESULT

These studies have shown that all genomes correspond to the latest updated version of the genome of T.variabilis ATCC 29413 and are characterized by a high degree of similarity and a minimum number of single-nucleotide substitutions.

When compared with known model bacteria, T.variabilis is characterized by higher level of conservatism in paralogical duplicated/multiplicated genes and loci. The bioinformatic analysis showed that the number of variable sites in such loci in T.variabilis genome is 2-3 times less than in the genomes of phylogenetically close heterocyst-forming cyanobacteria and 7-10 times less than in unicellular cyanobacterium Synechocystis PCC6803.These data indicate that the strain T.variabilis is a promising object for studying the processes of adaptation, DNA repair and other mechanisms of maintaining genome stability and low mutability level.

467 / DIVERSITY OF BENTHIC FORAMINIFERA FROM COASTAL SVALBARD

Keywords: *benthic foraminifera, sedimentary DNA, metabarcoding, Svalbard*

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Benthic foraminifera represent the main component of meiofauna in Svalbard Archipelago. Several studies analyzed the distribution and diversity of Svalbard foraminifera. However, all these studies are based exclusively on the morphological identification of specimens sorted from sediment samples. Here, we present the first assessment of Svalbard foraminifera diversity based on sediment DNA metabarcoding. We analyzed sieved and unsieved sediment samples collected at 15 stations situated in three fjords and two open marine areas in front of tidewater glaciers. We obtained a total of 5,968,786 reads that have been grouped in 1,384 ASVs.

More than half of the ASVs (51.7%) could not be assigned to any group in the reference database suggesting the high genetic novelty of foraminifera in the Svalbard coastal area. The sieved and unsieved samples resolved comparable communities, sharing 1023 ASVs, comprising over 97% of reads. Our analyses show that the foraminiferal assemblage differs between five localities, with communities distinctly separated between fjord and opened sea stations. Each locality was characterized by a specific assemblage, with only a small overlap in the case of open sea areas. Our study demonstrates a clear pattern of the influence of water masses on the structure of foraminiferal communities. We also observed changes in foraminiferal diversity depending on the impact of glacial meltwater. This high diversity and specificity of Svalbard foraminifera associated with water mass distribution indicate that the foraminiferal metabarcoding data can be a very useful tool for inferring present and past environmental conditions in the Arctic.

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484 / FABRICS AS FOMITES IN HEALTHCARE SETTINGS: ANALYSIS OF BACTERIAL COMMUNITIES ON HOSPITAL BEDSHEETS

Keywords: *BACTERIAL DIVERSITY, AMPLICON SEQUENCING, PATIENTS' BEDSHEETS, SEASONAL VARIATION*

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BACKGROUND

Textiles act as good substrates for microbial adhesion and growth. Microorganisms multiply rapidly on soft surfaces, form biofilms and can be transmitted from one fabric to another. Interactions between fabrics and pathogens increase the risk of nosocomial infections. Patients shed microorganisms into bedsheets and contaminate it. However, the impact on environmental factors on the bacterial community diversity on hospital fabrics is yet to be established.

OBJECTIVES

The present study was conducted to characterize the bacterial diversity on the bedsheets of patients over seven months (May-November 2019) in a primary healthcare unit, by employing molecular tools.

METHODS

Nucleic acid was extracted from the patches (polyester; cotton fabric) stitched on patient's bedsheets. First, the quantification of four bacterial phyla, viz. α-Proteobacteria, and ß-Proteobacteria, Actinobacteria, and Firmicutes, of significance in such settings, was done across different seasons (May-November 2019) by qPCR. Amplicon sequencing of 16S rRNA gene was performed.

RESULTS

Quantification by qPCR showed that the load of total bacteria, α-Proteobacteria, ß-Proteobacteria, Firmicutes, and Actinobacteria was found to be maximum in the month of June. Amplicon sequencing of 16S rRNA showed that the abundance of Firmicutes was maximum (65%), followed by Proteobacteria (30%) and Actinobacteria (3%) over the sampling period. Maximum abundance of different bacterial phyla was detected in the month of June. However, in the months of October and November, the load of different bacterial phyla was least. It can be concluded that the hospital fabrics harbour large bacterial diversity in healthcare settings, which is dependent on environmental conditions.

494 / IMPROVEMENT OF ENZYMATIC ACTIVITY OF THERMOALKALIPHILIC LACCASE (CTLAC) AND ITS APPLICATION FOR LIGNIN DEGRADATION

Keywords: *Laccase, Random mutagenesis, Lignin degradation, Lignocellulosic biomass*

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Laccase has been considered as a "green" catalyst due to its catalytic cycle: the reduction of one oxygen molecule and the concomitant oxidation of four substrates molecules with the production of two water molecules as the only by-product. Based on our previous research, a thermoalkaliphilic laccase, CtLac, showed versatile properties: high thermostability and resistance to the various surfactants, organic solvents, catalysis of the depolymerization of various lignocellulosic biomass with the enhanced production of high-value benzaldehyde chemicals. In the present study, a mutagenesis study was performed to improve the laccase activity of CtLac using random and site-directed mutagenesis in the lack of the protein structure. Enzymatic activity was determined through spectrophotometric assay via oxidation of 2,6-dimethoxyphenol and consumed oxygen measurement during enzyme reactions. Compared to wild-type, the mutant replacing Valine at 243 position by Aspartic acid (V243D) showed about 30% increased enzymatic activity under the optimal enzymatic activity conditions, 70^0 and pH 8.0. The kinetic constants kcat/Km and the oxygen consumption of V243D were 1.38 and 1.78 times higher than wild-type. After 3 hours of V243D treatment, the production of lignin monomers from rice straw was about 30% higher than wild-type. From these results, it could be assumed that Valine at the position of 243 might be one of the important amino acids contributing to the catalytic efficiency of CtLac.

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500 / BIOPROCESSING OF AGRICULTURE WASTE FOR THE PRODUCTION OF LIGNOCELLULOLYTIC ENZYMES AND THEIR IMMOBILIZATION OVER FLY ASH DERIVED

Keywords: *Lignocellulolytic enzyme, Agro-industrial waste, Immobilization, Saccharification*

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BACKGROUND

The enzymes are versatile biocatalysts in the industrial sector holds immense significance owing to ease of production. However, low stability and difficulty in recovering or recycling enzymes may increase production costs and limit their applications. To overcome this situation, enzymes are often immobilized onto solid insoluble carriers, which enhanced the enzyme stability, catalytic efficiency, shelf-life, and the scope for multiple reuses.

OBJECTIVES

Isolation of the lignocellulolytic microbes from various (extreme) ecosystems and to immobilize their lignocellulolytic enzymes over zeolite.

METHODS

To achieve effective transformation, potent microbes (mainly bacteria and fungi) were isolated from various niches and were screened qualitatively as well as quantitatively for different lignocellulolytic enzymes viz., filter paperase (FPase), endoglucanase (CMCase), β-D-glucosidase (cellobiase), xylanase, and laccase. Wheat straw and sawdust were used as substrates for the cost-effective production of hydrolases under solid-state fermentation (SSF). The enzymes produced were further immobilized over zeolite for their better stability and reusability.

RESULTS

Among different isolated microbial strains, Aspergillus fumigatus ZS-AG showed the highest enzymes activity viz., FPase, CMCase, cellobiase, and xylanase activity with 14.27, 30.78, 11.5, and 22.7 IU/ml respectively. At present immobilization was performed using functionalized zeolite as effective enzyme support for partially purified enzymes. The immobilized formulations were reusable and retained more than 75% of their original activity till the 5th cycle. The attainment of reusability after immobilization is one of the characteristic features which makes a biocatalyst attractive for industrial applications.

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502 / PLANT GROWTH-PROMOTING BACTERIA ASSISTED PHYTOTECHNOLOGY: A HOLISTIC APPROACH FOR THE REMEDIATION OF MARGINAL LAND

Keywords: *PGPB, Miscanthus × giganteus, Marginal land, Phytoremediation*

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BACKGROUND

Marginal land represents poor soil with low crop productivity, which is sometimes additionally contaminated. The exploitation of marginal land for normal agriculture is not possible but it is suitable for the cultivation of energy crops, especially Miscanthus × giganteus (Mxg), though, the harvest biomass value in such land is lower. The produced Mxg biomass can be converted to alternative energy like biofuel and biogas, or used for the production of other value-added products. It is a well-known fact that plant growth-promoting bacteria (PGPB) are beneficial for stimulating the overall development of plants even under stress conditions.

OBJECTIVES

To isolate novel indigenous multiple-abiotic-stress-tolerant PGPB that could be used for enhancement of Mxg growth and phytoremediation under the severe stress of metal-contaminated soil.

METHODS

Isolation and characterization of novel PGPB strains, tolerant to multiple abiotic stresses from metal-contaminated soil. The isolated strains were further evaluated for the biomass enhancement of Mxg grown in metal-contaminated soil and their influence on the phytoremediation process.

RESULTS

The isolated PGPB were highly resilient to diverse abiotic stresses, exhibiting tolerance to salinity (0–15%), pH (4–8), temperature (4–50 °C), and Pb (up to 1200 ppm). The association of PGPB with Mxg resulted in a significant impact on biomass enhancement. The results showed that PGPB treatment increased the growth process and leaves, stems, and roots biomass at harvest by 49%, 86%, and 76%, respectively. In the presence of PGPB, the translocation factor decreased, the phytoremediation process can be classified as phytostabilization.

ACKNOWLEDGEMENTS/REFERENCES

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504 / GLUCOSE INHIBITS FRUR-MEDIATED TRANSCRIPTIONAL ACTIVATION OF THE FRU OPERON IN VIBRIO CHOLERAE.

Keywords: *Vibrio cholerae, glucose, fructose, transcription regulatioin*

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In most bacteria, efficient use of carbohydrates is primarily mediated by the phosphoenolpyruvate (PEP):-carbohydrate phosphotransferase system (PTS) which concomitantly phosphorylates the substrates during import. Therefore, transcription of the PTS-encoding genes is precisely regulated by transcriptional regulators, depending on the availability of the substrate. Fructose is transported mainly through the fructose-specific PTS (PTSFru) and simultaneously converted into fructose 1-phosphate (F1P). We previously show that, FruR acts as a transcriptional activator of the fru operon and is indispensable for the growth of Vibrio cholerae on fructose. Several lines of evidence suggest that binding of the FruR-F1P complex to an operator which is located between the –35 and –10 promoter elements changes the DNA structure to facilitate RNA polymerase binding to the fru promoter. The PTS-dependent utilization of fructose is inhibited by glucose in Vibrio cholerae, but the underlying mechanism remains unknown. Here, we investigate the regulatory mechanism by which glucose inhibits the FruR-mediated transcriptional activation of the fru operon.

514 / MICROBIAL COMMUNITY STRUCTURE IN A CONSTRUCTED WETLAND TREATING CYANOTOXINS IN SURFACE WATERS

Keywords: *Microbial community, Bioremediation, Cyanotoxins, Natural-Based Solutions*

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BACKGROUND

Eutrophication often leads to harmful cyanobacterial blooms that may release cyanotoxins. When water scarcity occurs, vulnerable communities are forced to irrigate their crops with cyanotoxin-polluted water. Consequently, indirect or direct human consumption of cyanotoxins may happen, leading to cytotoxic pathologies. We propose the application of constructed wetlands (CWs) to treat water for irrigation. The hypothesis is that a vertical flow CW can remove cyanotoxins while preserving the nutrients in the eutrophic water.

OBJECTIVE

The microbiota in the CWs is the key player for the removal of pollutants. To understand cyanotoxin degradation processes, we investigate the structure of the CW microbial community, a gene associated with cyanotoxin degradation and possible degradation metabolites.

METHODS

We set up thirty-two vertical-flow CWs mesocosms with different treatments fed with synthetic eutrophic water containing microcystin-LR and cylindrospermopsin. We perform end-point water measurements, 16S amplicon community sequencing and mlrA qPCR. Furthermore, we use liquid chromatography coupled with high-resolution mass spectroscopy to screen for cyanotoxins and their metabolites.

RESULTS

The work is still ongoing. Removal rates are determined. Metabolites of cyanotoxins are being identified, as well as the metagenomics data is being treated. Several variable correlations were already found that explain the cyanotoxin removal dynamics in our systems. Initial data on the microbial community structure will be discussed at the conference. We strive to understand how the CW microbiome reacts and acts to ensure a safe design and operation of the CWs to treat cyanotoxins.

ACKNOWLEDGEMENTS/REFERENCES

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521 / SYSTEMS VIEW OF BACILLUS SUBTILIS PELLICLE DEVELOPMENT

Keywords: *Bacillus subtilis, biofilm, interfacial rheology, CLSM*

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INTRODUCTION

B. subtilis forms pellicles at the water-air interface. The formation of pellicles is usually studied in isolation from the rest of the activity in the liquid growth system and is not well understood. Thus far, the events in the bulk of the liquid growth medium were mostly ignored.

OBJECTIVES

The purpose of this study was to evaluate pellicle formation and development from the system's point of view.

METHODS

The development of B. subtilis PS-216 pellicle in a batch growth system was examined with real-time interfacial rheology and confocal laser scanning microscopy. During the lifecycle of the pellicle bacterial density at the water-air interface, water column, and solid-water interface was continuously monitored.

RESULTS

During pellicle development, we identified six key events that were accompanied by a major change in viscoelastic behaviour and morphology of the pellicle. Two biofilm structures (at the water-air interface and the bottom of the water column) were observed in the system. The viable bacteria were preferentially located in the pellicle, where increasing biomass reduced nutrient availability and resulted in a finite pellicle thickness. The pellicle collapse correlated with spore formation and reduced ability of the pellicle to support mechanical stress.

CONCLUSIONS

The results imply that pellicle formation is a multifaceted response to a changing environment induced by bacterial growth that causes population redistribution within the system, reduction of the viable habitat to the water-air interface, cell developmental and morphogenesis changes, and build-up of mechanical stress supporting structure.

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529 / CHARACTERIZATION OF BACTERIAL ISOLATES FROM TAILINGS POND WASTEWATER

Keywords: *tailings pond wastewaters, bacteria, heavy metals, antibiotics*

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BACKGROUND

Bacterial isolates from tailings pond wastewaters have a great potential for usage in bioremediation since they usually have a natural resistance to the presence of heavy metals present in concentrates and tailings.

OBJECTIVES

The objectives of this study were characterisation of bacterial isolates from tailings pond wastewater from mountain Rudnik (flotation processes polymetallic ore of lead, zinc and copper).

METHODS

Isolates were identified by using MALDI-TOF mass spectrophotometry. The growth ability at different temperatures in different pH and NaCl concentrations was examined by spectrophotometer (λ at 600 nm); the sensitivity to antibiotics and heavy metals was evaluated by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

RESULTS

Isolates were identified as Bacullus altitudinis, Bacillus cereus, Pseudomonas veronii, and Pantoea agglomerans. Tested isolates showed growth ability at both tested temperatures (22°C and 37°C). They are halotolerant, which corresponds to the habitat of the tested isolates. However, they were not tolerant to acidic pH. P. veronii and P. agglomerans showed resistance to Zn (MIC and MBC >20 mg/mL) and Pb (MIC and MBC >4 mg/mL). The lowest resistance was observed in the presence of Cd and Hg (MIC between 0.007-0.156 mg/mL). Isolated species showed sensitivity to tested antibiotics, especially for tetracycline. Other antibiotics acted in the range between <0.25-31.25 µg/mL. The isolates were resistant to the presence of selected heavy metals and sensitive to antibiotics, which is a desirable feature for potential usage.

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541 / BIMODALITY OF CURLI EXPRESSION IN ESCHERICHIA COLI CULTURES

Keywords: *curli expression, biofilm*

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Bacteria E. coli produce curli amyloid fibers as the key component of the biofilm extracellular matrix to gain protection against different environmental stress factors. The main regulator of curli synthesis is a transcriptional factor CsgD, which is under the control of stationary phase sigma factor σS and second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP). Thus, curli synthesis gets activated during the entry into the stationary growth phase. Previous studies on microcolony and submerged biofilms have shown that only a subpopulation of bacteria expresses curli, leading to heterogeneity of extracellular matrix synthesis. In the structured communities this effect can be explained by gradients of nutrients and oxygen or cellular aggregation. With the help of fluorescence labeling followed by flow cytometry analysis, we show that bimodal activation of curli expression also occurs in the well-mixed planktonic E. coli culture upon the entry into stationary growth phase, resulting in stochastic differentiation into two distinct subpopulations of curli-positive and curli-negative cells. Such stochastic differentiation is also observed on the single-cell level when cells are grown in the conditioned growth medium in the microfluidics device. With the help of gene knock-out approach we conclude that the regulation by c-di-GMP is not the origin of bimodal curli expression in E. coli, since the differentiation into distinct subpopulations of curli-positive and -negative cells still occurs when the control of CsgD expression by c-di-GMP is disrupted. Also, we show that the subpopulations of curli-positive and -negative cells exhibit different modes of their resistance against oxidative stress.

549 / TRANSCRIPTOME ANALYSIS OF THE EARLY ATTACK OF MYXOCOCCUS XANTHUS ON SINORHIZOBIUM MELILOTI

Keywords: *Myxococcus xanthus, Predation, Sinorhizobium meliloti, Transcriptomic*

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BACKGROUND

Bacteria in their environments interact with each other, so that the structure of ecosystems and the maintenance of diversity depend on what type of relationship is established between them. Among the antagonistic relationships, predation stands out, in which predatory bacteria look for the prey to kill and lyse it, using the by-products as food. One of the most efficient epibiotic predators is Myxococcus xanthus. Previously, we have reported that this myxobacterium is able to prey on several soil organisms, such as Streptomyces coelicolor (1) and Sinorhizobium meliloti (2), but the molecular mechanism that the predator uses to attack their prey remains unknown.

OBJECTIVES

To determine the transcriptional changes that M. xanthus undergoes in the first hours after contact with S. meliloti.

METHODS

RNA-Seq technology to examine the transcriptome in solid media at 2 and 6 hours after interaction.

RESULTS

During the initial phase of the attack the predator adapts some metabolic pathways and induces specific clusters and families of genes that help to penetrate the colonies and kill the prey. The analysis performed on biological process enrichment and differentially expressed genes indicate that M. xanthus modify its lipid metabolism, up-regulates some secondary metabolite pathways, increases the expression of genes involved in iron uptake, and up-regulates the expression of genes related to translation. Comparison with the peripheral-rods (3) and dynamic development transcriptomes (4) has shown that the main developmental up-regulated genes and genes that are up-regulated in peripheral rods are repressed early after contact the prey.

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558 / ROL OF COPPER AND IRON IN BACTERIAL PREDATION

Keywords: *Myxococcus xanthus, Sinorhizobium meliloti, Bacterial predation, Copper and iron*

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BACKGROUND

Myxococcus xanthus is a soil-predatory bacterium with complex social behaviour. This cooperativity allows it to prey on both prokaryotic and eukaryotic microorganisms. To carry out predation, cells use various mechanisms to kill their prey and obtain nutrients. It our lab, we have characterized many M. xanthus proteins involved in metal detoxification (1). However, it remains unknown whether such metal detoxification systems play a role in predation, as it has been found in eukaryotic predators. Moreover, we have demonstrated that copperaccumulates at the interface predator-prey when this myxobacterium preys on Sinorhizobium meliloti, another soil bacterium found in symbiosis with leguminous plants for nitrogen fixation, which is of great agricultural interest (2). In addition, the transcriptomic analysis of this interaction has revealed that genes involved in iron uptake increase their expression in both bacterial.

OBJECTIVES

To elucidate the role of copper and iron and their metal detoxification systems in the predatory activity of M. xanthus on S. meliloti.

METHODS

Construction and assay of predator and prey mutants in genes involved in metal trafficking and detoxification, studies of gene expression using lacZ fusions, transcriptomic analyses, and microscopy studies, among others.

RESULTS

M. xanthus uses copper detoxification systems to accumulate copper at the predation interface and generate oxidative stress to kill S. meliloti. In addition, this prey, S. meliloti, produces melanin to defend itself from oxidative stress (1). On the other hand, we demonstrate the importance siderophores production, and iron transport and uptake during this interaction.

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588 / TRANSMISSION OF ANTIBIOTIC RESISTANCE GENES VIA MOBILE GENETIC ELEMENTS IN DRUG RESISTANT CAMPYLOBACTER JEJUNI ISOLATES

Keywords: *Campylobacter jejuni, Antimicrobial Resistance, Mobile genetic elements, Whole genome sequencing*

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BACKGROUND

The antibiotic resistance coding genes (ARGs) carried by mobile genetic elements (MGEs) progressively cumulate and spread among zoonotic pathogens and contribute to the creation of extensively drug-resistant bacteria.

OBJECTIVES

The ARGs and the potential role of MGEs in the spread of AMR in drug-resistant Campylobacter jejuni isolates were tested in this study.

METHODS

In total 30 C. jejuni isolates resistant to erythromycin, tetracycline, gentamicin, ciprofloxacin, and ceftriaxone were included in this study. The whole-genome sequencing was performed using the Illumina MiSeq platform. De novo assembly was performed using the SPAdes genome assembler.

RESULTS

The different genomic approaches analysis revealed the prevalence of MGEs, including plasmids, pathogenicity islands, and bacteriophages in C. jejuni isolates. Most of the AMR and virulence factors were distributed within genomic island (GI) regions. Analysis of ARGs' composition based on GI revealed 18 GIs with the size ranging in length from 5.61 kb to 58.83 kb. In total, six GI (size 5.73 to 45.9 kb) were uniquely found in one of the ST-4447 (CCm33) isolates, which contains prophage CPS-53 integrase intS, flgE encoding flagellar motility, virB2, virB4, virB8, and virB9 genes.

The plasmid of 20.765 bp was identified in two C. jejuni isolates assigned to ST-21 (CC21) with high homology of repUS59 (pSSG1) plasmid sequence (FR824044) in the NCBI repository database. The nucleotide sequence comparison of a genomic island in CCm37 showed the presence of pTet plasmid with the tetO gene, L-asparaginase 2, flagellin A, and various homologous hypothetical genes.

592 / BIOCONTROL POTENTIAL OF PLANT GROWTH PROMOTING BACTERIA (PGPB) AGAINST MULTIPLE PHYTOPATHOGENIC FUNGI

Keywords: *antagonistic activity, biocontrol agents, plant growth promoting bacteria, phytopathogenic fungi*

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BACKGROUND

Phytopathogenic fungi are responsible for significant decreases agriculture yield. Although the use of fungicides boosts agricultural output greatly, but has a negative impact on the environment. As an alternative to fungicides, utilization of plant growth-promoting bacteria (PGPB) can inhibit plant diseases, as well as promote plant growth.

OBJECTIVES

The objective of this study was to obtain promising PGPB candidates as biocontrol agents against multiple phytopathogenic fungi.

METHODS

A collection of 35 bacterial isolates were screened for several traits (such as siderophores, hydrogen cyanide and ammonia production) to inhibit phytopathogen. The selected PGPB candidates were investigated antagonistic activity against four different phytopathogenic fungi using both dual culture growth inhibition method and agar well diffusion method using cell-free supernatant.

RESULTS

The results indicated that 62.9 %, 25.7 %, and 100 % of the PGPB isolates tested positive for siderophores, hydrogen cyanide, and ammonia, respectively. The findings of the dual culture growth inhibition tests revealed that 45.5 %, 27.3 %, 36.4 %, and 54.5 % of the 11 PGPB isolates tested could suppress the growth of Fusarium sp., Botrytis sp., Alternaria sp., and Cladosporium sp., respectively. Under the dual culture growth inhibition assay, only Pseudomonas protogenes ML15 demonstrated antagonistic activity against all tested phytopathogenic fungi. Pseudomonas protogenes ML15 and Pseudomonas fluorescens AF8I4 suppressed all tested phytopathogenic fungi in an agar well diffusion test. This study emphasizes the use of PGPB as biocontrol agents in the suppression of fungal infections, which is beneficial to agricultural extension agents and commercial producers.

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595 / MORPHOLOGY CHANGES IN BACTERIAL ISOLATE FROM PHARMACEUTICAL WASTEWATER UNDER THE EFFECT OF MACROLIDE ANTIBIOTIC

Keywords: *Pseudomonas putida, pharmaceutical wastewater, erythromycin, microscopic analysis*

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BACKGROUND

Of the large quantities of pharmaceuticals that enter the environment, antibiotics that act on microbial populations are the most common. Bacteria can develop the ability to biodegrade xenobiotics as a homeostatic response to the stress to which they are exposed by a particular compound. The acquisition of antibiotic resistance can lead to specific changes in bacterial metabolism or morphology that may be useful for bacterial survival under certain environmental conditions, e.g. in industrial effluents. Pharmaceutical wastewater may contain antibiotics as a group of active pharmaceutical ingredients. Therefore, autochthonous microorganisms found in such complex environments are considered to have adapted enzymatic potential. Furthermore, morphological changes in bacterial cells may also be an indicator of the antibacterial mechanism of action.

OBJECTIVES

In this study, the effect of various erythromycin concentrations on the morphology of the autochthonous bacterial isolate Pseudomonas putida from pharmaceutical industrial wastewater was investigated.

METHODS

The experiments were performed under batch conditions. The changes in cell size and shape were determined by microscopic analysis to investigate the effect of erythromycin on bacterial growth.

RESULTS

Microscopic analysis showed that filamentation of cells occurred at an erythromycin concentration of 200 mg/L, with cell length ranging from 6.86 to 19.57 µm during the exponential phase up to 36 hours. The autochthonous bacterial isolate has the ability to survive high concentrations of the macrolide antibiotic erythromycin, which manifests itself in cell morphological changes.

596 / INFLUENCE OF WASTE COMPOSITION AND INDIGENOUS MICROORGANISMS ON THE EVALUATION OF BIODEGRADABILITY PARAMETERS

Keywords: *organic fraction of municipal solid waste, indigenous microorganisms, biodegradability*

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BACKGROUND

In recent decades, increasing concern for the environment has driven the search for renewable and more environmentally friendly alternatives for the generation of energy and products. The organic fraction of municipal solid waste (OFMSW) is an abundant type of biowaste and is highly dependent on where and when it is collected in a given area. European and national legislation has focused on diverting OFMSW from landfill and stabilising it with various technologies. Aerobic and anaerobic indices can be used to evaluate the stability of solid waste samples. Biological treatment uses indigenous microorganisms that convert waste into resources in a native way, including composting or anaerobic digestion. The ratio between biological oxygen demand over 5 days (BOD5) and chemical oxygen demand (COD) indicates the degree of biodegradability of a waste.

OBJECTIVES

The objective is to investigate the effects of variability in OFMSW composition and microbial activity on biodegradability parameters and final outcomes in biological treatment selection.

METHODS

Physico-chemical and microbiological analyses were performed in eluates prepared from real OFMSW samples according to standard methods.

RESULTS

Based on the obtained experimental data from the eluate, biodegradability was evaluated by linear regression analysis. The results show high R2 values, ranging from 0.91 to 0.96. Increased activity of microorganisms is indicated by the dissolved oxygen values, which range up to 0.08 mg/L. The BOD5/COD ratio value is greater than 0.5 and the OFMSW is considered suitable for biological treatment.

615 / ASSESSMENT OF THE ACTIVE RESISTOME IN RUMEN MICROBIOME FROM CATTLE

Keywords: *Rumen, Resistome, Beef cattle, Stability*

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Recent research has documented the resistome in the rumen of ruminants and revealed that the type and abundance of antimicrobial resistance genes (ARGs) could be affected by diet and/or antibiotic treatment. However, most of these studies only assessed ARGs using metagenomics, and expression of the resistome and its biological role within the microbiome remains largely unexplored. In this study we aimed to assess the presence (metagenomic profiling) and expression (metatranscriptomic profiling) of ARGs in the rumen of 48 beef steers belonging to three breeds (Angus, Charolais, Kinsella composite hybrid) using both read-based and assembly-based approaches and ARG-OAP 2.0 pipelines. Sixty (including 20 plasmid-associated) ARGs were expressed which accounted for less than 1% of the abundance of the whole resistome, with tetW and mefA exhibiting the highest level of expression. The active resistome was less diverse in Kinsella composite hybrid than Angus, however, expression of ARGs did not differ among breeds. The total abundance of expressed ARGs was positively correlated with metabolic pathways and 'attenuation values' (a measurement of stability) of the active rumen microbiome. Moreover, Prevotella ruminicola 23, Ruminococcus sp. JE7A12, and Ruminococcus albus 7 were all identified as hosts of expressed ARGs. Our findings provide new insights into the active rumen resistome in vivo, which may inform strategies to limit the spread of ubiquitously found ARGs from the rumen to the broader environment without negatively impacting the key functional outcomes of the rumen microbiome.

ACKNOWLEDGEMENTS/REFERENCES

This work was financially supported by Ministry of Alberta Agriculture and Forestry (2015P008R and 2018F095R) as well as the NSERC Discovery Grant and the Genome Alberta "Omics" project held by LLG and TAM. In addition, TM, YT, and QD was supported by the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2017-FRI-04).

621 / WHOLE GENOME ANALYSIS OF M. INTRACELLULARE ISOLATES FROM ENVIRONMENT REVEALS GENETIC DIVERSITY AND THE EVOLUTION OF VIRULENCE

Keywords: *Mycobacterium intracellulare, Whole genome sequencing, Virulence factors, Host immune response*

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BACKGROUND

Nontuberculous mycobacteria (NTM) are ubiquitous bacteria that are widely distributed in natural environments. Mycobacterium intracellulare is an opportunistic pathogen that causes pulmonary NTM infections in a wide range of hosts. In a previous study, the distribution and antibiotic resistance profiles of NTM isolated from Korea were analyzed.

OBJECTIVES

Multidrug-resistant M. intracellulare isolates were analyzed by single-molecule sequencing to delineate the diversity of environmental strains with respect to phylogeny and pathogenesis.

METHODS

Whole genome sequencing and de novo assembly were performed for six M. intracellulare isolates and the type strain ATCC13950. To identify the host immune response induced by M. intracellulare isolates, the bacterial burden and cytokine expression were investigated in murine alveolar macrophages after M. intracellulare isolates infection.

RESULTS

Although some isolates were identified as M. intracellulare by conventional methods, complete sequence phylogeny suggested that they should be reclassified as M. intracellulare-related strains such as M. chimaera and M. paraintracellulare. M. intracellulare isolates and the type strain had virulence factors involved in the type VII secretion system and transporters. Antimicrobial resistance gene prevalence was not consistent with the drug susceptibility test results of the previous study. Differences in the burden of M. intracellulare and cytokine expression in macrophages are associated with genetic relevance. Whole genome sequencing of M. intracellulare isolates not only provides important insights into virulence factors and pathogenesis, but also helps to construct databases of complete sequences for M. intracellulare and related strains.

ACKNOWLEDGEMENTS/REFERENCES

This work was carried out with the support of “Cooperative Research Program of Center for Companion Animal Research (Project NO. PJ013985)” Rural Development Administration, Republic of Korea.

642 / CONSTITUTIVE PHENOTYPIC MODIFICATION OF LIPID A IN CLINICAL ACINETOBACTER BAUMANNII ISOLATES

Keywords: *lipopolysaccharide, phosphoethanolamine, pmrC gene*

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The degree of polymyxin B (PMB) resistance was measured using nine clinical Acinetobacter baumannii isolates recovered from healthcare facilities. All tested isolates possessed a multi-drug resistance phenotype against four different classes of antibiotics (meropenem, doxycycline gentamicin, erythromycin), except for PMB. Our matrix-assisted laser desorption ionization-time of flight mass spectrometry revealed that intrinsic lipid A modification by phosphoethanolamine (PEtN) incorporation was noticeable only in PMB-resistant (PMBR) strains. The presence of hexa- and penta-acylated lipid A occurred from loss of laurate (C12) acyl chain were identified in all PMB-susceptible strains, but not in the PMBR strains. A high level of the pmrC expression appeared to be responsible for PMBR constitutive phenotype. Reduced negative surface charge in PMBR strains was checked by zeta potential analysis. Fluorescence images using dansyl-PMB showed that PMBR strains had less tendency for PMB, a cationic antimicrobial peptide, binding to cellular surface. Sequence analyses of the pmrCAB operon in all tested nine strains uncovered that known and novel mutation only in PmrB structural protein might lead to constitutive phenotype in PMBR clinical strains.

643 / MORPHOLOGICAL AND PHYSIOLOGICAL ADAPTATIONS OF PSYCHROPHILIC PSEUDRATHROBACTER PSYCHROTOLERANS YJ56 UNDER STRESS CONDITIONS

Keywords: *extremophile, oxidative stress, cell elongation, branched filaments*

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Both culture-independent and culture-dependent analyses using Nanopore™-based 16S rRNA sequencing and terminal restriction fragment length polymorphism showed that short-term exposure of Antarctic soils to low temperature increased biomass with lower bacterial diversity and maintained high numbers of the phylum Pr006Fteobacteria, Firmicute, and Actinobacteria including Pseudarthrobacter species. The psychrophilic Pseudarthrobacter psychrotolerans YJ56 had a superior growth at 13ºC, but could not grow at 30 ºC, compared to other bacteria isolated from the same Antarctic soil. Unlike a single rod-shaped cell at 13ºC, strain YJ56 at 25ºC was morphologically shifted to a filamentous bacterium with several branches. Comparative genomics of strain YJ56 with other genera in the phylum Actinobacteria indicates remarkable copy numbers of the rimJ genes that are possibly involved in dual functions, acetylation of ribosomal proteins and stabilization of ribosome by direct binding. Two-dimensional gel electrophoresis analysis and extracellular H2O2 measurement suggested that cells experienced physiological stresses at 25ºC, which was supported by upregulation of chaperone proteins, GroEL and catalase, KatE in our proteomic analysis. Proteins involved in the assembly of 50S ribosomal proteins, and L29 in 50S ribosomal proteins was actively expressed at 13ºC, which suggested distinct roles of many ribosomal proteins under different conditions. This study highlights some of the strategies and different physiological states that Pseudarthrobacter sp. YJ56 has developed to adapt to the cold environment in Antarctica.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by the National Research Foundation of Korea (NRF) Grant (NRF-2020M3A9H5104237)

645 / UNDER-ICE BACTERIAL AND MICROEUKARYOTIC COMMUNITIES OF LAKE BAIKAL: DIVERSITY, FUNCTIONAL FEATURES AND COMPARISON OF FREE-LIVING

Keywords: *Under-ice period, Free-living communities, Particle-associated communities, Sequencing*

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BACKGROUND

The ice period is an important stage in the functioning of many lakes, including Lake Baikal. During this period, the spring peak of phytoplankton occurs, which is fundamental for the development of the entire reservoir in other seasons. Despite this, little is known about the diversity and metabolism of microorganisms living under the ice cover.

OBJECTIVES

The aim of this study was to compare the free-living and particle-associated bacterial fractions the during the under-ice phytoplankton bloom peak and in the summer season.

METHODS

Water sampling was carried out during the ice and summer periods. Identification and quantification of phytoplankton was carried out using microscopy. The samples were divided into two fractions: free-living (FL) (5–27 µm) and particle-associated (PA) (0.22–5 µm). A total of 64 16S rRNA samples and 32 18S rRNA samples were sequenced on a MiSeq Ilumina.

RESULTS

The bacterial community differed statistically between the FL and PA fractions; in addition, the communities differed seasonally. The FL community was taxonomically more diverse and stable than the PA community, which was most likely affected by changes in microeukaryotic structure.

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666 / VIABILITY OF BACILLUS SPECIES IN THE BIOLOGICAL SELF-HEALING CONCRETE PRODUCED WITH DIFFERENT CEMENT TYPES

Keywords: *Biological concrete, Bacterial viability, Cement type, Minimum inhibitory concentration*

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BACKGROUND

Due to the ability of some bacteria to precipitate calcium carbonate, the cracks in the concrete can be healed. The biggest challenge of developing self-healing concrete is to ensure the viability of bacteria spores. Bacteria must survive harsh conditions such as high pH values of the early age concrete and mechanical stress during concrete hardening.

OBJECTIVES

The viability of bacteria decrease drastically within several days after their incorporation into the biological concrete matrix. In this study, the influence of the cement type and composition on the viability of Bacillus strains was investigated.

METHODS

An expanded clay (EC) was impregnated with bacteria spores and nutrients under the vacuum. The concrete mix was obtained by mixing EC, sand, cement and water. The viability of spores was measured using the microbiological dilution-to-extinction method by CFU counting after plating on alkaline nutrient agar. The toxicity of metal oxides was assessed by determining the minimum inhibitory concentration (MIC) for Bacillus strains.

RESULTS

Out of five cement types tested, the best long-term viability of Bacillus pseudofirmus was obtained in a concrete mix using the white CEM-I cement. The study of the chemical composition of cement showed that it contains metal oxides with antimicrobial properties. Determined MIC values showed that Al_2O_3, Fe_2O_3, MgO, and TiO_2 are non-toxic for Bacillus pseudofirmus, Bacillus cohnii and Bacillus halodurans strains. The MIC values of ZnO varied between 12.5 and 25 µg/ml. ZnO was found to be the most toxic metal oxide and causes the death of bacteria in biological concrete.

ACKNOWLEDGEMENTS/REFERENCES

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670 / FUNGAL SOIL COMMUNITY CHANGES IN SLOVAK OLIGOTROPHIC GRASSLANDS IN RESPONSE TO MULCHING AND TRADITIONAL MANAGEMENTS

Keywords: *mycobiome, indicators, HTS sequencing, nan*

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In Slovakia, mulching (cutting of vegetation without removal of clippings) is used as a low-cost method for maintaining remote or abandoned grasslands. Our study focuses to assess the impact of a long-term mulching application to changes in biodiversity of the soil mycobiota in grassland ecosystems. We compared six different grassland management regimes applied over nine years on a sub-montane oligotrophic Nardus pastures in the Central Slovakia. The diversity of soil fungi was assessed using DNA metabarcoding of the ITS2 regions. We focused on a particular group of macrofungi which is characteristic of traditionally managed and undisturbed European grasslands that are collectively known as CHEGD fungi (the acronym of the constituent taxa). The relative abundance and diversity of CHEGD fungi was compared with the total fungal and plant diversity. CHEGD fungi and vascular plants diversity and richness were lower on plots where mulching or no management were imposed. This suggests that applying mulching would have a negative impact on grassland fungi. However, all treatments had some taxonomic unique units, indicating that combining of mulching with traditional managements can enhance overall local diversity of grasslands. Our results also suggest that the impact of mulching depends on the season when the mulching is applied and it might be reduced by combination with other management treatments. The high relative abundance and sensitivity of CHEDG fungi in oligotrophic grasslands to management treatments makes them excellent indicators of grassland natural quality and it highlight the ecological importance of this fungal group.

ACKNOWLEDGEMENTS/REFERENCES

This study is supported by grant of Slovak Research and Development Agency APVV 20-0257 and 20-0284

675 / DEGRADATION OF WASTE PLASTIC BY EVOLVED ENVIRONMENTAL BACTERIAL COMMUNITIES

Keywords: *Biodegradation, Plastic*

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BACKGROUND

Global plastic waste is a growing problem, it is predicted that 12,000 million metric tonnes of plastic waste will accumulate by 2050. Recycling will only combat some of this and an environmentally friendly alternative to landfill and incineration is needed. Microbial biodegradation of plastic is a promising solution for plastic waste.

OBJECTIVES

We have aimed to not only find bacteria capable of degrading plastic, but to advance this ability to create efficient biotechnological tools.

METHODS

Waste polystyrene was collected and inoculated into non-carbonated minimal media for two months and resulting evolved bacterial communities were assessed for plastic-degrading ability.

RESULTS

Bacterial communities present on the polystyrene originally showed little or no plastic degradation. However, after selective pressure of having only the waste polystyrene as a carbon source to survive, this led to evolved communities that were more efficient and quicker at degrading polycaprolactone (PCL) agar, a model polyester, showing they have evolved into enhanced plastic-degrading communities. These evolved communities use PCL as a sole carbon source and reduce the plastic weight. Some were also found to slightly reduce the weight of untreated polystyrene pellets. 16S sequencing of the best degraders found Pseudomonas stutzeri was highly prevalent. Comparative genomics of recent whole genome sequencing could reveal enzymes needed for plastic degradation. Directed evolution of environmental bacterial communities via this novel process using the selective pressure of plastic waste as the sole carbon source leads to more efficient plastic-degrading strains, which can be used as a valuable tool in plastic waste disposal.

ACKNOWLEDGEMENTS/REFERENCES

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677 / A MYXOCOCCUS XANTHUS TETR REPRESSOR REGULATES THE EXPRESSION OF GENES DURING PREDATION ON SINORHIZOBIUM MELILOTI

Keywords: *Bacterial predation, TetR regulator, Myxococcus xanthus, Sinorhizobium meliloti*

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BACKGROUND

Myxoccocus xanthus is a soil-predatory bacterium. In the presence of other microorganisms, they cooperate to prey on them by using diverse mechanisms. Although an increasing number of laboratories are working on bacterial predation, we are still far of understanding this process, especially regarding the regulators responsible for the expression of genes required to successfully kill the prey and feed on their macromolecules. Our group has carried out a transcriptomic analysis during predation of M. xanthus on Sinorhizobium meliloti, and the results obtained are shown in the poster by Pérez et al. In this study, we have identified that three different types of transcriptional regulators increase their expression in an early stage of predation: a TetR regulator, a two-component system, and two Crp regulators.

OBJECTIVES

We have focused on the characterization of the TetR regulator..

METHODS

We have used a variety of techniques, including predation assays with mutants, analyses of gene expression by transcriptomic experiments and lacZ fusions, microscopy and bioinformatic analyses, among others.

RESULTS

We have observed that TetR is responsible for the expression of at least one operon, which includes the gene for the regulator, indicating that it is autoregulated. In addition, we have demonstrated that this TetR functions as a repressor, and that it seems to respond to lipids. Moreover, we have also found that the expression of the operon encoding TetR is not up-regulated when M. xanthus prey on all the bacteria tested, but only on certain prey which share a number of common features.

ACKNOWLEDGEMENTS/REFERENCES

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678 / INFLUENCE OF TOXIC CYANOBACTERIA ANABAENA VARIABILIS ON THE GROWTH OF GREEN MICROALGA CHLORELLA SP.

Keywords: *Allelopathy, Cyanobacteria, Microcystin-LR*

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BACKGROUND

Allelopathy is a natural phenomenon that occurs in both freshwater and marine habitats, while organisms that have the ability to produce and realase allelopathic compounds participate in allelopathy interactions. Although allelochemicals can cause a stimulating effect on organisms, most authors describe the inhibitory activities of allelopathically active compounds.

OBJECTIVES

The objective of this study was to examine the allelopathic activity of the toxic cyanobacterium Anabaena variabilis. Effect of its cell-free filtrate on the growth of the green microalga Chlorella sp. and the outcome of co-cultivation these two strains were monitored.

METHODS

Cell-free filtrate was obtained by centrifugation and filtration of cyanobacterial cultures, while the co-cultivation of Anabaena variabilis and Chlorella sp. was performed under standard conditions in BG-11 medium. Growth of Chlorella sp. in cell-free filtrates was monitored spectrophotometrically in order to determine the concentration od chlorophyll a, while the cell number of Chlorella sp. from co-cultivation was determined using light microscopy in the Thoma counting chamber. Toxicity of the cyanobacterial strain Anabaena variabilis was determined by previously performed bioassays. Microcystin-LR toxin was identified in a methanol extract of this strain using the HPLC-DAD technique.

RESULTS

After 7 days of co-cultivation, it was observed that A. variabilis significantly reduced the growth, while cell-free filtrate also reduced the growth of Chlorella sp., but to a lesser extent. These results suggest that A. variabilis causes inhibitory effect on growth Chlorella sp. by the producing microcystin-LR or some other extracellular metabolite with allelopathic potential.

ACKNOWLEDGEMENTS/REFERENCES

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679 / PHOSPHATE SOLUBILIZING STRAINS OF PANTOEA BRENNERI AS AN ALTERNATIVE TO PHOSPHORUS FERTILIZERS AND CHEMICAL PESTICIDES

Keywords: *phosphate, Pantoea, PGPR, phytase*

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Environmental problems associated with the use of mineral fertilizers and chemical pesticides are forcing scientists to introduce new technologies in agricultural production that have the least negative impact on the environment, allowing them to maintain and increase the productivity of cultivated crops. The goal of this project is to develop a biotechnological basis for the use of new phytate-hydrolyzing PGP strains of Pantoea brenneri as biofertilizers and biopesticitdes.

The optimal growth parameters for P. brenneri strains were the following: 28 ºC, pH range from 6 to 9. High resistance of the strains to increased levels of salt in the environment was established. Wide range of PGP-treats were studied: strains were able to synthesize ammonia, cyanides, indoleacetic acid and siderophores of the cachetol-type, had proteolytic, chitinase and cellulase activities, fixed nitrogen and were able to mobilize soil phosphates. Earlier, we showed the ability of P.brenneri strains to hydrolyze soil organic compounds - phytates. Now we studied the ability of P. brenneri strains to mobilize a wide range of inorganic soil phosphates, presented in the form of tricalcium phosphates, hydroxyapatites, phosphorites and aluminum phosphates.

All studied strains were able to grow and produce halo-zones around the colonies on the agar media, containing one of these phosphorous sources. The solubilization indexes were determined, they were in the range of 2.0-5.0.

P.brenneri strains also exhibit growth-stimulating properties during seed treatment of wheat and potatoes, and are able to reduce the damage of potato tubers from Fusarium rot.

ACKNOWLEDGEMENTS/REFERENCES

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680 / MOLECULAR ANALYSIS OF MICROBIAL COMMUNITIES OF GROUNDWATER IN THE CITY OF MOSCOW SUBJECTED TO ANTHROPOGENIC IMPACT

Keywords: *microbial communities, springs, anthropogenic impact, molecular analysis*

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An important component of the ecosystem of the metropolis is groundwater, from which natural springs are formed. Deterioration of groundwater quality under the influence of anthropogenic factors is an important ecological problem. In this work, we explored five springs on the territory of Moscow (Russia) in spring and summer seasons. For each sample, physicochemical parameters (pH, temperature, Eh, elemental and ionic composition) were determined, and the composition of microbial communities was analyzed using 16S rRNA gene profiling. In total, 1071 species-level OTUs of Archaea and 10191 OTUs of Bacteria were identified in five springs. Archaea, mostly assigned to the Nanoarchaeota and ammonium-oxidizing Crenarchaeota, dominated in one spring; three microbial communities were dominated by Proteobacteria and one by Patescibacteria. Interestingly, Patescibacteria, comprising parasitic/symbiotic organisms with small genome size and limited metabolic capabilities, were found in all samples, accounted for about 20% of the communities and were represented by 3409 OTUs. Known pathogenic species were not found. Most of Bacteria belonged to aerobic heterotrophic lineages, although chemolithoautotrophic iron-oxidizing Gallionellaceae were abundant in one spring. Some springs contained hydrocarbon-degrading species, Alcanivorax, Dietzia and Thalassospira. Their presence indicated the occurrence of natural bioremediation processes in soils and groundwater. Halophilic microorganisms, including Halomonas sp., were abundant in spring samples, but were nearly absent in summer. Apparently, temporal abundance of halophiles reflected the wide use of salt-containing deicing chemical agents in winter. Overall, the results obtained indicate a relatively limited anthropogenic impact on both water quality and the composition of microbial communities.

ACKNOWLEDGEMENTS/REFERENCES

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684 / METAL-MICROBES INTERACTION DURING A PHYTOREMEDIATION EXPERIMENT TO RECOVER DIGESTATE AMENDED SOILS

Keywords: *Digestate, Trace Metals, Microbial Communities, Phytoremediation*

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Anaerobic digestion is emerging as the preferred treatment for municipal solid waste (MSW). The number of anaerobic digesters is increasing worldwide and a huge amount of digestate is being produced which, if inappropriately treated, can lead to serious environmental impacts. Digestate is a nutrient-rich material with soil reclamation potential, hence, current digestate management practice involves land application.

However, digestates are not entirely harmless. They can contain trace metals, which can be transferred to the soil ecosystem. There is a need of finding strategies for its safe use and land disposal. Phytoremediation is a cost-effective, ecologically responsible alternative often used to remediate soils contaminated with metals and can be an option for soil amended with contaminated digestate, where microorganism have also a key role.

Two non-food bioenergy crops (Panicum virgatum and Pennisetum alopecuroides) were evaluated for their capacity to recover soils amended with metal contaminated digestate. For that, 60 x 20 cm vertical soil column mesocosm experiments were performed to assess the impact of MSW digestate application and phytoremediation processes along time on: (i) metal migration along soil profile, (ii) plants growth performance and metals uptake capacity, and (iii) microbial community changes at different soil depths (through high-throughput sequencing of 16S analysis of soil/digestate samples).

Results will provide insights into metal-microorganisms interactions and microorganisms role during phytoremediation of metal contaminated digestate.

ACKNOWLEDGEMENTS/REFERENCES

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703 / DISTRIBUTION OF MICROBES INVOLVED IN METHANE CYCLE ALONG THE SALINITY GRADIENT

Keywords: *Methane cycle, Salinity gradient, 16S rDNA*

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BACKGROUND

By being the second most abundant greenhouse gas in the atmosphere with much higher retentive capacity than carbon dioxide, methane and its genesis by methanogens are being studied to reduce its emission into the atmosphere, thus mitigating global warming. Shallow lake ecosystems are one of the hot spots in natural global methane emissions [1]. The crucial role in regulating the amount of methane exiting the ecosystem have methanotrophs [2], and their occurrence depends on environmental conditions.

OBJECTIVES

The study's goal was to determine the spatial distribution of the most abundant methane cycle contributors in lake sediments of the coastal Lake Vrana. Since the lake is known to experience salinity intrusions via karstified ridge and subsurface canal Jugovir, we wanted to investigate whether salinity affects the microbial community involved in the methane cycle.

METHODS

Surface sediments were collected, of which seven represented the longitudinal transect of the lake while the rest were taken at the canal inlets or bays. Pore water ion concentrations and organic carbon and total nitrogen percentage were determined. 16S diversity was determined on the Illumina platform.

RESULTS

Detected methanotrophs belonged to five different orders, with the sulfate-dependent anaerobic ANME-1b being predominantly abundant in the entire lake, except on stations one and twelve, where aerobic methanotrophs Methylococcales were dominant. The most abundant order of methanogens was Methanomicrobiales with dominant genus Methanolinea, while detected sulfate reducers belonged to Desulfatiglandales, Desulfobaccales, and Desulfobacterales. Microbial community composition was influenced by sulfate concentrations, salinity, nitrogen compounds, and organic carbon.

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709 / BACTERIAL DIVERSITY OF PLITVICE LAKES CATCHMENT AREA DURING THE SUMMER SEASON

Keywords: *Plitvice lakes, bacterial diversity, 16S rDNA*

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BACKGROUND

The uniqueness of the Plitvice Lakes, a world-famous National Park, consists of the cascading lakes created by the constant biodynamic process of tufa barrier formation and growth. In addition to lakes, the biodiversity-rich aquatic ecosystem of National Park includes a large number of streams and rivers. Although it plays an important role in the tufa formation, the microbial community of this ecosystem is poorly researched (1), and due to increasing anthropogenic pressure, it is prone to changes.

OBJECTIVES

The aim of this study was to establish the structure and diversity of bacterial communities in aquatic ecosystems of Plitvice Lakes National Park. Therefore, we conducted research in the Plitvice Lakes catchment area during the summer season using a method that has not been used in this area so far.

METHODS

Water samples were collected in June 2019 at 24 points located at waterfalls connecting the lakes and at all surface streams and rivers belonging to the Plitvice lakes catchment area. The pair-end sequencing method of 16S rDNA was used for the taxonomic determination of the bacterial community.

RESULTS

The most abundant bacterial communities within the investigated area were affiliated to the phyla Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria, and Verrucomicrobiota. All these phyla are characteristic representatives of bacterioplankton in freshwater ecosystems. In general, bacterial populations collected at waterfalls were separated from other populations, with a significantly higher abundance of Actinobacteriota and the absence of Patescibacteria and Bdellovibrionota, found in stream and river samples.

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715 / BIOFILM CONSTITUENTS AS DETERIOGENS OF ANCIENT ROMAN MONUMENT MITREJ ABOVE ROŽANEC (SLOVENIA)

Keywords: *Biodeterioration, Biofilm, Relief, Biopitting*

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BACKGROUND

Mitrej above Rožanec is one of the most attractive archaeological monuments in Slovenia. The relief is believed to originate from the 2nd century, and the image of god Mitra carved into a limestone wall of an abandoned stone-quarrey has been preserved in its natural environment in the valley of chestnut forest Judovje.

OBJECTIVES

The principal purpose of the study was to characterize cyanobacteria, algae and fungi from biofilm cover on the surface of the monument in order to evaluate their biodeterioration impact and formulate an adequate conservation plan

METHODS

Biofilm was observed directly in the field with portable in situ microscope. Fragments of biofilm were collected via non-aggressive adhesive tape method and analyzed microscopically.

RESULTS

In situ observation of variously pigmented biofilm maths revealed complex community predominantly consisting of cyanobacteria, green algae, lichenized fungi, and mosses. Biopitting phenomenon was distinctly present on the surface of relief, usually with perforations filled by black endolithic lichen Verrucaria thalli.

Microscopic analysis showed dominance of phototrophs: Cyanobacteria, Chlorophyta and Bacillariophyta. The most diverse were Cyanobacteria of Gloeocapsa, Gloeothece and Chroococcus genera, followed by Aphanocapsa, Aphanothece, Gloeocapsopsis and Leptolyngbya. Chlorophyta of genera Chlorella, Desmococcus, Gloeocystis, Haematococcus and Trentepohlia were observed, as well as lichen photobionts in a mass. Aphanocapsa muscicola was the only taxon documented on almost all sampling sites. Of special note, for selecting adequate conservation methods and biocide treatment, is the presence of epilithic and endolithic lichens as pronounced biodeteriogens, as well as cyanobacteria with calcified sheets.

719 / A TRANSCRIPTOMIC STUDY OF THE THERMOPHILIC POLYHYDROXYALKANOATE-PRODUCING BACTERIUM SCHLEGELELLA THERMODEPOLYMERANS

Keywords: *Polyhydroxyalkanoates (PHA), Schlegelella thermodepolymerans, Thermophiles, Transcriptomics*

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BACKGROUND

Polyhydroxyalkanoates (PHAs) are biopolyesters naturally produced as intracellular granules by numerous microorganisms in response to stress and unbalanced nutritional conditions. PHA properties are comparable to polypropylene plastics that render them a biodegradable and renewable alternative to conventional plastics in a biobased production process employing cheap substrates. While PHA production is well-studied in several mesophilic bacteria, not much is known about PHA biosynthesis in thermophiles and their potential for the development of high-temperature bioplastic production on an industrial scale.

Schlegelella thermodepolymerans is a thermophilic bacterium that was proved to produce high amounts of polyhydroxyabutyrate polymers upon growing on xylose-rich media at 55°C (Kourilova et al., 2020). PHA production using xylose substrate is interesting as it is present in agricultural wastes, so the total PHA production costs can be decreased.

OBJECTIVES

In this study, the transcriptomics and different PHA genes expression were investigated to have a better knowledge of the metabolism of this bacterium.

METHODS

BLAST tools were used to detect the PHA genes. The accumulated intracellular PHA were investigated using a plate reader. Total RNA was then sequenced using Illumina techniques.

RESULTS

PHA genes were identified in the genome of Schlegelella thermodepolymerans. Four PHA production conditions were chosen that apparently induce PHA polymerization and depolymerization to investigate genome-wide differential gene expression levels using an RNA sequencing approach. From these results, we have a better knowledge of the metabolism of this organism that will help us to have better tools to improve PHA production in the future.

ACKNOWLEDGEMENTS/REFERENCES

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729 / THE INFLUENCE OF LIGHT ON TRANSLATIONAL ACTIVITY OF HOT SPRING BIOFILM

Keywords: *hot spring, biofilm, BONCAT*

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BACKGROUND

Methods for monitoring microbial activity in an environmental context are key to answering one of the most important questions in microbial ecology, namely how microorganisms respond to environmental stimuli. Bioorthogonal non-canonical amino acid tagging (BONCAT) is a technique that can be used to tag newly synthesised protiens in a variety of phylogenetically diverse microorganisms from different habitats, including hot springs. In these extreme environments, nutrient availability and the presence of light, along with high temperature, are the most important parameters affecting microbial communities.

OBJECTIVES

The aim of this study was to determine the effect of light on the number of metabolically active microorganisms in the biofilm sample from the geothermal spring Bizovacke toplice.

METHODS

The biofilm was incubated with the addition of L-homopropargylglycine (HPG) for 2 days in parallel with the presence and absence of light. After 24 and 48 hours, Cu(I)-catalysed azide-alkyne cycloaddition was performed to detect metabolically active cells. The sample was examined with an inverted microscope and cells were counted both manually and with daime software.

RESULTS

The presence of light increased the number of metabolically active cells compared to the sample incubated in the dark. As for the time of incubation, the longer incubation period resulted in higher translational activity in both samples.

730 / WASTEWATER BASED EPIDEMIOLOGY IN COUNTRIES WITH POOR WASTEWATER TREATMENT - SARS-COV-2 RNA IN SURFACE WATERS

Keywords: *SARS-CoV-2, COVID19, wastewater based epidemiology, the Danube river*

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BACKGROUND

Prokaryotic assays are of great interest in eco/geno-toxicology as they are short-term, simple and cost effective. Application of strains of Salmonella typhimurium with modifications such as increased cell wall permeability and lack of excision repair system is common for the detection of genotoxicity. Various eukaryotic models have been developed lately, such as fish derived cell lines, which can mimic the response of the aquatic organisms. Considering different level of cell organization among these models, difference in sensitivity is expected, yet only few studies address this topic.

OBJECTIVES

The major objective was to investigate genotoxic potential of extracts of surface water of the Danube and to compare the results obtained in applied prokaryotic and eukaryotic models.

METHODS

Surface water samples from 24 sites along the Danube River were extracted by LVSPE Horizon sampler providing relative enrichment factor (REF) of the extracts of 25,000x. For representative prokaryotic model the SOS/umuC assay with S. typhimurium TA1535/pSK1002 was used. Eukaryotic model comprised assessment of cytotoxicity by MTS test and assessment of genotoxicity by comet assay in ZFL (ZebraFish Liver) cells.

RESULTS

While none of the tested samples has induced SOS response, the result of MTS assay indicated that 11 of 24 samples were cytotoxic. In comet assay, genotoxic potential was found in 9 of 24 samples. Results indicated higher sensitivity of used eukaryotic model in comparison with prokaryotic. Applied prokaryotic assay is often suggested to be used as prescreening tool in diagnostics of genotoxic potential which by our opinion could be misleading.

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732 / OUTBREAK OF LEPTOSPIROSIS AFTER CYCLONE „TAMARA"

Keywords: *Leptospirosis, Bosnia and Herzegovina, Floodings*

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BACKGROUND

Leptospirosis is the most common zoonotic disease in our area. Various enviromental factors can increase incidence of leptospirosis.

OBJECTIVES

To investigated connection between heavy reinfall and floodings with the apperance of leptospirosis in our area.

METHODS

Retrospective study included 80 patients with leptospirosis. Data on precipitation were obtained from the official database of Federal Hydrometeorological Institute of BiH. OpenStreetMap (OSM) was used for spatial analysis; patients were geolocated and put on a map.

RESULTS

In the period between 01.01.2014 and 31.12.2014, 80 patients with leptospirosis confirmed by clinical and serological testing were hospitalized. Most of the respondents were male (67,5%), lived in the countryside (89%) and they were engaged in agriculture and animal husbandry (60%).

62,5% of respondents had a domestic animal or contact with flooded area (66,3%).

The increase in patients with leptospirosis in the study period was statistically significant after the flood ($p<0.01$).

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737 / BIOCONTROL POTENTIAL OF A NATIVE BACILLUS STRAIN AGAINST TRICHODERMA AGGRESSIVUM, THE CAUSAL AGENT OF AGARICUS GREEN MOULD

Keywords: *biocontrol, Agaricus, Trichoderma*

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In industrial mushroom production, highest crop losses are caused by the "green mould disease" caused by Trichoderma species responsible for devastating outbreaks. Fungicides represent the common control measure, while biocontrol is a more environment-friendly, cost-effective alternative to chemical control. We isolated and investigated bacteria from mushroom growing material and selected the most suitable biocontrol candidate based on a series of antagonism assays.

Extracellular enzyme and antibiotic production profiles of the selected Bacillus sp. strain SZMC 25431 were also assessed to investigate the mechanism of biocontrol. The strain proved to produce several variants of antibiotics, most dominantly surfactins, as well as several enzymes with lipases being the most dominant. The selected strain was further tested under simulated Agaricus cultivation conditions against T. aggressivum in three different concentrations. By weighing mushroom yield we determined, that highest biocontrol activity was achieved at an application concentration of 104 cells/g of casing material. The bacterial strain was also tested in growing houses with the bearing surface of 480 m2. Yield data were obtained by weighing the crops from mushroom growing houses with fungicide treatment alone, Bacillus treatment alone, and integrated treatment using both fungicides and Bacillus. The bacterial treatment increased crop yield by 21.95%, while the integrated treatment resulted in a crop increase by 26%. Our results suggest that the selected Bacillus strain can be potentially used for biocontrol in Agaricus cultivation. This work was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme through grant GINOP-2.2.1-15-2016-00006.

741 / IN VITRO ANTIMICROBIAL PROFILING OF CUCUMIS METULIFERUS PEELS

Keywords: *horned melon, antifungal activity, kiwano*

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BACKGROUND

Using bioactive compounds for creating antimicrobial products, many industry sectors invest in research and development of extraction of bioactives from a different source. One of the potential sources is kiwano or horned melon (C. metuliferus). Kiwano parts are considered as waste generated during fruit eating with currently little commercial value.

OBJECTIVES

Considering the above, the objective of this work was to evaluate the antimicrobial potential of the methanolic extracts of peels of five different C. metuliferus fruits originating from Serbia over ten referent cultures of microorganisms.

METHODS

The preliminary screening of antimicrobial activity was performed by the disk diffusion method, while the minimal inhibitory concentrations were determined by the microdilution method.

RESULTS

The tested extracts showed high antimicrobial activity against B. cereus, P. aeruginosa, S. cerevisiae, C. albicans, A. brasiliensis, and P. aurantiogriseum. Antimicrobial activity did not observe against S. aureus, E. faecalis, E. coli, and S. Typhimurium. Furthermore, the minimal inhibitory concentration of tested peel extracts was determined for all sensitive microorganisms. Based on the obtained results, the lowest minimal inhibitory concentration of 3.125 mg/mL was noticed in the case of P. aeruginosa, S. cerevisiae, and C. albicans.

On the other hand, the higher minimal inhibitory concentration of 25 mg/mL was determined for other tested microorganisms. In accordance with the gained results, it can be clearly pointed out that the C. metuliferus methanolic peel extracts could be possibly used as a promising antifungal agent for protection against different microbial strains.

ACKNOWLEDGEMENTS/REFERENCES

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764 / ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF PIGMENTED STREPTOMYCES SPP. CULTURE EXTRACTS

Keywords: *bacterial pigments , antimicrobial, cytotoxic, Streptomyces*

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BACKGROUND

Pigments from microbial origin have promising applications in food, cosmetics, textiles and therapeutics. Compared to other natural sources pigments from bacteria are more stable, safer, and could be cheaper to produce and extract. Bacterial genus Streptomyces has been known as a source of biologicaly active pigments that exhibit several effects such as antimicrobial and cytotoxic.

OBJECTIVES

The aim of this study was the isolation of pigment-producing Streptomyces strain from soil, optimization of growth parametres for pigment production and evaluation of antimicrobial and cytotoxic activity of extracted bacterial pigments.

METHODS

Isolation and characterization of pigment-producing Streptomyces strain from soil was done following the standard microbiological protocol, using four different growth media. The pigments were extracted by solvent extraction using ethyl acetate. Antimicrobial activity was analyzed using the disc diffusion test. Cytotoxic activity was tested on the HaCaT cell line following the standard protocol.

RESULTS

Extraction of pigments by solvent extraction resulted in crude pigment extracts with antimicrobial activities. In total pigments from 14 bacterial strains have been extracted. Antimicrobial activity was evident on Gram + bacteria Staphylococcus aureus (6 mm of inhibition zone at concentration 8.4 µg/ml and 4 mm of inhibition zone at concentration 1.1 µg/ml) as well as on the fungus Candida albicans (4 mm of inhibition zone at concentration 13 µg/ml). Pigments showed dose-dependent inhibiton of proliferation of HaCaT cells, with the lowest concentration at 25 µg/ml.

ACKNOWLEDGEMENTS/REFERENCES

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755 / SCREENING OF AHL LACTONASE ACTIVITY IN BACILLUS SPP. STRAINS ISOLATED FROM DIFFERENT NATURAL SAMPLES

Keywords: *AHL-lactonases, Bacillus*

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BACKGROUND

Through evolution, pathogenic bacteria developed a mechanism to increase their virulence to hosts by producing quorum sensing (QS) signaling molecules, primarily acyl homoserine-lactones (AHLs). Since its discovery, the QS phenomenon has been a promising target in biocontrol strategies involving quorum quenching (QQ) mechanisms. The great potential in this area lies in detection, characterization, and possible application of AHL-lactonases from Bacillus spp.

OBJECTIVES

This study aimed to test for the presence and activity of AHL-lactonases in natural Bacillus spp. isolates to find strains that could be used in QQ-mediated control of AHL-producing phytopathogens.

METHODS

The collection of 149 Bacillus spp. isolates, originated from the soil, manure and straw, were screened for the presence and activity of AHL-lactonases using the well-diffusion method. Violacein production by biosensor strain Chromobacterium violaceum CV026 was an indicator for AHL presence and degradation. PCR method was performed to detect the aiiA gene in selected isolates, with primers aiiA1-F and aiiA2-R

RESULTS

A total of 52 strains showed AHL-lactonase activity, which was confirmed by detecting a single 793 bp long band of amplified aiiA gene. Furthermore, a difference in AHL-lactonase production was perceived in Bacillus spp. isolates from different natural samples. The highest number of AHL-lactonase-producing Bacillus spp. were found in soil samples (65 %), followed by manure samples (33 %), while only one strain from straw demonstrated AHL-lactonase activity. The results suggest that AHL-lactonase is common in Bacillus spp. exceptionally in soil isolates.

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772 / MICROBIAL CONTAMINATION OF PHOTOGRAPHIC AND CINEMATOGRAPHIC MATERIALS IN ARCHIVAL FUNDS IN THE CZECH REPUBLIC

Keywords: *photographic and cinematographic materials, microbial contamination, archival funds of the Czech Republic, MALDI-TOF MS*

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BACKGROUND

Photographic and cinematographic materials have become an integral part of our lives over the past 200 years. They are art forms and a testimony to the past of human society and therefore need to be preserved for future generations.

OBJECTIVES

In this study we investigated the microbial contamination of 126 samples of photographic and cinematographic materials from 10 archival funds in the Czech Republic.

METHODS

Microorganisms were isolated from the light-sensitive layer by swabbing it with a polyurethane sponge. Microbial isolates were identified by MALDI-TOF MS (bacteria) or by phenotype testing and microscopy (fungi).

RESULTS

Bacterial contamination was more abundant and more diverse than fungal contamination, and both were significantly associated with archives. The most frequently isolated fungal genera were Cladosporium, Eurotium, Penicillium, Aspergillus and Alternaria. The most frequently isolated bacteria were Gram-positive genera such as Staphylococcus, Micrococcus, Kocuria, Streptococcus and Bacillus. This bacterial and fungal diversity suggests that air is the main vehicle of contamination. We also analysed the impact of the type of material used for the carrier (paper, baryta paper, cellulose acetate and nitrate or glass) or the light-sensitive layer (albumen, gelatine, collodion and other) on the level and diversity of microbial contamination. Carriers such as polyester and cellulose nitrate may have a negative impact on bacterial contamination, while paper and baryta paper may have a partially positive impact on both fungal and bacterial contamination.

ACKNOWLEDGEMENTS/REFERENCES

FUNDING

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775 / OPTIMIZATION AND MONITORING OF THE USE OF SPENT MUSHROOM COMPOST AS A CASING LAYER FOR MUSHROOM CULTIVATION

Keywords: *champignon*

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The two main points of champignon cultivation are good quality compost and casing layer. Casing layer has important role in fruiting body formation and possesses a high water retention capacity. In most cases the casing layer is consisting of peat. With peat mines being exhausted around the world and in Europe (including Hungary), the development of a new and effective alternative casing material is needed.

Our working hypothesis was that due to the outstanding fiber content of the spent mushroom compost, it can serve as an excellent raw material for the development of casing materials. We have tested the effectiveness of recomposted spent mushroom compost as casing layer in the cultivation of A. bisporus both in plant growth chamber and in industrial mushroom growing experiments. The growing chamber experiments showed that peat and recompost (1:1 mixture) as a casing layer gave yields similar to peat alone. The highest yields in the mushroom cultivation experiments were obtained with peat and a mixture of compost:peat (90:10). Our results suggest that the harvested mushroom compost has potential as applicable component of casing materials, which could be a valuable contribution to circular agriculture.

To ensure the production of a healthy crop, we also isolated, identified and selected Bacillus (15) and Pseudomonas (10) strains from the spent mushroom compost, which have the potential to be used for biocontrol in Agaricus cultivation.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme through grant 2020-1.1.2-PIACI-KFI-2020-00111.

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776 / IDENTIFICATION OF THE ARCHAEOME BY OPTIMIZING 16S RRNA AMPLICON SEQUENCING APPROACH

Keywords: *archaeal identification*

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BACKGROUND

Most studies investigating microbial community dynamics in different environments focus on bacteria. However, although often much less abundant in a microbial community, archaea can be of key importance. Nevertheless, the abundance of archaea is often overlooked or underestimated. A multitude of studies have developed primer pairs probing to specifically amplify the archaeal 16S rRNA gene. However, none of these methods were validated with a mock community. In addition, no DNAmock specifically designed to study archaea is available to date.

OBJECTIVES

This study aims to validate different primer pairs to estimate abundances of archaea in a microbial community.

METHODS

We developed five mock communities containing different mixtures of DNA of six archaeal and four bacterial species. Next, we evaluated nine primer pairs targeting different regions of the 16S rRNA gene and are estimated to amplify specifically bacteria, archaea or both.

RESULTS

Much variation among the different primer pairs was observed. Although some primer pairs could identify all species in the mock, the abundance of each strain could not be correctly assessed. Moreover, depending on the ratio between bacteria and archaea in the mock, several species could not be identified. Methods estimated to amplify only archaea were not specific for archaea and a low amount of reads belonging to bacteria were obtained. Specificity for archaea was obtained via a nested PCR approach. However, some biases towards specific species in the mock were observed, which should be eliminated further. Overall, our study shows the necessity of primer validation with a DNA mock.

782 / ENDOFUNGAL RHIZOBIUM SPECIES MEDIATED PROTECTION AGAINST ARSENIC TOXICITY TO COLONISED HOST PLANT

Keywords: *Arsenic, Toxicity, Rhizobium species, Host plant interaction*

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BACKGROUND

Arsenic (As) contamination is one of the common challenge in groundwater. Various studies suggested that As transfer from soil-crop-food is the major cause of As exposure to humans and livestock. Plant growth-promoting Rhizobia have been found involved in the induction of plant tolerance under various stresses. An endofungal Rhizobium sp. associated with fungus Serendipita indica deploy beneficial role in the plant growth promotion and tolerance against various biotic and abiotic stresses. In the current study, we have determined the role of endofungal Rhizobium sp. in protection of host plant Solanum lycopersicum (tomato) growth under As stress

METHODS

Arsenic tolerance of bacteria was done by OD595 and cfu analysis. Dip inoculation of Rhizobium sp. was done in sterilized tomato seeds under As exposure for seed germination and seedling growth analysis. Bacterial colonization was examined by RT-PCR and confocal imaging. Pot experiment was done for study of ROS generations, GSH, proline, pigment contents, photosynthesis, As bioaccumulation and mobilization.

RESULTS

Rhizobium sp. tolerated AsV upto 25 mM and its inoculation enhances tomato seed germination and seedling growth. A hyper-colonization of Rhizobium sp. was observed under As stress and resulted in modulation of GSH and proline content with reduced ROS, recovered pigment contents and photosynthesis. Bacterial colonization results in 40% increased As accumulation in root while a reduction in As translocation from root to shoot upto 89%. In conclusion, Rhizobium sp. association with host plant may improve plant health and tolerance against As stress with reduced As accumulation in the crop produce.

ACKNOWLEDGEMENTS/REFERENCES

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807 / UNCOVERING THE MICROBIAL COMMUNITIES IN GEOTHERMAL SPRINGS OF DARUVARSKE TOPLICE (CROATIA)

Keywords: *geothermal springs, biodiversity*

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BACKGROUND

Microbial communities in natural geothermal springs represent an excellent example of microorganisms inhabiting extreme environments. Geothermal ecosystems present poorly investigated habitats in Croatia. The biodiversity in such environments depends on temperature and pH, but also different nutrient concentrations. Nitrogen and sulfur, in particular, are often crucial factors in determining microbial community structure.

OBJECTIVES

The primary objective was to analyze the diversity of thermotolerant and thermophilic microorganisms in the geothermal springs of Daruvarske Toplice through different seasons, and to identify abiotic factors that shape the microbial community composition.

METHODS

Two hot springs (Antunovo vrelo and Ivanovo vrelo) and one well (Marijino vrelo) located in Daruvarske Toplice were selected for sample collection during three seasons. Physical-chemical parameters were measured in situ and in the laboratory. The 16S rRNA gene diversity was analyzed using high throughput sequencing techniques. All statistical analyses were performed in R.

RESULTS

Antunovo vrelo had the most stable community structure during all three sampling seasons, and was dominated by microorganisms affiliated with the phyla Crenarchaeota, Proteobacteria, and Nitrospirota. In contrast, most bacterial ASVs in Ivanovo vrelo were affiliated to Bacteroidota and Proteobacteria, while the bacterial community at Marijino vrelo was most diverse, with sequences of the phylum Proteobacteria dominating during all three seasons. Our results indicated that inorganic nitrogen and sulfur compound concentrations, along with correlating temperature and pH were the main drivers for microbial community composition.

ACKNOWLEDGEMENTS/REFERENCES

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814 / OPTIMIZATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR DETECTION OF ESCHERICHIA COLI IN FRESHWATER

Keywords: *LAMP, molecular diagnostics, pathogens, health risk*

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BACKGROUND

Contamination of water (and food) with fecal bacteria is a well-known common and diligent problem affecting public health and the local and national economy. One of the best indicators of fecal bacterial water pollution is Escherichia coli - which belongs to the group of enteric bacteria and most often creates diarrheal diseases which can have fatal consequences.

OBJECTIVES

This study aims to optimize loop-mediated isothermal amplification (LAMP) methodology - a powerful alternative to the polymerase chain reaction (PCR) - for the detection of pathogenic bacteria (Escherichia coli) from the freshwater samples.

METHODS

Water samples will be collected from rivers located at the territory of Vojvodina, Republic of Serbia. LAMP detection of E. coli from the freshwater samples will be performed by using WarmStart LAMP Kit (New England BioLabs) on the Genie® III, an instrument for isothermal nucleic acid amplification. Amplification will be performed using LAMP primers (F3, B3, FIP, BIP, LF, BF) for the malB gene. For comparison with Real-Time PCR - the same bacteria concentrations will be used for detection of E. coli, using F3 and B3 primers for the malB gene.

RESULTS

We have successfully detected E. coli from freshwater water samples. Once again, the LAMP has proven to be a powerful alternative to PCR methodologies in the rapid and efficient detection of pathogenic species.

ACKNOWLEDGEMENTS/REFERENCES

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834 / DESIGN AND DEVELOPMENT OF A HIGH-THROUGHPUT CONJUGATION PLATFORM

Keywords: *ANTIBIOTIC RESISTANCE, BACTERIAL CONJUGATION, ANTIBIOTIC RESISTANCE SPREAD*

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The increase of antibiotic resistance is one of the world's major health emergencies. In fact, it is estimated that in the year 2050 bacterial resistant infections will be the first cause of mortality in the world. Although the main effect of this huge problem is in medical contexts, its origin lies in the overuse and misuse in clinic, animal husbandry and agriculture, and the impact of these activities on the environment (O'Neill, 2016) (Cassini et al., 2019) (Murray et al., 2022).

Horizontal gene transfer (HGT) and, especially bacterial conjugation, is the principal mechanism responsible of the dissemination of antibiotic resistance genes among bacteria (Álvarez-Rodríguez et al., 2020). The result of this natural process is the appearance of multi-resistant bacteria that, when pathogenic, lead to a scenario of loss of effectiveness of antibiotics available.

Considering the importance of bacterial conjugation in this problem, an accurate quantification system of conjugation frequency will help (i) to evaluate the risk of multi-resistant bacteria in both clinical and environmental contexts; (ii) to study the impact of agro-livestock activities, emergent pollutants, climate change and other environmental factors (Epelde et al., 2018); and (iii) to analyse the effect of different strategies to solve or, at least alleviate, this problem.

As the number of samples in these studies is usually high, in this work we present the design, development and validation of a high-throughput platform to carry out a large number of conjugation frequency experiments accurately, using a simple and fast method.

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840 / FOLLOWING THE ROUTE OF VETERINARY ANTIBIOTICS TIAMULIN AND TILMICOSIN FROM LIVESTOCK FARMS TO AGRICULTURAL SOILS

Keywords: *veterinary antibiotics, feces, agricultural soils, anaerobic digestion*

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Veterinary antibiotics (VAs) are hardly metabolized in the animal body after administration and are mostly excreted in feces. Their use for soil fertilzation or energy production may facilitate VA dispersal impacting environmental quality and human health. We studied the persistence of two VAs, tiamulin (TIA) and tilmicosin (TLM) after their administration to pigs towards receiving environments. We asked the questions: (a) how different administration modes affect their excretion; (b) how anaerobic digestion and ambient storage affect VAs and vice versa; (c) how persistent are VAs in agricultural soils. TLM was detected in feces folds higher (4.27-749.6 μg g-1) compared to TIA (0.55-5.99 μg g-1), with both VAs peaking during the administration period followed by a gradual dissipation during withdrawal. Administration through water resulted in delayed or lower levels for TIA and TLM, compared with feed administration.

TIA and TLM (fortification levels 0.5, 5 and 50 μg g-1) dissipated gradually during manure stockpiling (DT50 5.85-35.9 and 23.5-49.8 days respectively). Both VAs persisted longer during anaerobic digestion (DT90 > 365 days) and negatively affected biomethanation at levels > 5 μg g-1. In direct soil application, TLM was more persistent than TIA with fumigation extending their persistence, suggesting a major role of soil microbiota in their degradation. Soil application of VAs through feces increased their persistence, probably due to sorption to organic fecal matter. Our results suggest that manure incorporation in agricultural soils of TIA/TLM treated pigs will likely disperse VAs in soil with yet unexplored consequences for the environment and human health.

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844 / FARM DAIRY SLURRY IRRIGATION: A MICROBIOLOGICAL PERSPECTIVE OF SOIL FERTILITY AND ENVIRONMENTAL IMPLICATIONS

Keywords: *dairy manure, soil functional diversity, soil enzymes*

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Dairy production has intensified during the last years concomitant with higher use of dairy slurries in agricultural soils to improve nutrient recycling. However, there is little information about the impact on soil microorganisms' diversity and their role in nutrient recycling when different dairy slurries are applied. This study evaluated the effects of the application of raw (RDS) and two-lagoons stabilized dairy slurry (LDS) compared to a non-fertilized (control) and urea fertilization on microbial diversity and enzyme activity, as well as fertility in soil planted with tall fescue. Treatments were distributed in four seasonal applications with consecutive fertilization/harvesting cycles of 45 days, simulating the typical management of forage cut from fescue. The results showed that dairy slurry applications increased soil organic matter (OM), Na, K and P. Meanwhile, the soils which received RDS applications had greater soil OM content than those receiving LDS; soils that received LDS had greater cation content. Soils treated with any dairy slurry showed higher dehydrogenase and phosphatase enzyme activities, respiration, nitrification, and N mineralization potentials. BIOLOG substrate utilization was affected by N-fertilized treatments compared with the control. Results from bacteria and fungi soil and effluents' community structure are being processed through 16S and ITS amplicon sequencing. Our study shows that the application of dairy farm slurry can enhance soil microbial metabolic activity in tall fescue swards and that the nutrient input applied may have been the driving force for these effects.

ACKNOWLEDGEMENTS/REFERENCES

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848 / GEOBIOLOGY OF ANDEAN MICROBIAL ECOSYSTEMS DISCOVERED IN SALAR DE ATACAMA, CHILE

Keywords: *Microbial mats, Microbialites, Endoevaporites, Extremophiles*

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The Salar de Atacama in the Chilean Central Andes harbors unique microbial ecosystems due to extreme environmental conditions. Combining X-ray diffraction analyses, scanning electron microscopy and molecular diversity studies, we have characterized twenty previously unexplored Andean microbial ecosystems in eight different lakes and wetlands from the middle-east and south-east regions of this salt flat. The mats and microbialites studied are mainly formed by calcium carbonate (aragonite and calcite) and halite, whereas the endoevaporites are composed predominantly of gypsum and halite. The carbonate-rich mats and microbialites are dominated by Bacteroidetes and Proteobacteria phyla. Within the phylum Proteobacteria, the most abundant classes are Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria. While in the phylum Bacteroidetes, the most abundant classes are Bacteroidia and Rhodothermia. Cyanobacteria, Chloroflexi, Planctomycetes, and Verrucomicrobia phyla are also well-represented in the majority of these systems. Gypsum endoevaporites, on the contrary, are dominated by Proteobacteria, Bacteroidetes, and Euryarchaeota phyla. The Cyanobacteria phylum is also abundant in these systems, but it is less represented in comparison to mats and microbialites. Regarding the eukaryotic taxa, diatoms are key structural components in most of the microbial ecosystems studied. The genera of diatoms identified were Achnanthes, Fallacia, Halamphora, Mastogloia, Navicula, Nitzschia, and Surirella. Normally, in the mats and microbialites, diatoms form nano-globular carbonate aggregates with filamentous cyanobacteria and other prokaryotic cells, suggesting their participation in the mineral precipitation process. This work expands our knowledge of the microbial ecosystems inhabiting the extreme environments from the Central Andes region, which is important to ensure their protection and conservation.

849 / EXPLORING BIODEGRADABILITY OF BACTERIAL CELLULOSE /POLYHYDROXYBUTYRATE BLENDS

Keywords: *Biodegradability, POLYHYDROXYBUTYRATE, BACTERIAL CELLULOSE*

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BACKGROUND

Mass-scale production and use of oil-based plastic worldwide have derived in a series of concerning environmental issues. Among those, global warming as the endangering of species and ecosystems, stand out. In an attempt to replace single-use, unrecyclable polymers, greener, environmentally-safer alternatives such as biobased polymers have emerged.1 Regardless of the benefits, a shift to biopolymers is slow thanks to production cost and mechanical characteristics in comparison with petroleum-derived packaging materials. Blending of biobased polymers has shown a favorable outcome improving mechanical properties suited for packaging purposes, such as thermal stability.2 Due to the modifications implied, degradability behaviour of novel polymeric mixtures such as bacterial cellulose in combination with polyhydroxybutyrate (BC/PHB) requires to be examined.3

OBJECTIVES

The aim of this study is to evaluate the ability of several microbial strains to utilize BC/PHB blends as a source of carbon and energy.

METHODS

Microbial strains were previously isolated from plastic-polluted soil samples. Mineral Salt Medium was used to test PHB/BC blends as sole carbon source (9 g/l $Na_2HPO_4 x 12H_2O$, 1.5 g/l KH_2PO_4, 1 g/l NH_4Cl, 0.2 g/l Mg-$SO_4 x 7H_2O$, 0.2 g/l $CaCl_2 x 2H_2O$). Tubes containing samples were inoculated with selected strains and incubated for 20 days at 30^0, 120 rpm. Samples were washed and dried and their degradation level was assessed by mass loss and scanning electron microscopy.

RESULTS

Significant loss of molecular weight was observed in samples inoculated with selected strains, meaning degradability was achieved in an important percentage proving the capability of strains to break down the proposed blend of PHB/BC.

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864 / STRUCTURE OF BIOFILMS OF THE BACTERIA ALCANIVORAX BORKUMENSIS GROWN WITH THE ADDITION OF DECANE, OCTANE AND GLYCERYL TRIOCTANOAT

Keywords: *Alcanivorax borkumensis, halloysite, biofilm, biodegradation of oil*

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BACKGROUND

Alcanivorax borkumensis (AB) is a widespread marine bacterium that uses petroleum hydrocarbons as its exclusive source of carbon and energy [1]. This species is dominant in seawater samples after oil spills [2]. Under certain conditions, AB bacteria are able to form thick biofilms at the oil-water interface [3]. Studies of this bacterium are relevant for the search for new developments in the field of oil degradation under conditions of high salt content (up to 12.5%) [2] and also in other areas, such as interspecies interactions [4].

OBJECTIVES

The objectives of the work was to study the structure of biofilms of AB bacteria grown on decane, octane and glyceryl trioctanoate as nutrient sources, using glass and halloysite substrates.

METHODS

The glass and halloysite substrates were laid out in 6-well plates, marine broth with decane, octane and glyceryl trioctanoate was poured into the wells and a culture of AB bacteria was added. The plates were cultured for 72 hours at a temperature of 30 ° C. Fixation, coloring and preparation for microscopic studies were carried out. The adhesion of the surface and the roughness of the sample, calculated by atomic force microscopy (AFM), the peak force error channel was investigated.

RESULTS

AB grew best with glyceryl trioctanoate on glass surfaces, and with octane and glyceryl trioctanoate on samples with halloysite substrates. The growth was more noticeable on substrates with halloysite nanotubes. The adhesion of biofilm surfaces is practically the same for different samples.

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05

874 / THE EFFECT OF BACTERIAL CELLS EXPOSURE TO NITROFURAN ANTIBIOTICS ON THE PROPERTIES ENVIRONMENTAL STRAINS

Keywords: *nitrofurans , antibiotics, environmental bacteria, cell morphology*

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Antibiotics constitute the largest proportion of pharmaceuticals produced globally. Due to increasing use, they have been increasingly released into the natural environment [1,2]. Despite unequivocal benefits provided for humans and animals, antibiotics may pose serious problems when released into the environment.

What is the most dangerous, their presence in the natural ecosystems promotes spread of antibiotic resistance in bacteria [3]. One of the main classes of widely used antibiotics which environmental impact is scarcely studied are nitrofuran derivatives which have been used as pharmaceuticals, food additives and conservatives [4,5].

Our research aimed to investigate the influence of selected nitrofuran antibiotics on the cell envelopes of newly isolated environmental bacterial strains.

Exposure of bacterial cells to nitrofurans induced significant changes in the cell's morphology and structure. The use of advanced microscopy techniques, such as atomic force microscopy or transmission electron microscopy, demonstrated that bacteria cultivated under the stress conditions showed, among others, a loss of their original shape. The cells also increased average surface roughness after exposure to nitrofurans.

The use of conventional microbiological methods made it possible to analyze changes in the permeability of bacterial membrane and cell surface hydrophobicity. For example, exposure of Sphingobacterium sp. to nitrofurantoin or furaltadone significantly increased membrane permeability while no change in hydrophobicity was observed. However, in case of Achromobacter sp. all parameters measured significantly changed upon exposure to both antibiotics. Despite visible bacterial adaptation to nitrofurans, prolonged presence of pharmaceuticals

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875 / METABOLOME PROFILING AND CHEMICAL DIVERSITY ASSESSMENT OF THE MARINE DIATOM SKELETONEMA COSTATUM

Keywords: *diatoms, metabolomics, cheminformatics*

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BACKGROUND

Skeletonema costatum is a marine diatom, with an ecological role in carbon dioxide sequestration, silicon cycling, and regulation of algal blooms. The metabolome depiction of S. costatum can provide molecular insights in its interaction with marine organisms and ecosystem functioning.

OBJECTIVES

A Metabolome Annotation Workflow has been developed for the identification of known compounds in S. costatum.

METHODS

Liquid Chromatography Tandem Mass Spectrometry (LC-MS2) samples from S. costatum were measured with parallel reaction monitoring (PRM) using a target list of all detected compounds created with the software Compound Discoverer by Thermo Fisher Scientific. The obtained MS2 spectra were then used as input for the Metabolome Annotation Workflow (MAW). The workflow utilizes (1) Spectral Database Dereplication using the R package Spectra and (2) Compound Database Dereplication using the software tools MetFrag and SIRIUS, to annotate features to molecule candidates. A suspect list of metabolites previously identified in S. costatum is used for candidate verification.

RESULTS

MAW output is a features list of distinct parent mass (m/z) and retention time (rt) with annotations from spectral and compound database entries which show high spectral and structural similarities. The most prevalent chemical classes annotated in this dataset are carboxylic acids, organooxygen and organonitrogen compounds, fatty acyls, and glycerophospholipids. These annotations will facilitate the Whole Metabolome identification of metabolites in S. costatum. With pathway enrichment analyses, compounds can be functionally connected to metabolic pathways of marine microbes and their ecological role in marine ecosystems.

ACKNOWLEDGEMENTS/REFERENCES

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881 / LANTHANUM INFLUENCES METHANOTROPHIC DIVERSITY IN AGRICULTURAL SOIL

Keywords: *Soil methane filter , Rare earth elements*

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Methane is a significant contributor to climate change, and soil methanotrophs represent a natural filter preventing its accumulation in the atmosphere. Rare earth elements, such as lanthanum (La), are applied in agriculture to increase productivity and crop quality. The role of lanthanides in one- and multi-carbon metabolism was only recently identified, and much remains unknown or poorly understood. Recently, specific types of lanthanides containing bacterial methanol dehydrogenases (XoxF-MDHs) were found. This opens up prospects for developing new approaches to regulate soil methanotrophs and associated microbiota.

The present study aimed to evaluate the shifts in the activity and composition of the soil methanotrophic communities in the microcosm experiments in response to La addition. Microcosms of the soddy podzolic soil were incubated in methane: air (10:90, v/v) atmosphere and 5 mg La/kg soil were added. The addition of La slightly increased soil methanotrophic activity. A metagenomics study has revealed the correlation between the abundances of the Methylococcaceae and Methylophilacea populations. In the La-amended samples, the quantity of Methylobacter was 4% and 6% after 1 and 2 months correspondingly. In native soil, it was undetected and has risen to 0.4% after methane incubation. Simultaneously, the content of methylotroph Methylotenera was 10 and 19% after 1 and 2 months. These data suggested that Methylotenera can obtain carbon from methane and that there may be selective La pressure for these organisms to work collectively with the methanotrophs. This interaction, and its dependence on La, may have important implications for the methane cycle in soils worldwide.

ACKNOWLEDGEMENTS/REFERENCES

This work has been supported by the grant the Russian Science Foundation, RSF 22-24-00418.

884 / GRADUAL CROP REPLACEMENT STRONGLY SHAPES SOIL FAUNA AND MICROBIAL COMMUNITIES IN LONG-LASTING TREE CROPS

Keywords: *crop replacement, bacteria, fungi, mesofauna*

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This project investigates the long-term effect of crop conversion from grapevine to apple orchard on chemical and biological (biodiversity) soil quality parameters. In South Tyrol (Northern Italy), we selected an agricultural field characterized by three close plots (less than 1 ha) where grapevine was replaced by apple cultivation 100, 50, and 4 years ago, respectively indicated as A-1922, A-1970, and A-2016. This unique setting allows us to investigate the effect of gradual orchard replacement avoiding same pedoclimatic characteristics.

We collected a total of 45 soil samples (15 replicates at each site). We assessed chemical-physical soil characteristics (e.g. pH, soil texture, soil organic matter, total nitrogen, total heavy metal content, and available mineral nutrient content). We investigated the soil biodiversity via DNA metabarcoding of the bacterial 16S rRNA gene, fungal ITS2 region and mesofauna COI gene. Overall, soil chemical composition was able to discriminate the plots and predict which history of conversion the samples went through (Anova on PCA components $p<0.01$, LDA with 88.89% classification accuracy). These patterns were reflected in the ecological organization of soil microbial and fauna communities. The alpha-diversity of the investigated communities showed significant changes across the sites (Kruskal Wallis $p<0.05$). Also, beta-diversity analysis showed a strong effect of the conversion time on the bacterial, fungal and soil fauna communities' composition (PERMANOVA p-value<0.05 for all kingdoms investigated). These results indicate that, in perennial tree crops, the story of a field must be carefully considered to enhance our understanding of microbial community assembly in agricultural soils.

886 / LINEAGES OF SALMONELLA NAPOLI CO-CIRCULATE IN HUMANS AND WILDLIFE IN NORTHERN ITALY AS SHOWN BY WHOLE GENOME SEQUENCING

Keywords: *Phylodynamic, Salmonella enterica serovar Napoli, Wild boars*

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BACKGROUND

Salmonella enterica serovar Napoli (S. Napoli) represents an emerging cause of human salmonellosis in Europe. Interestingly, no specific reservoirs of S. Napoli have been identified so far, environment has been suggested as an important source of infection. In Northern Italy, S. Napoli has been frequently isolated in wild-boar (Sus scrofa), while it was seldom isolated in livestock. Specifically, more than 8% of the S. enterica isolated in wild boar belong to this serovar. This suggests that wild-boar can act as a reservoir of S. Napoli by sustaining its enzootic cycle, thus contributing to human infections, either directly or indirectly through the environment.

OBJECTIVES

Identify S. Napoli lineages associated with wildlife and humans and assess the amount of cross-species transmission.

METHODS

We used a genomic approach, based on Bayesian phylodynamic, for inferring population dynamics and reconstructing transmission pathways of S. Napoli between wildlife and humans. The analysis was performed on a dataset composed of all S. Napoli isolates from humans and wild boars of the Province of Brescia (Lombardy, Northern Italy) in the 2009-2012 period. We used S. enterica serovar Typhimurium (S. Typhimurium) as a negative control, since it is a well-documented foodborne pathogen with negligible environmental transmission.

RESULTS

The topology of S. Napoli phylogeny, unlike S. Typhimurium, indicates a considerable amount of cross-species transmission over evolutionary time, with both species interspersed within lineages. Our results support the hypothesis that wild-boar represent a self-sustaining S. Napoli reservoir and a significant source of human infections.

889 / TRANSCRIPTOME LEVEL INTERACTIONS BETWEEN MYCOPARASITIC TRICHODERMA ATROVIRIDE AND THE ROOT ROT PATHOGEN ARMILLARIA OSTOYAE

Keywords: *Trichoderma, Armillaria, mycoparasitism, transcriptome*

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Armillaria species are among the economically most damaging soil-borne tree pathogens causing devastating root diseases world-wide. Bacterial and fungal biocontrol agents are promising environment-friendly alternatives to toxic chemicals to restrain and delimit the spread of harmful Armillaria activities in forest soils. Among fungal biocontrol candidates, Trichoderma species as mycoparasites may efficiently employ diverse antagonistic mechanisms against fungal plant pathogens.

In our study, plate assays were used to perform dual confrontation tests between a haploid A. ostoyae and a mycoparasitic T. atroviride strain. Dual RNASeq-based gene expression profiling was then performed to evaluate the transcriptome level interaction between Armillaria and Trichoderma. Multiple biocontrol mechanisms of T. atroviride were already activated even before physical contact. These included the production of carbohydrate-active enzymes (CAZys) and antibiotic secondary metabolites. The early transciptional impact of Trichoderma on the target pathogen before mycelial contact induced multiple defence reactions such as the production of quinolinic acid, a metabolite with antifungal properties. After physical contact, the CAZy expression profile of T. atroviride narrowed down mainly to fungal cell wall degrading enzymes with important roles in mycoparasitism (β-glucanases, chitinases), while further defence strategies were deployed by A. ostoyae, such as the overexpression of peptidases probably involved in detoxification. However, in spite of the multiple defense mechanisms of A. ostoyae, the interaction ended with Trichoderma overgrowing and killing Armillaria, indicated by the entire lack of Armillaria transcripts in the post-mycoparasitic stage of the interaction.

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900 / MICROBIAL AIR ANALYSIS IN THE ARCHIVES OF THE CZECH REPUBLIC

Keywords: *microbial air contamination, air analysis, Illumina MiSeq, cultivation methods*

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Cultural heritage objects often stored in archives or other facilities are commonly subject to biodeterioration caused by bacteria and microscopic fungi. One of the main factors that significantly influence this microbial contamination is the presence of microorganisms in the atmosphere. Therefore, this work aims to identify both bacteria and microscopic fungi present in the air of archives in the Czech Republic using two different approaches. A culture-dependent approach with multiple types of culture media was used to capture the broadest possible range of metabolically active and culturable species. In the case of low numbers of isolates, microorganisms were identified by MALDI-TOF MS. Otherwise, colonies were collected from agar plates with saline solution and DNA was isolated using the commercial PureLink Genomic DNA Mini Kit. Next, amplicons were prepared using two-step PCR and primers specific for the 16S rRNA or ITS region and further sequenced by Illumina MiSeq. A culture-independent approach was used to detect all microbial species in the air. The metagenomic DNA was isolated from fluorophore membranes using the commercial DNeasy PowerWater kit, and amplicons were prepared using the same approach as for the agars. Sequences were assigned to appropriate microorganism species using the R programming language and its DADA2 package. The impact of several factors (temperatures, relative humidity, filters in air conditioning) on microbiota were evaluated. Subsequently, the results obtained by the two approaches were compared. In the future, analysis of metabolically active species in the air by a culture-independent approach using RNA is also planned.

ACKNOWLEDGEMENTS/REFERENCES

This project is supported by grant no. DG18P02OVV062 of the Ministry of Culture of the Czech Republic.

902 / UTILIZATION OF PAPER INDUSTRY WASTE LIGNIN FOR PRODUCTION OF VALUABLE COMPOUNDS USING BACTERIAL CASCADE DELIGNIFICATION

Keywords: *paper industry, lignin, bacteria, value-added compounds*

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Paper industry is one of the largest consumers of wood biomass and while cellulose is used for pulp and paper production, lignin is often discarded in the form of wastewater. Lignin, as the richest source of renewable aromatic compounds, is therefore treated unsustainable. One of the reasons why lignin is used extensively is complex degradation, which is hard to optimize. The combination of strains forming co-metabolic pathways can be an eco-friendly and an effective solution of utilization of lignin waste that can simultaneously produce value added chemicals. While fungi have the ability to degrade lignin completely, bacteria can often oxidize only certain bonds in lignin polymer, which we can use to our advantage in creating a metabolic pathway for for production of value-added compounds.

To show paper industry waste can be utilized by a co-metabolic system comprised of three different bacterial strains, lignin degrading bacteria was isolated from decayed invasive plant species material, which was then immobilized on carriers along with vanillin and ferulic acid producing bacteria. The carriers containing strains were spatially distributed in a column, so the paper industry lignin waste was dropwise added and effluent was analyzed by the liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). By using this combined approach of different strains, over the period of 11 days, reaching the highest concentrations at day 8, several industrially relevant substances were determined among which were also vanillic acid, acetovanillone, hydroxyacetophenone in the effluent.

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906 / UNCOVERING THE MICROBIAL COMMUNITIES ASSOCIATED WITH PLASTIC FISHING NETS

Keywords: *Marine environment, Plastic, Microbial communities, Fishing gear*

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Fishing gears can be a source of marine plastic pollution . Once in the marine environment, biofilm can be formed on the surface of lost fishing nets (mainly composed of plastics) having distinct microbial communities attached to it. To better understand the ecological implications of such debris in the marine environments, an in situ experiment was conducted inside a maritime port, over one year. In this work we aimed to (i)survey the microbial communities associated with different types of fishing nets; (ii)evaluate microbial community changes over time and (iii)cultivate bacterial strains attached to the nets, in laboratory. Three types of new nets namely Braided Polyethylene, Braided Nylon and Thin Nylon, were placed underwater, 50 cm apart from each other. Samples of nets and surrounding seawater were collected, over time, for microbial community analysis by Next-Generation Sequencing (NGS). Also, net samples were collected after one month of experiment, for isolation of culturable microorganisms.

Over 120 bacterial strains were recovered and identified from the 3 nets, comprising 52 different bacterial genera. The genera Acinetobacter, Bacillus, Rhodococcus, Shewanella, Streptomyces and Vibrio were common to all 3 nets. Preliminary diversity results, indicate a clear distinction between the microbial communities attached to the nets and the ones present in the surrounding water. A deeper analysis on the diversity and taxonomic structure of these communities is currently being performed, considering the influence that different polymers, time, and water characteristics as nutrients, metals or hydrocarbons levels might have on the microbial communities attached to fishing nets.

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909 / PANORAMA: COMPARATIVE PANGENOMICS TOOLS TO EXPLORE INTERSPECIES DIVERSITY OF MICROBIAL GENOMES

Keywords: *pangenome, comparative genomics, genome plasticity, environmental microbiology*

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BACKGROUND

The last few years have seen the explosion of sequencing projects, leading to a considerable increase of genomes available in public databases. Comparative genomics approaches now use hundreds of genomes to analyze the diversity of a species. Many studies focus on the overall gene content of a species, the pangenome, to understand its evolution in terms of common and variable genes with regard to epidemiological or environmental data. Nevertheless, the processing of such a mass of data imposes a paradigm shift in knowledge representation and in the algorithms used.

OBJECTIVES

In this context, we developed PANORAMA, a bioinformatics toolbox, including new methodological developments for the comparative study of pangenomes.

METHODS

PANORAMA benefits from methods for the reconstruction and analysis of pangenome graphs, thanks to the PPanGGOLiN software suite (https://github.com/labgem/PPanGGOLiN), particularly for the identification of regions of genomic plasticity (RGPs) and their segmentation in conserved modules. PANORAMA allows inter-pangenome analysis, relying on the exploration and comparison of RGPs and modules predicted in different species. Moreover, the tool proposes functional annotations of these modules.

RESULTS

PANORAMA offers rapid and easy to use comparative analyses of pangenomes on several thousand genomes of different species. PANORAMA will help to understand the adaptive potential of bacteria and, with the exploration of functional modules in different species, provide a better understanding of the evolutionary dynamics behind the metabolic diversity of microorganisms.

911 / MICROSCOPE: A WEB PLATFORM FOR MICROBIAL GENOME ANNOTATION THROUGH PANGENOMIC AND METABOLIC ANALYSIS

Keywords: *microbial genomics, comparative analysis, pangenomes, metabolism*

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BACKGROUND

Large-scale genome sequencing projects are producing a vast amount of new information that is completely transforming our understanding of thousands of microbial species. However, despite the development of powerful bioinformatics approaches, full interpretation of the content of these genomes remains a difficult task for microbiologists.

OBJECTIVES

To address this challenge, we develop the MicroScope platform, an integrated Web platform for management, annotation, comparative analysis and visualization of microbial genomes (https://mage.genoscope.cns.fr/microscope). The platform enables collaborative work in a rich comparative genomic context and improves community-based curation efforts.

METHODS

Launched in 2005, the platform has been under continuous development. MicroScope provides analyses for complete and ongoing genome projects together with metabolic network reconstruction and transcriptomic experiments allowing users to improve the understanding of gene functions.

Besides automatic functional annotations, several tools allow analyzing a wide range of biological systems (antibiotic resistance, secondary metabolites, secretions systems, defense systems,...). The platform also has extensive functionalities to explore and compare metabolic pathways. Finally, recent functionalities allow users to perform comparative pangenomics on hundreds of genomes of the same species and to explore their content in regions of genomic plasticity.

RESULTS

MicroScope platform is widely used by microbiologists from academia and industry all around the world for collaborative studies and expert annotation. To date, MicroScope contains data for >16,000 microbial genomes, part of which are manually curated and maintained by microbiologists (>5,800 user accounts in January 2022). The platform is also a useful resource for academic training.

918 / STUDY OF THE BARK BEETLE IPS TYPOGRAPHUS BACTERIOME: UNRAVELLING BACTERIOME COMPOSITION AND ANALYSIS OF ITS ECOLOGICAL POTENTIAL

Keywords: *Bark beetle, Ecology, Metabolic, Diversity*

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The European bark beetle Ips typographus is formally considered as a secondary pest that usually affects dead and weakened trees in forests of central and northern Europe. Furthermore, due to climate change, massive outbreaks occur more frequently, resulting in the devastation of woodlands, thus, becoming a forest pest of great environmental and economic impact (García-Fraile, 2018).

It is proposed that the bacteriome might be playing key roles in the insect's ecology by providing nutrients, assisting in the degradation of complex polysaccharide polymers, or inhibiting pathogens, among other roles. In this sense, our objectives are to study this beetle bacteriome and infer the potential ecological role that they may be playing.

In this study, 124 bacterial strains were obtained from the inner part of I. typographus beetles in different life stages and identified by 16S rRNA sequencing. Additionally, a 16S metabarcoding sequencing was conducted. Results showed a relatively diverse microbiome dominated by Proteobacteria, also potentially non described taxa were detected.

The bacteriome potential to aid their host was in vitro evaluated: non-profitable for the beetle complex polysaccharides might be broken into simpler sugars by bacteria. Thereby, isolates were inoculated in mediums with CMC, xylan, pectin, or starch where presumptive carbohydrate-active enzyme activity were detected for most of the isolates. Also, more than half of the isolates showed siderophore activity in M9-CAS-AGAR medium, these molecules are important in due of their antibiotic capacity. In this line, 45 of the isolates showed direct entomopathogenic fungi antagonism when confronted in vitro.

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920 / CAVE THIOVULACEAE DIFFER METABOLICALLY AND GENOMICALLY FROM MARINE SPECIES

Keywords: *Cave microbiota, chemolithoautotroph, genome, novel phylogenetic clade*

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Life in Movile Cave (Romania) relies entirely on carbon fixation by bacteria. The microbial community in the surface water of Movile Cave's hypoxic air bells is dominated by large spherical-ovoid bacteria we identified as Thiovulum sp. (Campylobacterota). These form a separate phylogenetic cluster within the Thiovulaceae, consisting mostly of freshwater cave bacteria. We compared the closed genome of this Thiovulum to that of the marine strain Thiovulum ES, and to a genome we assembled from public data from the sulfidic Frasassi caves. The Movile and Frasassi Thiovulum were very similar, differing greatly from the marine strain.

Based on their genomes, cave Thiovulum can switch between aerobic and anaerobic sulfide oxidation using O2 and NO3- as electron acceptors, respectively. NO3-, is likely reduced to NH3 via dissimilatory nitrate reduction to ammonia using periplasmic nitrate reductase (Nap) and hydroxylamine oxidoreductase. Thus, Thiovulum, is likely important to both S and N cycles in sulfidic subterranean aquatic ecosystems. Additionally, we suggest that the short peritrichous flagella-like structures typical of Thiovulum are type IV pili, for which genes were found in all Thiovulum genomes. These pili may play a role in veil formation, connecting adjacent cells and the exceptionally fast swimming of these bacteria

ACKNOWLEDGEMENTS/REFERENCES

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923 / A NEW APPROACH FOR NICHE STRUCTURING BY SPATIAL ASSEMBLING OF MICROSIZED MICROBIAL 3D STRUCTURES

Keywords: *cell encapsulation, bioremediation, bacterial aggregates, microniches*

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BACKGROUND

The main form of existence of microorganisms in nature is within the different types of spatially oriented communities such as biofilms, microbial mats, aggregates, etc. These structures are extremely important since enable interspecies cross-feeding, intercellular interactions, niche separation resulting in lowering competition and increasing collaborative work.

OBJECTIVES

The aim of our research was to develop method for preparation of artificial spatially oriented structures that can enable remediation of environments polluted with inorganic pollutants.

METHODS

As a proof of concept, we prepared aggregates composed of a facultative anaerobic strain Shewanella oneidensis known for its ability to reduce many toxic metals, including vanadate to produce insoluble vanadyl (Carpentier et al. 2003). Using the methods of electrostatic modification of the cell surface described previously (Rybkin et al. 2019; Deev et al. 2021), we constructed artificial aggregates that were cultivated in a modified synthetic medium M9 containing vanadium (V) oxide.

RESULTS

The initial small aggregates start to grow further and after 7 days of cultivation the formation of an anaerobic zone approximately 10µm deep inside the aggregates was formed. At this depth the formation of precipitate of reduced form of vanadate, the vanadyl, was observed by optical microscopy as well as by using Energy-dispersive X-ray spectroscopy.

The data obtained are evidence that the method of synthetic formation of controlled aggregates allows us to control the metabolic activity of bacterial cells and makes a solid basis for further study of the processes occurring in such local anaerobic or other type of niches.

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938 / SYNTHETIC ANAEROBIC NICHES AND THEIR POTENTIAL ROLE IN MERCURY METHYLATION

Keywords: *mercury, bacterial aggregates, sulfate-reducing bacteria, microniches*

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BACKGROUND

It is well known that microbial competitive interaction can be decreased by niche separation. One example is an establishment of the oxygen gradient enabling survivability of strict anaerobes. Therefore, anaerobic niches can occur in well-aerated conditions by the interaction of anaerobes with aerobes, where aerobes scavenge oxygen and protect anaerobes.

OBJECTIVES

In order to test this hypothesis, we developed a synthetic approach for structuring aggregates composed of strict anaerobes (sulfate-reducing bacteria, SRB), and aerobic bacteria. Selected SRB are known to produce methylmercury (MeHg) and have been used to demonstrate the possibility of anaerobic processes occurring under aerobic conditions and to explain the phenomenon of autochthonous MeHg production in the aerated water column. (Lehnherr et al., 2011).

METHODS

Using the method similar to the Rybkin et al. (2019), we constructed and defined synthetic aggregates consisting of the strict anaerobic bacteria Desulfovibrio africanus and marine bacterium Cobetia marina. Aggregates were tested for anaerobic activity by cultivating in specific media. The spatial organization was studied using confocal microscopy. MeHg formation was determined by the cold vapor atomic fluorescence spectroscopy method (Liang et al.1994).

RESULTS

The aggregates retained their activity for up to ten days in batch cultures. By microscopy analysis we also observed self-organization, where C. marina cells grew outward while SRB were presented only in the center of the aggregate. The analysis of methylation activity showed a conversion rate of 0.89±0.2% from the initial concentration of spiked inorganic mercury resembling the activity in the strict anaerobiosis.

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946 / PREPARATION AND ANTIMICROBIAL EVALUATION OF BACTERIAL CELLULOSE/POLY(3-HYDROXYBUTYRATE) BLENDS ACTIVATED WITH CURCUMIN FOR BIOME

Keywords: *Antimicrobial, Bacterial Cellulose, Curcumin, PHB*

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BACKGROUND

New alternatives are urgently required to reduce the use of non-degradable single-use materials, drawing more attention towards the use of biodegradable polymers derived from renewables sources. However, these biopolymers still present some limitations to substitute commodity plastics, such as low thermal stability and poor mechanical properties, and they need to be improved by the development of new blends and composites. Blends of bacterial cellulose (BC) with poly(3-hydroxybutyrate) (PHB) show a promising alternative thanks to the unique mechanical properties of BC1,2 and the thermoplastic properties of PHB3. Furthermore, the addition of active compounds to provide antimicrobial properties is desirable to provide greater benefits in the use of these materials for medical and food packing applications.

OBJECTIVES

The aim of this work was to evaluate the antimicrobial performance of BC/PHB blends produced by several methods.

METHODS

BC was synthesized by Komagataeibacter medellinensis (ID13488), cultivated in HS liquid medium (2% glucose, 0.5% yeast extract, 0.27% Na2HPO4, 0.15% citric acid and 0.5% peptone) at 30°C, for 3 weeks in static conditions. BC/PHB blends incorporating active curcumin were produced by different methods (interpenetrating polymer networks, solution blending, extruding). Antimicrobial activity was tested in liquid culture against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) strains.

RESULTS

The resulting materials showed successful incorporation of curcumin into the BC/PHB blends. Bacterial growth reduction was observed. Resultant polymer blends showed potential to be adapted for food packing and biomedical applications.

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960 / IMPACT OF ORGANIC OLIVE AGROFORESTRY ON ASSOCIATED CEREAL OR LEGUME MICROBIOME NETWORKS IN THE SOUTH MEDITERRANEAN

Keywords: *Rhizosphere, Core microbiome, Co-occurrence networks, Agroforestry*

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Agroforestry has been identified as a promising strategy for climate change mitigation, but little is known about the belowground microbial communities of associated crops with such agroecosystem. Herein, we performed a microbial holistic integrated study using soil physicochemical properties and rhizospheric microbial community datasets to delineate the co-occurrence pattern of organic olive-agroforestry (AF) network compared to conventional cropping (C) network for two cereals (barley and durum wheat) and two legumes (chickpea and faba bean). Crops microbiomes were found to be exclusively shaped by the agroecosystem, with only marginal influence of host selection, whether cereals or legumes. Of the selected soil physicochemical properties, organic matter was the principal driver in shaping the soil microbial structure in the AF system. Additionally, agroforestry reduced cereal and legume rhizospheric microbial diversity, enhanced network complexity and leads to more stable beneficial microbial communities mitigating consequences of soil degradation. The co-occurrence network analyses indicated that the core microbial community in AF rhizosphere is more complex and established a higher number of interactions, suggesting that this latter is more stable and functional than conventional cropping. Taxa belonging to Tomentella, Funneliformis, Archaeospora, Entoloma, and Bovista genera were identified as potentially key ecological microbes. Overall, this study helps to understand the effect of AF on cereals and legume's core microbiome, especially in organic agriculture, thus providing much more accurate predictions to preserve soil microbial diversity.

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05

980 / THE ROLE OF BIOFILM IN PROTECTING FRUCTOPHILIC LACTOBACILLI BEE SYMBIONTS FROM AGROCHEMICALS STRESSORS

Keywords: *Fructophilic lactobacillus, biofilm, agrochemicals, honeybee symbiont*

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Honeybees are of great interest due to their contribution to human diets and the pollination service. In recent years, a severe decline has been reported with consequent enormous economic and ecological damages. Among the drivers of losses, the chronic exposure to cocktails of agrochemicals and pesticides is crucial.

Recent studies highlighted the role played by honeybee symbionts for the insect heath. Fructophilic lactobacilli are inhabitants of honeybee crop with important health promoting roles. The capability to synthesize biofilm enables them to colonize honeybee esophagus and proventriculus. However, they are still partially unexplored.

We investigated in vitro the effects of chemical stressors like phytosanitary products (associated with honeybee death) and pollen/plant secondary metabolites (ingested by bees as part of their diet) on both planktonic and biofilm growth of fructophilic lactobacilli strains isolated from honeybees crop. To this purpose, the work focused both on the preliminary phases of biofilm growth and on already developed biofilms. The results demonstrated that fructophilic lactobacilli biofilms were more tolerant to exposure compared to the planktonic counterpart. Moreover, the features of biofilm extracellular polymeric matrices vary depending on the exposition to different phytosanitary products.

We believe that the understanding of the reaction of key symbiotic microorganisms to chemical stressors conveyed by the diet can contribute to clarify the potential of fructophilic lactobacilli for the improvement of honeybees health, and to the development of future effective countermeasures against bees decline.

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987 / A CONVENIENT NUCLEIC ACID EXTRACTION PROCEDURE FOR WASTEWATER-BASED EPIDEMIOLOGY

Keywords: *Wastewater-based Epidemiology, SARS-CoV-2*

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BACKGROUND

The COVID-19 pandemic spurred investigation on the use of wastewater-based epidemiology (WBE) as a tool for monitoring the prevalence of COVID-19. The SARS-CoV-2 genetic signal is at low concentrations in wastewater, making sample concentration a prerequisite.

OBJECTIVES

We hypothesized that since the SARS-CoV-2 genetic material in the wastewater is primarily present in nucleoprotein complexes and not in intact virions, a direct capture method that renders total nucleic acid conducive to binding to affinity resin may be able to overcome cumbersome viral concentration steps.

METHODS

This led to development of a simple and rapid alternative to existing purification methods from wastewater.

RESULTS

The process recovered 63.13 ± 4.16%, 40.09 ± 10.89%, of HCoV OC43 and 229E. Using this workflow, we monitored wastewater samples from three wastewater treatment plants for one year using SARS-CoV-2 RT-qPCR. Pepper Mild Mottle Virus (PMMoV), a fecal indicator RNA virus present in wastewater was used as a normalization tool. SARS-CoV-2 variants of concerns (alpha, beta, gamma, delta) and enteric viruses (Norovirus GI, GII and Hepatitis A) were also analyzed. The workflow has a limit of detection of 1.6 GC/ ml with 40 ml of raw wastewater sample for SARS-CoV-2. Our analysis showed strong correlation between the SARS-CoV-2 load in wastewater and clinical cases declared by the municipalities. The alpha and delta variant started appearing in wastewater samples in March and May, 2021 respectively. Norovirus GII was detected in all samples and GI in 25% of the samples tested. HepA was not detected in any sample.

988 / ENHANCED GALLIUM AND INDIUM TOLERANCE AND ACCUMULATION BY ENGINEERED ESCHERICHIA COLI CELLS EXPRESSING METALLOTHIONEINS

Keywords: *bioaccumulation, metal recovery, metal resistance*

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BACKGROUND

Metallothioneins (MTs) are small cysteine-rich proteins important in sequestration of metals cadmium, zinc, copper and mercury.

OBJECTIVES

Development of genetically engineered Escherichia coli BL21 cells overexpressing MTs to improve the gallium (Ga) and Indium (In) recovery.

METHODS

MT gene was amplified from Pseudomonas aeruginosa in order to obtain four different variants of protein, cytoplasmic expressed MT with or without His tag and outer membrane MT with or without His tag. The ability of these MT proteins to confer Ga and In resistance to E. coli was evaluated comparing the growth of cell suspensions and through determination of Minimum inhibitory concentration (MIC). The accumulation of both metals into the engineered cells was determined by ICP-MS.

RESULTS

Comparing with the control strain BL21, the expression of cytoplasmic and membrane MTs increased the accumulation of Ga in 3.8±0.1 and 1.8±0.05 fold, respectively. In case of In, the expression of all types of MTs resulted in a similar increase (7 fold) of metal accumulation. MIC assays showed that in the presence of IPTG, the Ga and In MICs for the engineered strain were 3 mM and 0.75 mM, respectively, while the MICs of control BL21 were only 2 mM and 0.38 mM, respectively. Our results suggest that MTs play important roles in Ga and In detoxification and can be used to remove these critical metals through an environmentally friendly approach, which can be important for metal remediation and recovery.

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997 / HIGH-RESOLUTION CHARACTERISATION OF SHORT-TERM TAXONOMIC AND RESISTOME CHANGES IN WASTEWATER INFLUENT

Keywords: *Antimicrobial resistance , Wastewater, Metagenomics, 16S sequencing*

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BACKGROUND

Wastewater-based surveillance of population-level antimicrobial resistance (AMR) is gaining significant traction, but the impact of wastewater sampling methods on results is unclear.

OBJECTIVES

We characterised taxonomic/resistome differences between single-timepoint-grab and 24H-composites of wastewater influent from a large UK-based wastewater treatment work (WWTW).

METHODS

Hourly influent grab samples (n=72) were autosampled over three consecutive weekdays from a WWTW (population equivalent:223,435). Daily 24H-composites (n=3) were prepared from respective grabs. Metagenomic DNA was extracted from all samples (PowersoilTM) and 16S-rRNA sequenced (515F-806R primers, MiSeq). One composite and six grabs derived from day 1 underwent shotgun sequencing (NovaSeq). 16S-data was analysed using DADA2 and DESeq2 with zero-inflated model options; shotgun data using MASH and ResPipe.

RESULTS

For grabs, taxonomic abundances varied diurnally and ~12hrly; one grab completely missed Actinobacteria, which was otherwise ubiquitous (Fig.1A). Composites captured all phyla in grabs and resembled mean daily abundances. Grab 16S-taxonomic and overall genomic (MASH-distance) variability clustered into four time-periods; composites showed low taxonomic variability and clustered centrally between time-periods (Fig.1B).

Of 122 AMR genes (ARGs) identified across all day 1 samples, single grabs identified 90-101 (74%-83%) ARGs; the composite identified 101/122 (83%) ARGs (Fig.1C). The composite identified three ARGs not seen in any grab sample and single grab samples a median of 6 (IQR: 5-8) ARGs not seen in the composite (35/35 of these hits were at lateral coverage <0.5).

CONCLUSIONS

24H composite sampling optimises capture of overall daily wastewater taxonomic+ARG composition; grab samples are more convenient and potentially capture low-prevalence/transient targets but are less comprehensive.

1010 / A NEW ROLE OF DEDA FAMILY PROTEIN IN INDIUM EXTRUSION BY A RHODANOBACTER SP. STRAIN

Keywords: *gene mutation, indium bioaccumulation, gene complementation*

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BACKGROUND

DedA family protein is a superfamily of membrane proteins found in eukaryotes and prokaryotes but still poorly characterized.

OBJECTIVES

This work aims to use microorganisms able to resist and recover the critical metal Indium (In) as an eco-friendly approach. The study focus on the role of a DedA family protein YqaA from strain Rhodanobacter sp. B2A1Ga4 as a potential In transporter protein involved in In extrusion from the cells.

METHODS

An In-sensitive mutant of strain B2A1Ga4 (mutant B2) was obtained by random transposon mutagenesis. A mutant complemented with yqaA gene cloned into the plasmid pBBR1MCS-5 (B2_pyqaA) was obtained to confirm the role of protein YqaA. The In resistance, viability and accumulation was evaluated for native, mutant and complemented strains. The In amounts in the cells were analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

RESULTS

The mutant B2 exhibited higher In accumulation levels than the native strain. Even for longer periods of 4d of bacterial growth, mutant maintains the metal into the cells while the native strain extrudes In. Complemented strain B2_pyqaA accumulated significantly lower amounts of In compared to the control strain B2_p. The higher In accumulation by mutant cells comparatively to native or mutant complemented strains resulted in high cellular toxicity revealed by lower cellular viability and resistance to In.

The findings from this study support the role of YqaA as a membrane transporter involved in the In efflux process in this Rhodanobacter sp. strain.

ACKNOWLEDGEMENTS/REFERENCES

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1024 / A LINK BETWEEN CELL STRUCTURE AND GENOMICS IN THE GENUS ACHROMATIUM

Keywords: *Giant Bacteria, Polyploid, Heterozygous Bacteria*

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Achromatium is large and hyperpolyploid with cells containing ca. 300 different chromosomes. Using single-cell genomics, metagenomics, metatranscriptomics, and fluorescence in-situ hybridization, we showed that individual cells of Achromatium harbor genetic diversity typical of multi-species populations, far exciding that typical to single genera. These cells contain and express tens of transposable elements, which likely contribute to the unprecedented diversity observed in genome sequence and gene synteny.

We propose that the multiple chromosomes of Achromatium are not copies of its genome, making it the only known "heterozygous" bacterium. We propose that chromosomes occur in clusters, isolated in cytoplasmatic pockets trapped between the periplasmatic $CaCO_3$ crystals. Withing these pockets gene conversion leads to the stabilization of functional alleles. Upon cell division, these clusters are shuffled resulting in two daughter cells different from each other as well as from the mother cell. We could show Achromatium is common worldwide, spanning temperature, salinity, pH, and depth ranges normally resulting in bacterial speciation, yet unlike other taxa, Achromatium displays no clear phylogenetic separation. Furthermore, Achromatium contains a globally identical, complete, functional inventory regardless of habitat, but differences exist in gene expression patterns, likely transcribing only relevant genes. We, therefore, suggest environmental adaptation occurs by increasing the copy number of relevant genes across the cell's hundreds of chromosomes. The functional versatility of Achromatium, and its genomic features, reveal alternative genetic and evolutionary mechanisms, expanding our understanding of the role and evolution of polyploidy in bacteria while challenging the bacterial species concept and drivers of bacterial speciation.

1036 / DEVELOPMENT OF PLASTIC DEGRADING MICROBIAL CONSORTIA BY INDUCTION IN SOIL MICROCOSMS

Keywords: *Plastic biodegradation*

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Plastic pollution is a current environmental concern. Biodegradation is one of the best alternatives to solve this threat. Most studies employ pure microbial cultures to attempt plastic biodegradation with poor success, whereas microbial consortia can increase degradation efficiency. This work aimed to obtain new plastic-degrading microbial consortia from plastic contaminated soil by using sequential enrichment cultures on media containing linear low-density polyethylene (LLDPE). Microcosms made of soil with buried LLDPE films were incubated four months, and films were employed to obtain the consortia by sequential enrichment in culture medium with film or milled LLDPE as the sole carbon source. Enrichment cultures were incubated for one month with a monthly transfer to fresh medium until obtaining a stable consortium.

At each transfer, abundance and diversity of bacteria and fungi, as well as ligninolytic microorganisms, were measured. Consortia members were finally isolated, molecularly identified and characterized based on their enzymatic profile related to plastic degradation. Results showed a biodiversity reduction in each transfer, resulting in stable microbial consortia after four months. Consortia were composed of two bacteria and one yeast. Higher microbial counts were observed in milled LLDPE than in film leading to a plastic weight loss between 2.5% and 5.5%. Consortia members presented a wide range of enzymatic activities related to plastic degradation, highlighting the REBP5 or REBP7 strains, while other members with fewer activities, such as REBF1 or RBF8, could facilitate the action of the other consortium members. Microbial consortia selected have an outstanding potential for plastic biological treatment.

ACKNOWLEDGEMENTS/REFERENCES

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1037 / MECHANICAL EVALUATION OF BACTERIAL CELLULOSE-BASED BLENDS FOR BIODEGRADABLE FOOD PACKAGING APPLICATIONS

Keywords: *cellulose, mechanical, Polyhydroxybutyrate, biodegradable*

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BACKGROUND

Bacterial cellulose (BC) is the purest form of cellulose available and the most area-efficient since it is lignin, pectin and hemicellulose free and requires the area to grow1. These and many other mechanical and biocompatible properties make it a promising material in the quest for petroleum-based and non-degradable plastics-free substitutes. Particularly, its three-dimensional network structure allows interactions with a range of compounds providing potential activated functional attributes2. However, its drawback related to the high production cost and mechanical efficiency is a key factor that directs the efforts for improvements of the BC proprieties that could allow its viability as a sustainable alternative.

OBJECTIVES

The aim of this work is an assessment of the BC activated blends in order to compare mechanical properties against pure bacterial cellulose and an evaluation of its biotechnological application possibilities.

METHODS

Pure BC films were synthesised by Komagataeibacter (ID13488) in modified HS medium (2% w/v Glucose, 0.5% w/v Yeast extract, 0.5% w/v Bacterial peptone, 0.27% Na2HPO4 and 0.15% citric acid) for 3-4 weeks, at static conditions at 30°C. Previously prepared BC/polyhydroxybutyrate blends' mechanical properties such as cellulose concentration, density, water holding capacity and uniaxial tensile testing3 (apparent Young's module, elongation at the break) were examined..

RESULTS

The resulting data showed a significant difference between BC/polyhydroxybutyrate blends in terms of reinforcement tensile proprieties against pure bacterial cellulose, which indicates its potential for packaging application.

ACKNOWLEDGEMENTS/REFERENCES

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ACKNOWLEDGEMENTS

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1047 / SEDIMENT MICROBIAL COMMUNITIES THROUGHOUT SEAGRASS MEADOW DECLINE

Keywords: *seagrass meadow decline, sediment, Cymodocea nodosa, microbial communities*

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BACKGROUND

Seagrasses influence surface sediments forming a specific environment for diverse, abundant, and active microbial communities. Although a major decline in seagrass meadows has been reported in the Mediterranean Sea, little is known about how the sediment microbial communities underlying these meadows respond to such an event.

OBJECTIVES

The aim of this study was to characterize the sediment community composition of a declining Cymodocea nodosa meadow and assess the temporal dynamics of the community during the seagrass decline.

METHODS

Samples of surface sediments from a declining C. nodosa meadow were collected at monthly intervals from July 2017 to October 2018. In addition, samples were also collected from an adjacent non-vegetated sediment. Bacterial and archaeal community structure was determined using Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene.

RESULTS

Microbial communities were primarily stratified with sediment depth and secondly by area sampled. A clear dominance of bacterial over archaeal sequences was noted. Most archaeal sequences were classified as Nanoarchaeota, Thermoplasmatota, Crenarchaeota, and Asgardarchaeota, while the bacterial community consisted primarily of Desulfobacterota, Gammaproteobacteria, Bacteroidota, and Chloroflexi. Although C. nodosa reached a point where almost no leaves were present, a high stability of the sediment community composition was observed throughout the study period. Overall, it appears that the decline of the C. nodosa meadow had no or little effect on the temporal variation of the sediment microbial communities.

ACKNOWLEDGEMENTS/REFERENCES

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1050 / GENOME MINING TO UNRAVEL POTENTIAL METABOLIC PATHWAYS LINKED TO GALLIUM BIOLEACHING ABILITY OF BACTERIAL MINE ISOLATES

Keywords: *Bioleaching, Gallium, Genomes*

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BACKGROUND

Gallium is considered a high-tech Critical Metal, used in the manufacture of several microelectronic components containing either gallium arsenide (GaAs) or gallium nitride (GaN). The current high demand for this critical metal urges the development of effective recovery processes of gallium from low concentrated solutions or electronic recycling material.

OBJECTIVES

The ability to bioleach gallium from GaAs and GaN of strains: Rugamonas sp. A1-17, Sphingomonas sp. A2-49, Arthrobacter sp. A2-55, Rhodanobacter sp. B2A1Ga4, and Undibacterium sp. Jales W-56 was determined. Comparative genome analysis of these strains was also performed to identify potential functional metabolic features linked to the ability to bioleach gallium.

METHODS

Genomes were functionally annotated in terms of clusters of orthologous groups (COGs) using eggNOG-mapper v2 and were mapped to KEGG pathways by the KEGG Automatic Annotation Server. The bioleaching assays were performed in 20 ml of modified R2A medium containing 10 mg of sterile GaAs or GaN salts at 25°C, 150 rpm, for 21 days.

RESULTS

The COG category “Amino acid transport and metabolism (E) was the most abundant in all genomes (8.5-13%). The KEEG pathways analysis showed that most amino acids biosynthetic pathways were present in all genomes. Amino acids and peptides have been reported as chelating agents, interacting and mobilizing several metals and may play an important role in the Ga leaching mechanism of these bacteria. Overall a higher efficiency in gallium leaching was obtained with GaAS when compared to GAN, and strain B2A1Ga4 showed the highest efficiency to leach gallium (56%).

ACKNOWLEDGEMENTS/REFERENCES

Acknowledgements

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1051 / APPROACH TO THE ROLE OF MICROORGANISMS PRESENT IN LOW-RANK COALS FROM COLOMBIA DURING THE PROCESS OF HUMIC SUBSTANCES FORMATION

Keywords: *low-rank coal, humic acids, microorganisms realted to humic substances formation*

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Colombia is noted for the exploitation of thermal and metallurgical coal, but the coals that are classified as low-rank products do not have a particular use in the industry. During the formation of this resource, the humification process takes place where structures known as humic substances are developed, which stand out for their properties and potential application in the environmental and clinical fields due to their heterogeneity at the structural level.

The goal of this study was to analyze the relationship between the formation of humic substances from low-rank coals in the central zone of the country and the microbiota present in them. The samples were characterized by ASTM standards, humic acids obtained were analyzed by spectroscopy techniques. Microorganisms present in the coal samples were isolated, and the identification was made by sequencing the 16S gene for bacteria and the ITS region for fungi.

The coal samples analysis determined them as low-rank coal. Humic acids with a high degree of aromaticity and structures based on polyphenols and quinones were obtained. Eighty-four microorganisms were isolated, of which 59.5% are reported to be related to lignin and cellulose degrading enzymes production, in particular lignin peroxidase, Mn peroxidase and laccase. It was concluded that the formation of humic substances was initially related to the filamentous fungi and yeasts found, using the route of polyphenols and, subsequently, the degradation activity of humic products occurred mainly by prokaryotic organisms.

ACKNOWLEDGEMENTS/REFERENCES

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1060 / EXPLOITING METAL-MICROBE INTERACTIONS FOR RE-USE OF DIGESTATE WITHIN CIRCULAR ECONOMY

Keywords: *metal-microbes interactions, constructed wetlands, digestate, non-food bioenergy crops*

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Biogas plants receive inputs of different sources of carbon, nutrients, metals and other pollutants from large areas that result in a digestate that is a very complex and concentrated matrix. Exactly how to redistribute those components without causing imbalances in the (bio)geochemistry of receiving environments is one of the main questions arising regarding waste management. It is important to understand the effects digestate disposal on element-cycling and microbial communities in soils, as well as how metals interact with other (micro)pollutants in the environment. Therefore, it is necessary to investigate the effects of the disposal of solid and liquid digestate on soil microbial communities responsible for relevant biogeochemical processes.

Within the M2ex project "Exploiting metal-microbe applications to expand the circular economy", we are studying transfer and movement of metals from digestate into natural environments, and their interactions with plants and soil microorganisms. For that, two phytoremediation experiments were carried out in parallel: i) application of solid digestate to soils to promote the growth of non-food bioenergy crops, and ii) liquid digestate re-use after constructed wetland treatment. Microbial communities associated to digestate, soil and plant roots, collected along the experiments time, were characterized by using 16S rRNA gene amplicon sequencing and predictive functional profiling. Metal-microbe interactions will be explored in order to optimize bioremediation processes for metal uptake from soils and wastewaters amended with solid or liquid digestate, thus closing the loop of the circular metal bioeconomy.

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1063 / BIODEGRADATION OF PAROXETINE BY NATIVE BACTERIA IN NATURAL MEDIA MICROCOSMS

Keywords: *Bioremediation, Bioaugmentation, Native Microorganisms , Pharmaceuticals*

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In the last years, several technologies have emerged to remove pharmaceuticals. However, most of them target wastewater treatment plants and they are not suitable to be applied in natural environments.

This work aimed to assess the potential of native bacteria, selected from an estuarine sediment through an enrichment process [1], to biodegrade paroxetine in natural media microcosms. Microcosms were assembled with estuarine water and sediment, doped with 1 mg L-1 of paroxetine, and inoculated either with a bacterial consortium (composed by 10 different bacterial strains [1]) or with individual bacterial strains (Pseudomonas sp. or Acinetobacter sp.). Moreover, controls with paroxetine without bioaugmentation, sterile controls with paroxetine and controls only with estuarine water and sediment were assembled. Biodegradation experiment was conducted for 2 weeks, in static and dark conditions. Water samples were collected for paroxetine and nutrients analysis. Besides, sediment samples were collected for microbial community characterization (through NGS) and paroxetine analysis.

Results showed that paroxetine had a significant effect on natural microbial community structure, while the bioaugmentation process did not exert a significant change. Low paroxetine removal was observed in all bioaugmented treatments, being abiotic removal suggested as the main removal mechanism. Removal efficiency was likely affected by the low nutrient levels in the media, indicating that proper conditions should be selected to stimulate and enhance the bioaugmentation process.

More studies should be performed to optimize the combination of the bioaugmentation and biostimulation processes, along with the bacterial amount required to accomplish higher removal efficiencies of paroxetine in natural experiments.

ACKNOWLEDGEMENTS/REFERENCES

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1072 / ORGAN SPECIFIC ENDOSYMBIOTIC MICROBIOME OF EUROPEAN TICK IXODES RICINUS REVEALED BY TRANSCRIPTOMIC RNA-SEQ ANALYSIS.

Keywords: *Ixodes ricinus, microbiome, endosymbionts, RNA-seq*

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BACKGROUND

Tick Ixodes ricinus is a host of endosymbionts many of which are closely related to pathogens transmitted by ticks. These endosymbionts are likely to provide the host with their harmful, neutral of beneficial potential and may play role in nutritional adaptation, development, reproduction, defence against environmental stress and immunity.

OBJECTIVES

The aim of our research was to identify the diversity and activity of endosymbionts in individual organs of Ixodes ricinus ticks (unfed or fed after 24 and 72 hours) coming from checked breed of ticks and not infected with any pathogens either naturally or artificially and associate it with blood-feeding.

METHODS

Total RNA from individual organs of ticks (synganglion, ovary, salivary glands, gut) was sequenced with NEBNext ligation chemistry for Illumina. Quality of obtained libraries was verified on chip electrophoresis. Prepared paired-end libraries were sequenced (2x75) using Illumina NextSeq550 platform. Data analysis was carried out using bioinformatics tools available on the portal www.usegalaxy.eu.

RESULTS

The quality and quantity of bacterial and fungal community changed with blood-feeding and tissue type. In all analyzed tissues microbial transcripts were detected indicating microbial activity. As typical endosymbiotic fungal genera Malassezia, Saccharomyces, Botrytis, Tetrapisispora, Ustilago were detected. More than 300 active bacterial genera have been detected. Typical for synganglion and ovary of the unfed individual were Delftia, Gordonia, Rickettsia, Streptococcus. The activity of the microbiome in synganglion was at the same level after 24h, but increased after 72h of feeding, while in salivary glands and gut the opposite effects could be observed.

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1076 / ENVIRONMENTAL FATE OF THREE ANTIBIOTICS, SULFOMETHOXAZOLE, TIAMULIN AND TILMICOSIN, AND THEIR IMPACT ON THE MICROBIAL COMMUNITY

Keywords: *veterinary antibiotics, microbial community, resistome, mobilome*

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Use of animal manure for soil fertilization may facilitate veterinary antibiotic (VA) release, and antibiotic resistance gene (ARG) and pathogen dispersal, threatening environmental quality and human health. We studied, in two agricultural soils, the persistence and the effects of three VAs, sulfomethoxazole (SUL), tiamulin (TIA), and tilmicosin (TLM), on the soil microbial community. VAs were repeatedly applied in soils (3 cycles) either directly or through application pig feces containing VAs in environmentally relevant levels. We measured antibiotic dissipation, inorganic N soil pools, potential nitrification (PN); and the abundance of total bacteria, antibiotic resistance genes, mobile genetic elements and ammonia oxidizing bacteria and archaea (AOB/AOA) (with qPCR). We further analyzed the community composition of total prokaryotes, fungi and eukaryotes (focusing on protists) through high throughput amplicon sequencing. Out of the three antibiotics, only TIA showed accelerated biodegradation upon repeated application and only in the high pH soil (DT50 = 14 and 7.5 days in the first and third cycle respectively) and only under direct soil application. TLM consistently accumulated and SUL had a relatively stable dissipation pattern in both soils and application regimes. SUL negatively impacted PN, increasing ammonia as the application cycles progressed.

TLM did not show any adverse effects on PN, while TIA showed an adverse effect in the end of the last application cycle with varying responses between the two application regimes. Molecular analysis and high throughput amplicon sequencing analyses are on the way and will be presented in the conference.

ACKNOWLEDGEMENTS/REFERENCES

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1099 / VERMICOMPOSTING AMENDED WITH MICROORGANISMS AND BIOCHAR: PHYTOPATHOGEN RESISTANT SEEDBEDS FOR VEGETABLES AND HEAVY METAL POLLUTE

Keywords: *Biochar, Microorganisms, Saudi Arabia, Acrophialophora*

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BACKGROUND

Biochar can be used in numerous biotechnological applications due to its properties to adsorb beneficial nutrients and harmful pollutants.

OBJECTIVES

We aimed to treat heavy metal polluted organic wastes using vermicomposting process and produce a fertilizer that can be used in agriculture. We improved the process by adding biochar as well as microbial inoculum and biomass into household waste or sewage sludge before vermicomposting.

METHODS

The earthworm Eisenia fetida used in vermicomposting was included to accumulate heavy metals, biochar to adsorb heavy metals, and the microalga Navicula sp. or the mangrove fungus Acrophialophora sp. to promote plant growth in the final product used as a seedbed for Solanaceae vegetables. We carried out vermicomposting treatments to see the effect of different amendments. Final compost quality was analyzed for maturity. The earthworms were studied for their vitality, heavy metal accumulation, and metallothionein protein content to verify their role in the process. The compost was used as a seedbed for vegetables that were inoculated with a phytopathogen Pythium sp. known to cause root rot and destroy seeds.

RESULTS

Compost as seedbed promoted plant growth and reduced disease symptoms in leaves. In the treatment where E. fetida, 6% biochar, and Navicula sp. had been added, 90% of the seeds germinated, while less than 20% germinated in the control treatment. The experimental plants had acquired resistance against Pythium sp. The metagenomic profile of microbial communities will be reported.

1020 / BIO-FENTON APPLICATION FOR NON-SPECIFIC DEGRADATION OF CHLOROACETANILIDE HERBICIDES USING GLUCOSE OXIDASE AND FERRIC CITRATE

Keywords: *Alachlor, Bio-Fenton reaction, Chloroacetanilide herbicide, Hydroxyl radicals*

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There have been many reports on detecting residual chloroacetanilide herbicides and their degradation products in groundwater and river of different countries. Particularly, residual alachlor could cause a severe problem in the environment because of its high water solubility and low persistence in aerobic soil with a moderate degradation rate with a half-life of 2~3 weeks, which could lead to leaching to groundwater. Therefore, the degradation of residual alachlor in the environment is a persistently problematic issue. In this study, Bio-Fenton reaction supported by glucose oxidase (GOx) for producing H2O2 was applied to degrade persistent chloroacetanilide herbicides in the presence of Fe(III)-citrate at pH 5.5. There were a pH decrease to 4.3 and the production of 8 mM H2O2 and simultaneous consumption to produce •OH radicals which non-specifically degraded the herbicides.

Based on the HPLC analysis, the degradation rates followed the order acetochlor ≈ alachlor ≈ metolachlor > propachlor ≈ butachlor with the degradation percent of 72.8%, 73.4%, 74.0%, 47.4%, and 43.8%, respectively. Alachlor degradation was tested as a model compound to elucidate the Bio-Fenton degradation pathway.

During the Bio-Fenton degradation, alachlor was dechlorinated and filtered into catechol via the production of intermediates formed through a series of hydrogen atom abstraction and hydrogen oxide radical addition reactions. The current Bio-Fenton reaction leading to the production of •OH radicals could be applied for non-specific oxidative degradation to various persistent organic pollutants under in-situ environmental conditions, considering diverse microbial metabolic systems able to continuously supply H2O2 with ubiquitous Fe(II) and Fe(III) and citrate.

ACKNOWLEDGEMENTS/REFERENCES

This study was carried out with the support (PJ01497402 and PJ0162642021) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

1131 / OCCURRENCE AND ECOLOGICAL ROLES OF SYMBIOSIS-DERIVED KILLER TRAIT IN ASSOCIATIONS BETWEEN PARAMECIUM AND CAEDIMONAS

Keywords: *symbiosis, ciliates, R-body producing bacteria*

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BACKGROUND

Some Paramecium (Ciliophora) strains are known to acquire a killer trait. Coincubation of such cells with sensitive paramecia results in a rapid death of the latter. Ability to kill is conferred to the ciliates by their bacterial endosymbionts belonging to Caedimonas (Alphaproteobacteria) or Caedibacter (Gammaproteobacteria). Symbionts kill ciliates after being ingested. The mechanism of killer effect remains unclear, as well as the causes of resistance to it.

OBJECTIVES

Objectives were to describe killer traits associated with several Caedimonas varicaedens isolates, to study quantitative dependences and dynamics of killer effects, and to shed the light on the emergence of killer symbiosis.

METHODS

Several isolates of C. varicaedens from P. aurelia and P. caudatum were characterized by full rRNA-cycle approach [1]. Main methods used were DIC microscopy, DNA sequencing and FISH. Killer tests were done as described in [2].

RESULTS

We checked the possibility of horizontal transmission of symbionts and showed that they can be transferred from one ciliate to another during conjugation. One isolate transformed its host into a mate-killer. Caedimonas-associated killer traits were tested on a range of Paramecium species. We found that killer effects of different isolates were unequal. The killer strains had reciprocal resistance, though not absolute. The strains of the P. aurelia species complex and P. caudatum were mostly sensitive to the killer trait, while the strains of phylogenetically distant Paramecium species were resistant. We assume that Caedimonas-bearing ciliates acquire an advantage allowing them to win competition with uninfected paramecia normally present in the same environment.

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1142 / THERMOPHILIC MICROBIAL COMMUNITIES IN AEROBIC COMPOSTING BIOREACTORS FOR DEGRADATION OF BIO-BASED PLASTIC FILMS

Keywords: *bio-based plastics, PLA, PHBV, compost*

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Bio-based plastics (BPs), produced from renewable biomass sources, are currently increasing in products and applications, including polylactic acid (PLA) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). However, their biodegradability and environmental fate have not been fully investigated. In this study, we evaluated the degradation of PLA and PHVB films, as well as their modification by acetyl tributyl citrate (ATBC), calcium carbonate (CaCO3), and spray-dried lignin-coated cellulose nanocrystals (L-CNC) in aerobic composting bioreactors.

We characterized the microbial communities (bacteria and fungi) possibly involved in the degradation by quantitative PCR and sequencing. Plastic films were also analyzed by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). Generally, the results show that PLA/ATBC/CaCO3 and PHBV/ATBC/CaCO3 were degraded by more than 90% in 60 days and 30 days, respectively. Regarding PLA/ATBC/CaCO3, the abundance of bacteria and fungi was double compared to the control (PLA). Specifically, bacterial taxa such as Thermogemmatispora, Thermoflavifilum and Rubrobacter; increased after 2 months. In the case of PHBV films, microbial abundance was comparable to all treatments and the bacterial taxa S0134_terrestrial_group, Roseiflexus and Acidibacter highly proliferated after 1 month. Overall, PLA and PHBV that contained 10% ATBC and 10% CaCO3, representing the most promising biodegradable BPs.

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1151 / RCLS-MEDIATED SIGNAL TRANSDUCTION MODULATES VIRULENCE OF PSEUDOMONAS PUTIDA

Keywords: *Signal transduction system, sensor kinase, Pseudomonas putida, virulence*

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BACKGROUND

Signal transduction systems enable bacteria to sense diverse stimuli and adequately respond to them. Pseudomonas spp. has a remarkable ability to thrive in various environmental conditions and signal transduction systems have been considered as one of the cornerstones of their adaptability. In previous study, we described novel three-component system RclSAR in Pseudomonas putida WCS358. Sensor kinase RclS was shown to be positive regulator of integrase class 1 (intI1) gene transcription.

OBJECTIVES

The aim of this study was to elucidate the role of RclS-mediated signal transduction in development of P. putida WCS358 virulence phenotype.

METHODS

The AHL production of WCS358 WT and ΔrclS was detected by fluorescence assay using ethyl acetate AHL extracts. Biofilm formation was determined after crystal violet staining by measuring absorbance at 595 nm, while production of siderophore pseudobactin 358 was determined by measuring absorbance of obtained bacterial supernatants at 400 nm. Cytotoxicity of used strains was monitored by LDH assay, while adhesion ability was ascertained by comparing number of adhered bacteria relative to added bacteria. Both assays were performed using HaCaT keratinocyte cell line.

RESULTS

Inactivation of the rclS gene resulted in significant decrease in long-chain AHL production comparing to WT, while differences could not be detected for biofilm formation ability. Bacteria defective for RclS sensor kinase had elevated pseudobactin 358 production, higher cytotoxicity as well as higher adhesion ability relative to WT. These results indicate that sensor kinase RclS modulate P. putida WCS358 virulence.

ACKNOWLEDGEMENTS/REFERENCES

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1161 / ISOLATION AND SCREENING OF THE BACTERIA FOR SIMULTANEOUS ARSENIC OXIDATION AND NITRATE REDUCTION IN CONTAMINATED WATERS

Keywords: *Arsenic oxidation, Nitrate reduction, Electron communication*

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Arsenic and nitrate contamination of water bodies pose an immediate risk to human health besides being a serious environmental issue. Biological denitrification is the most important way for nitrate removal. Heterotrophic and chemoautotrophic microorganisms have been reported to oxidize As(III) under aerobic and anaerobic denitrifying conditions. To find the appropriate microbiome for an specific contaminated site is a critical step to carry out the desired bioremediation process. The objective of this study was to investigate the potential of native bacteria present in As-contaminated water to simultaneously oxidize arsenic and reduce nitrate. Ninety-four arsenic resistant strains were isolated using a chemically-defined medium supplemented with 50 mg·L-1 As(III). Pseudomonadaceae (Pseudomonas spp.), Comamonadaceae (Hydrogenophaga palleronii), and Nocardiaceae (Rhodococcus spp.) and Rhizobiaceae (Agrobacterium tumefaciens) were the most frequently identified among isolates. Amplification products of aoxB and aroA arsenite-oxidizing genes were observed in 37 and 32 isolates, respectively. Only three isolates had the complete set of denitrifying genes (nirS, nirK and narG, nosZ). Then, fifty isolates were selected and tested for simultaneous arsenic oxidation and denitrification. Concomitant As(III) oxidation and nitrate reduction in heterotrophic conditions was effective (oxidation >80% and 100% reduction) in five isolates, whereas two added isolates were positive under autotrophic conditions. Moreover, seven synthetic co-cultures (two isolates) were tested and compared with pure cultures, giving evidence of an interspecies electron communication between them. These results improve the feasibility for development of possible bioremediation strategies of co-polluted environments based on the use of co-cultures.

ACKNOWLEDGEMENTS/REFERENCES

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1164 / BIVALVES AS BIO-MONITORS OF HUMAN INTESTINAL PARASITES

Keywords: *Giardia, Bivalves*

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Giardia lamblia, a flagellated protozoan parasite, if ingested, infects the lumen of the small intestines in humans. G. lamblia is most commonly transmitted to humans via ingestion of contaminated food and water. Its presence in marine environment is relatively unorthodox. The goal of this study is to determine the prevalence of G. lamblia in Atlantic oysters (Crassostrea virginica) and ribbed mussels (Geukensia demissa) collected from Orchard Beach and Clason Point, Bronx, NY. Each year, the mussels were collected in the fall. Tissues were dissected, followed by DNA extraction and PCR analysis. Thus far, we found a marked fluctuation in the prevalence of G. lamblia in both bivalves from 2014-2018, ranging from no 4.5% to 42%, with no parasite detected in 2015 in the ribbed mussels collected from Orchard beach. In addition, a change in the genotype of G. lamblia was observed from year to year, with assemblage A being more frequent. Commercial bivalves were also examined for the presence of G. lamblia. A total of 30 oysters and 72 blue mussels (Mytilis edulis) specimens were purchased from a popular fish store located in the Bronx in the fall of 2019. G. lamblia DNA was detected in 20/30 of the oyster specimens with a resulting prevalence of 6.6%. On the other hand, G. lamblia DNA was detected in 9/72 blue mussels (12/5%). These data indicate that commercial bivalves are potential sources of G. lamblia infection. In conclusion, bivalves may be used as a bio-monitors of G. lamblia.

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1167 / BIOLOGICAL ACTIVITY OF LAKE MICROORGANISMS

Keywords: *enzymes, antimicrobial activity*

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Artificial lakes and soil are interdependent, what makes adjustments into water balance and diversity of microorganisms. Their microbial diversity are of interest, because closed lake has not rivers flowing out and there is no significantly change the species composition the microorganisms inhabiting of the bottom sediments. Microbiological investigation of unstudied ecosystems is important for discovering of new perspective bioactive compounds producers.

This study aimed to determine enzymatic and antimicrobial activity and identify the most active strains isolated from the water, biofilm and bottom sediments of lake.

After determination of cultural and morphological characters selected bacterial isolates were screened using agar plates for testing amylase, catalase, cellulase and lipase production. The antifungal and antibacterial properties were determined using reference strains, agar block method for antifungal investigations and microdilution method for antimicrobial testing.

Based on the obtained results of enzymatic and antimicrobial activity were selected the most active 4 bacterial strains from water samples, from bottom sediments 3 strains, and 5 strains from biofilm samples.

Due to antimicrobial potential selected isolates can be promising strains for its further research and applications in the industrial production of important biological compounds.

ACKNOWLEDGEMENTS/REFERENCES

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1168 / DEVELOPMENT OF LATERAL FLOW TEST FOR IDENTIFICATION OF ESCHERICHIA COLI O157:H7

Keywords: *Escherichia coli O157:H7, Detection Kit, Lateral Flow Assay, Water Safetyn*

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BACKGROUND

Lateral flow assay (LFA) is rapid detection system based on horizontal liquid flow that is low cost, sensitive, specific, easy to use. One of the most important microbiological pollutants threatening water safety is Escherichia coli (E. coli) O157:H7 bacteria. Development of LFA for detection of E. coli O157:H7 in water is critical for health, economy and military.

OBJECTIVES

Conventional methods such as Polymerase Chain Reaction and Enzyme-Linked ImmunoSorbent Assay used to detect E. coli O157:H7 are time consuming and require equipment and specialists. Instead of these methods, for the detection of it in water, it is aimed to develop LFA that can get results in a short time, can be used in the field and doesn't need equipment and specialists.

METHODS AND RESULTS

Monoclonal antibodies (mAbs) were immobilized to the nitrocellulose (NC) membrane, some of them were conjugated with gold nanoparticles and used in the conjugate pad. Optimizations were made regarding the stacking pad and NC membrane pretreatment, the drying conditions of the conjugate pad and the NC membrane, the conjugation method, AuNP sizes and pH conditions. When the bacteria was found in the water sample, the complex formed as a result of the interaction with the antibody bound to the gold nanoparticle was observed as a color change thanks to the optical properties of the gold and was reported a limit of detection of 10 colony forming units E. coli O157:H7. As a result of this, higher-sensitivity LFA for E. coli O157:H7 detection was developed.

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1176 / STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF MICROBIAL BIOFILMS OF THE SHULGAN-TASH CAVE

Keywords: *karst cave, Shulgan Tash, 16S rRNA, biofilm*

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BACKGROUND

Caves are extreme environments characterized by stable low temperatures, high humidity, scarcity of organic substrates, and lack of light. The impossibility of producing organic matter by phototrophic organisms is compensated by the abundance of chemoautotrophic and chemolithoautotrophic bacteria and archaea. Cave microorganisms have a broad metabolic potential and may be important in biotechnology and medicine.

OBJECTIVES

The objective of the study was taxonomic, structural and metabolic characterization of microbial biofilms found in aphotic parts of the Shulgan-Tash karst cave (Bashkortostan), located on the western slope of the Ural Mountains (Russia).

METHODS

Taxonomic profiling was performed based on sequencing of the V4 variable region of the 16S rRNA gene, and supplemented by morphological characterization using scanning electron microscopy. A functional reconstruction of metabolic pathways of the studied communities was carried out.

RESULTS

We described morphology and structure of 12 types of biofilms found in the Shulgan-Tash cave. Taxonomic profiling of the biofilms revealed members of the phyla Actinobacteria, Proteobacteria, Acidobacteria, Planctomycetes, and Chloroflexi. Actinobacteria prevailed in all studied biofilms. Among them, unknown representatives of Pseudonocardiaceae family dominated in most biofilms. Archaea were represented by the phyla Thaumarchaeota, Nanoarchaeaeota, and Euryarchaeota, among which Thaumarchaeota were the most abundant (>60%). The participation of cave microorganisms in the dissolution and precipitation of calcium carbonate was noted. Functional profiling of the Shulgan-Tash cave microbiome revealed its high potential for CO2 fixation, and predicted its roles in the oxidation of methane, nitrogen, and sulfur.

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The microbiological part of the work was carried out within the framework of the state task of the FRC KazSC RAS.

1180 / STARVATION RESPONSE OF SALMONELLA ENTERICA AT VARIOUS POPULATION DENSITY

Keywords: *responce to starvation , RNA-seq, persistence*

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BACKGROUND

Laboratory experiments with starving cultures indicate that a wide range of microorganisms show genetic traits that improve stability for oligotrophic conditions. However, it remains unclear to what extent laboratory studies of starvation survival in pure or mixed cultures can be extrapolated to stable microbial ecosystems in nature. Most laboratory studies related to adaptation to starvation have been performed on populations of high density while according to modern concepts bacteria are more likely to experience stress such as carbon and energy deficiency in low populations.

OBJECTIVES

The purpose of the experiment was to elucidate the mechanisms of long-term survival under starvation conditions at different inoculation densities.

METHODS

Within 150 days, we evaluated the dynamics of colony-forming units, genomic copies for cultures of Salmonella enterica subsp. enterica serovar Typhimurium str. 14028S of different inoculation density on minimal AB medium and their morphological features using electron microscopy. RNA-seq analysis was performed to identify metabolic pathways unique to cultures with low and high inoculation titers.

RESULTS

The ability to persist for a long time under conditions of carbon starvation for high titer cultures is probably due to the presence of many dead cells. At the same time, they demonstrated a gradual decrease in the number of CFU and genomic copies. Low titer cultures increased population density. The results of transcriptomic analysis show that low titer cultures express genes for biofilm formation, utilization of fatty acids and fatty acids of the cell membrane and exopolysaccharide matrix synthesis.

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1183 / VERTICAL ORGANISATION OF MICROBIAL COMMUNITIES IN SALINETA HYPERSALINE WETLAND, SPAIN

Keywords: *Ephemeral hypersaline lakes, Functional redundancy, Reverse redox gradient, archaeal biomarkers*

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Microbial communities inhabiting hypersaline wetlands, well adapted to the environmental fluctuations due to flooding and desiccation events, play a key role in the biogeochemical cycles, ensuring ecosystem service. In order to better understand the ecosystem functioning we studied soils microbial communities of Salineta wetland (NE Spain) in dry and wet seasons in three different landscape stations representing situations characteristic of ephemeral saline lakes: S1 soil usually submerged, S2 soil intermittently flooded and S3 soil with halophytes. Microbial community composition were determined according to different redox layers by 16S rRNA gene barcoding. We observed reversed redox gradient, negative at the surface and positive in depth, which was identify by PERMANOVA as the main factor explaining microbial distribution. The Proteobacteria, Gemmatimonadetes and Euryarchaeota phyla were dominant in all stations. Linear discriminant analysis effect size (LEfSe) revealed that the upper soil surface layer was characterized by the predominance of halophilic archaeal operational taxonomic units (OTUs) while the subsurface soil layer was dominated by Bacillus related OTU. Additionally, the potential functional capabilities involved in carbon, nitrogen and sulfur cycles were similar in all samples irrespective of the redox stratification, suggesting a functional redundancy. Our findings highlight the importance of biomonitoring and management of these wetlands whose extreme aridity and salinity conditions are exposed to irreversible changes due to human activities.

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1189 / ROLE OF AQUATIC BACTERIA IN THE PROTECTION AND GROWTH OF PISUM SATIVUM IRRITATED BY MICROCYSTIN-CONTAMINATED WATER

Keywords: *plant-growth-promoting-bacteria, irrigation water, toxic-cyanobacterial-bloom, cyanotoxin-tolerant bacteria*

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The aquatic environment could harbor plant growth-promoting (PGP) bacteria, as could soil. Bacteria with PGP potential could migrate into the rhizosphere via irrigation water and be able to adapt and thrive in this new environment. Our study aimed to investigate the role of bacteria co-evolving with cyanobacterial blooms in plant protection and growth under conditions of physiological and biochemical stress caused by cyanotoxins in irrigation water. Bacteria were isolated in three matrices heavily contaminated with cyanotoxins (microcystins -MCs) and inoculated in the greenhouse with peas irrigated with MC-LR at concentrations of 0, 50, and 100 ug/L. 16s rRNA characterization showed that the isolates were Starkeya novella isolated from cyanobacterial bloom colonies and water contaminated with cyanobacteria and Brevundimonas aurantiaca isolated from agricultural soil contaminated with cyanobacteria. Pots inoculation with the bacteria significantly affected all growth and yield parameters. Toxin increasing showed a significant adverse effect on morphometric and physiological parameters, such as photosynthesis, leaf quantum yield, and stomatal fluorescence. Interestingly, stress enzymes SOD, POD, PPO, Catalase were significantly reduced during bacterial inoculation compared to controls. The detoxification enzyme GST was also increased during inoculated bacterial consortia inoculation compared to the control and the other treatments. Overall, bacteria isolated from Microcystis aeruginosa toxic bloom and inoculated to pea seedlings conferred a significant growth advantage while regulating the expression of stress and detoxification enzymes and alternatively improving plant yield and quality parameters. The consortium of cyanotoxin-tolerant bacteria with PGP properties could provide a natural alternative for fertilizing soils irrigated with cyanotoxin-contaminated water.

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1193 / IMPACT OF MICROCYSTINS ON AQUATIC AND SOIL MICROBIOTA DYNAMICS: A FOCUS ON EXTRA- AND INTRACELLULAR MICROBIAL ENZYMES

Keywords: *cyanobacteria-contaminated waters, Soil enzymes, fluorometric method, Cyanotoxins*

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Cyanobacterial blooms have become a global threat due to toxins released into water bodies. These cyanobacteria-contaminated waters are used in agriculture without treatment. Nevertheless, effects of these cyanotoxins on aquatic and soil microbiota are not fully understood. This study investigates how these cyanotoxins affect the microbial activity of water and soil by measuring the microbial activities of key enzymes involved in biogeochemical cycles. Four enzymes were analyzed by the fluorometric method using MUB- linked substrates: arylsulfatase (ARS), β-glucosidase, N-acetylglucosaminidase (NAGase), phosphatase, and three others were quantified by colorimetry: dehydrogenases, proteases, and urease. Three Bacterial strains previously isolated in bloom colonies, water, and soil highly contaminated with cyanotoxins were inoculated into 48 soil pots, from which 24 soil had been sterilized and 24 not sterilized. These pots, placed in the greenhouse, contained pea plants in 5 replicates and were irrigated for three months with 0, 50, and 100 µg/L MC-LR. Finally, the soils were collected, and the soil's enzymatic activities and chemical properties were analyzed, including loss on ignition (LOI), total phosphorus, and carbon/nitrogen/sulfur analysis by combustion (NCS). The results obtained showed a significant difference in the decrease of all enzymatic activities when irrigating with high MC-LR concentration. Bacterial inoculation showed increased enzyme activities when triple bacterial inoculation was applied. Similarly, there was a positive correlation between bacterial inoculation and total phosphorus concentration and NCS. Our results indicate that MC -LR diminishes soil microbial activities. However, inoculation of soil with cyanotoxin-tolerant bacteria seems to counteract the adverse effects of these pollutants.

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1200 / ADDRESSING THE IMPACT OF TEMPERATURA AND PH ON VIBRIO HARVEYI MORPHOLOGY AND SURVIVAL IN THE TIME OF CLIMATE CHANGE

Keywords: *cell morphology, fluorescence microscopy, culturability, marine bacteria*

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BACKGROUND

Recent studies suggested a causal link between ongoing global warming and spread of invasive pathogenic species including those of the genus Vibrio. Our recent work was focused on study of strategies implicated in the temperature-dependent adaptation of V. harveyi [1] in seawater microcosms [2,3]. These studies revealed that such adaptation is accompanied by profound morphological and gene expression changes.

OBJECTIVES

The objective of the current study was aimed at determining the individual and combined effects of temperatura and pH on V. harveyi adaptation and survival in response to climate change.

METHODS

To assess the individual and joint impacts of temperature and pH, V. harveyi cells were incubated in artificial seawater microcosms at different temperatures (10oC to 30oC) and pH (7 to 8.5) for 3-4 weeks. The incubated V. harveyi populations were regularly examined by fluorescence microscopy to monitor cell size, number and viability after their staining using Live/Dead BacLightTM kit. In addition, culturability expressed as colony-forming units (CFU) was determined by spreading aliquots of V. harveyi populations on Marine Agar followed by incubation for 24 h at 26°C.

RESULTS

We found that more acidic pH, which corresponds to the current and predicted pH of natural aquatic systems being gradually influenced by climate change, affects V. harveyi growth and survival in a temperatura-dependent manner and the most profound changes are observed under suboptimal pH and temperature conditions. The results of this analysis and ongoing work on further characterization of V. harveyi adaptation using omics techniques will be presented.

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1472 / ZOONOTIC POTENTIAL OF ESCHERICHIA COLI STRAINS ISOLATED FROM HEALTHY CATTLE IN RUSSIA AND SLOVENIA

Keywords: *E. coli, cattle, virulence*

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BACKGROUND

Healthy cattle are a natural reservoir of potentially pathogenic Escherichia coli. E. coli strains isolated from animals without clinical signs are known to frequently carry virulence-associated genes (VAGs).

OBJECTIVES

The aim of our study was to perform a comparative analysis of virulence gene profiles and to evaluate the zoonotic potential of E. coli strains isolated from healthy animals from Russia and Slovenia.

METHODS

Non-clonal E. coli isolates from feces of healthy cows from industrial cattle farms and private farms in Russia (49 strains) and private farms in Slovenia (89 strains) were analyzed by PCR for the presence of 10 VAGs (stx2, hlyA, cnf1, fimH, iha, sfaDE, usp, APEC-ompT, iroN, and kpsMT) associated with intestinal and extra-intestinal diseases in animals and humans.

RESULTS

The most prevalent VAG among the analyzed E. coli strains from both countries was fimH, found in 87.8% of cattle strains from Russia and in 65.2% of cattle strains from Slovenia. In strains from both countries hlyA, stx2, usp, APEC-ompT and kpsMT were also found. The sfaDE and iroN genes were found only among the Russian strains, whereas the iha gene was found only among Slovenian E. coli. The cnf1 gene was not found among strains from either country. Strains containing genes and combinations of genes characteristic of extraintestinal and intestinal E. coli were found in Slovenian cattle strains and, to a greater extent, in Russian cattle strains.

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1525 / TRANSCRIPTOMIC ANALYSIS OF RHODOCOCCUS OPACUS R7 GROWN ON POLYETHYLENE BY RNA-SEQ

Keywords: *Rhodococcus genus, polyethylene, plastic biodegradation, RNA-seq*

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Plastic waste management has become a global issue, and among other plastics, polyethylene (PE) is the most abundant synthetic plastic worldwide, and low-density PE generates the largest volume of plastic pollution. Although PE is one of the most resistant to biodegradation for its properties, few bacteria can directly use PE as the sole carbon and energy source without any physical or chemical pretreatments.

In this context, an omic-approach contributed to unraveling the complex degradative system behind PE biodegradation to clarify the gene framework for PE biodegradation of Rhodococcus opacus R7. This strain was selected for its ability to grow on PE as the only carbon and energy source in a short range of time. RNA-seq uncovered genes putatively involved in the first step of PE oxidation. In-depth investigations through bioinformatic analyses and enzymatic assays on the supernatant of R7 grown in the presence of PE confirmed the activation of superficial or releasing extracellular enzymes genes encoding for laccase-like enzymes.

Moreover, the transcriptomic data showed candidate genes for the subsequent steps of short aliphatic chain oxidation including alkB gene encoding an alkane monooxygenase, cyp450 gene encoding cytochrome P450 hydroxylase, and genes encoding membrane transporters. PE biodegradative system was also validated by FTIR analysis on R7 cells grown on PE compared to malate condition indicating that PE induces metabolic changes, including longer lipid hydrocarbon chains in membranes, and a more intense beta-sheet band.

These results pose the basis for environmental and biotechnological applications contributing to plastic elimination.

1571 / SOIL MICROORGANISMS CHARACTERISTICS DURING PHYTODEGRADATION OF DIESEL AND PAH POLLUTION

Keywords: *phytodegradation, miscanthus, diesel, PAH*

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Second generation energy crop Miscanthus x giganteus (Mxg) is known for its ability to grow in marginal soils, high biomass yield as well as for the possibility to be transformed to various biobased products. It is accompanied by several environmental benefits including possibility of phytoremediation of different contaminants.

The current study focused on effect of Mxg cultivation on diesel and PAH degradation in soil and characteristics of soil microorganisms which are key players in phytodegradation process.

A two years pot experiment (2019 – 2020) was set with artificially diesel-spiked soil with concentration range 2 500 – 50 000 mg/kg, PAH (20 mg/kg) and combination of PAH and diesel. The diesel contamination was measured as C10-C40. Soil microbial biomass was determined regularly as PLFA and activity by respiration and dehydrogenase activity. Samples additionally analysed by qPCR (16S, AlkB, pahGN, pahGP markers) and next generation sequencing to identify the most abundant genera of bacteria.

It was found that degradation was improved in variants with miscanthus (mainly in the middle range of concentrations) although this difference was not accompanied by major changes in soil microbial parameters. The dehydrogenase activity and AlkB positively correlated with diesel concentration while total microbial biomass and respiration were mainly comparable across the concentration range. Also there was not significant difference between planted pots and controls with exception of mixed contamination with slightly higher quantity of marker AlkB and PLFA in planted pots.

1575 / BIOPROSPECTION FOR NOVEL FUNCTIONAL MODULES TOWARDS PLANT MONOTERPENE BIOTRANSFORMATION

Keywords: *Pseudomonas, monoterpenes, biotransformation*

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BACKGROUND

The biocatalysis of β-myrcene into industrially relevant value-added compounds, with enhanced organoleptic/ therapeutic properties, may be performed by the specialized enzymatic machinery of β-myrcene-catabolizing bacteria. In Pseudomonas sp. strain M1, the β-myrcene catabolic core-code was identified in a 28-kb genomic island (GI) [1,2].

OBJECTIVES

The lack of close homologs of this β-myrcene-associated genetic code and the limited molecular knowledge regarding its dissemination prompted a bioprospection of cork oak and eucalyptus rhizospheres, in northern and southern Portugal, to evaluate the environmental diversity of the myr+ trait.

METHODS AND RESULTS

Soil microbiomes were enriched in β-myrcene-supplemented cultures, from which a total of 150 β-myrcene-catabolizing strains were isolated, belonging to Alpha-proteobacteria, Beta-proteobacteria, Gamma-proteobacteria and Sphingobacteriia classes. Among these, the genome sequence of 43 selected isolates (including members of the genera Achromobacter, Acinetobacter, Burkhoderia, Citrobacter, Cupriavidus spp., Deftia, Lelliotia, Pseudomonas, Raoultella, Rhizobium, Serratia, Shigella, Sphingobacterium and Variovorax) was determined. Strikingly, the comparative genomics of the 43 genomes, against the strain M1 and phylogenetically closer references, detected the M1-GI in 11 new Pseudomonas isolates derived from different niches.

Currently, both wetlab (physiology, molecular biology) and in silico approaches (comparative genomics, gene ontology, etc) are being explored to further detail the diversity range of the genetic modules associated with myr+. Preliminary results of this work will be presented in the poster.

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1592 / MICROBIOLOGICAL ENVIRONMENTAL IMPACT OF NEW TOMATO CULTIVATION STRATEGIES (TOMRES PROJECT): A PUBLIC HEALTH PERSPECTIVE

Keywords: *microbial indicators, public health, microbial environmental impact, agronomic techniques Tomres project*

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Agricultural crops require high volumes of water and fertilizers. The objective of the TOMRES project (Horizon 2020, contract n. 727929) was the experimentation with new genotypes and agronomic techniques to increase multiple and combined stress tolerance in plants using tomato as a model. The evaluation of the hygienic-sanitary impact of the new cultivation methods was performed analysing soil samples collected pre- and post-cultivation during agronomic field trials.

Some microbiological analyses (22, 28, 37°C counts; Clostridia spores, faecal coliforms, E.coli, Salmonella), ecotoxicological assays and chemical analyses (heavy metals) were performed on soil sample extracts. To evaluate the impact a statistical comparison between the results of these analyses in the pre- and post-cultivation soil samples in each trial (t-test) and among the post-cultivation soil samples of the trials performed with different agronomic techniques (ANOVA) was done. The comparison between the pre- and post-cultivation samples revealed significant different behaviours of the microbial counts related to the different combination of the experimental agronomic conditions. A significant effect of the plant biostimulants application on some microbial indicators (total coliforms and total plate count) was found, with various trend, probably attributable to the different biostimulants in combination with the other agronomic practices. The results obtained showed that the agricultural practices analysed are promising as they limit the mobility of heavy metals, microbiological contamination and ecotoxicity. However, the level of environmental impact was strongly dependent on the type of soil and its possible over-fertilisation condition due to previous agricultural activities.

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1595 / ANTIBIOTIC RESISTANCE IN THE URBAN WATER CYCLE

Keywords: *antibiotic resistance, wastewater, drinking water*

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The urban water cycle, comprising wastewater and drinking water treatment, is particularly interesting to track the fate of antibiotic resistance in the environment and to assess the risks of its transmission to humans.

This study investigated the presence of antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) in the water of three wastewater treatment plants (WWTPs), a lagoon basin used as surface water pre-treatment and two drinking water treatment processes (DWTPs) for one year.

Influent and effluent samples of each plant were analysed for heterotrophic bacteria and ARB using R2A agar (without/with antibiotics, respectively). Media was supplemented with ampicillin, tetracycline and sulfamethoxazole. Moreover, a volume of all sample was filtered (0.22 µm polycarbonate filter) and total DNA was extracted (PowerWater DNA isolation kit). Subsequently, polymerase chain reaction was used to amplify specific genes (Blatem, TETA, SUL_II and SUL_III).

The results showed the ARB presence in almost all samples highlighting the spreading of these bacteria in the urban water cycle. As expected, higher ARB concentrations were encountered in the WWTPs, with a higher resistance percentage after wastewater treatment and after lagoon basin, suggesting a possible selection of ARB during these phases. TET(A) and Sul_II were the most widespread genes (56% and 64%, respectively), mainly in the influent and effluent of WWTPs and in the influent of DWTPs. In the drinking water the presence of Sul_II, Sul_III e Tet(A) genes was revealed. The results obtained suggest the need to evaluate the role of urban water cycle in the antibiotic resistance occurrence.

1659 / THE CONTRIBUTION OF PROTON ATPASE IN E. COLI GROWTH DURING MIXED CARBON SOURCES FERMENTATION AT DIFFERENT PHS

Keywords: *Fermentation, pH, proton ATPase, E. coli*

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BACKGROUND

FOF1-ATPase is the key enzyme of bioenergetics relevance during fermentation which operates ATP hydrolysis mode.

OBJECTIVES

The role of FOF1-ATPase in specific growth rate (μ) of bacteria grown under the fermentative conditions of glucose, glycerol and formate depending on external pH was studied.

RESULTS

It was shown that μ was 0.9 h-1 in wt at pH 7.5. In DCCD assays μ was decreased ~55 %, which indicates that FOF1-ATPase has a significant role in bacterial growth. Same result was obtained using DK8 strain lacking FOF1-ATPase. The involvement of FOF1-ATPase in bacterial growth properties was decreased at pH 6.5 as μ was reduced ~ 22% in the case of DCCD addition. μ was lower in DK8 strain by ~ 1.5 fold. Interestingly, μ was decreased at pH 5.5 in wt, but not in DK8, compared to pH 7.5 and 6.5. The inhibitory influence of DCCD on bacterial growth was not evident in both strains. This might be explained by that FOF1-ATPase possibly has such a conformation that inhibitors, such as DCCD are not able to bond or alternative mechanisms may function to regulate ΔμH+ which are targeted to resist at acidic pH.

To sum up, pH 7.5 is the optimum condition for E. coli growth during the co-fermentation of mixed carbon sources. Moreover, the impact of DCCD and contribution of FOF1-ATPase in growth properties is considerable at pH 7.5. Bacteria operate alternative mechanism, other than FOF1-ATPase, for regulation of ΔμH+ and survive under energy-limited conditions at acidic pH.

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1681 / OMICS ANALYSIS OF A DEEP OCEAN MARINE ALTEROMONAS SP. DURING METHYLMERCURY DETOXIFICATION

Keywords: *Methylmercury, Detoxification, Transcriptomic, Deep-ocean*

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BACKGROUND

Methylmercury (MeHg) is one of the most worrisome pollutants in aquatic systems which bioaccumulates and biomagnifies in the marine food web, and a potent neurotoxicant in humans exposed to it through fish consumption (9). Its biological detoxification is mediated by the mer operon, which contains two genes, merB and merA, responsible for the demethylation of MeHg and the reduction of inorganic mercury, respectively (1,2).

OBJECTIVES

While much emphasis has been put into studying contaminated sites, little is known about the prokaryotes carrying this operon living in pristine environments as the deep ocean. This study provides: the genome characterization of Alteromonas sp. ISS312, isolated from the bathypelagic Atlantic (3, 11) and the transcriptomic comparison of two conditions, with and without MeHg.

METHODS

Two culture conditions with our isolate (MeHg 5 uM and control) were used to obtain DNA and RNA at different stages of the growth curve (5, 13). DNA and RNA extraction, sequencing, assembly and differential expression analysis were subsequently performed (4, 12, 14).

RESULTS

The exploration of Alteromonas sp. ISS312 genome revealed that it harbors 4 mer operons, three of them containing merA gene and two of them the merB. Moreover, the comparative transcriptomics analysis showed that these merA/merB genes are within the most expressed genes at different expression level between them.

Our results also support the hypothesis that the mer operon is highly dynamic between distant related genomes, probably due to horizontal gene transference mediated by mobile elements such as transposases (6, 7, 8, 10, 11).

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1682 / STUDY OF CULTURED MARINE SEDIMENT ISOLATES AND CONSORTIA WITH POTENTIAL FOR MERCURY DETOXIFICATION

Keywords: *Mercury, Detoxification, Sediment, Isolation*

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BACKGROUND

Mercury (Hg) is one of the most worrisome contaminants in the aquatic environments, which bioaccumulates and biomagnifies through the food web, and reaches humans via fish consumption. The biological detoxification of Hg is mediated by the mer operon, which can be present in a wide variety of microorganisms (Barkay et al., 2003; Mason et al., 2012)

OBJECTIVES

Our goal was to find marine sediment bacteria and consortia with mechanisms of detoxification able to grow under high levels of mercury and characterize them.

METHODS

A culture collection of isolates and consortia from sediments of three Hg-contaminated sites was obtained under different culturing conditions, including a pre-treatment with HgCl2 (Rasmussen et al., 2008; Sanz-Sáez, et al., 2020). First of all, we analyzed the taxonomic assignment of the isolates through PCR amplification of the 16S rRNA marker. Secondly, we performed PCR screening for the merAB genes. Lastly, a subset of strains was exposed to different levels of Hg (5 concentrations of HgCl2 and MeHg) in order to calculate their MICs (Minimum Inhibitory Concentrations)(Sanz-Sáez, et al., 2021).

RESULTS

We obtained a collection of 1435 isolates and 456 consortia, and based on the analysis described above, we were able to identify several sediment bacterial isolates and consortia containing genes for Hg detoxification, which tolerated high mercury levels, up to 50uM of HgCl2 and 5uM of MeHg. Further analyses including the study of their genomes, growth curve characterization and biogeographical patterns, comparing them with metagenomes from the same sites, are in progress.

ACKNOWLEDGEMENTS/REFERENCES

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1755 / COMPARATIVE IMPACT OF MN2+ AND NI2+ ON THE MICROALGA CHLORELLA SOROKINIANA

Keywords:

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BACKGROUND

Manganese serves as a co-factor of more than 30 enzymes in microalgae, including the core components of energy and redox metabolism. Nickel is utilized as a co-factor of urease. Both of these transition metals represent important pollutants of aquatic ecosystems. The effects of Mn and Ni excess on microalgae are poorly understood.

OBJECTIVES

We analyzed and compared the impact of Mn2+ and Ni2+ on growth, mucilage release, and redox metabolism of Chlorella sorokiniana.

METHODS

The impact of a set of concentrations of Mn2+ and Ni2+ on growth of C. sorokiniana culture in 3N-BBM+V medium in the early stationary phase was evaluated by changes in optical density at 750 nm and biomass during 7 days treatment. Mucilage release was analyzed using SEM microscopy. Redox settings were analyzed by oxidation-sensitive fluorescent probe and assays for thiols.

RESULTS

Ni was more toxic than Mn and affected culture growth at lower concentrations. Microalgal cells started releasing mucilage polymers within 1 h of exposure to 1 mM Mn2+, whereas no mucilage was observed even at 24 h of treatment with equimolar Ni2+. The peak of reactive oxygen species production was reached faster for Ni2+ than Mn2+. Mn-induced drops in the concentration of reduced thiols showed a recovery after 1 h and 24 h. Ni2+-induced drop was irreversible. The observed differences between the impact of Mn and Ni may be related to different redox and coordinative properties and to higher capacities of microalgae to sequester Mn in relation to higher quotas than Ni that are required for normal function.

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1756 / OCCURRENCE, FATE AND BEHAVIOR OF MICROBIAL CONTAMINANTS OF EMERGING CONCERN (MCEM) IN AQUATIC ENVIRONMENTS

05

Keywords:

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BACKGROUND

There is an increasing concern regarding the evidence supporting the presence of pathogens of emerging concern in water systems receiving wastewater discharge and their transfer to food-webs, at levels known to cause adverse effects on the environment and on human health.

OBJECTIVES

This study aims at addressing the concern on the increasing presence of microbial contaminants of emerging (MCEM) in different aquatic environments collected from 2 cases of study, the Delta of Ebro and the Albufera (Spain). Thus, the occurrence of microbial contaminants including antibiotic resistance bacteria (ARB), antibiotic resistance genes (ARG), SARS-CoV-2, influenza viruses, human enteric viruses and viral indicators (crAssphage coliphages and pepper mild mottle virus (PMMoV)) were evaluated in sewage samples and aquatic environments.

METHODS

In order to assess the risk of aquatic environments as a source of microbial contaminants of emerging (MCEM), occurrence of SARS-CoV-2, influenza viruses, PMMoV and human enteric viruses have been be determined by RT-qPCR in sewage and surface samples. While the occurrence of crAssphage, and the most relevant ARG were determined by qPCR. In parallel, culture methods were used for Escherichia coli, ARB and coliphage determination.

RESULTS

Despite of the microbial load reductions detected in effluent compared to influent wastewaters (2-3 log10 on average), reductions of between upstream and downstream wastewater do not comply with the most recent European legislation

1767 / MICROBIAL RESPONSES TO ORGANIC POLLUTANTS: PFOA AND OCTANOIC ACID

Keywords: *PFOA, Octanoic acid, Low pH, E. coli*

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Perfluoroalkyl substances (PFAS), such as perfluorooctanoic acid (PFOA, C8), are man-made chemicals used in variety of consumer products and industrial applications. These are recognised as emerging contaminants of concern due to their widespread presence in the environment, wildlife and humans, their tendency to persist and bioaccumulate. Sparse information is available regarding the effect of these compounds on microorganisms and the possible associated knock-on effect on ecosystem services. In this study, we used E. coli as a model organism to investigate the effect of PFOA exposure. Furthermore, we examined the ability of E. coli to tolerate stress in the presence of PFOA using octanoic acid (a C8 non-fluorinated weak acid) as a control. To this end we carried out phenotypic characterisations of E. coli exposed to a series of sublethal conditions in the presence and absence of i) PFOA and ii) octanoic acid. We also performed an in vitro evolution experiment, where E. coli was sub-cultured daily in the presence of i) PFOA and ii) octanoic acid for 37 days at pH 5.2. PFOA and octanoic acid were found to negatively affect the growth of E. coli at pH 5.2. While E. coli developed increased growth phenotypes when continuously exposed to octanoic acid, no evidence of adaptation could be observed in the presence of PFOA. Furthermore, cells exposed repeatedly to PFOA at pH 5.2 could not be revived after freezing. Taken together, these results indicate that PFOA and octanoic acid have different mode of actions resulting in E. coli growth inhibition.

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1776 / MICROBIOLOGICAL DEGRADATION AND TRANSFORMATION OF COMPLEX REFINERY WASTE CAUSED BY VIOLENT DESTRUCTION OF INSTALLATIONS

Keywords: *bioremediation, toxic metals, total petroleum hydocarbons*

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BACKGROUND

In this study, we examined the model waste formed on the base of that in the NATO campaign in Serbia in 1999, where a refinery in Pančevo (RiP) city was bombed. A huge amount of material which contained crude oil and its derivatives, hydrodesulphurization catalyst (HDSc) that contained toxic metals molybdenum and cobalt was generated.

OBJECTIVES

The aim of this research was to investigate if a consortium of the microorganisms (CMOs) used in the process of bioremediation of soil contaminated with the sludge of the residual heavy fuel oil (SoRHFO) can change the chemical form of heavy metals from waste HDSc.

METHODS

We used the model mixture of pollutants that should fit the waste derived from RiP (SoRHFO and HDSc) and it also contained wood sawdust and river sand. Quantities of components of interest (total petroleum hydrocarbons–TPH, Co, and Mo) were taken to ensure that all of them were in the hazardous waste category.

The experiment was performed in polyvinyl-chloride containers (approx. 65 kg of the substrate) as pilot-plant and was treated with different conditions.

The CMOs was isolated from original refinery waste. Microorganisms were identified from them.

During the experiment, different parameters were analyzed.

RESULTS

It is noticed that the content of different fractions (hydroxides, sulfides, etc.) of examined toxic metals is transformed during time by microbiological interaction. Through the process, we observed a level of TPH degradation. High microbial activity is expressed in the transformation of the sand to clay, and the degradation of lignocellulose material.

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1033 / METAPROTEOGENOMIC ANALYSIS OF SALIVA SAMPLES FROM PARKINSON'S DISEASE PATIENTS ACROSS THE SPECTRUM OF COGNITIVE IMPAIRMENT

Keywords: *salivary microbiome, metaproteomics, parkinson's disease, cognitive impairment*

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BACKGROUND

One of the most common non-motor symptoms of Parkinson's disease (PD) is cognitive impairment (CI) that progressively develops on a spectrum from mild cognitive impairment (PD-MCI) to dementia (PDD). A better understanding of mechanisms and links underlying CI progression enables the the development and use of new prognostic tools for monitoring the CI state thus decreasing its burden to patients and their families. Saliva has already been investigated extensively in PD for exploration of potential biomarkers. However, the number of studies focusing on the potential relationship between saliva composition and CI in PD is very limited.

OBJECTIVES

In this study, we applied a metaproteogenomics approach by combining 16S rRNA gene amplicon sequencing and metaproteomics to identify candidate signature taxa and proteins in saliva that can differentiate CI stages in PD.

METHODS

We recruited a prospective cohort of PD-MCI (47), PDD (39), and healthy controls (HC, 26) groups in a multi-center study. We investigated the microbial composition of saliva by sequencing V3-V4 region of 16S rRNA gene and employed nano-LC-MS (Waters) to perform metaproteomics analysis.

RESULTS

Salivary microbiota profiles did not show significant difference in alpha diversity; however, there was a statistically significant difference in beta diversity between study groups. At the taxonomic level, we found differential relative abundances of the genera Lactobacillus, Moraxella, F0058 and Megasphaera between HC to PDD. At the functional level, Metaproteomics analysis revealed associations of several pathways with CI progression including glycolysis, dopamine receptor mediated signaling pathway and metabotropic glutamate receptor group II pathway.

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334 / ANTIMICROBIAL PROPERTIES OF LACTIPLANTIBACILLUS PLANTARUM STRAINS ISOLATED FROM TYPICAL HOMEMADE SOURDOUGHS FROM RHODOPE REGION

Keywords: *Lactiplantibacillus plantarum, RAPD - PCR, sourdough, bacteriocins*

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The current research focused in isolating and characterizing nine bacteriocinogenic Lactiplantibacillus plantarum species from homemade whole grain wheat sourdoughs manufactured following protocols typical for some Rhodope regions in South Bulgaria. 16S rRNA gene sequencing was carried out and nine isolates were identified as L. plantarum. Cluster analysis of RAPD-PCR revealed genetic diversity within the different strains.

Using PCR analysis with gene-specific primers, several genes of the plantaricin cluster were identified in the isolates. Plantaricine genes that were ampplified in the LAB genome varied between the strains. The key features for future industrial applications were evaluation of their antibacterial and antifungal properties.

The manifestation of antimicrobial activity against various pathogenic microorganisms is also an important criterion for the selection of potentially probiotic strains. Therefore, isolated nine bacteriocin-producing L. plantarum strains have been tested for activity against food-spoiling bacteria Bacillus subtilis.

They also showed antifungal activities against the fugal pathogens Aspergillus nidulans, Penicillium chrysogenum, Rhizopus stolonifer and/or Fusarium graminearum. The synthesized antibacterial bacteriocin was characterized based on the sensitivity to heat, increased pH, treatment with typsin and catalase.

ACKNOWLEDGEMENTS/REFERENCES

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344 / CHARACTERIZATION OF 5 BROAD-RANGE SALMONELLA BACTERIOPHAGES

Keywords: *Salmonella, Bacteriophage, whole genome sequencing, food microbiology*

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Non-typhodial Salmonella (NTS) is a major foodborne pathogen that causes millions of cases each year, worldwide. Antimicrobial resistance of pathogenic microorganisms is an emerging public health problem. Bacteriophages has been emerged as a viable alternative for biocontrol of Salmonella. They can be utilized for different applications from medicine to food safety. In this study, we isolated and characterized five bacteriophages that are able to lyse multi drug resistant Salmonella strains.

Phages were isolated from farms and wastewater in different cities, by using serotypes Enteritidis, Typhimurium, Infantis and Kentucky. Host-range of phages were determined against 36 drug resistant isolates representing 18 different serotypes. All phages were able to infect multiple serotypes and showed a broad host-range. Adsorption rate, latent period, and burst size of phages were determined. Morphology of phages were investigated with transmission electron microscopy (TEM). Phages were whole genome sequenced for further characterization. For all phages more than 95 % of particles were adsorbed in 10 minutes. Latent periods and burst sizes showed variance. Taxonomy of phages were determined. Enteritidis and Typhimurium phages were similar to Chi-virus and Jerseyvirus.

One of the Enteritidis phages were a jumbophage with over 240 kbp genome. One prophage that resembles Bacteriophage P2 were also extracted from sequences. Annotation of that phage revealed the presence of integrase gene. Infantis and Kentucky phages were Tequintavirus and Epseptimavirus, respectively. None of the phages had virulence or antibiotic resistance genes. The phages showed a potential to be utilized for Salmonella biocontrol agents.

357 / INVESTIGATING THE RISK OF TETRODOTOXIN (TTX) BIOSYNTHETIC VIBRIO IN IRISH SHELLFISH

Keywords: *Vibrio sp.*

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BACKGROUND

The marine pathogens Vibrio vulnificus and V. parahaemolyticus are causative agents of gastroenteritis in humans through the consumption of infected shellfish. Some strains of these pathogens have been seen to produce the potent paralytic neurotoxin tetrodotoxin (TTX). Generally, this toxin is of major concern in subtropical and temperate areas. However, with recent detection across Europe, TTX may pose a threat to European public health and aquaculture.

OBJECTIVES

This study investigates the presence and risk of toxigenic Vibrio within Irish shellfish production. In particular, this study aims to examine the presence of TTX biosynthetic Vibrio within Irish shellfish farms to enable investigation of the biosynthetic genes associated with TTX producing Vibrio.

METHODS

Shellfish harvesting and environmental monitoring is conducted once a month at mussel and oyster farms from Irish coastal waters for the duration of 12 months. Vibrio sp. are detected from samples using culture and genetic approaches and pathogenic isolates are determined using PCR. Currently, immunological assays are being developed for the identification of TTX producing Vibrio within samples. Furthermore, genetic analyses involving whole genome sequencing are being conducted to identify TTX biosynthetic genes in Vibrio.

RESULTS

Twenty-one V. parahaemolyticus isolates were detected from shellfish production sites along the West coast of Ireland. The isolates were present in sediment, oyster and mussel samples, but were not detected in seawater. Eleven isolates (52%) showed amplification by PCR of Type Three Section System 2 genes which are associated with disease in humans. Analysis of TTX production is ongoing.

446 / GROWTH RESPONSES AND GENETIC DIVERSITY OF BIOLUMINESCENT PHOTOBACTERIUM DURING REFRIGERATED STORAGE OF RAW CHICKEN MEAT

Keywords: *Photobacterium, chicken meat*

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BACKGROUND

Photobacterium is widely associated with marine environments, with certain species of the genus representing common fish spoilers. However, recent high-throughput approaches have revealed their abundance on raw chicken meat.

OBJECTIVES

To explore the presence, growth responses and genetic diversity of bioluminescent Photobacterium on chicken breast fillets during refrigerated storage.

METHODS

Chicken breast fillets (n=6, 3 independent batches) were analyzed microbiologically for the enumeration of total mesophilic bacteria, Pseudomonas spp., Brochothrix (B.) thermosphacta, lactic acid bacteria (LAB), Enterobacteriaceae and presumptive Photobacterium during storage at 4°C. Bioluminescent colonies were isolated from Marine Broth agar at the beginning, middle and end of storage. Their differentiation was achieved by RAPD and rep-PCR fingerprinting, while identification of presumptive Photobacterium isolates at genus/species level was performed by 16S rRNA, gyrB and fur genes sequencing.

RESULTS

Chicken meat spoilage was mainly driven by Pseudomonas (ca. 8.2 logCFU/g) followed by B. thermosphacta (ca. 7.5 logCFU/g), LAB (ca. 5.4 logCFU/g) and Enterobacteriaceae (ca. 4.8 logCFU/g). Bioluminescent bacteria were scarce at the beginning of storage, detected in one out of three batches at ca. 2.0 logCFU/g. At later stages of storage they were encountered at relatively high populations (5.3 - 7.0 logCFU/g) in samples from two batches, but below the detection limit in the third batch. In this latter case, these were sporadically encountered throughout storage at populations of up to 3.9 logCFU/g. The genotypic characterization of bioluminescent bacterial isolates resulted in the assignment of the diverse DNA fingerprints to Photobacterium genus.

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447 / BACILLUS CEREUS, YEASTS, MOULDS AND AFLATOXIN B1 IN RICE SAMPLES COLLECTED FROM MARKET IN ZENICA, BOSNIA AND HERZEGOVINA

Keywords: *food contamination, hygiene, microorganisms, mycotoxins*

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BACKGROUND

Many of microorganisms and them metabolites can cause food poisoning and exposure to metabolites is a public health concern due to their carcinogenic, acute and chronic effects.

OBJECTIVES

The aim of this study was to determine the prevalence of Bacillus cereus, Yeasts and moulds and aflatoxin B1 in rice marketed in Zenica, Bosnia and Herzegovina.

METHODS

A total of 47 rice samples were collected from markets and analyzed according to International standards for detection of B. cereus (ISO 7932:2013), and Yeasts and Moulds (ISO 21527-1/21527-2:2009). Enzyme-linked immunosorbent assay (ELISA) was used to detect aflatoxin B1.

RESULTS

Among 47 rice samples (36 rice samples, seven rice products and four cooked rice samples) were studeid for the presence of B. cereus, yeasts and moulds and AFL B1. Long grain of rice was found to have the highest prevalence (30.0%) of B. cereus compared to other type of rice (white rice - 16.6% and integrale rice - 14.3%), respectively. In all rice samples occurence of B. cereus were range from <10 to 320 cfu/g. Yeasts and moulds were detected in seven samples in the range from <10 to 1400 cfu/g. Three samples were positive in the same time on B. cereus and moulds. One sample showed contamination with AFL B1 (2.96 ppb) and also this sample had moulds in concetration of 1400 cfu/g.

CONCLUSIONS

It is important to educate food handlers about their responsibilities for food safety and train them on personal hygiene policies and basic practices for safe food handling.

495 / FERMENTATION FEATURES OF ASPERGILLUS ORYZAE DURING MEJU FERMENTATION REVEALED BY GENOMIC AND METATRANSCRIPTOMIC ANALYSES

Keywords: *Aspergillus oryzae, metatranscriptomic analysis*

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Aspergillus oryzae is one of the important microorganism in East Asia, because of polysaccharide and protein degradation abilities. In Korea, A. oryzae is usually identified in various fermented soybean foods, and also used as starter of meju. However, fermentation features of Aspergillus oryzae during soybean fermentation is not fully elucidated.

The aim of this study is to investigate the fermentation features of A. oryzae during entire meju fermentation periods based on genomic and metatranscriptomic analyses of A. oryzae KG15.

Meju were collected from local meju manufacturers in South Korea, and Aspergillus oryzae strains were isolated. High quality genome of A. oryzae KG15 was obtained, and gene annotation and KEGG analysis was conducted. Metatranscriptome was obtained from meju inoculated with A. oryzae KG15.

In genome of A. oryzae KG15, 11766 genes were predicted and 4333 genes were assigned to KEGG categories. Genes categorized to 'translation' and 'carbohydrate metabolism' showed the highest expression, and genes categorized to 'glycolysis/gluconeogenesis' showed the highest expression in 'carbohydrate metabolism' category. Gene expression in 'glycolysis/gluconeogenesis' and 'starch and sucrose metabolism' showed decrease during fermentation periods, and gene expression in 'pentose phosphate pathway' and 'fructose and mannose metabolism' showed increase during fermentation periods, which suggested the change of metabolism because of nutrient depletion. Starch and cellulose degradation genes showed the highest expression in early fermentation days. Furthermore, protease genes also showed high expression in early fermentation days, which suggested the macromolecule degradation occurs actively in the early fermentation stage of meju.

512 / GROWTH KINETICS OF REGULATORY MUTANTS PROVIDES UNIQUE INSIGHTS INTO THE BIOLOGY OF SALMONELLA TYPHIMURIUM DURING MILK SURVIVAL

Keywords: *Salmonella Typhimurium, bovine milk, camel milk*

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BACKGROUND

Milk, though anti-microbial in nature, causes salmonellosis globally. Therefore, Salmonella can adapt to the antimicrobial environment and multiply in the milk matrix. Camel milk is shown to be more antimicrobial than bovine milk. There are many reports on how to rapidly detect Salmonella in milk. Unfortunately, reports on how Salmonella adapts and survives in the antimicrobial environment of milk are very rare.

OBJECTIVES

We aimed at obtaining a deep understanding of how Salmonella survives in milk. For this, we used wild-type Salmonella Typhimurium and a collection of 16 isogenic regulatory mutants, each representing a stress environment Salmonella faces during its survival in milk.

METHODS

We spiked S. Typhimurium and the mutants into both bovine and camel milk to obtain the growth kinetics of each mutant in both milk matrices and subjected the data to stringent statistical evaluation.

RESULTS

Salmonella can rapidly adapt, survive and multiply in both milk matrices, and the difference in growth kinetics between both milk matrices is statistically insignificant, except in late stationary phases – confirming camel milk is not anti-Salmonella, as reported earlier. Further, the growth patterns of 16 isogenic regulatory mutants of S. Typhimurium show that careful modulation of iron metabolism is important for the survival of the pathogen in milk, besides anaerobic and extracytoplasmic stress adaptation. To our knowledge, our data provide the first demonstration of the stress factors encountered by Salmonella during survival in milk, a theme that can be further extrapolated for developing strategicdesigns to mitigate milk-borne salmonellosis.

ACKNOWLEDGEMENTS/REFERENCES

The authors thank United Emirates University Program for Advanced Research (UPAR - Grant Code G00003478) for funding this project

520 / EFFECT OF UNDER-VACUUM AND MODIFIED ATMOSPHERE PACKAGING ON PDO STELVIO CHEESE MICROBIOTA

Keywords: *Dairy, Next-generation sequencing, Volatile organic compounds*

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The consumers' preference for portion-packed cheeses is increasing, as they provide a quick way to pick them up at the grocery stores. These easy-to-buy products have a large surface area exposed to light and oxygen and are prone to undesirable microbiological and biochemical changes. The aim was to characterize the PDO Stelvio cheese and to determine the shelf-life and stability of under-vacuum and modified atmosphere packaged portions at 4-6 °C for three months. Unpackaged cheese was used as the control. We investigated the microbiota through culture-dependent and independent methods, volatile compounds, proteolysis. Both core and under-the-rind sections were analyzed to find any spatial distribution of the microbial populations. Cheese samples were dominated by mesophilic lactobacilli and mesophilic lactococci (ca. 6-8 Log cfu/g), whereas thermophilic streptococci were ca. 4-5 log lower. Genotyping by RAPD-PCR generated 20 and 15 clusters for mesophilic lactobacilli and mesophilic cocci, respectively. All strains belonged to the species Lacticaseibacillus casei, L. paracasei, L. rhamnosus and Lactococcus lactis subsp. lactis. Illumina-MiSeq next-generation sequencing analysis of 16S and ITS showed that Lactococcus and Debaryomyces had the highest relative abundance; the microbial composition was stable during three months storage, with no significant differences among the cheese sections over time. All samples had a similar proteolysis profile regardless of storage time and packaging conditions. A total of 168 volatile compounds have been identified, including terpenoids, alcohols, aldehydes, ketones, esters, carboxylic acids, lactones, and aromatic compounds. A multivariate analysis will show microbial interactions and metabolic activities linked to the aroma signature.

ACKNOWLEDGEMENTS/REFERENCES

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524 / FEEDING WITH SUSTAINABLY SOURDOUGH BREAD HAS THE POTENTIAL TO PROMOTE THE HEALTHY MICROBIOTA METABOLISM AT THE COLON LEVEL

Keywords: *gut microbiota, sourdough, SHIME, metabolome*

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The value of sustainably food processing in contributing to healthy intestinal microbial functions is emerging. The sourdough fermentation fits with the most traditional and sustainable bread making. We industrially manufactured traditional baker`s yeast (BYB) and sourdough (t-SB30) breads, which first underwent to microbiological, biochemical, textural, and nutritional characterization. According to questionnaires, we selected 40 volunteers adhering to Mediterranean diet. Two highly representative fecal donors were selected based on statistically elaborated data on their fecal microbiota composition and metabolome.

Two Twin Mucosal-Simulator of the Human Intestinal Microbial Ecosystem (Twin M-SHIME) were separately run under feeding with BYB and t-SB30. Bread feeding did not affect the phylum and family composition of both donors. Linear discriminant analysis of the genus core microbiota showed only few significant fluctuations of Lactobacillus and Leuconostoc relative abundances according to feeding with BYB and t-SB30, respectively. Compared to BYB, the content of all SCFA and some derivatives significantly increased through feeding with t-SB30. The same was found for the content of free amino acids.

The bread characterization made possible to identify the main features responsible for these effects. Compared to BYB, t-SB30 had much higher contents of resistant starch, peptides and free amino acids, and showed an inhomogeneous microstructure. We believe that the most efficient approach to investigate the potential of a staple food component was used, excluding interferences from other dietary factors and attenuating human physiology overlaps. The daily consumption of sourdough bread has the potential to promote to the healthy microbiota metabolism at the colon level.

ACKNOWLEDGEMENTS/REFERENCES

REFERENCE

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561 / ENVIRONMENTAL SOURCES ALONG NATURAL CAVE RIPENING SHAPE THE MICROBIOME AND METABOLOME OF ARTISANAL BLUE-VEINED CHEESES

Keywords: *cheese microbiome, metabolome, whole metagenome sequencing, metagenome assembled genomes*

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BACKGROUND

Artisanal Protected Designation of Origin (PDO) cheeses are highly valuable premium products due to their unique sensory attributes. Microorganisms colonising processing environments can have a significant impact on food quality and safety.

OBJECTIVES

To describe the impact that ripening in natural caves has on the microbial successions and various cheese quality markers on Cabrales PDO cheese.

METHODS

Content in volatile compounds and biogenic amines, and microbiome composition through whole-metagenome sequencing (WMS) were monitored on cheeses from three producers that employed the same cave for cheese ripening, while one of the producers ripened one batch of cheese in three different caves. Cheese samples were taken at initial (before cave ripening), mid-term and final ripening stages. Moreover, raw materials and environmental samples from dairy facilities and caves were also taken for WMS analysis.

RESULTS

Both the producer and cave in which cheeses were ripened significantly influenced the cheese microbiome and metabolome. The cheese microbiome was significantly determined by the microbiome of caves, which were a source of Brevibacterium, Corynebacterium, Staphylococcus, Tetragenococcus and Yaniella, among others, as demonstrated through source-tracking and the characterization of 613 bacterial metagenome assembled genomes. Some taxa (e.g., T. koreensis, T. halophilus) were for the first time found in high abundance in cheese, associated with the occurrence of various metabolites. Overall, we demonstrate that processing environments can be a source of non-starter microorganisms with a relevant role in the ripening of artisanal fermented foods and that microbiome-related technologies can be used for the authentication of PDO food products.

ACKNOWLEDGEMENTS/REFERENCES

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546 / TOWARDS A MORE SUSTAINABLE MUSHROOM PRODUCTION: ELUCIDATING NUTRIENT FLOWS IN COMPOST

Keywords: *fungi, bacteria, stable isotopes, edible mushrooms*

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The mushroom Agaricus bisporus, also known as champignon, is the most widely consumed mushroom across the world. Being rich in nutrients and a source of bioactive compounds, its demand is predicted to rise. Although mostly sustainable, the production of A. bisporus is characterized by a high amount of spent compost waste due to a sub-optimal nutrient usage by the mushroom (ten Have et al., 2003; Grimm et al., 2021).

We investigated to which degree the nutrient uptake of A. bisporus is influenced by the microbiome of the compost. To do so, we traced nutrients labeled with stable isotopes (13C-glucose and 15N-ammonium) in the compost system during the cultivation of A. bisporus at different spatial scales. Isotope mass ratio spectrometry (IRMS) was used to determine the bulk abundance of label in the compost layers, nematodes and phospholipid-derived biomarkers (PLFAs), while nanoscale secondary ion mass spectrometry (nanoSIMS) and genomic analyses were used to zoom in spatially or within specific taxonomic groups. This approach allowed tracing nutrient flows from the macro to the nano scale (Fig. 1).

Our results showed that the carbon and nitrogen utilized by the mycelium during mushroom formation originated mostly from the top half of the compost. Moreover, the initial microbial abundance was positively correlated with the carbon uptake of A. bisporus mycelium. However, this correlation was not evident when the compost was fully colonized by the mycelium. These findings can help optimize the industrial production of A. bisporus.

ACKNOWLEDGEMENTS/REFERENCES

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FUNDING

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583 / CHANGES IN THE MICROBIOTA OF PRAWNS TREATED WITH HIGH-HYDROSTATIC PRESSURE, HEAT AND BACTERIOCIN AS-48

07

Keywords: *Biodiversity, Seafood*

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BACKGROUND

Adequate processing and preservation of seafood is crucial to minimize economic losses and risks of food poisoning. Boiled praws are a type of seafood which is eaten without further cooking and therefore its microbiological aspects deserve greatest attention.

OBJECTIVES

To determine the impact of high-hydrostatic pressure (HHP) treatments applied singly or in combination with moderate heat and the biopreservative bacteriocin AS-48 on the bacterial diversity of boiled praws during refrigerated storage. .

METHODS

Boiled prawns were treated by high-hydrostatic pressure (250, 300 or 350 MPa) applied at 22 ºC or 45 ºC, singly or in combination with bacteriocin enterocin AS-48. Prawns were refrigerated stored for 20 days. Total DNA was extracted periodically and bacterial diversity was estimated using amplicon sequencing.

RESULTS

The microbiota of untreated boiled praws was composed mainly by Proteobacteria (Pseudoalteromonas, Aliivibrio, Vibrio and Psychrobacter). The relative abundances of these groups decreased during storage while Firmicutes (Carnobacterium, Brothothrix, Peptostreotpcoccaceae), Fusobacteriota (Psychrilyobacter) and Campylobacterota (Malaciobacter) increased. The main group in the pressurized samples was Psychrobacter. Other genera (such as Carnobacterium and Alkalibacterium) also had higher relative abundances towards end of storage, depending on treatment. The results indicated that HHP treatments have a great impact on the microbiota of boiled prawns and on the changes in the microbiota during refrigerated storage.

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598 / EFFECT OF MILD HEAT TREATMENT COMBINED WITH ESSENTIAL OIL ON E. COLI GROWTH IN VITRO AND IN FRESH FRUIT JUICE

Keywords: *juice fruit, E.coli, Flow cytometry, safety*

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E. coli is a pathogen frequently involved in fruit juices outbreaks, despite the intrinsic low pH of fruit juices. Alternative food preservation technologies are emerging to develop safe food products with extended shelf-life and preserved nutritional and organoleptic characteristics in line with consumers' changing demand.

In this study, the efficacy of a mild heat treatment in the presence of Origanum vulgare essential oil (OEO) on E. coli ATCC 25922 was assessed by traditional culture-based and Flow Cytometry (FCM) with the aim to achieve understanding into viability and cells physiology (Viable But Non Culturable state) analyses. Bacterial survival ratio in vitro and in a fruit juice challenge test was estimated by plating culture on MacConkey agar, and by FCM with SYBR Green

I/Propidium Iodide double-staining. Results obtained in vitro showed E. coli ATCC 25922 inactivation by increasing the treatment temperature (55 C, 60 C, 65 C). Interestingly we highlighted an overestimation of the dead population using the culture-based method and the preponderance of injured cells with a potential resuscitation detected only by the FCM analysis. The challenge test on fruit juice at its own pH (3.8) and buffered at pH 7, displayed a bactericidal action of OEO and a higher efficiency of the mild heat at 65 C for 5 min combined with OEO aimed at food preservation.

Mild heat treatment can enhance the antimicrobial activity of the OEO by promoting the formation on the vapor phase of the oil's volatile compounds, thus showing the potentiality of this approach to guarantee fruit juices safety.

ACKNOWLEDGEMENTS/REFERENCES

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631 / STABILITY, GROWTH AND DECREASE OF BACTERIA IN FIBRE-BASED PACKAGING MATERIALS

Keywords: *food packaging, packaging material, microbial growth, Bacillus*

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Microorganisms can be found ubiquitously and were previously shown to be present also in various types of packaging materials. Although the microbial community of fibre-based packaging materials has been studied extensively for years, the interaction of bacteria with the surrounding packaging matrix still needs more research.

Therefore, this study investigated the survival of food relevant species e.g. E. coli, S. aureus, B. cereus as well as the environmental strains Cytobacillus firmus and Niallia circulans (directly isolated from packaging material) and bacterial spores of B. cereus and B. subtilis. All strains were separately spiked into sterilized and homogenized packaging material samples of different fibre types.

The stability of the added bacteria differed between packaging material samples. Respecting on the different fibre types, some packaging samples appeared to supply more nutrients for bacteria than others resulting in prolonged bacterial stability, higher growth rates and spore germination. Furthermore, none of the strains could grow in all included samples. Rather, the use of fibre based packaging materials as a nutrient is no universally applicable strain characteristic, but depends on the bacterial strain and the type of packaging material.
Moreover, one sample strongly reduced all added bacteria within 24h suggesting antimicrobial activity.
Bacterial spores could only germinate in one sample.

Although further investigations are needed, this knowledge is essential for the development of packaging materials and their application as food packaging to understand and control bacterial growth in packaging material.

ACKNOWLEDGEMENTS/REFERENCES

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651 / MECHANISMS OF GLOBAL EPISTASIS AND THE PREDICTABILITY OF EVOLUTION IN MICROBIAL METABOLISM

Keywords: *Acid resistance, Population heterogeneity, Evolution*

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Acid stress is one of the most common and important stresses that foodborne pathogens can encounter. Acidity can be intrinsically present in the food matrix or can be imposed as a preservative. Moreover, to infect the human host, pathogens should also pass and survive the extreme acid conditions in the stomach. Escherichia coli, a species harboring many important foodborne pathovars, appears to be well-equipped to withstand severe acid shock, although the more fundamental causes of this behavior remain to be established.

Closer examination of this trait revealed that acid resistance within clonal E. coli populations is highly heterogeneous, with some cells being predisposed to survive severe acid exposure. At the population level, E. coli acid resistance also proved to be a readily evolvable trait, which furthermore tended to coincide with cross-resistance against heat as well. The genetic underpinnings of both the heterogeneous behavior and the adaptive evolution with regard to acid stress resistance could be established and validated.

Our results are of particular importance for understanding the survival of low infectious dose foodborne pathovars of E. coli that can become faced with (sudden) acid stress in foods as well as in the human stomach.

667 / ANTIBACTERIAL EFFECT OF DRIED AROMATIC HERBS AND THEIR EXTRACTS ON CHEESE STARTER CULTURE IN VITRO - PRELIMINARY RESULTS

Keywords: *aromatic herbs, cheese, starter culture, antibacterial effect*

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The application of natural compounds from aromatic herbs in cheese production has been studied mainly in the context of improving cheese quality. Their antimicrobial capacity should also be considered from hygienic and technological aspects.

In this work, the effect of cold-sterilized Dalmatian aromatic herbs - sage (Salvia officinalis L.), immortelle (Helichrysum italicum (Roth) G. Don), rosemary (Rosemarinus officinalis L.) and their extracts - against commercial cheese starter culture Lactobacillus helveticus/Lactobacillus lactis in pasteurized milk was tested.

Dried herbs were irradiated (8 kGy, P = 5.23 Gy/s) at the panoramic Co-60 source in the Radiation Chemistry and Dosimetry Laboratory of the Ruđer Bošković Institute in Zagreb, Croatia. Herbal extracts were prepared according to the Soxhlet protocol, filtered, and evaporated in rotary vacuum. Different concentrations of sterilized herbs and extracts (0.25, 0.5, and 1% v/w) were added to pasteurized milk inoculated with a starter (103 CFU/mL) and incubated at 30 ºC for 24 hours.

Lactobacillus helveticus/Lactobacillus lactis reached a counts of 7.4 log CFU/mL in pasteurized milk at 30 ºC/24h. When dried herbs were applied, the growth of the milk culture was not affected, regardless of the concentrations applied. The higher the concentration of sage and immortelle extracts, the lower the number of starter cultures, which was, however, within the growth rate of the control sample. These preliminary results show that dried sage, immortelle and rosemary and their extracts have no negative effects on the targeted starter culture Lactobacillus helveticus/Lactobacillus lactis, which is preliminarily important for their use in pilot cheese production.

682 / INDUSTRIAL WASTE FROM GANODERMA LUCIDUM EXTRACTS PRODUCTION: CHEMICAL COMPOSITION, BIOACTIVITY AND POTENTIAL APPLICATION

Keywords: *Industrial waste, Ganoderma lucidum, yogurt*

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BACKGROUND

Being an important medicinal mushroom, Ganoderma lucidum (GL) finds its main application in the clinical, pharmaceutical, and nutritional industries. It has been traditionally utilized for the treatment of numerous alignments, including stomach cancer, bacterial infections, and nervous disorders. Nowadays, GL has extensively been used as a functional ingredient and a natural preservative with increased application in alcoholic beverages production. More than a hundred Ganoderma-based products are part of the modern world market. However, the majority of these products are based on GL extract or fruiting body powder. Consequentially, several thousand tons of industrial solid waste from GL are annually collected worldwide. Thus, it would be of interest to examine the potential usability of GL residues.

OBJECTIVES

Chemical characterization and bioactivity of dried GL solid residues remaining after industrial hot-water (GLW) and alcohol extract (GLE) production were analyzed. Additionally, sensory acceptable yogurts supplemented with GLW were designed.

METHODS

Polyphenolic profiles were assessed by HPLC analysis. Cytotoxicity against human cell lines, colon carcinoma (HCT116), and melanoma (Hs294T) was established by MTT assay. Yogurts were prepared with 2%GLW and tested for in situ anti-Escherichia activity and lactic acid bacteria (LAB) viability. Trained panel assessed palatability.

RESULT

GLW and GLE are valuable sources of polyphenols: rutin, p-hydroxybenzoic and chlorogenic acid. Decrease viability of cells were detected in a concentration-dependent manner. Acceptable sensory properties of yogurt were established. In comparison to plain yogurt, reduced viability of E. coli strains: 0157:H7 and ATCC35218 were noted in GLW yogurt. High LAB viability was maintained.

ACKNOWLEDGEMENTS/REFERENCES

This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, contract number 451-03-9/2021-14/200051.

708 / APPLICATION OF LACTIC ACID BACTERIA FROM KIMCHI IN SWORD BEAN SOURDOUGH TO IMPROVE THE QUALITY OF GLUTEN-FREE RICE BREAD

Keywords: *Red sword bean, Sourdough, Gluten-free rice bread, Kimchi lactic acid bacteria*

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Rice is one of the popular grains that are commonly used in celiac diets, but lack of gluten makes it difficult to mimic the viscoelastic property. Red sword bean (Canavalia gladiata) can be a good source of protein and starch to increase the volume of gluten-free rice bread (GFRB) with high antioxidant capacity. And sourdough can be used to make better quality of bread. During sourdough fermentation, lactic acid bacteria (LAB) produce a lot of metabolites such as organic acids which improve its flavor, texture, and delay retrogradation.

This study delved into the influence of different sword bean sourdough (SBS) made with LAB from Kimchi (KLAB) on the physicochemical properties of GFRB. The sourdoughs were made with each Latilactobacillus sakei KCKM 0741, Lactiplantibacillus plantarum KCKM 0745, Leuconostoc mesenteroides KCKM 0751 and spontaneous fermentation. GFRBs were prepared by replacing rice flour with 20% of sword bean flour (SBF) and 4 types of SBS. Specific loaf volume, textural properties, color and total phenolic contents (TPC) of GFRB were analyzed. Application of SBS prepared with KLAB greatly influenced on the structure of bread revealing higher values of specific loaf volume and yellowness and low values of hardness compared to control, GFRB prepared with SBF. Among the bacteria species, fermented with Lactiplantibacillus plantarum showed the highest TPC. This study revealed the possibility of application of SBF and KLAB in preparation of sourdough to improve the physicochemical characteristics of GFRB.

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713 / BIOACTIVITY AND PALATABILITY OF YOGURT ENHANCED WITH GABA-PRODUCING STRAIN AND INDUSTRIAL WASTE FROM MEDICINAL MUSHROOM

Keywords: *GABA, Medicinal mushroom, Industrial waste, Yogurt*

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BACKGROUND

Ganoderma lucidum (GL), Phellinus linteus (PL), and Phellinus igniarius (PI) are economically important medicinal mushrooms used as a dietary supplement and/or a remedy for the treatment of various diseases, including gastroenteric disorder and depression. As culinary purpose goes, PL and GL are seen in pastry, cookies, and yogurts. However, majority of developed products include extract or fruiting body powder. Consequentially, a large quantity of residues from industrial processing remain as solid waste. Yogurt could be supplemented with residues and used as a carrier for gamma-aminobutyric acid (GABA)-producing bacteria. GABA is an antioxidant and neurotransmitter linked to anti-depressive effects. Such yogurt with potential psychobiotic properties could find value in dietary therapy.

OBJECTIVES

Yogurts fermented with GABA-producing bacteria and supplemented with GL, PL, and PI residues remaining after industrial alcoholic extract production, were subjected to investigation of bioactivity and sensorial acceptance.

METHODS

Twelve lactic acid bacteria (LAB) were tested for GABA producing potential by GAD colorimetric assay. Yogurts fermented with Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus reuteri were supplemented with 2% GL and 4%PI/PL residues. LAB viability and in situ anti-Escherichia activity, monitored on three E. coli strains: 0157:H7; GN289; ATCC35218 were tested. Molecular docking was conducted. The trained panel assessed sensorial acceptance.

RESULTS

L. reuteri DSM17938 possesses the highest GABA producing potential. Acceptable sensorial properties were described to GL yogurt. In comparison to plain yogurt, reduced viability of E. coli GN289 and ATCC35218 were noted in yogurt fermented with PL and GL resuides, respectively. High LAB viability was maintained.

ACKNOWLEDGEMENTS/REFERENCES

This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, contract number 451-03-9/2021-14/200051.

740 / EXPLORING LACTOCOCCAL PLASMIDS FOR THE IDENTIFICATION OF NOVEL FUNCTIONALITIES

Keywords: *lactococci, starter culture, dairy fermentation, plasmidomes*

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Lactococcus lactis and Lactococcus cremoris represent bacterial species of substantial economic and industrial importance due to their extensive use in the production of fermented food products. The technological necessity for starter strains with enhanced characteristics, which at the same time can be utilised to meet consumer demands for unique products with advanced or special organoleptic properties, highlights the importance of shedding light into previously unknown plasmid-encoded lactococcal functionalities.

In this study, the genomes of 53 lactococcal strains were sequenced by using a combined Pacific Biosciences RS II sequencing and Illumina approach. The plasmidomes were extracted and annotated by using tools such as Pfam and HHpred. On average each strain contained six plasmids, while their sizes ranged from 1.1 to 178 kb. The lactococcal plasmids were subsequently analysed for the presence of novel plasmid-encoded traits of biotechnological relevance.

ACKNOWLEDGEMENTS/REFERENCES

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743 / GENOMIC CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS REVEALS NOVEL TYPES OF THE VSAß GENOMIC ISLAND

Keywords: *Staphylococcus aureus , Enterotoxin, Typing, vSaß*

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BACKGROUND

Staphylococcus aureus is a widespread opportunistic human and animal pathogen, able to produce a variety of heat-stable enterotoxins that can lead to staphylococcal food-poisoning outbreaks. In addition to enterotoxin coding genes, S. aureus harbours the vSaβ genomic island that encodes numerous virulence-associated factors. The genetic composition of vSaβ is variable among strains.

OBJECTIVES

The aims of this study were to characterize S. aureus milk and cheese isolates based on their enterotoxin-gene profile and genetic composition of vSaβ region, and to stablish their phylogenetic relationship

METHODS

90 isolates from fresh milk and cheese were recovered during 2021, and their DNA was isolated with NucleoSpin Tissue kit. Whole genome sequencing was performed by Illumina NextSeq. RAST and Prokka programs were used for genome annotation and NAuRA for enterotoxin gene detection. vSaβ types were determined by comparative analysis of the genetic content within each island. Phylogenetic relationship was determined based on SNP analysis.

RESULTS

Eleven enterotoxin-gene profile were identified among isolates. Classical A, B and C enterotoxin genes were identified in 92.5% of the isolates, and in almost 100% non-classic enterotoxin genes. A total of 47 isolates were classified in previously described vSaβ types: type IX (44), type III (2) and type V (1). The other 43 isolates could not be classified by this scheme, and 20 possible novel vSaβ types were identified among them. A clustering correlation was observed between SNP based phylogeny and vSaβ island typing.

ACKNOWLEDGEMENTS/REFERENCES

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749 / DNA RELEASE BY THE LACTOCOCCAL PHAGE TP901-1 REQUIRES A THREE-COMPONENT GLYCOSYLATION SYSTEM OF ITS HOST

Keywords: *Lactococcus cremoris, TP901-1, DNA release, Glycosyltransferase*

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The first step in phage infection is the recognition of, and adsorption to, a receptor located on the host cell surface. This reversible host adsorption step is commonly followed by an irreversible event and the phage DNA is subsequently delivered into the bacterial cytoplasm. The molecular components that trigger this event are unknown for most phages of Gram-positive bacteria. In the current study, we present a comparative genome analysis of three mutants of Lactococcus cremoris 3107, which are resistant to the P335 group phage TP901-1 due to mutations that affect TP901-1 DNA entry.

Through genetic complementation and phage infection assays, we identified a novel lactococcal three-component glycosylation system (TGS) required for TP901-1 DNA infection. Major cell wall saccharidic components were analyzed, but no differences were found. However, heterologous gene expression experiments combined with infection assays indicate that this TGS is involved in the glucosylation of a cell envelope-associated component that acts as the trigger for DNA release by TP901-1. A cell-envelope saccharide modification has not been implicated in the DNA delivery process of a Gram-positive phage so far.

ACKNOWLEDGEMENTS/REFERENCES

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763 / INSIGHTS INTO THE RELEVANCE OF BACILLUS CYTOTOXICUS AS A FOODBORNE PATHOGEN

Keywords: *enterotoxins, Vero cell assay, mashed potato powder*

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INTRODUCTION

Bacillus cytotoxicus belongs to the Bacillus cereus group. The first B. cytotoxicus was isolated in the context of a severe food poisoning outbreak leading to fatal cases of diarrheal disease. Subsequent characterization of the outbreak strain led to the conclusion that this Bacillus strain was highly cytotoxic and eventually resulted in the description of a novel species, whose name reflects the observed toxicity: B. cytotoxicus. However, only a few isolates of this species have been characterized with regard to their cytotoxic potential and the role of B. cytotoxicus as a causative agent of food poisoning remains largely unclear.

PURPOSE

The aim of this study was to gain further insights into the toxicity of B. cytotoxicus to allow for conclusions on the relevance of the organism as a foodborne pathogen.

METHODS

Nineteen B. cytotoxicus isolates were obtained from mashed potato powders and characterized by toxin gene profiling for nhe, cytK, and ces, as well as by Vero cell cytotoxicity assays.

RESULTS

All isolates harbored the cytK1 (cytotoxin K1) gene and species-specific variants of the nhe (non-hemolytic enterotoxin) gene. The isolates exhibited low or no toxicity towards Vero cells. A high prevalence (95%) of B. cytotoxicus in mashed potato powder was observed. Isolates from mashed potato powder harbored cytK1 and nhe genes but exhibited no or low cytotoxicity in a Vero cell assay. Our findings indicate that while B. cytotoxicus are highly prevalent in mashed potato powder, their cytotoxic potential is likely lower than initially assumed.

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779 / IMPACT OF THE DNA ISOLATION ON THE DIVERSITY OF BACTERIOME AND RESISTOME OF BEAN SPROUTS DETERMINED BY NANOPORE SEQUENCING

Keywords: *spread of antibiotic resistance, food safety, nanopore sequencing, barcoding*

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BACKGROUND

Antimicrobial resistance (AR) is a health problem that threatens the global ability to treat bacterial infections. The food chain and food production facilities contribute to the connection between animals, plants, and humans, which can increase the risk of spreading antibiotic resistance. Sprouts are known for their high loads of microorganisms, and because they are consumed raw, they can serve as a transmission route for antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARG) to the gastrointestinal microbiome.

Nanopore sequencing (NS) is a portable next-generation sequencing device developed by Oxford Nanopore Technologies (UK), which generates long sequencing reads with a lower cost per sample and an analysis in real time.

OBJECTIVES

Our goal was to compare the impact of three different methods of DNA isolation and barcoding on the diversity of the bacteriome and resistome of a sample of bean sprouts determined by NS using a ligation sequencing kit.

METHODS AND RESULTS:

To confirm the presence of ARG in individual genera predicted by NS using MinKNOW and EPI2ME of the Enterobacteriaceae, Pseudomonadaceae, and Moraxellaceae family were then analyzed. These isolates obtained by culture methods and identified by MALDI-TOF MS were genotyped using ERIC-PCR and REP-PCR techniques to determine clonal isolates. PCR protocols designed for the detection of ARG in these bacterial genera (especially genes encoding beta-lactamases and efflux pumps) showed that the predicted occurrence of some ARG in concrete species is deeply influenced by these ARG specifications in the used CARD database.

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809 / BIOFILM FORMATION OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM HEALTHY CHICKENS AND QUAILS FOR HUMAN CONSUMPTION

Keywords: *biofilms, coagulase-negative staphylococci, multidrug-resistance, poultry*

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Staphylococci are recognized as one of the most frequent causes of biofilm-associated infections both in humans and animals. It has been showed that food of animal origin can carry coagulase-negative staphylococci (CoNS) and, besides being able to cause infection, CoNS can also cause food poisoning. Therefore, we aimed to evaluate biofilm formation of methicillin-resistant CoNS isolated from chickens and quails slaughter for human consumption.

A total of 75 staphylococci were isolated from chickens and quails including Staphylococcus sciuri, S. lentus and S. cohnii. All strains carried the mecA gene and almost all had a multidrug-resistant profile. The biofilm formation of all isolates was carried out by the microtiter assay and statistical analysis of the results was performed.

All strains had the ability to form biofilms. A correlation was observed between the staphylococci species and the production of biofilm. In general, S. cohnii strains produced a more biofilm mass than S. sciuri and S. lentus isolates. Furthermore, S. lentus isolates were the least biofilm producers with a statistically significant difference when compared to S. sciuri and highly significant when compared to S. cohnii. However, no statistically significant differences were observed when comparing the several levels of multidrug resistance with the biofilm production.

ACKNOWLEDGEMENTS/REFERENCES

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891 / BUTYRIC ACID PRODUCING CLOSTRIDIA IN RAW MILK: A PREVALENCE STUDY

Keywords: *Cheese spoilage, Butyric acid, Clostridium tyrobutyricum, Raw milk*

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Butyric acid producing clostridia (BAPC) are spore-forming bacteria with the ability to cause late blowing defects (LBD) during cheese ripening. As this quality defect can result in major economic losses, knowledge on the entry routes of BAPC into raw milk is crucial, but limited. To acquire new insights into the prevalence of BAPC in the barn environment, a study on the concentration of BAPC-spores in raw milk samples from eight dairy farms on five consecutive seasons was conducted. At each farm, pooled quarter milk samples (PQM) of individual cows and bulk tank milk samples (BTM) were collected. The sampling-design allowed to not only cover data on farm-level, but also on single-cow-level. Additionally, information on management styles of each farm (e.g. cow-cleanliness) was gathered. The BABC-spore concentration of a total of 631 PQM-samples and 40 BTM-samples was determined using the AMP-6000 Most Probable Number approach with a selective medium. This study provides new insights into BAPC-spore concentrations on single-animal-level. Interestingly, low variation of BAPC-spore counts among cows of the same farm was observed. Furthermore, seasonal shifts in the PQM- and BTM-BAPC-spore concentration were apparent: spore concentrations were significantly lower in summer than in winter/spring. Also, a strong-positive correlation between BAPC-spore concentrations in PQM and BTM samples was noticed and the average cow-cleanliness appeared to be a good indicator for BAPC-spore concentrations in raw milk. The results of this study highlight the importance of good farm management practices and the relevance of regular raw milk testing for BAPC-spores to ensure cheese quality.

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936 / YEASTS OBTAINED BY A NON GMO STRATEGY TO MINIMIZE ETHYL CARBAMATE IN SHERRY WINES

Keywords: *Urethane*

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During Sherry base wine alcoholic fermentation, diverse metabolites are produced by the yeasts responsible for the process, such as urea. The reaction of that metabolite with the ethanol in the medium is which generates ethyl carbamate spontaneously. Ethyl carbamate is a carcinogen compound belonging to the group 2A. This is the reason why its concentration in alcoholic beverages is being increasingly regulated, being therefore necessary to obtain yeasts that produce less urea concentration.

Urea is produced by yeasts in the arginine metabolic pathway. If any of the permeases which introduce L-arginine into the yeast (CAN1p, ALP1p or GAP1p) is inactivated, this route will not be carried out and, therefore, urea will not be produced, with the consequent decrease in the ethyl carbamate concentration.

To achieve this, a fermentative yeast modification through directed evolution has been developed, using a toxic analogue of L-arginine, L-canavanine, to exert a selective pressure. This has made it possible to subsequently select those yeasts that were resistant to the introduction of L-canavanine since it would be indicative that any of the permeases mentioned before is inactive. Five putative yeast variants obtained from the parental yeast strain and susceptible of having any of the L-arginine permeases inactive have been obtained.

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940 / MOLECULAR IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF LISTERIA MONOCYTOGENES STRAINS

Keywords: *prevalence, serotype identification, antimicrobial resistance, artisanal products*

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Listeria monocytogenes is a pathogen of concern for fermented sausages (FS), since it can adhere and form biofilms in food contact surfaces and overcome the processing steps, being able to survive in the final products. Besides, L. monocytogenes possesses genes that confer innate resistance to some antimicrobials commonly used to prevent and control listeriosis infections.

The aims of this study were to characterize and evaluate the antimicrobial susceptibility of L. monocytogenes strains isolated at Spanish small-scale factories producing fermented sausages.

Samples of raw meat (RM, n=30), Salchichón (n=30) and food contact surfaces (FCS, n=72) collected at two Andalusian factories of FS were microbiologically analysed for L. monocytogenes detection by using ISO methods. Presumptive colonies were identified by MALDI-TOF and further confirmed and characterized by 16S rRNA gene sequencing. Finally, the antimicrobial susceptibility of the confirmed strains to 12 antibiotics was evaluated using the Disk Diffusion Method.

L. monocytogenes was detected in 26.7, 33.3 and 4.2% of RM, Salchichón and FCS, respectively. PCR results indicated that all RM isolates belong to serotype 4b, 4d and 4e and all FCS isolates belong to serotype 1/2a. Regarding Salchichón isolates, 1/2a, 1/2b, 4b, 4d and 4e serotypes were identified, indicating that the products could have been contaminated by both RM and FCS. The L. monocytogenes isolates were sensible to most of the tested antibiotics, excepting clindamycin, to which the majority were resistant. The results of this study deepen knowledge on serotype variability and antimicrobial resistance of L. monocytogenes strains found in FS factories.

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969 / GENETIC DISSECTION OF A PREVALENT PLASMID-ENCODED CONJUGATION SYSTEM IN LACTOCOCCUS LACTIS

Keywords: *Lactococci, Conjugation, Mutagenesis, Co-mobilisation*

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Natural gene transfer processes, and in particular conjugation, are believed to play a major role in driving evolution of lactic acid bacteria and provide a much-needed tool for developing genetically enhanced strains in a natural, non-genetically-modified manner. Plasmid pNP40, which was first identified nearly forty years ago in Lactococcus lactis subsp. lactis biovar diacetylactis DRC3, encodes functions such as heavy metal-, bacteriophage- and nisin-resistance, as well as horizontal transfer ability by conjugation.

We report an optimized conjugation protocol for this plasmid, yielding a transfer frequency that is approximately 4,000-fold higher than those previously reported in literature, while we also observed high-frequency plasmid co-mobilisation. Individual mutations in 18 genes that encompass the presumed conjugation cluster of pNP40 were generated using single-strand-DNA recombineering to evaluate the role of each gene in the conjugation process. A possible transcriptional repressor of this conjugation cluster, the product of the traR gene, was identified in this manner. This mutational analysis, paired with bioinformatic predictions as based on sequence and structural similarities, allowed us to generate a preliminary model of the pNP40 conjugation machinery.

ACKNOWLEDGEMENTS/REFERENCES

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974 / PROBIOTIC PROPERTIES OF BACTERIA ISOLATED FROM BEE POLLEN AND BEE BREAD

Keywords: *bee pollen, bee bread, probiotic properties, Bacillus*

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BACKGROUND

Our previous research revealed that Bacillus strains isolated from bee pollen and bee bread exhibit high anti-microbial activity against human pathogens.

OBJECTIVES

Our present study aimed to evaluate the probiotic characteristics and the antioxidant activity in vitro of two strains isolated from bee pollen and bee bread.

METHODS AND RESULTS

Isolates were cultured under aerobic conditions at 37°C for 24 hours in LB medium.

Bile salts and acid tolerance tests were performed on the isolates. Both selected strains showed 95% of survivability in broth supplemented with 0.3% oxgall bile and the survival rate in broth with pH=2 ranged from 47-58%.

In addition, antibiotic disk susceptibility tests and blood hemolysis assay were executed. Eight antibiotics were selected – gentamicin, azithromycin, ciprofloxacin, clindamycin, trimethoprim, rifampin, linezolid and chloramphenicol. Both isolates were sensitive for these antibiotics and furthermore, blood hemolysis assay showed that tested strains exhibited gamma-hemolysis.

The evaluation of auto-aggregation and adhesion to hydrocarbons also was determined. Auto-aggregation ability for both strains varied from 24 to 31% after 4h and from 62 to 70% after 24h. A marked difference in adhesion was observed - adhesion to chloroform, ethyl acetate and xylene for both strains was in the range of 38-41%, 31-37% and 25-35%, respectively.

Moreover, the DPPH radical scavenging activity assay was performed. Antioxidant activity of both strains varied from 66 to 71%.

ACKNOWLEDGEMENTS/REFERENCES

Pełka, K.; Worobo, R.W.; Walkusz, J.; Szweda, P. Bee Pollen and Bee Bread as a Source of Bacteria Producing Antimicrobials. Antibiotics 2021, 10, 713. https://doi.org/10.3390/antibiotics10060713

994 / UNDERSTANDING THE INTRA STRAIN VARIABILITY OF CAMPYLOBACTER JEJUNI SUSCEPTIBILITY TO ULTRAVIOLET (UV) LIGHT

Keywords: *Campylobacter, Ultraviolet light, Whole genome sequencing*

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UV light-emitting diodes (UV-LEDs) are efficient disinfection technologies, with potential application in the food industry. However, variations in the inactivation kinetics between different strains within the same bacterial species have been reported when UV light was applied. This study aimed to evaluate and investigate the susceptibilities of 9 C. jejuni strains towards repeated treatment with UV light, with altered susceptibility further investigated using whole genome sequencing (WGS). Eight C. jejuni strains, isolated from different sources and a reference strain (NCTC11168) were cultured and incubated microaerobically in Mueller Hinton broth at 42°C for 48 h. Bacterial suspensions were serially diluted to a concentration of ~5 log CFU/mL in a 20-mL suspension of milk (UHT, 2% fat) and maximum recovery diluent (MRD) in a 1:4 ratio (v:v). Suspensions were poured into petri dishes and treated with a UV-LED device for 1 to 11 min at 280 nm.

Untreated samples were used as controls. Serial 10-fold dilutions were prepared after treatment, plated on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated microaerobically for 48 h at 42°C. Different inactivation kinetics were observed between the strains of which three showed reductions greater than 1.5 log CFU/mL and one strain was particularly resistant to UV light with a maximum reduction of 0.39 CFU/mL. Moreover, isolated colonies of the 9 C. jejuni strains which survived repeated UV treatment were sequenced using the Illumina platform. Comparison of bacterial genomes is expected to identify genomic similarities or dissimilarities linked with tolerance to this antimicrobial treatment.

ACKNOWLEDGEMENTS/REFERENCES

The present work was supported by the Teagasc Walsh Fellowship program, Department of Agriculture, Food, and Marine (DAFM) under the Food Institutional Research Measure (FIRM) program [Grant number: DAFM/17/F/275].

998 / ROLE OF NTRC IN EARLY PHASE OF BACTERIAL GROWTH

Keywords: *starvation biology, gene regulation*

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Enteric bacterial pathogen like E. coli and Salmonella often experience fluctuating nutrient conditions, as they cycle between their host and external environment. In external environment, these enteric bacterial pathogens exposed to extreme conditions, including extreme nutrient starvation. These bacterial pathogens evolved nutrient scavenging systems to survive and thrive under nutrient limitations.

NtrC protein is a part of one of such nutrient scavenging system. Earlier reports showed that NtrC protein facilities cells with nitrogen scavenging system that activate in low nitrogen condition. Here, we report the role of NtrC protein in early phase of bacterial growth, lag-phase, where ample amount of nitrogen (ammonia) is present. The ntrC mutation caused 8 hours long, extended lag-phase in S. Typhimurium. The molecular analysis of ntrC mutant reveals that, mutation has supressed the expression of nitrogen metabolism genes that led to overall slow metabolism. The extended lag-phase of ntrC mutant, is the reflection of slow metabolism in ntrC mutant. Therefore, we report that in addition to the role of NtrC during nitrogen starvation. NtrC is also required in early phase (lag-phase) of bacterial growth in minimal media, containing ample amount of nitrogen source.

ACKNOWLEDGEMENTS/REFERENCES

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1008 / APPLE PECTIN AS A POTENTIAL PREBIOTIC IN INFANT NUTRITION

Keywords: *pectin, infant, nutrition*

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During early life of infants, the gastrointestinal tract with its microbiota is a complex ecosystem which plays an important role in their growth and development. Pectin is a prebiotic dietary fiber which affects the gut microbiota and shows local intestinal and systemic effects.

The aim of this study was to research the influence of apple pectin in infant nutrition, its impact on selected bacteria of the infant's gastrointestinal tract, gas production and total antioxidant capacity by electrochemical determination of changes in redox potential.

In vitro influence of pectin on selected Bifidobacterium and Lactobacillus was anaerobically tested during 72h. Selected bacteria, isolated from infant's feces, collected from term vaginally delivered infants, as well as standard strains, were incubated in the selective medium with 0.5 % apple pectin. The changes in redox capacity of media were measured before and after fermentation by voltammetry and pH. Additionally, production of gases by microorganisms was measured using the MicroOxymax respirometer.

Apple pectin had a positive effect on the growth of Bifidobacterium and Lactobacillus of the infant's gastrointestinal tract as well as the standard strains, while there was a slight reduction in the pH of the media. In vitro fermentation of pectin by selected bacteria showed increase in redox capacity of media. The cumulative production of CO2 also slightly increased during fermentation. These results indicate that pectin could be effective prebiotic in infant nutrition.

ACKNOWLEDGEMENTS/REFERENCES

The authors would like to thank the Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No: 451-03-9/2021-14/200026) for financial support.

1019 / AMPLICON AND SHOTGUN METAGENOMICS TO UNRAVEL THE MICROBIOME OF THE GREEK CHEESE SFELA

Keywords: *cheese, amplicon sequencing, shotgun metagenomics, metagenome assembled genomes*

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BACKGROUND

Sfela is a Greek protected designation of origin (PDO) white-brined cheese, produced in Messinia and Laconia provinces of Peloponnese. It is produced from ovine or mixture of ovine and caprine milk and it is often called "cheese of fire" due to the cooking of the curd during production. It is a semi-hard cheese similar to Feta cheese and it ripens in brine for at least three months.

OBJECTIVES

In this study, we attempt a first characterization of the microbiome of a number of Sfela cheese samples.

METHODS

We used amplicon sequencing and shotgun metagenomics.

RESULTS

16S rDNA amplicon sequencing revealed the bacterial presence in the samples up to the genus level. The main bacteria identified were lactic acid bacteria (LAB), however important differences were observed among the samples. In at least one case the prevailing bacteria were different members of the Lactobacillaceae family and the Streptococcus genus, while in the other samples the Lactococcus genus prevailed. Shotgun metagenomics allowed the identification at the species level. One sample was dominated by Streptococcus thermophilus followed by several lactobacilli, like Levilactobacillus brevis, Latilactobacillus curvatus, Lactobacillus delbrueckii, Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, etc. In another sample, Lactococcus lactis was the prevalent species followed by Lactiplantibacillus plantarum and Latilactobacillus curvatus. In a third sample, Tetragenococcus halophilus and Lactococcus lactis shared the bacterial population, while yeasts were present with Debaryomyces hansenii showing highest abundance. Further analysis of the shotgun data revealed several metagenome-assembled genomes (MAGs) and important functional properties of the microbiomes related to cheese technology.

ACKNOWLEDGEMENTS/REFERENCES

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call SUPPORT FOR REGIONAL EXCELLENCE (MIS 5047289)

The evaluation of auto-aggregation and adhesion to hydrocarbons also was determined. Auto-aggregation ability for both strains varied from 24 to 31% after 4h and from 62 to 70% after 24h. A marked difference in adhesion was observed - adhesion to chloroform, ethyl acetate and xylene for both strains was in the range of 38-41%, 31-37% and 25-35%, respectively.

Moreover, the DPPH radical scavenging activity assay was performed. Antioxidant activity of both strains varied from 66 to 71%.

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1059 / OIL PRESS CAKES AS A NOVEL SUBSTRATES FOR INNOVATIVE PLANT-BASED FERMENTED FOODS

Keywords: *press cakes, fermented food*

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BACKGROUND

Oil press cakes are valuable by-products of pressings oils from oilseeds. However, they should not be treated only as a by-product of limited value, as they also contain a number of valuable nutrients and bioactive. It seems particularly important to expand the possibilities of their application in the food industry due to their beneficial nutritional values. The development of a larger assortment of vegan foods based on press cakes may be a response to the growing market demand, due to the growing trend for a plant-based diet.

OBJECTIVES

Based on presented ideas the project ProBioVege* was developed. The aim of the project is to develop a group of functional products in the form of fermented snacks, plant milks and spray-dried instant products, produced from selected oil cakes (flaxseed, amaranth, black cumin, hemp, camelina, sesame, sunflower and evening primrose). Moreover, mold-ripened cheese alternatives (Camembert and Roquefort) are developed.

MATERIAL AND METHODS

The project includes development of products using mixed starter cultures (yogurt and kefir) as well as selected probiotic strains, determination of their physicochemical properties, content of bioactive compounds and their durability during refrigerated storage, and survival of microorganisms in the simulated digestive tract. Moreover a broad spectrum of bioactivity including: antioxidant, antimicrobial properties of products obtained in relation to selected pathogens determining their potential in the prevention of food poisoning, and effects on enzymes involved in civilization diseases will be studied.

*Project ProBioVege financed by The National Centre for Research and Development grant number LIDER 41/0141/L-11/19/NCBR/2020

1116 / POLYAMIDE NONWOVEN NANOFIBROUS MATERIALS AS PROMISING ACTIVE FOOD PACKAGING

Keywords: *active food packaging, nanomaterials, food microbiology*

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Packaging is the main protection of food from external contaminants, including microorganisms. Currently, active food packaging (AFP) is considered one of the most promising technologies providing effective barrier and eliminating microbial risks. For its production, nonwoven nanofibrous materials (NMs) functionalized with various active substances can be used. Our long-term aim is to develop NMs functionalized with nature-origin substances applicable as AFP providing a protection of the final consumer from foodborne microbial infections and intoxications.

According to a designed comprehensive methodology, we analyzed interactions between electrospun polyamide (PA) NMs functionalized with 2.0 wt% of natamycin, green tea extract, and rosemary extract, respectively, and food pathogens Escherichia coli, Listeria monocytogenes, Salmonella enterica, and Staphylococcus aureus. We focused on PA´s permeability for bacterial cells and antibacterial and antibiofilm properties. The results showed i) excellent retention of all the tested PAs reaching up to 6.4 log10removal, and ii) a significant ($p \leq 0.05$) inhibition of both bacterial growth and biofilm formation (up to 3 log10suppression) by the functionalized PAs.

Further, the PAs were tested as a packaging of chicken meat both non-inoculated and inoculated with L. monocytogenes; the functionalized PAs prolonged microbial and sensory quality of the chicken samples stored at 7 °C for 7 days. The PA NM functionalized with green tea extract was determined the most effective one. The results confirmed the potential of PA NMs in packaging applications. Subsequent research should be focused on their mechanical properties.

ACKNOWLEDGEMENTS/REFERENCES

The study was supported by the grant OP PIK CZ.01.1.02/0.0/0.0/16_084/0009936

1147 / NOVEL BIOTECHNOLOGICAL APPROACHES TO GROW MEDICINAL MUSHROOMS (MMS) ON MAIZE RESIDUES

Keywords: *Medicinal Mushrooms, corncobs valorization, beta-glucans*

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BACKGROUND

maize landraces, an important source of genetic variants for adaptation to specific environments, are characterized by high nutritional value. Their small-scale farming cultivation allows the exploitation of residual corncobs for alternative uses.

Medicinal mushrooms (MMs) are gaining attention for their content of bioactive compounds relevant for human health.

OBJECTIVES

the research aims at developing high value-added ingredients with nutritional properties by growing MMs on corncobs of traditional Lombard varieties. Investigation will highlight whether and to what extent MMs bioactivities are maintained up to the production of the functional ingredient.

METHODS

maize varieties of different cob colour (Spinato di Gandino, Rostrato Rosso di Rovetta, Spinoso Nero Valle Camonica, Fiorine di Clusone) and the B73 inbred line were grown; harvested cobs were characterized for fiber and secondary metabolites contents, then used. Solid State Fermentations (SSFs) were set-up employing the MMs Pleurotus ostreatus, Ganoderma annularis, Flammulina velutipes and Lentinula edodes: trials were first carried out in small jars and subsequently in boxes equipped with a semipermeable gas membrane. Fungal growth was measured acquiring the SSF surface image, applying a pre-processing step. beta-glucans were determined through the Mushroom-Yeast β-glucan assay kit (Megazyme).

RESULTS

Pleurotus and Ganoderma proved the best growing performances on investigated corncobs. Image analysis highlighted that 50 and 80% of corncob surface of Spinoso Nero was covered by Pleurotus in 10 and 40 days incubation, respectively. Higher beta-glucans content was detected in isolated P. ostreatus, however their amount in the final ingredient will strictly depend on mycelial extension onto corncobs

1155 / CHARACTERIZATION OF PLASMID-MEDIATED ANTIBIOTIC RESISTANT BACTERIA FROM COOKED STREET FOOD SOLD IN OSUN STATE, NIGERIA

Keywords: *sequencing, plasmid DNA, resistance genes, 16SrRNA*

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BACKGROUND

Rapid urbanization increases demand for street foods though street foods are identified as carriers of antibiotic-resistant bacteria.

OBJECTIVES

The objectives of the study are to determine the plasmid-mediated resistance genes and characterize the antibiotic-resistant bacteria isolated from cooked street vended food sold in Osun State Nigeria using standard techniques.

METHODS

Seventy-five bacteria present in cooked foods were isolated on nutrient, Mannitol salt and Mueller-Hinton agar as well as identified using standard procedures. Antibiotic multidisc containing cefuroxime, gentamicin, cefixime, ofloxacin, augmentin, nitrofurantoin, ciprofloxacin and ceftazidime were used to study the resistance patterns. Plasmid curing and DNA extraction procedures were carried out on the obtained resistant bacteria. Isolates carrying multiple antibiotic resistance genes on their plasmid were amplified through PCR techniques (16s rRNA) and identified through NCBI (National Center for Biotechnology Information) blasting. A complete plasmid DNA of an identified bacteria was then sequenced through Illumina techniques and analyzed using the snapgene and geneious prime bioinformatics tools.

RESULTS

From the results of this study, a high level of antibiotic resistance was observed to ceftazidime and cefixime (99%) while ciprofloxacin (92%) and ofloxacin (88%) showed high susceptibility. Out of the antibiotic-resistant bacteria, 37% carried OXA2 and 21% carried CTX-M resistance genes on their plasmids. Bioinformatics analysis of complete plasmid sequence of identified bacteria through assembly, annotation and map showed the presence of more resistance genes and enzymes catalyzing different reactions. Sequencing techniques and bioinformatics tools utilized in this study provided personalized information for better understanding of the identified bacterial genome.

ACKNOWLEDGEMENTS/REFERENCES

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1201 / DETERMINATION OF PROBIOTIC GENES AND BIOGENIC AMINES PRODUCED BY LACTOBACILLUS ACIDOPHILUS PNW3

Keywords: *bioactive peptide, ornithine decarboxylase, phylogeny, insertion sequence*

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The genome of Lactobacillus acidophilus PNW3 was assessed for probiotic and safety potentials. The genome was completely sequenced and assembled using SPAdes. This was thereafter annotated with NCBI prokaryotic genome annotation pipeline (PGAP) and rapid annotation using subsystem technology (RAST).

Further downstream assessment was determined using appropriate bioinformatics tools. The production of biogenic amines was confirmed through HPLC analysis, and the evolutionary trend of the strain was determined through the Codon Tree pipeline.

The strain was predicted as a non-human pathogen. A plethora of encoding genes for lactic acids and bioactive peptides production, adhesion molecules, resistance to the harsh gut environmental conditions, and improvement of the host metabolism, which are putative for important probiotic functionalities, were located at different loci within the genome. A bacteriocin predicted to be helveticin J was identified as one of the secondary metabolites. The maximum zone of inhibition exhibited by the crude bacteriocin against STEC E. coli O177 was 21.7±0.58 and 24.3±1.15 mm after partial purification (250 g/mL). Three coding sequences were identified for insertion sequences and one for the CRISPR-Cas fragment.

The protein-encoding sequence for Ornithine decarboxylase was found within the genome. The L. acidophilus PNW3 presents important features categorizing it as a viable and safe probiotic candidate, though further safety investigations are necessary. The application of probiotics in livestock-keeping would ensure improved public health and food security.

1509 / APPLE POMACE AS CARRIER FOR STARTER CULTURE USED IN PRODUCTION OF YOGURT INTENDED FOR DEPRESSION TREATMENT: NEW APPROACH

Keywords: *apple pomace, yogurt, depression*

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BACKGROUND

Improvement of environment quality and dietary therapy is achieved by utilizing fruits by-products in food formulation. The immobilization of yogurt starters on apple pomace flour (APF) and its application as an affordable product for yogurt preparation emerged recently as an innovative approach. Dietary therapy that includes ferments is seen as potential adjunctive treatment for depression that could potentially reduce relapse rates.

OBJECTIVES

APF was used as a carrier for lactic acid bacteria (LAB) serving purpose to produce yogurt enriched with antioxidants and dietary fiber. Yogurts or their extracted whey were tested for antioxidative effect and activity on gastrointestinal pathogens and neuroblastoma cells (SH-SY5Y). Depressed patients evaluated sensory acceptance of APF yogurt.

METHODS

APF granulation was accomplished by a fluidized-bed. LAB viability was estimated by the spread plate technique. Yogurts were prepared with starter immobilized on APF. Effect on Escherichia strains was tested in situ. The effect of whey on SH-SY5Y was tested by MTT assay. TPC, DPPH and FRAP antioxidant assay was conducted. Depressed patients evaluated yogurt taste.

RESULTS

High LAB viability was maintained (>log 8). Increased cell viability was detected after treatment with whey extracted from APF yogurts in comparison to plane ones. Acceptable sensory properties were established. APF yogurts as a majority of apple and dairy products could be a favorable medium for growth of some E.coli strains. Prevention of E.coli contamination is essential. Antioxidant activity was detected. Estimated price of granulated APF (3g) necessary for APF yogurt production in home condition (100mL) is 0.05 €.

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1524 / CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCAL ENTEROTOXINS IN RAW MILK FROM NORTHERN PORTUGAL

Keywords: *Staphylococcus aureus, Raw cow's milk, Staphylococcal enterotoxins, MRSA*

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BACKGROUND

Staphylococcus aureus can cause sub-clinical mastitis in dairy cows and produce staphylococcal enterotoxins (SEs) that can be shed into the milk supply chain, leading to food poisoning in humans.

OBJECTIVES

This study aims to investigate the prevalence and diversity of S. aureus and staphylococcal enterotoxins in raw cow's milk samples collected in the main dairy region of mainland Portugal.

METHODS AND RESULTS

S. aureus were isolated according to ISO 6888-1:2021 methodology and then characterized by spa typing, antimicrobial susceptibility, virulence genes and antibiotic resistance genes. Of 100 milk samples, S. aureus were identified in 53.0%, with t529 (17.7%) and t1403 (16.1%) being the predominant spa types. SEs (SEA-SEE) were immunoenzymatically (ISO 19020:2017) detected in one sample (1.0%). Among S. aureus isolates, 35.5% were resistant to at least one antimicrobial agent, with penicillin resistance being the highest (32.3%) followed by tetracycline (24.2%). Moreover, 8.1% of the isolates were identified as methicillin-resistant S. aureus (MRSA). Regarding virulence genes, 46.8% harboured at least one enterotoxin-encoding gene, and seg was the most frequently detected (41.9%) followed by sei (40.3%), sec (6.5%), seh (4.8%), and sea (1.6%). Isolates phenotypically classified as MRSA were carriers of the mecA gene (8.1%), whereas 4.8% enterotoxigenic strains also harboured the toxic shock syndrome toxin-1 (tsst-1) gene. Our study confirms that raw milk can be a zoonotic source of enterotoxigenic and MRSA strains, and there is a need for broader SEs screening to control transmission along the food chain and prevent food poisoning outbreaks.

ACKNOWLEDGEMENTS/REFERENCES

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1542 / APPLICATION OF PHOTOACTIVATED ZNO NANOPARTICLES TO MICROBIAL CONTROL OF STRAWBERRIES

Keywords: *ZnO nanoparticles, Strawberries, Photocatalysis, Visible light*

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BACKGROUND

It is well documented that most harmful pathogenic microorganisms are able to develop high resistance to conventional chemical fungicides and disinfectants. During the past decade, the emphasis on postharvest fruit protection has shifted from using chemicals to various alternative techniques including physical antimicrobials. To this end, the development of novel nano-sized antifungal and antibacterial agents seems to be promising.

METHODS

The antimicrobial activity of ZnO nanoparticles (ZnO NPs) in combination with visible light (λ = 405 nm) against Escherichia coli O157:H7, Listeria monocytogenes ATCL3C 7644, Enterococcus faecalis MSCL 302 and Botrytis cinerea was investigated. Afterwards, the antimicrobial efficiency of photoactivated ZnO NPs was examined on the surface of strawberries.

RESULTS

Data indicate that photoactivated ZnO NPs have strong bactericidal activity against all investigated bacteria in-vitro, achieving more than 7 log reductions in bacterial counts. Results reveal remarkable photoinactivation (33-58%) of B. cinerea by ZnO NPs at concentration 1×10-3 – 5×10-3 M and incubation time 24h. Scanning electron microscopy images of treated bacteria indicated cell lysis and confirmed substantial morphological changes in microfungus.

Photoactivated ZnO NPs can reduce the microbial contamination of strawberries by 1.4 log without harmful effects on their antioxidant capacity. As the result, shelf-life of treated strawberries was extended by 2 days.

These ZnO NPs properties can potentially be applied as an effective antimicrobial agent to prolong shelf-life of most perishable strawberries inhibiting spoilage by microorganisms. By no means, further studies should be performed to understand the pathways of cell destruction after photoactivated ZnO NPs treatment.

1567 / LIBERATION OF PHENOLICS FROM SORGHUM SAMPLES USING AN ENZYME COCKTAIL WITH CELLULOLYTIC AND LIPOLYTIC ACTIVITIES

Keywords: *phenolics, antioxidant, sorghum*

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Phenolic compounds are important secondary metabolites in plants. In addition, these molecules have beneficial effects on the human health through antioxidative, antimicrobial and anti-inflammatory properties. Majority of phenolics can be found in carbohydrate-ester and carbohydrate-glycoside forms in plants decreasing their bioavailability.

However, treatment with cellulase and lipase enzymes can be an ecofriendly strategy to liberate these conjugated phenolics. Sorghum is an important and widely produced plant worldwide. Although the sorghum has considerable amount of phenolic esters/glycosides, extraction of them by cellulase and/or lipase enzymes is rarely investigated.

An enzyme-assisted approach using a cellulolytic and lipolytic cocktail from Rhizomucor miehei was applied to enrich phenolics from three different sorghum samples.

The enzyme cocktail was produced in a wheat-bran based solid state fermentation system and was partially purified by gel filtration before use. During enzyme treatments, a mass of 1 g grounded grain was treated with 10 mL of enzyme cocktail, and the mixtures were incubated for 7 hours at 50 °C. Samples were taken at predefined intervals, then, total phenolic content (TPC) and antioxidant activity measurements were carried out.

Results showed increased TPC during the reaction which was generally the largest in the 1st hour of the treatment. Moreover, the antioxidant capacity has increased in parallel to the TPC in most samples.

In conclusion, the cellulase/lipase treatment applied had a positive effect on the release of antioxidative phenolics from sorghum grain samples.

ACKNOWLEDGEMENTS/REFERENCES

This research was supported by the NKFI FK134886.

1599 / NITRATE REDUCING STARTER CULTURE AND NATURAL NITRATE SOURCES AS A "CLEAN LABEL" ALTERNATIVE IN MEAT PRODUCTS

Keywords: *clean label, meat products, nitrite*

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Clean label products have been introduced in the market with the goal of ensuring product safety having in sight the maintenance of environmental and public health.

This study aimed to assess a clean label alternative to nitrite addition in cooked ham manufacturing by exploiting natural plant sources of nitrate as the substrate for a nitrate reductase-producing starter culture. Cooked hams were prepared at a laboratory scale using minced pork leg and water, salt, sodium tripolyphosphate, carrageenan, and the following plant extracts in combination with the starter culture were used: i) dried beetroot and paprika; ii) fresh spinach or radish cooking water. Cooked ham positive and negative controls were performed with sodium nitrite (150 mg kg-1) and no nitrate/nitrite addition, respectively.

After processing, the cooked hams were submitted to several microbiological and physicochemical analysis. A typical reddish-pink color formation was observed in hams with the starter culture combined with fresh spinach or radish cooking water, although combined with beetroot and paprika, the starter culture was unable to produce a proper color development.

Regarding texture, similar results were observed for hams prepared according to the traditional processing or with fresh spinach cooking water in combination with the starter culture, being both products microbiologically stable during at least 10 days of storage at 4 °C.

Although preliminary, these results highlight the feasibility of combined spinach cooking water and nitrate reductase-producing starter culture as a promising clean label solution to the addition of chemical nitrite in cooked ham production.

ACKNOWLEDGEMENTS/REFERENCES

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346 / ETHANOLIC EXTRACTS OF LAMIACEAE SPECIES INHIBIT THE BACTERIAL INFECTION OF HUMAN LUNG FIBROBLASTS AND STIMULATE CELL MIGRATION

Keywords: *Lamiaceae, Extracts, Antibacterial activity, Wound healing*

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BACKGROUND

The increasing bacterial resistance to antibiotics poses a serious global threat. For that reason, the research into plant-derived antibacterial agents has become progressively more interesting, particularly in regard to their capacity to prevent the delay or inability of wound closure and healing.

OBJECTIVES

The aim of this study was to evaluate i) the antibacterial potential of ethanolic extracts (70% ethanol) of 18 Lamiaceae species against four Gram-negative and three Gram-positive bacterial strains; ii) the effect of these extracts on Pseudomonas aeruginosa PAO1 infection of human lung fibroblasts (MRC-5), and iii) the effect on MRC-5 cell migration.

METHODS

The antibacterial activity was tested by minimal inhibitory concentration (MIC) assay. The effect on bacterial infection of MRC-5 cells was determined using the invasion assay, while the cell migration was assessed with Scratch assay.

RESULTS

The tested Gram-positive bacteria, especially Bacillus subtilis, are more sensitive to the effects of ethanolic extracts than the Gram-negative bacteria. Moreover, the tested extracts significantly inhibited the invasion of Pseudomonas aeruginosa PAO1 during MRC-5 infection, and most of them also displayed promising stimulating potential on MRC-5 migration in a disrupted cell monolayer. Finally, the results of IBR (integrated biomarker response) analysis highlighted Salvia officinalis ethanolic extract as the most active one, which is in accordance with the existing data suggesting that this plant represents an effective antibacterial and wound-healing agent commonly used in traditional medicine. Altogether, these results are expected to foster further studies that will focus on combating bacterial resistance to antibiotics using natural products.

ACKNOWLEDGEMENTS/REFERENCES

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347 / DO ETHANOLIC EXTRACTS OF LAMIACEAE SPECIES USED IN FOLK MEDICINE HAVE ANTIBIOFILM ACTIVITY ON PSEUDOMONAS AERUGINOSA PAO1?

Keywords: *antibiofilm activity, Lamiaceae, Pseudomonas aeruginosa, ethanolic extracts*

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BACKGROUND

According to World Health Organization, 70-95% of the population chooses folk medicine as their primary approach for health maintenance. In addition to their healing properties and application in folk medicine, Lamiaceae plants are often valued in cookery as spices and food preservatives.

OBJECTIVES

Hence, the aim of this study was to examine the antibiofilm activity of 12 ethanolic extracts of Lamiaceae species on biofilm formation and the degradation of existing biofilm of Pseudomonas aeruginosa PAO1 (ATCC 15692).

METHODS

The crystal violet staining method was used to evaluate the newly formed bacterial biofilms. Four concentrations (double dilutions) of plant extracts (starting conc. 2500 µg/mL), solvent control (ethanol, starting conc.15%), and positive control (streptomycin, starting conc. 12.5 µg/mL) were tested.

RESULTS

All ethanol extracts showed antibiofilm activity. However, the strongest activity was observed for Hyssopus officinalis, Melissa officinalis, Mentha piperita, and Ocimum basilicum, where the viability of bacteria in the biofilm after treatment was about 60% mainly at each tested concentration. Teucrium chamaedrys extract exhibited the strongest activity by degrading about 60% of biofilm (about 40% viability) at all concentrations except at the lowest tested one. Promising results were also observed for M. piperita (at each of the tested concentrations) and O. basilicum (at the highest tested concentration), where the viability of bacteria in the biofilm after treatment was reduced by about 40%. The remaining extracts showed a slightly lower effect on the degradation of the previously formed biofilm of P. aeruginosa PAO1.

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354 / ANTIBIOTIC SUSCEPTIBILITY AND PHYSIOLOGICAL HETEROGENEITY OF ESCHERICHIA COLI IN BLADDER EPITHELIAL CELL INFECTION MODEL

Keywords: *antibiotic tolerance, stress response, fluorescent bioreporter, uropathogenic Escherichia coli*

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BACKGROUND

The major causative agent of cystitis is uropathogenic Escherichia coli (UPEC). During infection, different pathogen subpopulations can be distinguished: bacteria attached to the bladder cell, internalized, and free extracellular bacteria. It has been shown that besides pathogen internalization, stress response and/or dormancy, induced by the surrounding environment, can contribute to increased drug tolerance of infecting bacteria.

OBJECTIVES

The aims of this study were to establish an in vitro model of infection of bladder epithelial cells with UPEC and to study different bacterial subpopulations by looking at their susceptibility to antibiotics, stress response, and dormancy.

METHODS

Human bladder cell-line HTB-9 was infected with UPEC CFT073 strain. Bacteria were carrying a plasmid with two fluorescent reporter-proteins: constitutively expressed GFP and SOS-induced mScarlet-I enabling to measure the response to fluoroquinolone antibiotics. At specific time points, samples were collected and analyzed by flow cytometry and/or confocal microscopy. Bacterial viability was assessed through colony forming units (CFU) counts, and the growth resumption speed of individual cells was followed by using a ScanLag.

RESULTS

In the infection model, attached cells could be discriminated from internalized by using fluorescent membrane dye MM-64 (Panel A and B). Long-term antibiotic treatment confirmed that intracellular bacteria were the least susceptible. Short-term and low-concentration antibiotic treatment revealed that extracellular attached and non-attached bacteria respond differently to fluoroquinolones (Panel C). The high phenotypic and physiological heterogeneity indicates that, in addition to intracellular bacteria, adherent bacteria may play an important role in how the infection responds to antibiotic treatment.

379 / FUNGAL CONTAMINATION OF MAKE-UP PRODUCTS AND ASSESSMENT OF ALCHEMILLA HYBRIDA (L.) L. EXTRACTS AS POTENTIAL COSMETIC INGREDIENTS

Keywords: *make-up, fungi, natural preservatives, Alchemilla hybrida*

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BACKGROUND

Make-up products are not required to be sterile but need to be carefully designed to ensure microbiological safety for the consumers. Pathogenic bacteria and fungi must be absent, and density of non-pathogenic species must be low and stable. Synthetic preservatives are effective but often seen as undesirable, with consumers preferring "organic" make-up supplemented with natural preservatives i.e. plant extracts.

OBJECTIVES

The main aim of the research was to assess fungal contamination of unused and used make-up products, and to evaluate Alchemilla hybrida (L.) L. extracts phenolic content, antioxidative and antifungal properties to gain insight into their applicability as make-up ingredients.

METHODS

Culturable mycobiota was obtained using several nutrient media and characterized based on macromorphological features and ITS and β-tubulin gene sequencing. Total phenolic content, free radicals scavenging (DPPH test) and antifungal (microdilution method) potential of A. hybrida root and aerial part EtOH extracts was determined.

RESULTS

Contaminated samples had fungal loads in range of from 0.2 x 102 to 4.9 x 102 CFU g-1. A total of 18 fungi, mainly Ascomycota, were isolated. The most prevalent species were of Aspergillus and Cladosporium genera, while Meyerozyma guilliermondii and A. tubingensis were the most commonly isolated fungi. Root extracts were richer in phenolic compounds (0.244 mg mL-1) and had more pronounced antioxidant (IC50 0.082 mg mL-1) and antifungal properties (MIC: 0.104 to 1.667 mg mL-1; MFC: 0.208 to 2.5 mg mL-1). The obtained activities could gain the applicative value of using A. hybrida root extract as natural preservatives in cosmetics.

385 / INCREASED CORDYCEPIN BIOSYNTHESIS IN REJUVENATED CORDYCEPS MILITARIS STRAINS IS ASSOCIATED WITH DECREASED CNS1 AND INCREASED CNS

Keywords: *Cordyceps militaris, Cordycepin, Bombyx mori, Tenebrio molitor*

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BACKGROUND

The insect pathogenic fungus Cordyceps militaris (Ascomycota, Hypocreales) is considered as a highly valuable species due to its ability to produce cordycepin, i.e., a potent bioactive compound. Cordycepin biosynthesis is regulated by a multiprotein complex encoded by the Cns1 and Cns2 genes acting together; Cns2 converts 3'-dAMP to the compound 2'-C-3'-dA which is further converted to cordycepin by Cns1 through a reduction reaction. Although large-scale cultivation of C. militaris is successfully performed, culture degeneration tremendously affects the yield and the quality of the ascomata produced.

OBJECTIVES-METHODS

In this study, we worked with C. militaris degenerated commercial strains that were unable to produce fruitbodies in standard rice-based solid substrates. By supplementing those substrates with dried Bombyx mori (Lepidoptera, Bombycidae) pupae or Tenebrio molitor (Coleoptera, Tenebrionidae) larvae, we aimed to rejuvenate the degenerated strains and reverse their fruitbodies production incapacity.

RESULTS

Our results showed that degenerated strains produced fruitbodies exclusively in insect-enriched substrates. In particular, T. molitor dried larvae at a concentration of 10 % (w/w) increased ~6 times the biosynthesis of cordycepin in rejuvenated ascomata of C. militaris. Moreover, expression analysis on the aforementioned ascomata, showed increased Cns2, decreased Cns1 and increased gltA, zwf and pfkA mRNA levels compared to the ascomata of the non-degenerated parental strain. Experimental data suggested a differential substrate-specific regulation of cordycepin biosynthesis genes with the most important factor being the transcriptional ratio of Cns2:Cns1. Moreover, new insights are provided considering the importance of insect-additives in commercial production of C. militaris.

ACKNOWLEDGEMENTS/REFERENCES

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388 / INTRACELLULAR PROTEIN TARGETS OF NEOSARTORYA (ASPERGILLUS) FISCHERI ANTIFUNGAL PROTEIN 2 IN CANDIDA ALBICANS

Keywords: *Neosartorya (Aspergillus) fischeri antifungal protein 2, Candida albicans, Protein target*

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BACKGROUND

Nowadays the incidence of fungal infections caused by antifungal drug-resistant strains is continuously increasing, which hampers the patient management. The small, cysteine-rich, highly stable antifungal proteins secreted by filamentous fungi provide a possible solution to this problem, such as the Neosartorya (Aspergillus) fischeri antifungal protein 2 (NFAP2). Our previous studies indicated that NFAP2 is effective against antifungal drug-resistant Candida albicans isolates both in vitro and in vivo.

OBJECTIVES

The understanding the mode of action of NFAP2 is required for reliable therapeutic application, therefore, we investigated its intracellular protein targets in C. albicans.

METHODS

To do this, we used constraint-based genome-scale metabolic models, a technique that offers a biologically realistic way to explore a large-scale, empirically calibrated fitness landscape. Our landscape included the fitness of hundreds of thousands of genotypes, defined by the presence or absence of specific reactions, in several nutrient environments.

RESULTS

Our result indicated that NFAP2 is able to interact with triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and enolase 1 of C.albicans SC 5314. These proteins are important enzymes involved in glycolysis.simulations. Our results have implications for our understanding of global epistasis and, more generally, the predictability of evolution.

ACKNOWLEDGEMENTS/REFERENCES

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440 / POTENTIAL OF PLANT ORIGIN COMPOUNDS AS THERAPEUTIC AGENTS

Keywords: *antibiotic-resistance, cell membranes, antioxidant activity, antimicrobial activity*

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BACKGROUND

Plants are promising sources of biologically active compounds. Due to the unique geography and climatic conditions, plants growing in Armenia have a high ability to synthesize active secondary metabolites, so they are of great interest (1).

OBJECTIVES

The aim of our research was the investigation of biological activity of extracts of different plants, represented in high-altitude Armenian landscape.

METHODS

DPPH, TBARs, FRAP tests were used to study the antioxidant potential of extracts. Antimicrobial activity was determined using disk-diffusion and broth-dilution methods. The specific growth rate (μ), generation succeeding factor (G) and the FoF1-ATPase activity was evaluated. Different Gram-positive and Gram-negative bacteria were applied as test-microorganisms.

RESULTS

The 80% ethanol extracts of Ribes nigrum, R. rubrum, Rumex obtusifolius, Hypericum alpestre, Origanum vulgare leaves possess high antioxidant potential and, depending on concentration, are able to suppress the growth of Escherichia coli ATCC 25922, kanamycin-resistant E. coli pARG-25, ampicillin-resistant E. coli DH5α-pUC18, Salmonella typhimurium MDC 1754, Enterococcus hirae WT ATCC9790, Bacilllus subtilis A1. In the presence of plant-born substances, μ can decrease up to 2.5 times with increasing of G. Our recent studies showed also the influence of plant extracts on the FOF1-ATPase activity in E. hirae and in E. coli different strains, including antibiotic-resistant ones (2-5).

Obtained data will deepen the basic knowledge concerning the role of cell membranes, redox status in cells, and will open a new possibility for solution of the antibiotic-resistance problem. The results will make a contribution to the global search for new antimicrobials.

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501 / ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF SOME PLANT CRUDE EXTRACTS

Keywords: *Antibiotic-resistance, Armenian flora, Ficus carica*

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BACKGROUND

Emergence of microorganisms antibiotic-resistance urged to the search for a new antimicrobial compounds. The ability of some plant metabolites to act as antioxidant and antimicrobial agents is a valuable property in mitigating the spread of bacterial resistance (1).

OBJECTIVES

The aim of our research was to explore antibacterial and antioxidant capacity of several plant leave (represented in high-altitude Armenian flora) alcoholic extracts.

METHODS

DPPH-assay was used to evaluate antioxidant property. Antimicrobial activity was determined using disk-diffusion and broth-dilution methods. Different Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains were applied as test-microorganisms. The specific growth rate (μ) and generation succeeding factor (G) of kanamycin-resistant Escherichia coli pARG-25 in the presence of plant extracts was evaluated. The quantity of total phenols and flavonoids were determined using Folin-Ciocalteu and aluminum chloride colorimetric assays respectively (2-3).

RESULTS

According to obtained data the extracts of Vaccinium myrtillus, Ribes nigrum, Vitis vinifera, Teucrium polium possessed considerable antioxidant properties. Investigated extracts showed antibacterial activity against tested bacteria (including endospore-producing strains), but only Ficus carica leave extract suppress the growth of kanamycin-resistant E. coli. In the presence of F. carica extract the μ of kanamycin-resistant E. coli decrease op to 24 % in comparison to the control, and G was increase up to 33 %. All investigated extracts were rich of phenolic substances.

The obtained data show that these plants can be a source of biologically active compounds for food/feed industry and can contribute to the process of searching of new antimicrobial preparations.

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506 / MIMIVIRUS PROTECTS AND COMPACTS ITS GENOME IN A 30 NM HELICAL STRUCTURE

Keywords: *Mimivirus, Cryo-EM*

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Mimivirus, the prototype of the Mimiviridae family of giant dsDNA viruses possesses icosahedral 0.45 µm diameter capsids covered by a dense layer of glycosylated fibrils composing the Mimivirus 0.75 µm wide virion. The giant size and thickness of the virions makes difficult to access the information on the organization of the 1.2 Mb genome the membrane-limited nucleoid, inside the virion. We managed to extract and purify from Mimivirus virions a nucleoprotein complex organized as ~30 nm width helical structure we coined Mimivirus genomic fibre. Cryo-EM and proteomics analysis of this complex structure revealed that the genome was encased into a helical protein shell surprisingly composed by the same GMC-type oxidoreductases also composing the glycosylated fibrils decorating the capsid. The genomic fibre corresponds to a 5- or 6-start left-handed helices, with the DNA-strand lining the protein shell interior. Proteomic analysis of the genomic fibre identified RNA polymerase subunits and oxidative stress proteins which could correspond to the dense macromolecules observed by cryoET in some segments of genomic fibre. This would suggest that the RNA polymerase could be ready for immediate transcription upon unwinding of the genomic fibre, as the nucleoid is transferred in the host cytoplasm.

523 / GENOME SEQUENCE-BASED CHARACTERISATION OF PLASMIDS FROM STRAINS HELD BY THE UK'S NATIONAL COLLECTION OF TYPE CULTURES

Keywords: *Plasmids, Enterobacteriaceae*

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BACKGROUND

The National Collection of Type Cultures (NCTC) is a unique resource, composed of almost 6,000 medically relevant bacterial strains, maintained and developed by the UK Health Security Agency (UKHSA). The genomes of approximately 3,000 NCTC strains were recently long-read sequenced, with the genomic reads, assemblies and annotations uploaded to the European Nucleotide Archive (ENA) database under BioProject PRJEB6403. This genomic data provides an unparalleled opportunity to assess the plasmid content of the sequenced strains, for many of which this information is not currently definitively known.

OBJECTIVES

To comprehensively determine the plasmid content of select Enterobacteriaceae strains held by the UK's National Collection of Type Cultures.

METHODS

Here, we utilise a series of bioinformatic tools to identify and classify contigs of plasmid provenance from select NCTC whole genome assemblies, specifically those derived from strains belonging to Escherichia coli and Klebsiella pneumoniae species. Genome assembly optimization (e.g. adding short read information) was performed to investigate whether the current genomic assemblies adequately reflect the true plasmid content of the strains in question, and to assess whether plasmid sequences are captured accurately, in their entirety, and are circularised. Work was also carried out to assess the AMR gene content of these plasmids.

RESULTS

The resulting dataset details the most comprehensive description of the plasmid population of the Escherichia coli and Klebsiella pneumoniae strains held by the NCTC carried out to date, revealing NCTC bacterial strains to be a rich source of diverse plasmids, containing both previously described and putatively novel structures.

536 / ROLE OF E. COLI POTASSIUM TRANSPORTERS IN PROTON / POTASSIUM FLUX DURING MIXED CARBON FERMENTATION AT PH 7.5

Keywords: *potassium transporters, fermentation, Escherichia coli*

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The cytoplasmic potassium concentration in E. coli is balanced by the activity of Kdp, Trk, Kup K+ transporters and other mechanisms. It was investigated the proton/potassium flux in E. coli wild type (wt) and mutants with defect of K+ transporters during fermentation of mixed carbon sources (glucose, glycerol, formate) at alkaline pH. The influence of the proton ATPase specific inhibitor N,N'-dicyclohexylcarbodiimide (DCCD) on the mentioned process was also studied. The experiments were done using electrodes sensitive to proton and potassium ions.

It was shown that the K+ flux in wt cells was ~0.22 mM min-1 during the utilization of glucose. Moreover, DCCD completely suppressed the flux of K+ in the assimilation of both glucose and glycerol. Proton flux in wt cells was ~1.91 mM min-1 during glucose uptake. The DCCD had no effect on both direction and rate of the proton flux. Uptake of K+ under glycerol utilization was accompanied by outflux of H+. In mutant lacking Kup and TrkA transporters K+ flux was not detected during consumption of glucose and glycerol. Moreover, H+ flux decreased by ~9.5 fold in mutant compared to wt. In mutant lacking KdpA transporter K+ uptake increased ~1.7 fold. The absence of the K+ flux in the presence of DCCD suggests that potassium uptake is associated with proton ATPase activity.

Thus, Kup and Trk systems are responsible for K+ uptake and KdpA for K+ outflux under the fermentation of glucose, glycerol and formate at pH 7.5.

ACKNOWLEDGEMENTS/REFERENCES

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548 / THE ROLE OF E. COLI HYDROGENASE-1 IN PROTON FLUX DURING GLUCOSE UTILIZATION AT PH 7.5

Keywords: *E. coli, hydrogenase-1, glucose concentration*

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Hydrogenase-1 (Hyd-1) in E. coli encoded by hya operon is one of the reported four membrane-bound hydrogenases (Hyd).

In this study proton fluxes in mutants lacking large and small subunits of Hyd-1 during fermentation of 2 g L-1 and 8 g L-1 glucose were investigated. Proton flux was determined by registering the changes in H+ activities in the external medium using selective pH electrode and expressed in mmol min-1 per 109 cells per unit. BW25113 wild type and hyaA, hyaB, hyaC mutant strains was used.

In wild type total proton flux was ~1.16 mmol min-1 upon addition of 2 g L-1 glucose, and it was higher in hyaA, hyaB, hyaC mutants, respectively, ~1.92, ~1.47 and ~1.63 fold. DCCD-sensitive fluxes were significantly different in hyaA and hyaC mutants, but not in hyaB. Under the given condition the contribution of FOF1-AT-Pase in wild type was 28%, and differed in all mutants constituting 37%, 18% and 35%, respectively, in hyaA, hyaB and hyaC mutants.

When 8 g L-1 glucose was used no significant differences between wild type and mutants were observed. Namely, total proton flux in wild type was ~1.89 mmol min-1, it was similar in hyaB and hyaC and increased ~1.28 fold in hyaA mutant. Interestingly, the contribution of FOF1-ATPase was ~50% in wild type and all tested mutants.

Taken together it can be concluded that role of Hyd-1 in proton flux is expressed in the presence of low glucose which is dependent on FOF1-ATPase.

ACKNOWLEDGEMENTS/REFERENCES

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556 / INORGANIC NANOPARTICLES ALTER GROWTH AND ANTIPATHOGENIC EFFECTS OF PROBIOTIC LACTOBACILLUS SP. STRAINS

Keywords: *antimicrobial nanoparticles, nanoparticles-microbiota interaction, probiotics for pathogen modulation*

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BACKGROUND

Nanoparticles (NPs) are investigated in the last years for infection control. They possess important antimicrobial properties and could deliver bioactive compounds in specific sites. However, they could interfere with beneficial bacteria, such as microbiota and probiotic strains. As such species impact on the host health and disease, their interaction with antimicrobial NPs should be considered when designing nanostructured antimicrobials.

OBJECTIVES

The aim of this study is to investigate in vitro interactions of NPs and Lactobacillus sp probiotic strains, highlighting their impact in growth and antipathogenic behavior.

METHODS

Two types of NPs which are widely encountered in health care and personal use products, and also studied for antimicrobial applications, namely Fe3O4, and Ag NPs were analyzed in five Lactobacillus sp isolates, obtained from gut microbiota (n=3) and homemade yogurt (n=2). All NPs were physico-chemically characterized by SEM, TEM and FTIR spectroscopy. Minimum inhibitory concentrations (MICs) were established by microdilution method, while antipathogenic effect of probiotics was investigated by adherence and virulence attenuation of opportunistic pathogens Staphylococcus aureus and Pseudomonas aeruginosa.

RESULTS

NPs showed round shape and variable size, ranging 10-100 nm, depending on their type. The results demonstrated that, while Fe_3O_4 NPs show a low impact in modulating the growth and antipathogenic behavior of Lactobacillus sp. isolates, Ag NPs may seriously impact on these strains, when utilized in very low amounts (lower than 0.2mg/mL). Subinhibitory concentrations interfered antipathogenic activity of the probiotic strains, demonstrated by adherence competition to the host cells in vitro in Gram-positive and Gram-negative tested bacteria.

ACKNOWLEDGEMENTS/REFERENCES

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567 / ANTIMICROBIAL ACTIVITY OF EXTRACTS OF NATURAL PRODUCTS – A SOURCE OF POTENTIAL ANTIMICROBIAL ACTIVE COMPOUNDS

Keywords: *Antimicrobial activity, Natural products , Antimicrobial active compounds*

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BACKGROUND

Antimicrobial resistance (AMR) has been identified as a global problem for public health. The increasing incidence of AMR has increased the importance of discovering new therapeutic options for treating MDR bacterial infections. Natural products are nowadays an important source of compounds with biological activity and this includes the antimicrobial activity. This study evaluates the antimicrobial activity of natural extracts against Gram-positive and Gram-negative bacteria and fungi.

MATERIALS/METHODS

Aqueous and hydro-alcoholic extracts of 14 natural products, that include plants and food processing by-products, were evaluated. Antimicrobial activity of 500mg/ml extracts was screened in different agar methods (incorporation, surface spreading and disk-diffusion) against S. aureus ATCC®25923, E. coli ATCC®25922, and C. albicans ATCC®10231. A growth inhibition diameter greater than the one containing only solvent was considered as a positive result. Positive results were submitted to broth microdilution method in order to determine the minimum inhibitory concentration (MIC).

RESULTS

From de 14 extracts evaluated, 4 of them, showed antimicrobial activity. Activity against S. aureus was positive for Gunnera tinctoria, olive pomace and coffee silverskin extracts. Considering C. albicans only Gunnera tinctoria showed positive results. Considering MIC results analysis, Gunnera tinctoria showed the best results for S. aureus (1,5625mg/ml) and C. albicans (0,7813mg/ml), and olive pomace was better for E. coli (62,5mg/ml). predictability of evolution.

CONCLUSIONS

These exploratory results show that natural products can be an important source of antimicrobial compounds, highlighting the need of more studies to evaluate which chemical components from each positive extract are responsible for the antimicrobial activity.

ACKNOWLEDGEMENTS/REFERENCES

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577 / MULTIPLE FUNCTIONS AND FORMS OF THE ESCHERICHIA COLI YIH CASSETTE

Keywords: *yih cassette, sulfoquinovose and lactose degradation, multifunctionality*

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BACKGROUND

The ompLyihOPQRSTUVW cassette (further, the yih cassette) of Escherichia coli K-12 MG1655 participates in the degradation of sulfoquinovose (SQ), a derivative of glucose. Recent results in our laboratory demonstrated the possible connection of several yih genes with lactose utilisation. We intend to examine the role of yih genes in an alternative pathway of lactose catabolism in more detail and analyse the evolution of the yih cassette.

OBJECTIVES

We aim to study the evolution of the yih cassette and explore the possibility of its multifunctionality.

METHODS

The phylogenetic analysis allowed us to determine patterns of the yih genes co-localization. RNA-sequencing and qRT-PCR were performed to assess the expression of yih genes in E. coli str. K-12 MG1655 and E. coli str. Nissle 1917 strains during growth on glucose (a baseline condition), lactose, and SQ.

RESULTS

According to the phylogenetic analysis, the yih cassette exists mainly in the Enterobacteriales order as one of two forms, long (e.g., ompLyihOPQRSTUVW in E. coli str. K-12 MG1655) or short (e.g., yihTUVW in E. coli str. Nissle 1917). Our qRT-PCR results demonstrated a distinct activation of yih genes of the long cassette during growth on sulfoquinovose and a slight activation on lactose. In contrast, yih genes of the short cassette did not respond to the differences in these carbon sources. Differential expression analysis results were consistent with these observations. We suggest that the long yih cassette is responsible for the SQ and lactose degradation. The short variant might be involved in other metabolic pathways.

ACKNOWLEDGEMENTS/REFERENCES

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589 / ZOOMING IN ON THE FISSION YEAST KINETOCHORE DURING MITOSIS: A SUPER-RESOLUTION STUDY

Keywords: *Mitosis, Kinetochore, super-resolution microscopy, Schizosaccharomyces pombe*

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The segregation of sister chromatids to the daughter cells is a vital process, which needs to be tightly regulated. Here, a multi-protein complex called kinetochore, which serves as a force-sensing linker between the centromere in chromosomes and microtubules originating from the spindle pole body plays a pivotal role. Thus, understanding the architecture and regulation of this complex is essential.

Here we present the in situ architecture of several inner and outer kinetochore proteins of the eucaryotic fission yeast at a sub 20 nm resolution using multi-color single-molecule localization microscopy (SMLM) [1]. By imaging each kinetochore protein of interest (POI) in reference to cnp1CENP-A at the centromere and sad1 at the spindle pole we determined protein distances within the complex using Bayesian inference and the stoichiometry of individual POIs per sister chromatid as a first output.

Using this technique, we discovered that while the outer kinetochore subcomplexes are highly conserved among different organisms in distances and stoichiometry, the inner kinetochore architecture is highly variable on which subcomplexes play a primary and rescue role [2,3]. Furthermore, we confirm the in vitro measured stoichiometry and distances in various crystal structure studies in vivo and show an overlap of the NDC80c and MIND complex in the outer kinetochore [4-7]. Additionally, we show that while two different theories for the location of the DASH complex in the outer kinetochore exist [9], either at the NDC80c Loop region or at the NDC80c HEK domain, the fission yeast DASH ring localizes at the NDC80c Loop region.

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597 / IMPACT OF TOTIVIRIDAE L-A DSRNA VIRUS ON SACCHAROMYCES CEREVISIAE HOST: TRANSCRIPTOMIC AND PROTEOMIC APPROACH

Keywords: *dsRNA virus, Host gene expression, Virus proteomics*

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BACKGROUND

Totiviridae L-A virus is a persistent Saccharomyces cerevisiae dsRNA virus. It encodes the major structural capsid protein Gag and Gag-Pol fusion protein, responsible for virus replication and encapsulation. These features also enable the copying of satellite dsRNAs (called M dsRNAs) encoding a secreted toxin and immunity to it (known as killer toxin). Viral capsid pore presumably functions in nucleotide uptake and viral mRNA release. During cell division, sporogenesis, and cell fusion, the virions remain intracellular and are transferred to daughter cells.

OBJECTIVES

Our objectives were to identify numerous changes in the transcription profile of S. cerevisiae genes influenced by the presence of the Totiviridae L-A virus and identify host proteins associated with structural proteins of the virion.

METHODS AND RESULTS

High-throughput RNA sequencing was employed to uncover the transcriptional regulation related to the Totiviridae L-A virus followed by multiple bioinformatics analyzes. Transcriptional responses to L-A infection were similar to those induced upon stress or availability of nutrients. It also delves into the connection between the cell metabolism and L-A virus-conferred demands to the host transcriptome by uncovering host proteins that may be associated with intact virions. To better understand the virus-host interaction, we applied differential proteomic analysis of virus particle-enriched fractions of yeast strains that harbor either complete killer system (L-A-lus and M-2 virus), M-2 depleted or virus-free. Our analysis resulted in the identification of host proteins, associated with structural proteins of the virus (Gag and Gag-Pol).

ACKNOWLEDGEMENTS/REFERENCES

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599 / UNRAVELLING THE MICROBIAL DYNAMICS OF JEEVAMRUTHA - A TRADITIONAL BIODYNAMIC FORMULATION

Keywords: *Organic formulation, Jeevamrutha, microbial dynamics, Physicochemical characteristics*

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BACKGROUND

Jeevamrutha (JA) is a traditional liquid organic formulation used widely by Indian farmers for several decades. In this study, we aim to understand the dynamics of physicochemical characteristics and microbial composition of JA.

METHODS

JA was prepared using cow dung, cow urine, jaggery, pulse flour, soil, and water. The formulation was incubated at 35±2 ºC for 12 days, with stirring at regular intervals. The physicochemical characteristics of JA were assessed using standard protocol. The microbial dynamics of the formulation on 0, 4, 8, and 12 days of incubation was determined by 16S rRNA gene amplicon analysis.

RESULTS

Fluctuations in the pH, EC, and dissolved oxygen (DO) of JA was recorded during the incubation period. A reduction in pH was accompanied with an increase in EC value. Further, a reduction in DO was observed as incubation progressed. The analysis of the 16S rRNA results revealed that during incubation there wasa shift in the microbial community from the obligate anaerobes to the facultative and aerotolerant anaerobes. The PCoA analysis indicated that JA harbour structurally diverse microbes. Also, the 0-day and 4-day samples recorded closer alpha diversity and rarefaction curves.

CONCLUSION

This study reveals that JA is a fermentative type of formulation consisting of several structurally and functionally diverse microbes. As JA incubation progressed, continuous changes in the physico-chemical properties were observed, resulting in temporal succession of growth and metabolic activities of different microbial communities present in JA.

ACKNOWLEDGEMENTS/REFERENCES

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600 / GLOBAL PROFILING OF THE E. COLI RNA AND PROTEIN COMPLEXES BY SIZE EXCLUSION CHROMATOGRAPHY

Keywords: *Grad-seq, size exclusion chromatography, RNA-binding protein, small RNA*

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Diverse biological processes depend on stable cellular protein complexes, often mediated with RNAs. To extend our knowledge about the number and mechanisms of bacterial protein/RNA complexes, we have recently performed an omics approach that relied on the separation of cell lysate using glycerol gradient (Grad-seq), which has guided the discovery of novel global-acting bacterial RNA-binding proteins (RBPs) such as ProQ and KhpB. However, the Grad-seq approach requires a long operation time for ultra-centrifugation and considerable efforts for manual fractionations, which sometimes makes Grad-seq intractable and non-reproducible.

To overcome these drawbacks, we have exploited size exclusion chromatography (SEC) as a complement to glycerol gradient to separate RNAs and proteins in E. coli cell lysate. Our new SEC approach simplifies complicated workflows associated with the Grad-seq mentioned above. SEC fractionation followed by RNA-seq and LC-MS/MS separated and recovered ~85% of all E. coli transcripts and ~60% of the proteins, comparable with the numbers identified by Grad-seq.

Interestingly, SEC outperformed Grad-seq in terms of resolution in a lower molecular weight range where many known RBPs are sedimented. We validated the resolution of SEC based on the distribution of a translational repressor CsrA and two CsrA-binding sRNAs CsrB and CsrC. Indeed, CsrA shows more distinct migrations depending on the binding to either CsrB or CsrC in SEC than in glycerol gradient. Thus, we show that chromatographic separation streamlines and improves the global profiling of bacterial protein/RNA complexes.

ACKNOWLEDGEMENTS/REFERENCES

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637 / BIOETHANOL TO HYDROCARBONS PROCESS VIA ZEOLITE-BASED CATALYST

Keywords: *renewable energy*

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BACKGROUND

Bioethanol is a perspective renewable motor fuel, especially obtained from lignocellulosic residues. But, to be more effective, and have a broad perspectives for applications, it needs to be converted to hydrocarbons as the most used motor fuels [1]. Currently, the development of new methods and technologies for obtaining fuel components and substrates for petrochemistry, based on renewable biomass, is extremely urgent.

OBJECTIVES

Bioethanol from lignocellulose was obtained after fermentation of substrate pretreatment and enzymatically hydrolyzed substrate followed by membrane concentration (to the factor of 7-15) and chemical catalysis. MFI (alumni-silicate zeolites of the pentasil family, Si/Al = 30) showed especially high activity in the conversion of ethanol to aliphatic and aromatic hydrocarbons C3-C12.

METHODS

Microbial ethanol obtaining from lignocellulosic wastes; chemical catalytic methods for hydrocarbons; gas chromatography and HPLC methods of detections.

RESULTS

Since the main problem of its use in industrial scale is the fast coking followed by activity loss, we will present in this new study the possibility to operate in stable continous conditions during up to 100 hours. An original Au-Cu/Al2O3/MFI catalyst was developed that provides high activity (100% ethanol conversion at 330oC) and selectivity (desired products yield - 96%) in ethanol conversion to hydrocarbons C3-C12. Catalyst operation method was suggested that provides stable work during at least 100 h with intermediate regeneration with water vapor treatment followed by reduction with hydrogen.

ACKNOWLEDGEMENTS/REFERENCES

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640 / PHOTODYNAMIC INACTIVATION OF ACINETOBACTER BAUMANNII AND STENOTROPHOMONAS MALTOPHILIA BIOFILMS

Keywords: *photodynamic therapy, Acinetobacter baumannii, Stenotrophomonas maltophilia, biofilms*

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BACKGROUND

Infections caused by antibiotic-resistant bacteria is a global problem, urgently requiring new antibacterial agents or alternative strategies. Antimicrobial photodynamic therapy (aPDT) is a modern biophotonic technology based on the interaction of a non-toxic photosensitizer, oxygen and light. The advantage of aPDT is non-invasive approach and that bacteria have little ability to develop resistance.

OBJECTIVES

To investigate, for the first time, whether chlorophyllin-, riboflavin-, and δ-aminolevulinic acid (ALA)-based aPDT can efficiently inactivate antibiotic-resistant Acinetobacter baumannii, Stenotrophomonas maltophilia and their biofilms.

METHODS

The absorption changes of riboflavin and chlorophyllin were analyzed after LED illumination (402nm or 440nm). The formation of ALA-induced porphyrins in the bacterial samples was followed by using fluorescence spectroscopy. Aliquots of bacterial suspensions (~107 CFU/ml) were incubated with 0.015mM Chl, 0.011mM riboflavin and 1mM ALA in the dark. For the photoinactivation, 200µl of the samples were illuminated in sterile flat-bottom wells for different periods. The antibacterial effect was evaluated using the microdrop method. The surviving cell populations were counted, deriving the mean, and expressing it as log10 (CFU/ml).

RESULTS

The riboflavin and chlorophyllin photomodifications were assessed under the conditions subsequently used for photoinactivation. A. baumannii planktonic cells were more sensitive to riboflavin-aPDT, while biofilms were more efficiently inactivated by chlorophyllin-aPDT. S. maltophilia cells were more susceptible to chlorophyllin-aPDT compared to riboflavin-aPDT. ALA-induced endogenous porphyrins can inactivate both pathogens under light exposure.

In summary, chlorophyllin-aPDT, riboflavin-aPDT and ALA-aPDT can be used as a potential antimicrobial treatment for A. baumannii and S. maltophilia inactivation both for planktonic and biofilm cells.

ACKNOWLEDGEMENTS/REFERENCES

This research was funded by the European Social Fund under the No 09.3.3-LMT-K-712 “Development of Competences of Scientists, other Researchers and Students through Practical Research Activities” measure.

652 / THE EFFECT OF GLUCOSE CONCENTRATION ON METABOLITE ACCUMULATION IN ESCHERICHIA COLI AT ALKALINE PH

Keywords: *Fermentation, Glucose concentration, Escherichia coli*

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For the biotechnological point of view, it is essential to reveal the substrate concentration effect on microbial metabolism. During fermentation of sugars metabolite accumulation or substrate consumption can activate or inactivate fermentative pathways.

HPLC by C18 column with specified method (sample flow 0.4 ml/min for 42 min at 60oC, mobile phase – acidified deionized water) has been applied to determine and measure glucose and end-products up to 72nd h of bacterial growth. Organic matters were determined by RI detector.

~11 and ~44mM glucoses were added to the E.coli BW25113 strain. After 6th and 24th h substrates were consumed, respectively~11mM and~44mM concentrations. 11 mM glucose utilization brought to both~10mM formate and acetate maximal yields in 7th and 8th h. During the utilization of~44mM glucose~21mM formate and~18.9mM acetate maximal yields were observed in 24th h. Here 1:1 ratio is for these two acids.

In both cases formate starts to be utilized back. While in~44mM glucose assay ratio of metabolite were; ethanol(1.5):-formate(1):acetate(1): Succinate was produced in insignificant quantities in ~11mM assay, while in ~44mM glucose concentration of succinate has reached up to~7.7mM but in 72nd h of growth. Interestingly, lactate was utilized after producing during fermentation of~11mM glucose, but not of~44mM glucose, showing stable concentration during the further growth.

It can be concluded that depending on glucose concentration, metabolites produced may subject to further fermentation or accumulation, which gives new approaches in microbe metabolism regulation for organic waste management.

ACKNOWLEDGEMENTS/REFERENCES

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658 / THE USE OF VITAMIN AND MICROELEMENT SUPPLEMENTS DURING THE COVID-19 PANDEMIC IN SERBIA

Keywords: *SARS-CoV-2, vitamin D, vitamin C, zinc*

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BACKGROUND

Vitamins and microelements are crucial for the proper function of the immune system. However, there is still no scientifically confirmed benefit of their use in coronavirus disease 2019 (COVID-19) prevention or treatment.

OBJECTIVES

The study aimed to evaluate the intake of supplements containing vitamins and microelements as well as factors affecting the supplementation in the Serbian population during the SARS-CoV-2 pandemic.

METHODS

This cross-sectional observational study collected self-reported information from respondents of the general population regarding their use of dietary supplements containing vitamins and/or microelements. An online questionnaire was used for data collection, during 2021. Among others, the questions aimed to identify a perception of COVID-19 severity, a change in micronutrient consumption, knowledge about supplements characteristics and belief on their effects in COVID-19 treatment.

RESULTS

Most of our respondents (n=229) use micronutrient supplements (93.4%), and more than a half (56.1%) increased their intake during the SARS-CoV-2 pandemic, particularly of vitamin D, vitamin C, zinc, and selenium. Supplements were used more often by females, younger adults, and those who adhered to preventive measures against the virus. The odds for the change in supplementation habits increased due to the external influence, in particular, 3.39 times (95%CI: 1.09-10.59; p=0.036) with the information from a scientific article, and 5.38 times (95%CI: 1.79-16.20; p=0.003) when advice came from a medical professional. Supplement use appears to be commonly recommended by physicians and pharmacists in Serbia.

ACKNOWLEDGEMENTS/REFERENCES

The first two authors contributed equally.

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663 / ANTIFUNGAL ACTIVITY OF PEPTIDES DESIGNED ON THE Ý–CORE OF TOMATO PLANT DEFENSINS

Keywords: *Plant defensin, Antifungal activity, Synthetic peptide*

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BACKGROUND

The prevention and reduction of the enormous crop losses caused by plant pathogenic fungi represent a worldwide challenge. The small molecular weight, cysteine-rich antifungal defensins and their synthetic peptide derivatives represent promising candidates to develop novel plant and crop protection strategies.

OBJECTIVES

Previous studies demonstrated that the presence of the evolutionarily conserved γ-core region (GXC-X[3-9]-C) is important for the antifungal effect of plant defensins, and synthetic peptides spanning the γ-core region have in vitro antifungal activity. We investigated the antifungal effect and mechanism of eight peptide derivatives designed on the γ-core region of novel Solanum lycopersicum L defensins.

METHODS

The antifungal efficacy of synthetic γ-core peptide derivatives against Botrytis cinerea SZMC 21472, Cladosporium herbarum FSU 1148, Fusarium oxysporum f.sp. lycopersici CBS 123668, and Fusarium oxysporum SZMC 6237J was determined in a microdilution susceptibility testing method. Fluorescence-activated cell sorting (FACS) coupled with propidium iodide staining was applied to investigated the antifungal mechanism of the most effective peptide derivatives on the most susceptible fungal strain, C. herbarum FSU 1148.

RESULTS

The highly positively charged and hydrophilic γ-core peptide derivatives were able to effectively inhibit the growth of the tested plant pathogenic filamentous fungi with different efficacy (minimum inhibitory concentration: 12.5 - 400 µg/ml). FACS analysis indicated that the antifungal active peptides destruct the plasma membrane integrity of C. herbarum FSU 1148 which results in cell death.

ACKNOWLEDGEMENTS/REFERENCES

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711 / PPRA REGULATES DNA REPLICATION INITIATION IN MULTIPARTITE GENOME HARBOURING BACTERIUM DEINOCOCCCUS RADIODURANS

Keywords: *Multipartite genome, Replication Regulation, Radioresistance, PprA*

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BACKGROUND

Mechanism underlying DNA replication regulation is very poorly understood in multipartite genome harbouring bacteria including D. radiodurans (2 Chromosomes and 2 plasmids). PprA plays role in DNA repair, genome segregation, cell division and radioresistance of D. radiodurans. ΔpprA exhibits increased ploidy of genome.

OBJECTIVES

To study the role of PprA protein in regulation of DNA replication in D. radiodurans.

METHODS

Interaction of DnaA and DnaB with origin of replication (oriC) was monitored using Electrophoretic-Mobility Shift Assays. Interaction of PprA and their DNA binding/polymerisation mutants with DnaA and DnaB was studied by Bacterial-Two Hybrid System and Co-Immunoprecipitation. Impact of replication inhibitor (Hydroxyurea, HU) on γ-radiation survival of D. radiodurans was determined. We also studied the effect of PprA expression on growth and cell morphology of E. coli.

RESULTS

Deinococcal DnaA showed sequence-specific interaction with oriC of D. radiodurans as well as E. coli albeit with different affinity. The C-terminal domain of DnaA is involved in oriC recognition. DnaB showed ATP hydrolysis dependent unwinding of dsDNA substrate in 5′→3′ direction only. Wild-type and DNA-binding/polymerisation mutants of PprA suppressed the assembly of DnaA-DnaB complex at oriC by interacting with DnaA and DnaB.

Trans-expression of PprA in E. coli caused cell growth inhibition and cell elongation. The replication arrest by HU has made faster recovery of D. radiodurans after γ–radiation exposure than ΔpprA, which has suggested the roles of PprA in DNA replication regulation by modulating the activities of replication initiation proteins and thereby contributing to extraordinary radioresistance in D. radiodurans.

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726 / THE SECRETED FORM OF THE VIRULENCE FACTOR INLB AFFECTS THE INNATE IMMUNE RESPONSE ON LISTERIA MONOCYTOGENES.

Keywords: *InlB, Listeria monocytogenes, innate immune response*

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The L. monocytogenes virulence factor InlB mediates active invasion into mammalian cells via interactions with surface receptors c-Met and gC1q-R. Both receptors present on macrophages and epithelial cells. c-Met controls cytoskeleton rearrangements, cell proliferation and migration. Both receptors involved in regulation of the immune response. The virulence factor InlB presents in two forms, attached to bacterial surface and released. Phylogenetically defined substitutions were described in InlB that affect L. monocytogenes virulence.

In this work, we were interested whether InlB could affect the innate immune response and whether this response would depend on phylogenetically defined InlB isoforms. We used InlB isoforms specific for different L. monocytogenes clonal complexes including lineage I clonal complex CC1 and lineage II clonal complex CC7 (InlBCC1 and InlBCC7, respectively). The thermophoretically defined dissociation constants of InlB with c-Met were 7.4 and 58.7 nM for InlBCC1 and InlBCC7 respectively. The dissociation constants of InlB with gC1q-R were 7.4 and 10.2 for InlBCC1 and InlBCC7, respectively.

Pretreatment of human macrophages with purified InlB affected macrophage response on subsequent L. monocytogenes infection in an isoform dependent manner. Macrophage pretreatment with InlBCC1 reduced L. monocytogenes uptake and improved L. monocytogenes reproduction within macrophages for the strain expressing InlBCC1 but not InlBCC7. Pretreatment with InlBCC7 did not affect the efficiency of bacterial uptake and intracellular reproduction. Obtained results suggested that interactions of InlB and c-Met affect macrophage response on L. monocytogenes infection, and the effect is different for distinct InlB isoforms.

ACKNOWLEDGEMENTS/REFERENCES

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773 / TRICHOMONAS VAGINALIS AND MYCOPLASMA SPECIES: A FASCINATING CONSORTIUM IN THE VAGINAL MICROENVIRONMENT

Keywords: *Trichomonas vaginalia, Mycoplasma girerdii, Mycoplasma hominis, endosymbiosis*

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BACKGROUND

Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract causing the most common non-viral sexually transmitted disease worldwide. T.vaginalis establish a symbiotic relationship with Mycoplasma hominis, influencing the protozoan growth rate, energy production and pathogenicity. Interestingly, a new uncultured Mycoplasma species, Candidatus Mycoplasma girerdii, has been recently described almost exclusively in the vagina of women with trichomoniasis. Metagenomic analyses have shown some features of this new bacterium, but the nature of its association with T.vaginalis and the possible influence on protozoan have not yet been explored.

OBJECTIVES

Investigate the association between T.vaginalis and M.girerdii, on the capability of the bacterium to influence the protist pathobiology and evaluate the interaction and the possible competition with M.hominis to infect trichomonad cells.

METHODS

The relationship and effects of Mycoplasma presence on the pathobiology of protists were studied through the development of isogenic T.vaginalis strains with and without M.girerdii, to evaluate in vitro the impact of the bacterium on protozoan pathobiology. Moreover, the molecular basis of Trichomonas-Mycoplasma symbiosis was analysed by transcriptomic profiling of isogenic T.vaginalis strains.

RESULTS

M.girerdii influences the growth and cytopathic activity of T.vaginalis, besides previously demonstrated for M.hominis associated with protist. These in vitro results are supported by transcriptomic data showing major shifts in parasite gene expression in response to Mycoplasma species, including metabolic enzymes and virulence genes. Our data support the hypothesis that the presence and quantitative ratios of endosymbionts may contribute to modulating T.vaginalis virulence, reinforcing the idea to consider pathogenic entities as microbial ecosystems.

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781 / COMMON FEATURES AND INTRA-SPECIES VARIATION OF CUTIBACTERIUM MODESTUM STRAINS

Keywords: *Cutibacterium modestum, Propionibacterium humerusii, new species, genome*

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Cutibacterium modestum is a new species coined in 2020 as the fifth species of genus Cutibacterium, which include Cutibacterium acnes. The species is predicted as a minor but common member of skin microbiome and includes a group tentatively named as “Propionibacterium humerusii”.

The description of the species has been provided only with a single strain. To establish the characteristics of C. modestum and search for possible disease-related subtypes, we investigated the biochemical characteristics of eight live strains and performed in silico comparison of nine genomes. The common features, which included morphology of Gram-stain positive short rods, negativity of phenylalanine arylamidase, and several unique MALDI-TOF MS spectral peaks, were considered useful in laboratory identification. Pairwise comparisons of the genomes by in silico DNA-DNA hybridization showed similarity values of 98.1% or larger, which were far higher than the subspecies cutoff of 79–80%. 16S rRNA gene sequences of thirteen isolates and genomes were identical.

Their recA gene sequences were identical except for two strains, HM-510 (HL037PA2) and Marseille-P5998, which showed unique one-gene polymorphisms. Spectra of MALDI-TOF mass spectrometry showed slight differences in the presence of m/z 10,512 (10 kD chaperonin GroS) and three other peaks, further clustering the eight isolates into three subtypes. These results indicated that these isolates did not separate to form subspecies-level clusters, but subtyping is possible by using recA gene sequences or MALDI-TOF mass spectrometry spectra. Also, this work confirmed that a group “P. humerusii” is included in C. modestum.

803 / DIVERSITY OF COAGULASE-NEGATIVE STAPHYLOCOCCI AND S. AUREUS CLONAL LINEAGES IN NOSTRILS AND BUCCAL MUCOSA OF CAMELS

Keywords: *S. aureus, coagulase-negative staphylococci, antimicrobial resistance, camels*

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Several different species of animals host staphylococci as normal microbiota and can be a source of staphylococci zoonotic infections. People with routine or occupational exposure to infected/colonized animals are at risk of a potential transmission. Therefore, we aimed to investigate the presence of S. aureus and other staphylococci in camels used for recreational purposes as well as their antimicrobial resistance, virulence factors and genetic lineages.

A total of 116 samples were collected from 58 healthy camels (nose and mouth) from different farms located in the Canary Islands, Spain. The swabs were incubated onto Mannitol Salt agar and ORSAB plates. The antimicrobial susceptibility testing was performed against 14 antimicrobial agents. The presence of virulence genes was studied by PCR. Multilocus sequence typing, spa- and agr-typing was performed in all S. aureus isolates.

From the 58 camels tested, 42 (72.4%) staphylococci were isolated of which 11 S. aureus, 13 S. lentus, 12 S. sciuri, 3 S. xylosus, S. epidermidis, S. hominis and S. chromogenes. Interestingly, camels had staphylococci either in the mouth or in the nose, but in no case was it possible to isolate the same species of staphylococci from both the nose and mouth of the same animal. Staphylococci isolates were susceptible to all antimicrobial tested except for ciprofloxacin and fusidic acid. All S. aureus isolates harbored the hla, hlb and hld virulence genes. Regarding the molecular typing, S. aureus were ascribed to 3 sequence types (STs) and 4 spa-types. All S. aureus isolates belonged to agr type III.

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828 / PREVALENCE OF FAECALIBACTERIUM PRAUSNITZI, CHRISTENSELLA SPP. AND ROSEBURIA SPP. STRAINS IN ELEVEN-GUT MICROBIOTA

Keywords: *gut microbiota, genomics, diet*

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BACKGROUND

Gut microbiota are both considered central to cause many disorders and able to hold the key to new therapies. An understanding of the microbiome composition will help us understanding their role. Existing studies comparing gut microbial composition have shown that Faecalibacterium prausnitzii, Christensenella spp. and Roseburia spp. are well represented in healthy people (Immun Ageing 18, 2 (2021). https://doi.org/10.1186/s12979-020-00213-w).

OBJECTIVES

A preliminary study was conducted in order to highlight Faecalibacterium prausnitzii, Christensenella spp. and Roseburia spp. in the gut microbiota of general population volunteers having a vegetarian or wholesome well-balanced diet.

METHODS

Eleven donors meeting the criteria were selected. Antibodies were used to select the targeted bacteria and their sorting by "(Fluorescence Activated Cell Sorting) FACS" flow cytometry performed. A screening of the isolates using PCR primers (targeted firmicutes phylum) was then made. Strain identification was obtained in first intention by 16S rRNA gene sequencing, followed by WGS Illumina sequencing for further analysis.

RESULTS

Three hundred eighty-eight colonies were selected from the eleven donors for a further study. Their distribution was as follow: 54 Faecalibacterium prausnitzii, 73 Christensenella minuta and 261 Firmicutes. No Roseburia was detected. The 16S rRNA gene analysis of isolates belonging to the phylum Firmicutes revealed strains classified as Ruminococcus sp., Faecalicatena sp., Oscillospira sp., Lachnospira sp. and Butyrivibrio sp. Differences in the intestinal microbial composition of the eleven donors were observed depending on the diet.

837 / METAGENOMIC ANALYSIS OF FUNCTIONAL BILE ACID GENES AND BILE ACID COMPOSITION IN FAECAL SAMPLES OF BILE ACID DIARRHOEA SUFFERERS

Keywords: *Bile Acid Diarrhoea, Microbiome*

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Bile Acid Diarrhoea (BAD) is a widespread gastrointestinal disease, estimated to affect 1% of the population in the United Kingdom alone. BAD is associated with excessive bile acid synthesis secondary to a gastrointestinal or idiopathic disorder, therefore, decreasing bile synthesis can lead to reduced symptoms. Bacteria are essential in transforming primary bile acids into secondary bile acids. The profile of an individual's bile acid pool is central in maintaining bile acid homeostasis as bile acids regulate their own expression. Indeed, examining the gut microbiome to determine the bile acid transforming potential of the microbiota could help elucidate key bacterial strains potentially responsible for causing BAD.

BAD is often misdiagnosed as irritable bowel syndrome (IBS), with up to a third of IBS sufferers having BAD. Human stool samples from 26 BAD patients and 25 healthy donors (controls) were provided by collaborators at the University Hospitals Coventry & Warwickshire (Prof. Arasaradnam). Firstly, the microbiome was analysed following DNA extraction and shotgun sequencing at a depth of 50 million reads per sample.

Metagenomic data was used to analyse both the taxonomic structure of the microbiome, with targeted analysis of BSH gene and BAI genes used to determine the microbial potential for secondary bile acid synthesis. Secondly, the bile acid composition for each sample was determined using UPLC-MS.

Here, we will report the results from the analysis of the human faecal microbiome and bile acid profiles and explore the role of the microbiome in bile acid homeostasis in BAD.

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860 / BACTERIAL POPULATION OF A TOP-RANKING HIGH BACKGROUND RADIATION AREA

Keywords: *High Background Radiation Area, Firmicutes, culture-dependent*

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BACKGROUND

High background radiation areas (HBRAs) are unique regions which emit elevated levels of natural radiation: greater than the world average of 0.07mSv/yr. Chavara-Neendakara placer deposit present at the southwestern coastal region of India, ranks second in terms of its average effective dose (~9mSv/yr) due to the presence of monazite sand. Other HBRAs include the coastal regions of Espirito Santo and Morro Do Forro in Brazil, Yangjiang in China and Ramsar and Mahallat in Iran A wide variety of ecosystems have been thus far investigated in depth to understand their microbiome and its interaction with the environment; but no information is available on HBRA.

OBJECTIVES

The present study is a prelude to understand the microbiota inhabiting the high background radiation area of Chavara-Neendakara (India) and its dynamics.

METHODS

A culture-dependent approach involving isolation on culture media, characterization through microscopy and identification using 16S rDNA amplicon sequencing has been undertaken to study the bacterial population. Further, BLAST analysis and statistical calculations were performed to further understand the microbiome and compare with those from other habitats.

RESULTS

35 bacterial species belonging to 22 genera were identified. Shannon-Simpson diversity-predominance indices, revealed an over-riding presence of Firmicutes (~60%) in the HBRA. Results of comparison with T22 Trench of Chernobyl, contaminated with radionuclides, the Uranium ore deposit of Domiasiat, (India) and other habitats encourage us to hypothesize that the long-term exposure to radiation may have impacted on the HBRA microbial population, leading to the predominance of Firmicutes, a matter of ecological and evolutionary significance.

ACKNOWLEDGEMENTS/REFERENCES

Financial support from the Ministry of Earth Sciences, Government of India is gratefully acknowledged.

879 / UNEXPECTED EFFECTS OF CAMP-CRP AND UXUR ON BIOFILM FORMATION IN ESCHERICHIA COLI

Keywords: *E. coli, biofilms, UxuR, cAMP-CRP*

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BACKGROUND

The problem of biofilm formation is widely known especially for uropathogenic E.coli, but regulation of this process is not clear yet. Our aim is to find key regulators of biofilm formation in E.coli acting in different conditions. Here we focus on UxuR, regulator of hexuronate metabolism which besides overall influence on the host microbiome, also inhibits flagellar genes, and global regulator cAMP-CRP which was predicted as a regulator of biofilm formation.

OBJECTIVES

The main objective is to identify the key regulators of biofilm formation in E.coli

METHODS

2D electrophoresis, HPLC-mass-spectrometry, biofilm assays, RNA-seq

RESULTS

Effects of cAMP-CRP on biofilm formation were dependent on glucose and aeration. In anaerobic conditions, the crp deletion led to dramatically increased biofilm formation, whereas in aerobic conditions this effect was much less pronounced. Deletion of uxuR in some experiments decreased biofilm development, which corresponded to the expression data. However, in other experiments the effect was just opposite. We thus made proteomic analysis, and in the first experiment observed extensive synthesis of flagellar proteins in the duxuR derivative, but no Flg-or Fli- proteins were visible in the second. This was surprising but at the same time correlating with our RNA-seq data where huge activation of fli-and flg-genes was detected in two out of three experiments. We suggest that the biofilm formation by E.coli may be influenced not only by temperature and aeration but also by the population composition of the colonies, as it sometimes happens within the host intestine.

ACKNOWLEDGEMENTS/REFERENCES

Work was supported by RSF 18-14-00358

894 / MICROBIOLOGICAL AND HISTOLOGICAL ANALYSIS OF TONSIL TISSUE FROM PATIENTS DIAGNOSED WITH CHRONIC TONSILITIS

Keywords: *tonsillitis, biofilm, Staphylococcus aureus*

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If chronic tonsillitis (CT) is treated surgically, the long-term risks of infections and respiratory diseases are higher, making of high importance to find an alternative for tonsillectomy. In order to isolate and identify the causing agents of CT, a total of 79 postoperative palatine and adenoid tissue samples were obtained from the ENT Clinic, KBC Zvezdara. A total of 82 bacterial species were isolated and identified by MALDI-TOF. They belong to the genera Streptococcus, Staphylococcus, Micrococcus, Rothia, Enterobacter and Stenotrophomonas. Analysis of the incidence of bacteria isolated from tissue samples showed the highest prevalence of Streptococcus oralis (27/33%), followed by S. aureus (17/21%) and Streptococcus parasanquinis (16/20%). Histological analysis of tissue samples was conducted in order to detect the presence of CT-causing bacteria. In addition to interfollicular hyperplasia, colonies of species S. aureus and Stenotrophomonas maltophilia were detected in histological material. The presented results confirm that the colonizers of tonsils tissues can be both G (+) and G (-) bacteria. The presence of S. aureus in the tonsillar tissue even after the antibiotic therapy may be related to its ability to form biofilm. Our study demonstrated that among 17 S. aureus isolates, 13 displays more pronounced biofilm forming ability than the reference strain S. aureus ATCC 11632, and only 4 isolates display lower biofilm forming ability. The presence of biofilm might be the reason for the recurrence of infection. Therefore, a searching for new antibiofilm therapeutics in CT therapy is of great importance.

ACKNOWLEDGEMENTS/REFERENCES

The authors thank to Serbian Ministry of Education, Science and Technological Development for the financial support (grant number 451-03-9/2021-14/ 200007).

896 / ISOLATION AND GENOMIC CHARACTERISATION OF NOVEL WEBERVIRUSES

Keywords: *Bacteriophage, Functional genomics, Klebsiella, Comparative genomics*

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BACKGROUND

Webervirus is a genus of lytic bacteriophages (phages) in the family Drexlerviridae. Webervirus phages infect clinical Klebsiella spp. and exhibit depolymerase activity on many of their hosts. Seven new Webervirus phages were isolated while looking for phages that infect multi-drug-resistant Klebsiella spp.

OBJECTIVES

To characterise the host range and activity of these 7 phages and determine their genetic relationship with respect to other members of the genus Webervirus. To identify Webervirus genomes represented in recently published metagenome-assembled genome (MAG) datasets.

METHODS

Phages were isolated from sewage samples, before their genomes were sequenced and images generated using transmission electron microscopy (TEM). The genomes were compared to publicly available Webervirus genomes using the tools ViPTree, VIRIDIC and vConTACT2. Portal protein and large terminase subunit genes were aligned to confirm the genus Webervirus was monophyletic. PhageClouds was used to find Webervirus genomes within MAG datasets, with hosts predicted using the CRISPR Spacer and Database and Extraction Tool, and HostPhinder. A pangenome analysis was undertaken with 106 high-quality/complete genomes to better understand genomic diversity within the genus Webervirus.

RESULTS

TEM revealed the 7 phages had a Siphoviridae-like morphology. Their genomes were complete and circular. vConTACT2 and phylogenetic analyses confirmed that the 7 newly isolated phages were part of the genus Webervirus. Inclusion of MAGs in data analyses (VIRIDIC, vConTACT2, Roary) showed the genus was more diverse than previously recognized, with pangenome analysis a useful tool for delineating taxa within the family Drexlerviridae.

914 / EXTRACELLULAR ATP IS A SIGNALLING MOLECULE IN GUT BACTERIA

Keywords: *Extracellular ATP, Intestinal bacteria , Host-bacteria interaction, Signalling molecule*

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BACKGROUND

In animal and plant cells extracellular ATP (eATP) functions as a signaling molecule. Whether bacteria can sense and respond to eATP was unknown, but intestinal bacteria are exposed to eATP at low concentration under homeostatic conditions and at elevated concentration during inflammation, with variations depending on their niche.

OBJECTIVES

We investigated the role of eATP as a signalling molecule in intestinal bacteria.

METHODS

We used a commercial fluorescent reporter library in the commensal E. coli, in which each reporter strain carries a plasmid containing a single promoter region of E. coli, which controls the expression of GFP. We screened this library for a response to eATP in rich and minimal medium. We performed untargeted intracellular and extracellular metabolome analysis using LC-MS on commensal E. coli upon exposure to eATP. We transformed a selection of eATP-regulated reporters into other gut bacteria including pathogenic bacteria, to test their ability to respond to eATP.

RESULTS

We found that eATP induced a strong and extensive transcriptional response in commensal E. coli. eATP could both positively and negatively modulate promoter activity in a dose dependent manner. The response to eATP was very different in rich and minimal medium, suggesting that the environment determines the signalling network to eATP. E. coli stimulated with eATP showed a significant metabolic modification as compared to non-stimulated bacteria. Finally, the bacteria tested were all able to sense and respond to eATP, suggesting ability to sense and respond to eATP is a conserved feature in bacteria.

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958 / ACTIVITIES OF ANTIOXIDANT ENZYMES IN MYCELIUM OF FUNGUS PHYCOMYCES BLAKESLEEANUS

Keywords: *Antioxidant enzymes, fungus*

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Phycomyces blakesleeanus is a strict aerobic filamentous fungus often used as a model system in studies of physiology, genetics, environmental sensing, and metabolism. As all other aerobic organisms, this fungus faces the toxic effects of oxygen-reactive species, but data about its antioxidative defense systems are scarce. The aim of this research was to examine the activities of three antioxidant enzymes during different phases of growth. The fungus was grown in two ways, in Petri dishes, and on a shaker in Erlenmeyer flasks. The activities of superoxide dismutase (SOD) and peroxidase (POD) were determined spectrophotometrically, while the activity of catalase (CAT) was determined polarographically with a Clark-type oxygen electrode. The highest activities of SOD were noticed in mycelia grown in Erlenmeyer flasks in the stationary phase of growth. In mycelia grown in Petri dishes, the highest activities of POD (0,014U/mg protein) and CAT (20,63 U/mg protein) were noticed in the early exponential phase. The activities of these two enzymes decreased with mycelial growth. In mycelia grown in Erlenmeyer flasks, POD and CAT showed similar behavior, but differences in activities between exponential and stationary phases were smaller and the highest activities were noticed in the mid-exponential to stationary phase of growth (0,011U/mg protein for POD and 17,21U/mg protein for CAT). High activities of these two enzymes indicated increased production of H_2O_2 and pointed out the importance of this phase for mycelia grown in this way.

ACKNOWLEDGEMENTS/REFERENCES

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983 / MITOCHONDRIAL GENOMES PROVIDE INSIGHTS INTO THE EVOLUTION OF SACCHAROMYCOTINA YEASTS

Keywords: *Comparative mitochondrial genomics, Fungi, Synteny, Molecular dating*

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Saccharomycotina yeasts consist of genetically diverse, but morphologically similar species within the kingdom of Fungi and are important to human everyday life. This work investigates the evolutionary relationships among these yeasts from a mitochondrial (mt) genomic perspective, by performing a comparative study of 155 yeast mt genomes representing all major phylogenetic lineages of Saccharomycotina. In detail, this study investigates the genetic elements (i.e., introns, gene clusters, intergenic regions, GC islands), genetic mechanisms (i.e., recombination, gene shuffling) and evolutionary events (i.e., genetic code changes) under the prism of molecular dating.

Findings from this study suggest that mt genome size diversity is the result of a ceaseless random process mainly based on genetic recombination and intron mobility. Gene order analysis revealed conserved syntenic units and many occurring rearrangements, which can be correlated with major evolutionary events as shown by the phylogenetic analysis of the concatenated mt protein matrix. For the first time, molecular dating indicated a slower mt genome divergence rate in the early stages of yeast evolution, in contrast with a faster rate in the late evolutionary stages, compared to their nuclear time divergence. Genetic code reassignments of mt genomes is a perpetual process happening in many different parallel evolutionary steps throughout Saccharomycotina evolution. Overall, this work showed that phylogenetic studies that employ the mt genome of yeasts reveal major evolutionary events that complement data from nuclear based analyses.

ACKNOWLEDGEMENTS/REFERENCES

* Authors 1, 2 and 3 have contibuted equally to this work.

1026 / FILAMENTOUS PHAGE PF4 RE-SENSITIZE PSEUDOMONAS AERUGINOSA TO ANTIBIOTICS

Keywords: *Pseudomonas aeruginosa, Pseudomonas phage Pf4, ceftazidime, tetracycline*

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More than 60% of Pseudomonas aeruginosa strains are infected with filamentous bacteriophages, but they are still not examined in a great extent. The best-known P. aeruginosa filamentous phages are Pf1, Pf4 and Pf5. The aim of this study was to determine the effect of sub-inhibitory concentrations of antimicrobials on Pf4 prophage expression in the PAO1 strain, as well as effect of this phage on phenotype of other P. aeruginosa strains. Using q-RT-PCR, the expression of Pf4 was checked after exposure to different classes of antibiotics.

Colony PCR was used to confirm the integration of Pf4 into the genomes of other strains of P. aeruginosa strains harboring other filamentous phages. The effect of Pf4 phage on the phenotype of the new hosts was tested by a number of microbiological methods. Sub-inhibitory concentrations of ciprofloxacin and mitomycin C enhanced production of Pf4 for approximately 50% during first and sixth hour of exposure, respectively.

Gentamicin and ceftazidime had no effect on Pf4. All tested strains were successfully infected with Pf4. After comparing the properties of Pf4 infected and uninfected strains, it was found that Pf4 significantly reduces cell auto-aggregation, pyocyanin and pyoverdine production. However, Pf4 significantly increased swarming motility and biofilm production in all strains. Surprisingly, Pf4 successfully increased the antibiotic susceptibility of LESB58 and it has been re-sensitized to ceftazidime and tetracycline. The results indicate filamentous phages can spread certain bacterial virulence factors among P. aeruginosa, but also implicate potentially new strategies to re-sensitize bacteria to conventional antibiotics.

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1031 / "PSEUDOMONAS VIRUS PFLES" - CANDIDATE FOR A NEW SPECIES AND GENUS OF THE FAMILY INOVIRIDAE

Keywords: *Pseudomonas phage PfLES, LESB58, Inoviridae, CoaB protein*

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The most studied Pseudomonas aeruginosa phages belong to order Caudovirales, but filamentous phages are very interesting and insufficiently researched group, belonging to order Tubulavirales and family Inoviridae. In addition to two accepted species: Pf1 and Pf3, there are other Pf1-like prophage that are integrated into the genomes of P. aeruginosa bacteria, including Liverpool epidemic strain LESB58, prevalent among cystic fibrosis patients. The objective was to confirm production of PfLES and partially characterize this filamentous phage.

The multiplied PfLES phages were confirmed using TEM, DNA isolation and confirmation of replicative form (RF) by PCR. In addition, purified virions were used to infect PAO1 strain and infection was confirmed by PCR. The genome and key PfLES proteins (CoaA, CoaB and Zot) were analyzed in silico and phylogenetic relationships with other similar phages was determined using Mega X. The results showed that RF of this prophage is present in LESB58, and TEM confirmed that filamentous virions were successfully produced. The PfLES virions contained ssDNA and infected PAO1, as confirmed by PCR.

The analysis of genome and key phage proteins showed that it is similar to Pf1 phage, but belongs to potentially new species of the Inoviridae family. The CoaB and Zot of PfLES58 phage proteins showed significant differences compared with the proteins of other filamentous phages, indicating new genus. Finally, as PfLES prophage produces virions, its involvement in virulence of LESB58 should be further examined.

ACKNOWLEDGEMENTS/REFERENCES

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1040 / THE PREVALENCE OF ONCOGENIC VIRAL INFECTIONS AND HLA-G POLYMORPHISMS IN HEAD AND NECK CARCINOMA TISSUES

Keywords: *Human papilloma viruses, Epstein-Barr virus , polyomaviruses, HLA-G polymorphisms*

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BACKGROUND

It has been suggested that oncogenic viruses and HLA-G polymorphisms may be associated with the development Head and Neck squamous cell carcinoma (HNSCC).

OBJECTIVES

To investigate the prevalence of infections and co-infections with Human papillomavirus (HPV), Epstein-Barr virus (EBV) and BK and JC polyomaviruses and HLA-G polymorphisms in HNSCC.

METHODS

100 HNSCC tissue samples were analyzed. HPV and EBV were detected by single, Nested and Real-time PCR. Seminested PCR was used for polyomaviruses. Sanger sequencing method and Nested PCR were used for HPV and EBV genotyping, respectively. Determination of HLA-G types was done by Sanger sequencing method.

RESULTS

HPV was detected in 27%, EBV in 20%, BKV in 3% and JCV 0% of samples. Overall frequency of high-risk HPV infection was 84%. The most prevalent type was HPV 16 (57.1%). Genotype EBV-1 was more frequent compared to EBV-2 (93.75% vs. 6.25%). Single infection was detected in 28% of samples with a significant prevalence of HPV infection. Co-infections were confirmed in 11% of tissues. HPV/EBV co-infection is the most common but without a difference in the frequency of co-infection type. Six HLA-G types were identified, where *01:01:01 is the most common (56.66%), with no difference between HLA-G types and the presence of single infections and co-infections.

CONCLUSION

The association of oncogenic virus infections with HNSCC has been demonstrated, but without the correlation between HLA-G polymorphisms and presence of oncogenic virus infections and co-infections in HNSCC.

1041 / SYNERGISTIC ANTIBIOTIC-PHOTODYNAMIC THERAPY COMBINATION INACTIVATING OPPORTUNISTIC PATHOGEN STENOTROPHOMONAS MALTOPHILIA

Keywords: *Stenotrophomonas maltophilia, Synergistic antibiotic-photodynamic therapy*

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BACKGROUND

Stenotrophomonas maltophilia – a newly emerging multidrug resistant opportunistic pathogen causing serious concern worldwide. Trimethoprim-sulfamethoxazole (TRP-SFX) and fluoroquinolone class antibiotics (e.g. ciprofloxacin (CIP)) are the main treatment options for S. maltophilia infections, although resistance to these first choice antibiotics is already emerging. Antimicrobial photodynamic therapy (aPDT) is a modern biophotonic technology which can be used as alternative to antibiotics or in combination with them. However, there is no information about synergistic antibiotics and aPDT approach used for S. maltophilia inactivation.

OBJECTIVES

To investigate antibiotic and photodynamic therapy synergism in inactivating clinical S. maltophilia isolates.

METHODS

Two clinical multidrug-resistant S. maltophilia isolates were used in this pilot study. In order to identify conditions needed for partial bacteria growth inactivation different TRP-SFX (0.5-8mg/L) and CIP (0.5-4mg/L) concentrations were used. Natural photosensitizer chlorophyllin concentrations (1.5-150µM) and illumination time (0-30min) used for aPDT (402nm LED light, irradiance 35W/cm2) inactivation were tested. Inactivation effects using combinations of sublethal antibiotics concentration and aPDT conditions were evaluated. Bacterial growth and viability were evaluated using optical density measurements and counting colony forming units.

RESULTS

Sublethal TRP-SFX and CIP concentrations needed for S. maltophilia inactivation were identified. Optimal illumination time and photosensitizer concentration for aPDT were selected. Both CIP and TRP-SFX together with chlorophyllin-based aPDT were shown to work synergistically and reduce bacterial growth more efficiently than antibiotic and aPDT separately. Results indicate that combination of first choice antibiotics TRP-SFX and CIP with chlorophyllin-based aPDT can be used for the inactivation of antibiotic-resistant S. maltophilia isolates.

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1081 / VIRULENCE IN BACILLI ISOLATED FROM A ROMAN AMPHORA

Keywords: *Bacilli*

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Eight still viable bacterial strains belonging to the Bacillus genus have been isolated from a Roman amphora dating back to the IV-V century found still intact and sealed in an archaeological site of Aquileia. The whole-genome sequencing of these bacteria was then carried out in order to precisely identify the correct taxonomy and in order to identify the presence of possible genetic differences with the current bacteria. Furthermore, following the functional annotation of the genome, factors of antibiotic resistance and virulence have been identified.

The identification of these factors in bacteria dating back to an era where the use of antibiotics was not yet widespread, is important in order to be able to reconstruct the evolution of antibiotic resistance over time. While some strains were found to be comparable with isolated bacteria in the modern era, some bacteria were found to be unclassifiable with current standard strains by comparing them with ANI and dDDH.

1134 / DIFFERENCES IN THE PRETERM INFANTS GUT MICROBIOME IN THREE AUSTRIAN NEONATAL INTENSIVE CARE UNITS

Keywords: *premature infants, gut microbiome, neonatal intensive care units*

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BACKGROUND

The first weeks in life are crucial for the development of the infant´s microbiome. For preterm-born infants, the neonatal intensive care units' (NICU) environment including nutrition and medication are mainly shaping their microbiome. The influence of these factors was compared in 56 infants born in three Austrian NICUS following different regimens of prophylaxis against necrotizing enterocolitis (NEC) regarding medication, probiotics, and nutrition (1).

OBJECTIVES AND METHODS

The gut microbiome developments during the first three weeks of life was investigated beyond 16S rRNA gene amplicon-sequencing by shot-gun metagenomic and metabolomic analysis.

RESULTS

Our results indicate that the different regimens lead to great differences between the gut microbiomes, with samples from one NICU being highly divergent. Differences could be observed in composition, development and functional profiles of the gut microbiomes. For example, alterations in the metabolism of human milk oligosaccharides (HMOs) could be observed and matched to metabolomic data.

Furthermore, multiple antibiotic resistances could be detected and correlated with specific bacterial genera. The main genera carrying antibiotic resistance (AR) genes were Escherichia-Shigella, Streptococcus and Enterococcus, whereas the probiotic genera Bifidobacterium and Lactobacillus did hardly carry any ARs.

By evaluating relative abundances and replication rates a higher colonizing potential of probiotic Bifidobacterium was observed compared to probiotic Lactobacillus.

Beyond the bacterial part of the gut microbiome here but also the viral, fungal, and archaeal parts respond to the different regimens.

Our results contribute to a better understanding of the impact of NEC regimens on the overall preterm infants' gut microbiome development.

ACKNOWLEDGEMENTS/REFERENCES

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1140 / EFFECT OF FOOD SUPPLEMENTS ON GUT MICROBIOTA IN OBESE PATIENTS: PRELIMINARY RESULTS OF ZIMBA TRIAL

Keywords: *Gut microbiota, Food supplement, Pediatric obesity, Zinc*

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BACKGROUND

Human bodies harbor a diverse community of microbes that together compose the human microbiota. Non-digestible carbohydrates can highly modify the composition and function of gut microbiota. Prebiotics are a group of nutrients that are degraded by gut microbiota and their relationship with human health has been an area of increasing interest in recent years.

OBJECTIVES

Zimba project is a placebo-controlled clinical trial that study the efficacy of the association of Zinc, Myoinositol and GOS in respect to the administration only GOS (placebo) in pediatric obesity.

METHODS

DNA was extracted from stool using the DNeasy PowerFecal Pro kit. The bacterial 16S DNA libraries were prepared using the Microbiota solution B kit. The amplicon pool was sequenced using the Nano Kit v2 kit on MiSeq platform. Raw sequences were processed by MicrobAT software. Statistical analysis was performed using both MicrobiomeAnalyst and R softwares.

RESULTS

The preliminary results on microbiota characterization of 27 enrolled patients showed a decrease in biodiversity in active treated patients in respect to placebo ones, probably due to Zn. Moreover, the prebiotic intake induced a significant increase in Firmicutes, essential for a healthy microbiota homeostasis, such as Dorea longicatena, Clostridium colstridioforme and Faecalibacterium sp. and the decrease in Bacteroidetes. Concluding, further analyses linked to the clinical and metabolic responses will be necessary to validate the proposed mechanisms at the base of the microbiota modulation.

1144 / EVOLVABILITY OF ESCHERICHIA COLI BACTERIA WITH DIFFERENT FITNESS BACKGROUND

Keywords: *systems biology, origin of replication, adaptability, multi-omics*

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Adaptation through the fitness landscape may be influenced by the gene pool or expression network. However, genetic factors that determine the contribution of beneficial mutations during adaptive evolution are poorly understood. In this study, we show that the fitness gains of two Escherichia coli strains with different fitness backgrounds converge even during a short period of experimental evolution. Populational genome sequencing revealed various mutations with different allele frequencies in evolved populations.

When we introduced the mutations to the ancestral E. coli strains, beneficial effects tended to be lower in the ancestor with higher initial fitness. Replication rate analysis showed that the replication index does not correlate with the growth rate. Besides, the degree of transcriptional change was proportional to the fitness gain. Thus, the evolutionary trajectories of bacteria with different fitness backgrounds can be complex and counterintuitive. Notably, transcriptional change is a major contributor of diminishing returns epistasis.

ACKNOWLEDGEMENTS/REFERENCES

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1150 / DETERMINATION OF HORTICULTURAL FUNGAL PATHOGENS SENSITIVITY TO PLANT OILS

Keywords: *Alternaria, Botrytis, Colletotrichum, sensitivity*

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Horticultural plants and production are especially favorable for fungal infections. Some of the most severe and widespread horticultural pathogens are Botrytis spp., Colletotrichum spp. and Alternaria spp. Fungi of mentioned genera have a broad host range and are economically crucial on berry fruits and vegetable yields.

In the context of new pesticide reducing strategies, the development of efficient and environmentally friendly crop protection approaches is demanded. Substances extracted from plants may help avoid problems caused by fungicides, such as environmental pollution or pathogen resistance. Thus, in our study, we aimed to determine the susceptibility of Botrytis cinerea, Colletotrichum acutatum, and Alternaria species to plant extraction products. Hydrodistillation and subcritical CO2 extraction were used to produce the oils from various plants.

Experiments were conducted by adding tested oils to potato dextrose agar medium at 200-1800 µL L-1 concentrations and inoculating Petri dishes with mycelium plugs. Incubation temperature was at 22-25°C, depending on the pathogen. Biological parameters of the pathogens were determined after incubation.

The result revealed that the highest sensitivity of horticultural pathogens C. acutatum, B. cinerea, and Alternaria spp. to thyme oil was determined as this oil completely reduced pathogens’ colony growth and sporulation under laboratory conditions. Meanwhile, fungi sensitivity to other plant oils, like coriander, clove, and sage varied and depended on the investigated concentrations. Summing up the results, plant oils that cause fungi sensitivity can be considered for further investigation in vivo.

1152 / EVOLUTION TOWARDS PATHOGENICITY: PHYLOGENOMIC INSIGHTS INTO BRUCELLACEAE

Keywords: *Pseudochrobactrum, Proteobacteria, Evolution, Brucellaceae*

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BACKGROUND

Brucellosis, an infectious disease caused by Brucella spp., is one of the most extended bacterial zoonosis worldwide. Brucella belongs to α-Proteobacteria, which includes environmental bacteria as well as others that have evolved to stealthy intracellular pathogens, such as Brucella. Gaining knowledge into this evolution is crucial for a better understanding and control of the disease.

OBJECTIVES

To understand the genomic differences between the true pathogen Brucella, and Ochrobactrum and Pseudochrobactrum, phylogenetically close genera that are mainly free-living bacteria. The comparison was focused on P. algeriensis(1), a recently described Pseudochrobactrum species isolated from lymph nodes of cows, a natural host of Brucella.

METHODS

Genomes were sequenced using PacBioRSII, assembled by GenoScreen and annotated with RASTtk. Bioinformatic tools available in PATRIC were used. Lipid, lipopolysaccharide (LPS) and cationic peptide resistance profiles were performed following previously described procedures(2,3).

RESULTS

Genomic analyses suggest that Pseudochrobactrum, as Ochrobactrum, conserves the lipidA structure of Brucella LPS, a trait that could delay immunity activation. In addition, Pseudochrobactrum contains putative genes for lipid modifications, probably explaining their resistance to cationic peptides. Finally, P. algeriensis, unlike other species, presents the genomic capacity of producing a rhamnose based LPS, characteristic of ancestral Brucella, and conserves part of the operon coding for the T4SS, a mechanism involved in intracellular multiplication of Brucella. This work supports the notion that LPS and lipid modifications might be evolutionary conserved mechanisms of resistance in Brucellaceae. Furthermore, these results find out P. algeriensis as a good model for studying the niche adaptation in Brucellaceae.

ACKNOWLEDGEMENTS/REFERENCES

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1154 / IMPROVING THE CURRENT BRUCELLOSIS VACCINE REV1 BY CORRECTING ITS STREPTOMYCIN RESISTANCE

Keywords: *Brucella , Vaccine, Streptomycin*

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BACKGROUND

Brucella melitensis, the etiological agent of ovine and caprine brucellosis, is the major cause of human brucellosis. Animal vaccination is essential for its control and eradication. However, B.melitensis Rev1 (the only effective vaccine in small ruminants) is abortifacient when applied to pregnant sheep, virulent to humans and resistant to streptomycin (Strp), antibiotic of choice for treating human brucellosis.

OBJECTIVES

To understand the mechanisms of resistance of the Rev1 vaccine to streptomycin and to correct this resistance to obtain a safer vaccine.

METHODS AND RESULTS

Strp resistance in bacteria is associated with mutations in 16SrRNA and proteins of the 30S ribosomal subunit and with 16SrRNA modifying enzymes, thus we compared the corresponding genes in Rev1 and B.melitensis16M (wild-type and Strp-sensitive [Strps]). We identified point mutations in Rev1 rpsL (encoding the 30S-S12 protein) and rsmG (encoding a methyltransferase acting on G527 of 16SrRNA) leading to P91L and P81R changes, respectively.

We demonstrated the involvement of the mentioned mutations in Rev1 Strp resistance by inserting the corresponding B.melitensis16M genes into Rev1. A Rev1ΔrsmG::Tn7BmersmG construct (Rev1StrpS), carrying the 16MrsmG, was selected due to its Strp sensitivity and in vitro stability. Rev1StrpS showed attenuation in BeWo trophoblasts and THP-1 monocyte-derived-macrophages. In mice, Rev1StrpS protected as well as Rev1 against B.melitensis, showing less residual virulence and abortifacient effect. In pregnant sheep, Rev1StrpS did not cause abortions or perinatal deaths, nor was it excreted. Thus, Rev1StrpS is a potential vaccine against ovine brucellosis, safer than Rev1, whose protective efficacy in ruminants needs to be evaluated.

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1175 / THE MICROBIOME AND THE SENSE OF SMELL: A STORY ABOUT THE ROLE OF OUR MICROBIAL INHABITANTS IN OLFACTORY PERFORMANCE.

Keywords: *microbiome, olfaction, nose*

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BACKGROUND

The ability to smell is mediated by olfactory neurons in the ceiling of the nose, which is inhabited by numerous microorganisms. In general, microbes are intertwined with human health and disease by communicating with human body cells.

The loss of smell is an incisive event, caused by mechanical impact, infection, disease, or occurs subtly during ageing. It affects psychological, social and behavioral performance and impacts the quality of life tremendously.

In this study, we assume that the nasal microbiome plays not only an important role in olfactory function and dysfunction but also in olfactory.

OBJECTIVES

We aim to identify and understand the role of the microbiome in olfaction to increase therapeutic opportunities and by that reduce the burden of olfactory dysfunction and increase life quality again.

METHODS

This study compares the nasal and stool microbiome of normosmic (normal olfaction) and dysosmic (impaired olfaction) participants via 16S rRNA gene sequencing. Specific samples were selected for further metatranscriptomic and metabolomic analyses. Additionally, these microbiome measures are correlated with neuroimaging biomarkers to further investigate the interaction of the nasal microbiome with olfactory performance.

RESULTS

In general, first analyses of the microbial diversity and composition but also dietary data reveal trends as well as significant differences based on the olfactory performance of the participants. Interestingly, alterations in these results were observed by looking at intact cells only via propidium monoacid treatment (PMA). Together with functional MRI scans of the brain, these findings extend and support the work of our published pilot study (Ref1).

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Abstract Image: nasal mucus sample, red arrows: bacteria-like structures, unpublished image by C. Kumpitsch

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The authors declare that they have no competing interests.

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1520 / EMERGENCE AND RE-EMERGENCE OF VECTOR-BORNE DISEASE IN WESTERN BALKAN AND SERBIA - GLOBAL HEALTH PROBLEM AND POSSIBLE SOLUTIONS

Keywords: *parasitology, vector-born disease, epidemiology*

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Vector-borne parasitic diseases, in recent years have emerged or re-emerged worldwide causing global health and economic problems that involve humans, livestock, companion animals and wild life. The ecology and epidemiology of vector-borne diseases are affected by the interrelations between comprising the pathogen, the host (human, animal or vector) and the environment.

Global climate change, urbanization and pollution of the environment, large-scale migrations of the population at the global level have significantly affected the prevalence and biodiversity of vectors of these diseases. In recent years, the presence of many vector arthropods and insects has been recorded in the Western Balkans, which were not established earlier. Insects of the genus Culicoides were identified along with the advent of BTD.

A large number of new species of ixodid ticks have been identified, along with the appearance of anaplasmosis and ehrlichiosis in animals and humans in the first decade of the 21st century. At the same time, the presence of Aedes albopictus was consequent outbreak of the West Nile virus. The return of Phlebotomus sp. caused epidemics of leishmaniasis again. These data point to the need for wider monitoring of vectors, monitoring the movement of vector-borne diseases and the establishment of multidisciplinary research by physicians, veterinarians, biologists and other professionals involved in the chain of control of these diseases. Also, international organizations and government agencies in cooperation with research institutions should be involved in the fight against these diseases and implement a coordinated global approach to the prevention of vector-borne diseases.

1536 / CHANGES IN THE COMPOSITION OF RAT PERITONEAL CELLS AND THEIR RESPONSE TO STIMULATION BY SELECTED GUT MICROBIOTA DURING COLITIS

Keywords: *microbiota, experimental colitis, E.coli*

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BACKGROUND

Deregulation of the immune response to microbiota or pathogens, and increased intestinal permeability have been proposed as disease-driving mechanisms in colitis. Since peritoneal macrophages guard the sterility of peritoneal cavity from bacterial leakage from the gut, it is plausible to assume that peritoneal macrophages are involved in colitis development.

OBJECTIVES

The objective was to investigate changes in the composition of peritoneal cells and their response to stimulation by selected gut microbiota during colitis.

METHODS

Seven days following induction of colitis with intrarectal instillation of ethanol or trinitrobenzenesulfonic acid (TNBS, 10mg/kg or 40mg/kg), peritoneal cells of Dark Agouti (DA) rats were isolated and subjected to flow cytometry. The amount of IL-6 and TNF-α produced by adherent cells was determined by ELISA following in vitro stimulation with LPS and commensal E.coli and Enteroccocus spp.

RESULTS

Instillation of ethanol or TNBS (10 and 40 mg/kg) increased the proportion of CD11bintCD4low monocytes and decreased the proportion of resident CD163+MHCIIlo macrophages and CD163-MHCIIhi macrophage/dendritic cells. In vitro treatment with Enterococcus spp. was superior over LPS and E.coli in increasing macrophage TNF-α release in all but saline-injected control rats. In vitro treatment with E.coli exceeded the level of LPS stimulation in inducing macrophage IL-6 release in saline- and ethanol-injected rats. It may be concluded that changes in the composition of peritoneal cells during colitis and, subsequently, their selectively altered response to gut commensals may perpetuate or modulate inflammation during disease development (Supported by Ministry of Education, Science and Technological Development Republic of Serbia, Contract 451-03-9/2021-14/200177).

1537 / THE HUMAN GUT VIROME USING OXFORD NANOPORE SEQUENCING TECHNOLOGY IN TWO INDIGENOUS POPULATIONS FROM MALAYSIA

Keywords: *Virome, Metagenomics, Nanopore, nan*

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The human gut contains a complex microbiota dominated by bacteriophages but also containing other viruses and bacteria and fungi. There are a growing number of techniques for the extraction, sequencing, and analysis of the virome but currently no standardized protocols. This study established an effective workflow using the Oxford Nanopore Sequencing Technology for virome analysis to investigate the virome of stool samples from two understudied ethnic groups from Malaysia: the Jakun and Jehai Orang Asli. By using Oxford Nanopore Sequencing Technology, we successfully captured close to full-length viral genomes.

The virome composition of the two indigenous Malaysian communities were remarkably different from those found in other parts of the world. Additionally, plant viruses found in the viromes of these individuals were attributed to traditional food-seeking methods. This study establishes a human gut virome workflow and extends insights into the healthy human gut virome, laying the groundwork for comparative studies.

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1538 / THE INTRIGUING ROLE OF ANTI-COMMENSAL BACTERIA ANTIBODIES IN SERA OF COLITIC RATS

Keywords: *microbiota, anti-commensal bacteria antibodies, experimental colitis*

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BACKGROUND

A variety of commensal bacterial taxa elicit serum IgA responses resulting in protection against polymicrobial sepsis, whereas anti-commensal IgG may have deleterious role in gastrointestinal and systemic inflammation.

OBJECTIVES

The aim was to determine the systemic levels of specific antibodies directed to autologous E.coli in rats of Albino Oxford (AO) and Dark Agouti (DA) rat strains during colitis.

METHODS

Rats were intrarectally injected with ethanol or trinitrobenzenesulfonic acid (TNBS, 10 or 40mg/kg) whereas controls received saline in the same manner. Sera were tested for the level of anti-E.coli antibodies of IgG1, IgG2a, IgG2b and IgA classes by ELISA.

RESULTS

Both rat strains developed colitis, but the degree of colon necrosis and hyperemia were slightly greater in DA than in AO rats and the survival rate was significantly lower in DA relative to AO rats. Among saline-treated controls, the levels of IgG1, IgG2a, and IgG2b anti-E.coli antibodies were comparable between rat strains, whereas the amounts of IgA were significantly higher in DA compared to AO rats. Development of colitis significantly increased the amount of anti-E.coli antibodies of IgA and IgG2a classes in sera of AO rats, and those of IgG2a and IgG2b classes in sera of DA rats. Hence, mostly beneficial role of IgA and probably deleterious role of IgG2b antibodies directed to commensal E.coli during colitis may be suggested, whereas the role of anti-E.coli antibodies of IgG2a classes remains intriguing. (Supported by Ministry of Education, Science and Technological Development Republic of Serbia, Contract 451-03-9/2021-14/200177).

1555 / INTERACTION OF THE MAP3773C PROTEIN OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS WITH THE DNA OF FERROPORTINA 1 DE RATON

Keywords: *Map337c protein, docking, ferroportin 1*

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Mycobacterium avium subsp. paratuberculosis (MAP), bacteria that causes Johne's disease. The map3773c gene is located in the LSP 15 region in MAP. MAP3773c is a FUR (Ferric uptake regulator) protein, is one of the regulators of iron homeostasis in MAP. The Fur protein is in general a 17–21 kDa polypeptide that acts as an iron-dependent transcriptional regulator that regulates the expression of several iron responsive genes.

They bind to unique sequences (iron boxes) present in the upstream promoter region of their own responsive genes, using ferrous iron as a co-regulator. The affinity of MAP3773c in the iron boxes of regulated regions with CACCTTACAGGCAGAAAAG map sequence and bacteria iron boxes GATAATGAGTCTAATCATTATC. Our objectives: determine in silico and in vitro the affinity/binding of interaction of MAP3773c in iron boxes of map3773c and iron box. Determine in silico and in vitro the binding interaction with FPN 1.

In this study we present an in silico result using docking software to predict the possible outcome and then in vitro studies (EMSA), in result, these regions determined there is a high affinity with bacterias ΔG=-743.24 and the micobacterias ΔG-755.72 . Also, we analyzed the interaction with guest proteins like FPN 1 of mice to determine the role of map3773c in paratuberculosis pathology. In FPN 1 we located two possible boxes, micobacterias and bacteria in the coding region and we found a high affinity for Fur box in bacterias ΔG=-779.5 and Fur box in micobacterias ΔG=-657.54.

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1560 / BIODETERIOGENIC MICROORGANISMS FROM ROMANIAN HERITAGE OBJECTS — COMPLEX CHARACTERIZATION AND ALTERNATIVE MITIGATING SOLUTIONS

Keywords: *cultural heritage objects , biodeteriogenic microorganisms, hydro alcoholic plant extracts*

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BACKGROUND

Biodeterioration of cultural heritage objects is a major problem that requires the rapid development of optimal prevention strategies. Microorganisms involved in biodeterioration are mainly fungi and bacteria which are characterized by highly diversified enzymatic equipment involved in aesthetic/structural alteration.

OBJECTIVES

The aim of the present study is characterization of biodeteriogenic microorganisms isolated from the surface of cultural heritage objects (17th-19th centuries) from the south-central region of Romania and to test the efficacy of eco-friendly solutions as alternative for preventing biotedeteriogenic process.

METHODS AND RESULTS

A total number of 65 fungal and bacterial strains isolated from wooden and stone churches from Transylvania (Arad and Hunedoara counties) were taxonomically identified using Maldi-TOF mass spectrometry and characterized from the perspective of the production capacity of enzymes/ organic compounds involved in biodeterioration.

The alternative solutions based on hydroalcoholic plant extracts (Curcuma longa, Rosmarinus officinalis, Galega officinalis, Rhus coriaria) were tested against selected microbial strains to demonstrate their broad-spectrum of antimicrobial activity against planktonic cells and inhibitory effect upon their biodeteriogenic machinery mediated by enzymes/ organic acids production. The plant extracts composition was determined by HPLC, GC-MS and antioxidant activity by DPPH, CUPRAC, FRAP, TEAC methods. The plant extracts efficiency was correlated with microbial strains taxonomy/ isolation source and the established MIC value was determined between 3,125% and 25%. The plants extracts harbored thiosulfinate, flavonoids and polyphenols as major compounds with anti-adhesive/ anti enzymatic activity.

This eco-friendly solutions described in this study show promising potential for the conservation/ safeguarding of tangible cultural heritage.

ACKNOWLEDGEMENTS/REFERENCES

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1610 / ANTI-BACTERIAL ACTION OF LEGO-LIPOPHOSPHONOXINS: THE ROLE OF HYDROPHOBIC MODULE LENGTH

Keywords: *LEGO-Lipophosphonoxins, antibiotic resistance, membrane active compounds*

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Over the past two decades there has been a dramatic increase in antibiotic resistance of many bacterial strains. Antibiotic resistance originates mostly from bacterial tendency to mutate or from horizontal gene transfer. However, the bacterial membrane is less prone to such changes, thus being promising anti-bacterial target. Based on that, we developed membrane active compounds, LEGO-Lipophosphonoxins (LEGO-LPPOs), by assembling simple building modules together. According to our previous results with earlier generations of LPPOs, these molecules have a great potential in antibacterial treatment1.

The aim of our research was to investigate the action mechanism of three chosen LEGO-LPPOs molecules which mutually differ in length of their hydrophobic module by one C atom. The molecule with longest hydrophobic module proved to be the most active. We determined minimum inhibitory concentrations (MIC) on Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacteria and quantified the effect of LEGO-LPPOs on membrane potential by using voltage-sensitive fluorescence probe DiSC3(5).

Next, we studied permeabilizing LEGO-LPPOs activity on living cells by measuring the influx of propidium iodide (PI). Lastly, we examined pore-forming activity on planar lipid membranes. We observed slower membrane permeabilization of E. coli with MIC value about four times higher than for S. aureus. That implies that the outer membrane might electrostatically entrap the LEGO-LPPO molecules and prevent their partitioning into the cytoplasmic membrane. We suggest LEGO-LPPOs form membrane active oligomers that are stimulated by high negative membrane potential mostly in growing bacteria.

ACKNOWLEDGEMENTS/REFERENCES

REFERENCE

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1677 / DEVELOPMENT OF PHOTOACTIVE SUPRAMOLECULAR ASSEMBLIES WITH MACRO-N-BIS-HETEROCYCLIC BIS-IMIDAZOLIUM STRUCTURE: NEW PHOTOTHERAPY T

Keywords: *phototherapy, anti-infectious, bacteria, virus*

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BACKGROUND

The development of photoactive molecular systems, with application in phototherapy, opens the way to the treatment of many pathologies, including those of infectious origin – bacterial or viral, using light. Our recent work carried out in the laboratory, concerning the preparation of potential macro-N-bis-heterocycles bis-imidazolium ligands NHC (N-heterocyclic carbene) in coordination with transition metals (Ag (I)), has shown interesting intrinsic photophysical and biological properties of these molecules.

OBJECTIVES

The objective of this work is the development of original photosensitizers based on original N-macro-bis-heterocyclic carbene ligands and the metal cation complexes of isolated compounds. The focus of the biological characterization of the compounds is placed on the evaluation of the anti-infectious properties of the molecules, in presence or absence of activating irradiation.

METHODS

We evaluated the antibacterial and antiviral properties of the compounds, as well as the evaluation of their impact on the host cells. The photo-physical (fluorescence emission, 1O2 production, photoacoustic properties), the physico-chemical and structure-activity relationship properties were also analyzed.

RESULTS

We synthesized novel water soluble homoleptic bis-heterocyclic bis-imidazolium macrocycles with bipyridine or bithiazole units (L1-L6), as well as interesting reaction intermediates and derivatives, for direct application in anti-infectious phototherapy (aiPT). The compounds have shown promising bioactivity, particularly interesting in the case of the complexes with transition metals. These observations concern particularly the production of singlet oxygen (1O2), the yield of which can reach 84% for some of the macrocycles obtained, as well as the antibacterial activities evaluated on Gram-positive and Gram-negative pathogenic bacteria.

1679 / VIRULENT AND PROBIOTIC ESCHERICHIA COLI STRAINS HAVE DIFFERENT MOTILITY PATTERNS AND SURFACE ADHESION

Keywords: *motility patterns, Escherichia coli, adhesion*

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BACKGROUND

Bacterial motility provides the ability for bacterial dissemination and surface exploration, apart from a choice between surface colonisation and further motion. It is assumed that microorganisms that have the same size and structural characteristics should possess similar movement patterns.

OBJECTIVES

We characterized the movement trajectories of pathogenic and probiotic Escherichia coli strains (ATCC43890 and M17, respectively) at the landing stage and its correlation with adhesion patterns and efficiency. A poorly motile strain JM109 was used as a control.

METHODS

Bacteria were injected into microfluidic chamber 30 µm in height to make videos. The obtained videos were processed using software Plasma-4.0 to reconstruct bacterial trajectories. The adhesion efficiency was estimated as an amount of adhered bacteria to a plastic (and HEp-2 cell) surface. Microphotographs of the attached bacteria were made to investigate the adhesion patterns.

RESULTS

The motion behavior near surfaces drastically varied between the strains, correlating with adhesion patterns. We consider two bacterial strategies for effective surface colonization. The horizontal strategy demonstrated by the M17 strain is characterized by collective directed movements within the horizontal layer during a relatively long period and non-uniform adhesion patterns, suggesting co-dependence of bacteria in the course of adhesion. The vertical strategy demonstrated by the pathogenic ATCC43890 strain implies the individual movement of bacteria mainly in the vertical direction, faster transition from bulk to near-surface swimming, and independent bacterial behavior during adhesion, providing uniform distribution over the surface.

1683 / SYNONYMOUS MUTATIONS IN THE GENE ENCODING THE RNA CHAPERONE HFQ AFFECT FITNESS, YIELD AND DUPLICATION RATE OF ESCHERICHIA COLI

Keywords: *codon usage, fitness, Hfq, transcriptomic*

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BACKGROUND

The genetic code is redundant, which means more than one codon can decode the same amino acid (synonymous codons). The choice of synonymous codons in each gene is not random, since it can affect efficiency of translation, folding and levels of the encoded protein. In bacteria, translation is regulated partly by sRNA-mRNA interaction in which the RNA chaperone Hfq plays a crucial role specially in stress response. In E. coli while hfq mRNA is biased to optimal (preferred) codons, regions of mRNAs that interact with sRNAs have a bias for low usage (non-preferred) codons (1).

OBJECTIVES

Evaluate the effect of codon bias in Hfq. We carried out the replacement of optimal to non-optimal codons in certain regions of the gene encoding Hfq in E.coli strain BW25113.

METHODS

Based on the ribosome profile of hfq mRNA, two regions were randomly mutated and the mutant genes were replaced in the genome of E. coli giving rise to a collection of mutants.

RESULTS

Analyses of the mutant cells revealed that replacement of codons affected the fitness, growth rate and yield of the cells. Thus, natural codon bias for optimal codons in hfq not necessarily implies that the function of the protein is optimized. Probably other effects of the synonymous codons in the gene expression are taking place. For instance, the level of the mRNA was affected in some mutants without affecting protein levels. Transcriptomic analysis of mutant cells as well as functional and structural properties of mutant Hfq are in progress.

ACKNOWLEDGEMENTS/REFERENCES

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1721 / A COMPARISON OF STANDARD VS CRUDE RNA EXTRACTION PROTOCOLS FOR DETECTION OF SARS-COV-2 ACROSS TWO COMMERCIALLY AVAILABLE RT-QPCR

Keywords: *SARS-CoV-2, RT-qPCR, extraction*

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BACKGROUND

Covid-19 has emerged, spread and become a global health problem. Rapid detection and isolation of SARS-CoV-2 positive individuals from public is vital. In this sense, extraction procedures have a key role in the duration and efficiency of SARS-CoV 2 Rt-qPCR testing. In this study, two RT-qPCR kits (Bio-speedy and Lilif) and two extraction protocols (complete RNA extraction (COEx) and crude extraction (CREx)) were compared across various parameters by establishing an experimental setup.

OBJECTIVES

The aim was to analyse the effects of different extraction procedures on the outcome of SARS-CoV-2 diagnostic tests.

METHODS

RNA extraction was performed on clinical and quality control samples both by COEx and CREx protocols, followed by validated RT-qPCR tests by using Lilif and Bio-speedy kits. Each sample undergone 4 types of setups (CREx-Bio-speedy vs. COEx-Bio-speedy vs. COEx-Lilif vs. CREx-Biospeedy). Ct values, amplification efficiencies and diagnostic performances of two extraction protocols and kits were analysed and compared.

RESULTS

Amplification efficiency of the kits, which is desired to be between 90-110%, were found to be 107.86% and 82.23% for Lilif and Bio-speedy, respectively. Despite statistically significant differences between mean Ct values (T=6.411, p=0.000, 95% CI of the difference= 3.461 to 6.577), there were no difference in the final diagnostic outcome when two suggested protocols were compared (CREx/Bio-speedy vs. COEx/Lilif). Comparative analysis of Ct values obtained from four experimental set-ups indicates comparable reliability of the CREx and COEx protocols with specifically designed RT-qPCR kits. However, CREx was found to require lower hands-on-time, cost and laboratory workload.

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1761 / PEER COMMUNITY IN: A FREE ALTERNATIVE TO EVALUATE, VALIDATE (AND PUBLISH) PREPRINTS

Keywords: *open science, open access, transparent publication, peer reviewed*

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The Peer Community in (PCI, https://peercommunityin.org) project offers an alternative to the current system of publication - which is particularly expensive and not transparent. PCI is a non-profit scientific organization building communities of researchers handling the evaluation (through peer-review) and recommendation of preprints in their scientific field. Each PCI is a group of several hundred recommenders, acting as editors, recommending preprints based on peer-reviews making them complete, reliable, and citable articles, without the need for publication in 'traditional' journals. Evaluations and recommendations by a PCI are free of charge.

The preprint recommendation is a citable, referenced, article discussing the study, and is published along with all the editorial correspondence (reviews, recommender's decisions, authors' replies) on the PCI website. The preprint itself remains on the original preprint server and can be submitted to any journal, including one of many PCI-friendly journals accepting the PCI review process as their own. Alternatively, authors can publish cost-free in the newly established "Peer Community Journal", an open access diamond journal launched in 2021.

The first Peer Community In was founded in 2017: Peer Community in Evolutionary Biology (PCI Evol Biol). To date there are 14 PCIs, including PCI Infections, PCI Ecology, and PCI Genomics, offering a publication outlet to scientists working in different fields of microbiology. More than 1200 scientists from around the world have already joined as PCI recommenders. The PCI initiative won the 2020 LIBER award for library innovation of the European League of Research Libraries.

1827 / THE GLOBAL POINT PREVALENCE SURVEY (PPS) OF ANTIMICROBIAL CONSUMPTION AND RESISTANCE: RESULTS OF ANTIMICROBIAL STEWARDSHI PROGRAM ON SURGICAL PROPHYLAXIS FROM 2017 TO 2021 IN MONTENEGRO- LONGITUDINAL STUDY

Keywords: *antimicrobial consumption, hospital, point prevalence survey, quality indicators*

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BACKGROUND

Montenegro is one of the developing European countries, with high consumption of broad-spectrum antibiotics. The national antimicrobial stewardship program was targeted on education of healthcare workers and general population, but it was partially aborted because of COVID19 pandemic. Global PPS allowed us to follow an improvement in antimicrobial prescription in Montenegrin hospitals through indications, such as surgical prophylaxis, which is generally one of the commonest.

METHODS

PPS in Montenegro was conducted once yearly, in 2017 in 8 Montenegrin hospitals and in 2021 in 10 Montenegrin hospitals. The survey included all inpatients receiving an antimicrobial on the day of PPS. Data collected included details on the antimicrobial agents, reasons and indications for treatment as well as a set of quality indicators. A web-based application is used for data-entry, validation and reporting as designed by the University of Antwerp (www.global-pps.com).

RESULTS

Data included 1019 adult and 181 paediatric patients in 2017, and 799 adults and 177 paediatric patients in 2021, admitted to Montenegrin hospitals and treated with at least one antimicrobial on the day of PPS. Surgical prophylaxis was prescribed in 19.7% (236 patients) in 2017 and 9.9% (97 patients) in 2021. The commonest indications for prophylaxis were gastrointestinal and obstetric or gynaecological (table 1). In 2017 the compliance to guidelines in the treatment of patients at surgery wards was 39.2% and in 2021 74,4% ($\chi 2=3.63$, $p=0.157$).

CONCLUSIONS

Guidelines for adequate antimicrobial use in Montenegro were developed and shared, and the adherence to guidelines improved trough years, but broad spectrum antibiotics are still widely used for surgical prophylaxis. New antibiotic stewardship interventions should help continuation of improvement.

397 / PSEUDOMONAS SPP. VS. TUMORIGENIC RHIZOBIUM SP. – BIOCONTROL OF CROWN GALL DISEASE

Keywords: *Biological control, Pseudomonas, Bacillus, crown gall*

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BACKGROUND

Atypical Rhizobium sp. were identified as a causative agent of crown gall on rhododendron and blueberry in Germany. Considering the lack of efficient measures against crown gall disease, evaluation of innovative biocontrol measures would greatly contribute to sustainable agriculture.

OBJECTIVES

Identification of candidate bacterial strains that could be employed for biological control of the novel crown gall bacteria belonging to the ”tumorigenes” clade.

METHODS

Antimicrobial activity of six antagonistic Pseudomonas and Bacillus strains were tested in vitro and in vivo against pathogenic strain Rhizobium sp. rho-6.2. The in vivo experiment, using co-inoculation and preventive inoculation strategies, was performed in controlled greenhouse conditions on tomato plants as a model system in four replicas and randomized.

RESULTS

The whole cultures of two antagonistic Pseudomonas strains were the most efficient against pathogenic Rhizobium sp. rho-6.2 in co-inoculation strategy with reducing tumor size of 92.86%. Contrary, in the preventive treatment same Pseudomonas strains were less efficient (15.38 and 30.77%). Despite the high in vitro antimicrobial activity of Bacillus strains their in vivo activity was less pronounced in preventive treatment (up to 15.38%), while in co-inoculation strategy was more prominent (42.86%). Consortium based on Bacillus and Pseudomonas strains, was increased biocontrol activity up to 38.6% of tumor's reduction. The selected Pseudomonas strains could be further tested as an alternative strategy for the biocontrol of crown gall disease and potential involvement of quorum quenching mechanism will be determined.

ACKNOWLEDGEMENTS/REFERENCES

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416 / EFFECT OF CHROMOHALOBACTER SALEXIGENS ANJ207 ON THE DIFFERENTIAL EXPRESSION OF SALT STRESS RESPONSIVE GENES IN WHEAT

Keywords: *Wheat transcriptome, Differential expression, Salinity stress responsive genes*

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BACKGROUND

It is generally confirmed that bacteria actively interrelate with plant host and posses beneficial effect on growth as well as allow tolerance to various biotic and abiotic stresses also for their adaptation. But still the mechanism of communication and adaptation to the plant environment by plant growth promoting bacteria are not well known.

OBJECTIVES

In this study we examined the influence of Chromohalobacter salexigens ANJ207 on the growth of wheat plant under the saline stress.

METHODS AND RESULTS

To address the effect of C. salexigens ANJ207 on wheat to salinity stress tolerance, wheat salt sensitive seed variety HD2733 were inoculated with C. salexigens ANJ207 and grown with 100 mM NaCl under sterile condition. Total RNA were isolated and RNA library Prep Kit was used to prepare high quality libraries and paired end sequencing reads of read length 151bp was generated with Ilumina HiSeq-X seqeuncing platform. Differential analysis was performed against control sample vs treated by using raw transcript count and ballgown pipeline. Transcripts with fold change value >1 consider as up regulated, ≤-1 down regulated.

Wheat plants under salt stress showed total 1651 significant gene whereas 273 genes up-regulated and 160 genes down regulated. Many genes from chlorophyll metabolism, and flavonoid pathways were differentially expressed within inoculated plant under salt stress. A total 13 genes for stress responsive transcription factors and secondary metabolites were also detected; which are noticed to take part in biosynthesis of cell wall along with antioxidants.

ACKNOWLEDGEMENTS/REFERENCES

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431 / PHYTOPLASMA GENOTYPE-DEPENDANT EFFECTS ON SPECIALIZED METABOLISM OF GRAPEVINE LEAVES

Keywords: *phytoplasma, specialized metabolism, phenolics, flavescence dorée*

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Phytoplasmas (genus Candidatus Phytoplasma) are a diverse group of bacterial intracellular pathogens causing significant damage in agriculture worldwide. However, due to inability of obtaining a pure phytoplasma culture in vitro, they remain insufficiently investigated. With the aim of studying the interactions between plants and phytoplasmas, grapevine (Vitis vinifera; Vv) and flavescence dorée phytoplasma (FDp; ribosomal group 16SrV), one of the main causative agents of grapevine yellows disease and a quarantine pathogen in Europe, were chosen as a model pathosystem. Leaves of symptomatic and asymptomatic plants from Continental Central Croatia were sampled three times during the development of symptoms. Using triplex real-time PCR assay, infection of Vv by FDp was confirmed in symptomatic plants.

To assess the genetic structure of phytoplasmas, map gene was amplified and sequenced followed by phylogenetic analysis. In order to investigate changes in concentration of specialized metabolites between infected and uninfected Vv leaves, high performance liquid chromatography and spectrophotometric assays were used. Two different mapFD-genotypes (M38 and M54) were identified among analysed samples. Metabolomic analyses revealed a high dynamics of specialized metabolite concentrations (metabolite groups and individual compounds) during the development of infection.

Furthermore, results point to a phytoplasma genotype-dependant effect on specialized metabolism of infected Vv leaves, as suggested by different patterns of change in phenolics concentration in investigated time points between plants infected with two different phytoplasma genotypes. The results contribute to the understanding of grapevine response to infection with different FDp genotypes and reveal potential protective mechanisms of Vv against phytoplasma infection.

475 / ANTIFUNGAL ACTIVITY EVALUATION OF NINE BACTERIAL STRAINS ISOLATED FROM RUBUS ULMIFOLIUS SCHOTT

Keywords: *Botrytis cinerea, Blackberry, Endophytic bacteria, Biopesticides*

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In agriculture, biopesticides have risen as a powerful weapon against phytopathogenic fungi. Those are made of beneficial microorganisms which possess antifungal activity able to limits fungal growth.

In this work, endophytic bacteria were isolated from Rubus ulmifolius Schott and nine strains which showed beneficial effects for plants were selected. Antifungal activity was tested against Botrytis cinerea employing in vitro, in vivo and in silico assays. To further selection of those with better characteristics for developing new biopesticides.

Regarding methodology, several in vitro assays were performed to detect different antifungal activities, including production of siderophores, lytic enzymes, volatiles compounds and direct attack to the fungus. In in vivo assays, tomato leaves and blackberry fruits were singled-inoculated with Botrytis and doubled-inoculated with both fungus and bacteria, to study the bacterial antifungal effect in plants. Finally, genomic DNA was extracted, sequenced by Illumina technique, and analyzed using KofamKOALA, dbCAN and antiSMASH tools. These can identify gene functions, carbohydrate-related genes and secondary metabolites, respectively; allowing a thorough understanding of their genomes.

As results, some strains exhibited a significative reduction in fungal growth. The most remarkable were Bacillus subtilis, Pseudomonas silesiensis and Inquilinus ginsengisoli, showing positive results in all the performed assays. Genomic analysis of these strains found specific related genes, such as bacillaene, a polyene antibiotic; chitinases and β-glucanases, which can disrupt fungal cell wall; and volatile compounds like terpenes.

In conclusion, these strains may be a suitable option for the development of new biopesticides against B. cinerea and their application in blackberry fields.

ACKNOWLEDGEMENTS/REFERENCES

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569 / AN IN-DEPTH CHARACTERIZATION OF THE CARBOHYDRATE-ACTIVE ENZYMES FROM BACILLUS ALTITUDINIS PS213

Keywords: *Microbial Genomics, Carbohydrate-active enzymes (CAZy), Plant biomass degradation*

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BACKGROUND

The need for clean energy, renewable resources, and circular economy has fostered the research in plant biomass degradation (PBD) catalyzed by microorganisms. The bovine rumen is among the best studied environments where PBD takes place. In this organ, the plant cell wall components (a combination of cellulose, hemicellulose and pectin) is enzymatically digested by carbohydrate-active enzymes (CAZymes) secreted by the microorganisms living in it.

OBJECTIVES

In this study, we report the complete circular genome sequence of a Bacillus altitudinis strain PS213, with annotation of the secreted CAZymes encoded in its genome and describe in depth their 3D structure.

METHODS

The genome was assembled using Unicycler from a combination of long and short reads (Illumina MiSeq and ONT). The predicted proteins were submitted to dbCAN to identify the putative CAZy. The 3D structure of secreted plant cell wall degrading enzymes was estimated using AlphaFold, and compared to experimentally determined structures to infer substrate binding specificity and catalytic mechanism.

RESULTS

The genome assembly resulted in a single contig of length 3.896.825 with GC%=41.2. It encoded 3955 proteins (avg protein length 287.6 aa), resulting in a coding ratio of 87.6%. We found 97 CAZy proteins, including 24 proteins presenting a secretory signal peptide and 21 proteins annotated as hypothetical proteins. The identification and in-depth description of known and novel CAZymes from B. altitudinis PS213 pave the way toward the improvement of plant biomass degradation processes with multiple possible biotechnological applications in the treatments of different wastes, feed stocks and composting materials.

581 / SALINE WATER IRRIGATION LOWER DATE PALM (PHOENIX DACTYLIFERA) ROOT-ASSOCIATED FUNGAL DIVERSITY AND ALTER COMPOSITIONAL PATTERNS

Keywords: *Arid agroecosystem, Date palm (Phoenix dactylifera), Irrigation source, Soil salinization*

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The arid-ecosystem is characterized by higher temperature and evaporation rate with poor water availability. Therefore, the use of saline groundwater for irrigation is rising, which further increases soil salinization. Soil salinity is a critical environmental factor affecting the root-associated fungal communities, playing a critical role in host plant growth. Here, we investigated the impact of saline water irrigation on the date palm (Phoneix dactylifera) root-associated fungal communities using metabarcoding of the ITS2 marker. Root samples were collected from date palm farms located in Abu-Dhabi, where saline and non-saline water were the sources of irrigation. We found that saline water irrigation altered the fungal community composition and affected by the electrical conductivity and pH of irrigated water. Of total 411 OTUs, only 16.7% were shared where 25.3% and 57.9% were strictly found in samples where the irrigation source was saline and non-saline, respectively. The fungal richness was significantly lower where saline water was the source of irrigation. Ascomycota was the most abundant taxa, and their abundance and occurrence were relatively lower where saline water was the irrigation source. In contrast, the proportion of Glomeromycota (arbuscular fungi) was relatively higher where saline water was used for irrigation.

The most abundant OTUs identified as Fusarium delphinoides, Sordariales sp., Magnapothiopsis sp., and Pleosporaceae sp. were completely absent where saline water was the irrigation source and contrasting patterns were observed for Coprinopsis sp. This study strongly corroborates that irrigation with saline water alters the date palm root-associated fungal communities, and specific fungal taxa are favored.

718 / DIVERSITY OF FUNGI AND OOMYCETE SPECIES PREVALENCE IN CROPS IRRIGATION, TREATED WATER AND HYDROPONIC SYSTEM IN KUWAITI FARMS

Keywords: *Fungi and oomycete, irrigation system, oxford nanopore technologies, environmental DNA*

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Kuwait is located in a critical arid environment, and it has limited freshwater resources in addition to scarce amounts of brackish groundwater. Agriculture in this country mainly depends on desalinated water obtained from a sea water distillation factory to satisfy fresh water demands and irrigation with desalinated water is the most common method used for watering crops.

Many crops can get diseased and plant pathogens are estimated to cause around 12.5% of global crop losses. Here we present the use of a rapid advanced method for detection of all possible fungal and oomycete plant pathogenic species in three different water systems used on Kuwaiti farms. The Oxford Nanopore metabarcoding protocol was used to identify and quantify the fungal and oomycete biome of water that was sampled from their irrigation systems: 1) an open water source, 2) a hydroponic system, and 3) treated recycled water.

For all three water systems, the results revealed differences in taxonomic diversity at each level. The open water source system showed the greatest diversity in the identified ITS reads, while the least diverse environment was observed in treated recycled water. Despite the different levels of taxonomic diversity, the largest group in each water irrigation system were fungi from the Orbiliaceae family. This group contains many nematophagous fungi and hence it would be interesting to investigate whether many nematodes are thriving in the water systems and whether these are plant pathogenic species.

722 / UNDERSTANDING THE SURFACE MICROMORPHOLOGY AND CHEMICAL COMPOSITION OF THE EPICUTICULAR WAX OVER THE LEAVES OF XEROPHYTES

Keywords: *Epicuticular wax, wax micromorphology, wax composition*

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BACKGROUND

Plants synthesise wide variety of non-starch polymers having low or no solubility in water, such as the epicuticular waxes. Waxes are essential structural and fundamental element composed of very long chain hydrocarbons including n-alkanes, aldehydes, esters, alkanols, etc, which impart hydrophobicity, water impermeability, making the surface superhydrophobic. Similar to plant-based polymers, synthetic plastics also exhibit similar properties such as water insolubility, durability, and resistance to physical, chemical, or biological degradation. Since plastics are derived from crude oil, the chemical bonds linking the monomeric units of the plastics are found to be similar to natural polymers.

OBJECTIVES

The study aims to examine the micromorphology of epicuticular wax and chemical composition of two xerophytic plants namely, Salvadora persica and Calotropis procera. Since, the cuticle components show chemical similarities to plastics, this study allows to build a hypothesis that phylloplanes of cuticle-rich plants could be explored for the isolation of phytopathogenic microbes towards their plastic degradation ability.

METHODS

Scanning electron microscopy (SEM), Confocal microscopy and gas chromatography with mass spectroscopy (GC-MS/MS) were used for characterizing the morphology and chemical composition of the epicuticular wax of selected plants.

RESULTS

SEM established platelet and granule forming crystalline microstructure with trichomes over the surface of S. persica and C. procera, respectively. Confocal microscopy displayed the presence of cuticle as a distinct layer with thickness of 5 µm and 10 µm for S. persica and C. procera, respectively. GC-MS/MS analysis showed long chain alkanes as a major constituent of the wax for both the plants.

ACKNOWLEDGEMENTS/REFERENCES

Facilities provided by the Birla Institute of Technology & Science, Pilani, Pilani Campus are gratefully acknowledged.

742 / INFLUENCE OF GRAPEVINE PHENOLOGICAL STAGE AND VIRUS INOCULUM COMPOSITION ON THE REPLICATION OF GLRAV-3

Keywords: *GLRaV-3, grapevine, relative expression*

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BACKGROUND

Grapevine performance can be impacted by more than 80 viruses and virus-like agents, with grapevine leafroll-associated virus 3 (GLRaV-3), as one of the most dangerous viruses affecting grapevine performance [1]. Mixed infections occur naturally in grapevine populations and GLRaV-3 is usually accompanied by other viruses. Impact of grapevine viruses on GLRaV-3 replication is still unknown due to lack of studies on this subject.

OBJECTIVES

Relative GLRaV-3 quantification in grapevine of different phenological stages inoculated with GLRaV-3 in combination with other grapevine viruses.

METHODS

Four inoculums containing GLRaV-3 accompanied by different combination of other economically important grapevine viruses were inoculated by green grafting on 41 indicator plants during the summer season. Phloem tissue was collected in two different phenophases: the dormancy which occurred five months post inoculation, and the beginning of bud swelling which occurred eight months post inoculation. Total RNA was extracted using a commercial kit followed by the synthesis of cDNA. By using SYBR green qPCR, relative concentration of two genomic GLRaV-3 regions: ORF1a and CP was assessed by normalizing their Ct values with those of housekeeping genes [2].

RESULTS

Relative concentration of GLRaV-3 was generally higher during the dormancy stage, but did not depend on the inoculum composition. It varied significantly in the bud swelling stage where inoculum harbouring the highest number of viruses (six) showed significant differences in comparison to other inoculums. The GLRaV-3 concentration at this stage appears to depend more on inoculum composition and infection duration than on sampling phenophase.

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745 / CULTURABLE SEED, ROOT AND RHIZOSPHERIC BACTERIAL DIVERSITY OF THE VARIOUS SUGAR BEET HYBRIDS

Keywords: *Endophytic bacteria, Microbiome, Sugar beet, Hybrids*

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BACKGROUND

The plant microbiome has been recognized for direct or indirect plant growth capacities, protection of host plants against phytopathogens and ability to increase plant's tolerance to abiotic stresses.

OBJECTIVES

Determination of endophytic bacterial communities in the roots and seeds of various sugar beet hybrids, including their surrounding rhizosphere in order to better understand sugar beet bacteriome and to explore it's plant-growth-promoting abilities.

METHODS

Root, seed and rhizosphere samples from five different sugar beet hybrids (Eduarda, Tibor, Tajfun, Koala and Cercospora-resistant) were used for the isolation of plant-associated bacteria. Different formulations of nitrogen-free selective media were used for the isolation of diazotrophic bacteria.

RESULTS

A total of 140 isolates were obtained from all hybrids, including 14, 26 and 47 unique species from the rhizosphere, roots and seeds, respectively. With a count of 80 isolates in total, seeds were the most bacterially abundant, especially for Koala and Eduarda hybrids. The rhizosphere of all hybrids contains Lysinibacillus macroides, while Bacillus subtilis was unique as a common species for all seeds. Moreover, Curtobacterium pusillum was a unique species for the Cercospora resistant, Eduarda and Koala seeds' hybrids.

No similarities were observed for the isolates from the roots of all hybrids simultaneously. In general, the rhizosphere of all hybrids was dominated by the Bacillus and Lysinibacillus, while roots were abundant in Paenibacillus, Curtobacterium, Mycetocola, Knoellia, Neorhizobium, Microbacterium, Rhodococcus, Massilia, Rathayibacter and Arthrobacter species. The species from genera Bacillus, Lysinibacillus, Kocuria, Sanguibacter, Pantoea, Glutamicibacter, Pseudomonas, Erwinia, Providencia and Pseudoclavibacter were prominent as seed endophytes.

ACKNOWLEDGEMENTS/REFERENCES

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797 / PATHOGENIC BACTERIAL POPULATION CAUSING SOFT ROT ON CABBAGE: CASE STUDY IN FUTOG (SERBIA)

Keywords: *cabbage, soft rot, Pectobacterium*

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BACKGROUND

Futog (Vojvodina, Serbia) is known for the five-century long tradition of cabbage cultivation, which is grown in two forms during the season, summer and autumn. Pectobacteriaceae causing soft rot disease are among the most destructive pathogens, influencing the yield and quality of this crop. These pectolytic bacteria cause maceration of attacked tissues, often followed by specific odor caused by the colonization of saprophytic bacteria that inhabit damaged tissues.

OBJECTIVES

Within this work, we aimed to identify soft rot-causing bacteria observed on two summer cabbage hybrids (Cheers F1 and Hippo F1) grown in Futog in 2021.

METHODS

Crystal Violet Pectate (CVP) medium was used for bacterial isolation from six collected cabbage samples showing soft rot. The pathogenicity of isolates was checked on cabbage heads by making holes at the top and filling them with bacterial suspensions. Multilocus sequence typing (MLST) of cabbage isolates was performed by sequencing of proA and dnaX genes. Additionally, the Neighbour-joining phylogenetic analysis was performed with concatenated sequences.

RESULTS

Isolations resulted in the prevalence of pit-forming colonies on CVP. Water-soaked lesions followed by a specific odor appeared on cabbage heads 24 h after inoculation with all tested isolates, and complete head destruction occurred after seven days. MLST performed with genes proA and dnaX, identified isolates as Pectobacterium carotovorum on hybrids Cheers F1 and Hippo F1 and Pectobacterium versatile only present on Hippo F1, indicating combined infection on this hybrid. On the phylogenetic tree, P. versatile isolates were separated from P. carotovorum isolates, clustered into four groups.

802 / NEW DATA ON PRESENCE OF CANDIDATUS PHYTOPLASMA SOLANI ON POTATO IN VOJVODINA PROVINCE

Keywords: *potato, Candidatus Phytoplasma solani, 16S rRNA*

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BACKGROUND

Bačka (Vojvodina, Serbia) is one of the main potato-growing areas in Serbia. The presence of the stolbur-associated symptoms in the form of reddening and leaf rolling, shortened internodes, and aerial tuber formation were frequently noticed on the potato plants. Therefore, the identification of the causal agent of the disease was initiated, considering that presence of phytoplasmas was not earlier reported in Maglić locality.

OBJECTIVES

This research aimed to identify the causal agent of the phytoplasmas' disease in potato field (unknown cultivar) in locality Maglić (Bačka) in 2021.

METHODS

Samples for the identification within the survey site consisted of pooled multiple diseased plants (leaves and tubers). Genomic DNA from the ten pooled samples was extracted using the Cetyltrimethyl ammonium bromide (CTAB) method. Identification was performed with polymerase chain reaction (PCR) using universal phytoplasma 16S rRNA primer pair P1/P7. The obtained sequences were checked for identity with the deposited strains in the NCBI database using the BLASTn search tool. Further, Neighbour-joining (NJ) tree was made to reconstruct the phylogeny of potato strains in relation to deposited strains in the NCBI database.

RESULTS

PCR amplification performed with primer pair P1/P7 resulted in amplicon generation at 1800 bp for three (two from tubers and one from leaves) out of a total of ten tested samples. BLASTn analysis revealed 100% identity of potato strains with deposited strains of Candidatus Phytoplasma solani, the 16SrXII-A ribosomal subgroup. NJ phylogenetic analysis grouped potato strains in the same tree cluster with NCBI deposited strains of Ca. Phytoplasma solani.

903 / CHANGES IN RHIZOSPHERE MICROBIAL COMMUNITIES OF DIFFERENT PLANT CROPS UPON DIFFERENT APPLICATION OF BIOWASTE ELUATES

Keywords: *Plant biostimulant, Soil biodiversity*

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High-throughput sequencing (HTS) of phylogenetic amplicons can be applied to assess bacterial and fungal composition changes, in response to different treatments. This approach was used to assess the soil microbial composition and allowed the understanding of which components of the bacterial and fungal communities were either stimulated or inhibited by the application of a biowaste eluate obtained from the industrial production of microorganisms.

The eluate was applied in different ratios (0% 5% 30%) along with standard N fertilization practices, in order to reduce the chemical input in crop production. The objective of the research was to evaluate if the eluate applications affected the rhizosphere microbial composition and whether the eluate promoted beneficial microorganisms. The trials were carried out in both greenhouses and fields studying three different crop plants. Soil samples were collected at three different times and the total DNA was extracted.

HTS analysis comprised PCR amplification of bacterial and fungal amplicons, addressing the entire population for both kingdoms. Amplicons were sequenced with Illumina MiSeq technology, and the sequences obtained were analyzed with bioinformatic tools and used to determine microbial α-diversity and taxonomic composition. Results indicate that there is a strong correlation in the microbial community in the same plant group and in the rhizosphere or bulk soils. Other preliminary results obtained from bioinformatic analysis seem to indicate that rhizosphere composition is influenced by the eluate application.

921 / BACTERIAL-BASED BIOFERTILIZER WITH THE CAPACITY TO ENHANCE CROP YIELD AND QUALITY OF WHEAT (TRITICUM DURUM) AND MAIZE (ZEA MAYS)

Keywords: *Biofertilizer, Crop, Plant-Bacteria interactions, Sustainable*

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The challenge of feeding a growing population along with minimizing our environmental impact drives us to develop more sustainable methods to improve crop yields. Cereals are the largest source of carbohydrates, protein, vitamin, and minerals in human nourishment, within them, maize and wheat are two of the most important. In this sense, bacterial based biofertilizers have been proposed as a more sustainable method to increase crop yield and quality (García-Fraile, P. 2015; Menéndez, E., 2017).

Our objective is to formulate a bacterial based biofertilizer that improves crops yield and quality while reducing dependence on inorganic fertilization by substituting or complementing them.

For this purpose, 245 bacterial strains were obtained from lentil and durum wheat soils and roots (La Armuña, Salamanca, Spain). A set of in vitro tests were performed to evaluate isolates PGP potential, including inorganic phosphorus and potassium solubilization and siderophore, biofilms, auxins, and acc-deaminase production. The most promising candidates were chosen for in vivo testing in wheat and maize seedlings. Finally, 3 strains belonging to Bacillus, Azospirillum, and Rhizobium genus were selected as candidates for field experiments.

These candidates were inoculated in wheat and maize seedlings in plots in an experimental field and harvested when dry. Regarding to production, all three bacterial inoculated treatments yields were better than those of unfertilized plots, and similar to those inorganically fertilized. Among quality parameters, results were variable among treatments, but in general parameters such as energy content, grain density or protein content were observed improved when treated with bacterial inoculants.

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932 / SELECTION OF RICE PHYLLOSPHERE BACTERIA TO INDUCE SYSTEMIC TOLERANCE TO THE WATER STRESS IN PLANTS

Keywords: *Rice, Phyllosphere, Drought, Bacillus megaterium PB50*

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This study assessed eight phyllosphere bacterial strains for their direct and indirect plant growth-promoting (PGP) traits and osmotic stress response and tested them in a plant growth experiment. Measured direct PGP traits included the production of phytohormones, such as indole-acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and cytokinin. In addition, hydrogen cyanide and siderophore production and antagonistic activity against the rice foliar pathogens Pyricularia oryzae and Helminthosporium oryzae were assessed as indicators of indirect PGP activity. The bacterial strain's osmotic stress response was evaluated by treating their growth medium with PEG 6000 (11%, 21%, and 32.6%) and measuring bacterial growth as well as IAA, GA, proline, and glycine betaine (GB) contents. Statistical analysis revealed that Bacillus megaterium PB50, Bacillus altitudinis PB46, and Bacillus endophyticus PB3 had higher PGP activities and osmotic stress responses. The best three strains were foliar sprayed on rice plants maintained in pots with physical drought stress for ten days during the flowering stage. Leaf samples from each treatment were collected and analyzed for total sugars, proteins, proline, malondialdehyde (MDA), antioxidant enzymes, phenolics, potassium, calcium, abscisic acid, and indole acetic acid, as well as stress-related gene expression (LEA, RAB16B, HSP70, SNAC1, bZIP23, and AP2/ERF). The results revealed that B. megaterium PB50 altered stress-related biochemical properties and gene expression in rice during drought conditions. Overall, this research suggests that foliar application of B. megaterium PB50 can induce tolerance to drought stress in rice.

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941 / THE ROLE OF WATER IN THE EPIDEMIOLOGY OF TOMATO BROWN RUGOSE FRUIT VIRUS

Keywords: *ToBRFV, water-mediated transmission, epidemiology*

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Viruses have become increasingly important in terms of their incidence and economic impact, causing nearly half of all emerging plant diseases. Currently, one of the most economically damaging viruses is tomato brown rugose fruit virus (ToBRFV), which emerged in tomatoes in Israel and Jordan around 2014. Following its emergence, outbreaks of ToBRFV have been recorded around the world. ToBRFV represents a serious threat to its main host plants, tomatoes and peppers. As for other tobamoviruses, the main route of introduction of ToBRFV into new countries and regions is through infested seeds and infected planting material. Due to its physical and biological properties, ToBRFV is particularly stable and can be easily mechanically transferred to other plants within the same production object. Extreme virion stability enables ToBRFV, once it has entered the greenhouse, to be resilient to eradication using existing disinfection strategies.

RNA of ToBRFV has been detected in wastewaters, rivers, and in irrigation systems. However, water-linked epidemiology of ToBRFV is not yet known and is therefore being investigated as part of our project.

The release of ToBRFV from the plant into irrigation water has been established. We are currently investigating how long ToBRFV remain infectious in water at room temperature and whether ToBRFV from water can infect healthy plants through their roots. This study will fill the knowledge gaps in the epidemiology of the virus and provide a reliable risk assessment to identify critical points for monitoring and control.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by Slovenian Research Agency (grant number L4_3179).

956 / MYCOVIRUSES AS A POSSIBLE ALTERNATIVE TO SYNTHETIC FUNGICIDES AGAINST BOTRYTIS CINEREA

Keywords: *Biocontrol*

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Mycovirus are viruses that infect most phytopathogenic fungi as Botrytis cinerea. Recent studies confirm that some mycoviruses found in B. cinerea can reduce its virulence. This biological phenomenon is called hypovirulence and it makes mycoviruses a promising strategy as antifungal.

The main goal of this study is the detection and the identification of mycoviruses in wild strains of B. cinerea. In second place, we propose the performance of a bioassay to study hypovirulence in these strains. The aim of these trials is to provide information to support the development of alternative mycovirus-based control methods that reduce the pathogenicity of this fungus. To detect and identify mycoviruses, polymerase chain reaction-based assays were performed to identify them on agarose gels. However, this study is very complex due to the lack of highly conserved sequence in these mycoviruses. Furthermore, to test hypovirulence in work strains, two bioassays were performed in model plants infected with spores of the five strains under study.

The presence of mycoviruses was demonstrated in some of the B. cinerea strains. In addition, the results obtained in bioassays were consistent with the conclusion reached from molecular study results. Some results were inconclusive due to the difficulties associated to working with this type of viral material, consequently, massive sequencing techniques are required to successfully identify mycoviruses in B. cinerea.

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991 / EFFECTS OF PLANT PROBIOTICS AND BIOCHAR APPLICATION ON RHIZOSPHERE BIODIVERSITY AND MAIZE GROWTH IN THE FIELD

Keywords: *microbial consortia, biochar, maize, rhizosphere microbiome*

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BACKGROUND

Many efforts are made in recent years in developing new microbial consortia inoculants not only for growing crops sustainably but also for preserving soil health. In this context, the question of whether natural soil microbiomes are negatively or positively affected by adding foreign microorganisms needs to be addressed.

OBJECTIVES

The main objective of the present work is to exploit the potential use of microbial consortia, developed within the frame of the Horizon 2020 SIMBA project, for sustainable crop production of maize and to assess the impact of their application in field on indigenous rhizosphere microbiota.

METHODS

Microbial consortia were applied alone or in association with Arbuscular Mycorrhizal Fungi (AMF) and Biochar in open field under conventional and organic management. The bacterial community composition and diversity in maize rhizosphere soil was investigated at different maize growth stages and with different fertilization levels via Illumina 16S MiSeq sequencing and bioinformatic analysis.

RESULTS

The application of SIMBA's microbial consortia had clear effects on biomass growth, yield and N content of maize, especially at lower fertilization levels while did not significantly affect species diversity and richness of native rhizospheric microbial communities. A significant change in rhizospheric microbiota diversity was found following application of commercial Micosat F1, AMF and biochar as well as increased fertilization level. Overall, our results suggest that SIMBA microbial consortia may be effectively exploited as biofertilizer in sustainable maize cultivation without altering the biodiversity or the resident microbiota, thus removing risks of long-term impacts on natural soil microbial biodiversity.

ACKNOWLEDGEMENTS/REFERENCES

This research was funded by the European Union's Horizon 2020 Research and Innovation Programme under Grant agreement No. 818431 (SIMBA, Sustainable Innovation of Microbiome Applications in Food System).

Annamaria Bevivino acknowledges funding from the Italian project "Creazione di un HUB italiano a supporto della partecipazione dell'Italia alla Global Soil Partnership ed alla rete di eccellenza europea sulla ricerca sul suolo—SOIL-HUB" (D.M. 37072/7303/18—28/12/2018, D.M. 35851—5/112019) and the European Union's Horizon 2020 research and innovation programme under grant agreement no. 652615 (European Joint Programme SOIL).

1058 / LEGUME PLANT PEPTIDES AS SOURCES OF NOVEL ANTIMICROBIAL MOLECULES AGAINST PATHOGENS

Keywords: *Legumes, NCR peptides, antimicrobial activity, nan*

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A major issue currently facing humanity is antibiotic resistance, and multidrug-resistant (MDR) strains are emerging rabidly. While our environment is full of plants that have evolved large numbers of antimicrobial peptide during its evolution, we still did not explore these peptides very well. In legume plants from the inverted repeat lacking clade (IRLC), there is an interesting gene family that encodes nodule-specific cysteine-rich NCR peptides that has a remarkable antimicrobial activity. In our work we aim to investigate the antimicrobial potential of 109 synthetic NCR peptides from M. truncatula, M. sativa, Pisum sativum, Galega orientalis and Cicer arietinum against gram-positive and gram-negative bacteria and Candida albicans.

We chemically synthesised the NCR prptides. Antimicrobial activity assays were done to detect the minimal bactericidal and fungicidal concentration of these NCRs. Several assays were performed to study the potential of these peptides beside the mechanism of action of it, such as Biofilm inhibition assay, membrane permeability assay, In vitro transcription/translation assay and gel retardation assay. And finally Hemolysis assay on human blood cells was conducted

Our findings indicate that:

- the Antimicrobial activity of NCR peptides requires net positive charge and depends on the amino acid sequence.

- NCRs inhibit biofilm formation and degrade biofilms of C. albicans.

- NCR peptides cause membrane permeability and cell death.

- NCR peptides have no or only weak effects on bacterial translation.

- NCR peptides can form complexes with DNA.

- Hemolysis Assay did not reveal toxicity of the tested legume NCR peptides.

ACKNOWLEDGEMENTS/REFERENCES

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1070 / METAGENOMIC AND CULTUROMIC ANALYSIS IN BLACKBERRY AND BLUEBERRY PLANTS

Keywords: *Metagenome, Blackberry, Blueberry, Core Microbiome*

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Microbial biodiversity plays an important role in agriculture since microorganisms help plants to develop and maintain soil quality. In fact, the higher the soil biodiversity is, the higher is crops efficiency.

The objective of this work is to study the microbial biodiversity associated to blackberry and blueberry, from both rhizosphere and roots, to unravel plant-bacteria interactions, with the aim of developing new biofertilizers that help to achieve a more sustainable agriculture.

Regarding methodology, total DNA was extracted from rhizosphere and roots from both blue- and black-berry samples and 16S rRNA gene region was massive sequenced. Amplicon-based metagenomes were processed with QIIME2. Sequence quality filtering, microbiome composition and diversity analysis were performed with this program. Additionally, endophytic and rhizospheric bacteria were cultured, selected and isolated to further identification, which was accomplish by using MALDI-TOF analyses and 16S rRNA gene based identifications.

Metagenomics analyses showed that rhizosphere is higher diverse than roots, which possess an enrichment of specific families. Regarding blackberry samples, there are high abundancy of Pseudomonadaceae and Rhodanobacteraceae. As for blueberry, this abundance is observed in Rhodanobacteraceae and Burkolderiaceae. Also, it was tried to isolate members of the core microbiome and found that 24.3 and 31.1% of total isolated bacteria in blackberry belong to anterior families respectively. Same happens in blueberry plants, with 4.3 and 13.2% respectively.

In conclusion, plants may be able to select surrounding microorganisms for stablishing microbial communities which benefit them. Thus, isolation of bacteria from this groups may be interested in the development of new biofertilizers.

ACKNOWLEDGEMENTS/REFERENCES

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1094 / CRISPR/CAS9 BASED GENE STACKING IN PLANTS FOR VIRUS RESISTANCE USING SITE-SPECIFIC RECOMBINASES

Keywords: *CRISPR/Cas9, Genome editing, Gene stacking, recombinases*

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Gene stacking emerged as potential approach for integrating multiple genes in plants. During present study, recombinase technology has been used for site-specific gene stacking. Target vector (pG-Rec) was designed to engineer a predetermined specific site in plant genome whereby genes can be stacked repeatedly. Using Agrobacterium-mediated transformation, pG-Rec was transformed into Coker-312, Nicotiana tabacum and Nicotiana benthamiana. The transgene analysis of target lines was conducted through junction PCR. The transgene positive target lines were used for further transformations to site-specifically stack two genes of interest using Bxb1 and PhiC31 recombinases. Cas9 driven by multiplex gRNAs was site-specifically integrated into target lines and determined by junction and real-time PCR. Resulting plants were used to stack second gene of interest (AVP3 gene for enhancing plant growth). The addition of genes is simultaneously achieved with removal of marker genes for recycling with t next round of gene stacking. Consequently, transgenic marker-free plants were produced with two genes stacked at specific site. These transgenic plants can be potential germplasm to introduce resistance against various strains of cotton leaf curl virus (CLCuV) and abiotic stresses. The results of the research demonstrate gene stacking in plants, a technology that can be used to introduce multiple genes sequentially at predefined genomic sites. The current climate-change scenario highlights the use of such technologies to solve gigantic environmental issues in single step. After evaluating virus resistance, the lines can be a primer to initiate stacking of genes in Cotton for other traits and molecular breeding with elite cotton lines.

ACKNOWLEDGEMENTS/REFERENCES

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1156 / IDENTIFICATION AND COPPER SENSITIVITY STUDYING OF XANTHOMONAS STRAINS ISOLATED FROM PEPPER

Keywords: *Xanthomonas, Pepper, Copper sensitivity*

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Control of Xanthomonas species, causing pepper bacterial spot, is based on a frequent use of copper-based bactericides, which usually lead to occurrence of resistant bacterial strains. A total of 188 strains were isolated from diseased pepper plants in 52 localities in Serbia and Montenegro during the period of last three decades. Based on the BOX-PCR profiles, 74 strains were selected for studying biochemical, physiological and molecular traits. The strains, formed yellow, convex and mucoid colonies on yeast extract-dextrose-CaCO3 (YDC) medium and were Gram-negative, strictly aerobic, oxidase negative, catalase positive, hydrolyzed gelatine and esculin but not starch, and did not grow at 37°C. They were further identified by PCR analysis, using primer pair BS-XeF/BS-XeR (Araújo et al., 2012; Koenraadt et al., 2009), resulting in a single band of 173bp, characteristic for Xanthomonas euvesicatoria.

Copper sulphate sensitivity was studied by spotting the strains' suspension droplets (10 µl) on the surface of sucrose peptone agar (SPA) plates, amended with either 100 or 200 ppm of the bactericide. All of the strains grew on SPA plates containing 100 ppm of copper sulphate, indicating tolerance to this concentration of the bactericide. However, after initial growth, 200 ppm of copper sulphate stopped further development of the colonies. This explains low efficacy of the copper based protection of pepper from bacterial spot and urge plant bacteriologists to search for the alternatives.

ACKNOWLEDGEMENTS/REFERENCES

This study was funded by the contract between Ministry of Education, Science and Technological Development, Republic of Serbia and Faculty of Agriculture (Contract No. 451-03-9 / 2021-14 / 200116)

1197 / PSEUDOMONAS SYRINGAE PV. SYRINGAE AS CAUSAL AGENT OF BACTERIAL CANKER OF BLUEBERRY IN SERBIA

Keywords: *Pseudomonas syringae pv. syringae, bacterial canker, blueberry*

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Blueberry production in Serbia has been increasing recently due to market demands and export potential. During late spring 2021, in an orchard in west part of the country, a 2-year-old highbush blueberry plants (Vaccinium corymbosum) cv. Draper were suffering of young twigs and flower buds necrosis. The incidence of disease was 80%, while 10% of the plants died.

Beneath the branches bark, wet lesions and dark brown discoloration of the tissue were observed. In some plants, cankers surrounded the stem, causing shoot-tip dieback and necrosis of the buds. A few weeks before the symptoms development, morning frosts and low daily temperatures were recorded in this area. Pathogen isolation was carried out on King's medium B. After 2 to 3 days of incubation at 27°C, ten isolates producing a fluorescent pigment were selected for further characterization. The isolates were Gram, arginine-dihydrolase and oxidase negative and levan positive.

They induced hypersensitive response on tobacco leaves, and showed no pectinolytic activity on potato slices. Based on the biochemical-physiological characteristics, including PCR detection of syrB gene and analysis of 16s rRNA and gyrB gene sequences, isolated strains were identified as Pseudomonas syrinage pv. syringae. In order to confirm pathogenicity of the strains, 2-year-old blueberry plants were inoculated by bacterial suspension (conc. 106 CFU/ml). Three weeks after, symptoms corresponding to these of natural infection were observed and the pathogen was reisolated. This was the first occurence of bacterial canker of highbush blueberry caused by P. s. pv. syringae in Serbia.

ACKNOWLEDGEMENTS/REFERENCES

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-9/2021-14/200010).

09

1488 / INOCULATION WITH SOIL BENEFICIAL MICROORGANISM MODULATE GROWTH AND VOLATILOMA OF ARTEMISIA ANNUA PLANTS GROWN IN CONTROLLED CO

Keywords: *soil beneficial microorganism, artemisia, volatilome*

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Artemisia annua L. (Family: Asteraceae) is an annual herbaceous plant native of the Hunan region (China), well known to produce a wide range of secondary metabolites, including various VOCs (Volatile Organic Compounds) and artemisinin, an important bioactive compound used against malaria Plasmodium as a good alternative to conventional treatments. Since many studies reported that arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB) can affect plant growth and metabolism, the aim of this work was to evaluate the effects of different AMF species and PGPB strains, used alone or in combination, on two Artemisia annua micro-propagated clones (CL7 and CL26) grown for about two months in controlled conditions. Plant growth parameters, leaf pigment concentration (spectrophotometric analysis), artemisinin production (HPLC analysis) and volatilome composition (GC-MS) were investigated.

Results showed on one hand that the two clones differentially responded to the inoculation with the same microorganism and, on the other hand, that different microorganisms differently affected the same clone, either in terms of biomass production or photosynthetic pigment concentration. Compared to previous studies, the present leaf volatilome investigation allowed to identify a much higher number of molecules (approximately 120), some of which recorded for the first time in A. annua. The identified compounds belonged to the alkenes, alcohols, aldehydes, acids, ketones, ethers, esters, and percentages of molecules in each class varied according to the used inocula, showing that inoculation with different microrganisms resulted in specific plant odors.

ACKNOWLEDGEMENTS/REFERENCES

This research is original and had financial support of the Università del Piemonte Orientale.

09

1605 / BACTERIAL COMMUNITY COMPOSITION OF CROWN GALL TUMORS ON RHODODENDRON

Keywords: *Phytopathogenic bacteria, tumorigenic Rhizobiaceae species, Agrobacterium, Rhizobium*

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BACKGROUND

Crown gall is an important plant disease caused by tumorigenic Rhizobiaceae species. The formation of tumors on different parts of the host plant is the characteristic symptom of the disease. Crown gall tumors are not only inhabited by the pathogen; they seem to be dynamic ecological niches inhabited by diverse microorganisms.

OBJECTIVES

The objective of this study was to explore diversity of bacterial community inhabiting crown gall tumors on rhododendron, to gain a better understanding of the pathobiome of this ecological niche.

METHODS AND RESULTS

The bacterial community inhabiting crown gall tumors on rhododendron was analyzed by using both isolation-based and cultivation-independent methods. Representative isolates were subjected to whole-genome sequencing (Illumina NextSeq and PacBio RS II) and comparative genomics analysis. Total bacterial community of crown gall tumors was analyzed by amplicon sequencing using Illumina MiSeq system targeting different gene markers.

Results obtained indicated that tumorigenic Rhizobium sp. and nonpathogenic Agrobacterium spp. and are cohabitants and highly abundant members of the bacterial community in rhododendron tumors. Whole-genome analysis suggested that tumorigenic Rhizobium sp. strains isolated from rhododendron tumors are most closely related to Rhizobium tumorigenes. Interestingly, these strains carry tumor-inducing (Ti) plasmid that differs from those described so far and most likely represents a novel type of Ti plasmid. Furthermore, nonpathogenic Agrobacterium spp. strains formed diverse and novel lineages within the genus Agrobacterium. Genome analysis suggested that these nonpathogenic strains harbor a novel type of opine-catabolic (OC) plasmid responsible for utilization of opines, compounds synthetized in crown gall tumors.

ACKNOWLEDGEMENTS/REFERENCES

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1685 / INVESTIGATING CYANOBACTERIAL DIVERSITY IN WETLANDS WITH DIFFERENT SALINITY AND OIL POLLUTION

Keywords: *Cyanobacteria, Cyanobiaceae, Leptolyngbyaceae*

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BACKGROUND

Cyanobacteria are known as a very diverse group of prokaryotes and a kind of gram-negative bacteria, which are generally autotrophic containing photosynthetic pigments. Cyanobacteria are in a wide range of habitats; for example, sea, freshwater, soil, biological soil crusts, snow, saline environments, and so on.

OBJECTIVES

This paper aims to characterize three water samples of lagoons around Qom, Iran, which contain different percentages of salt and oil pollution from specific depths along specific latitudes and longitudes, as well as the amount of salt, pH and TPH. Moreover, all elemental analyzes of water samples are determined.

METHODS

50 liters of each sample were filtered sequentially using a 5-micron pre-filter as well as a 22.0-micron polycarbonate filter using a peristaltic pump. The genome sequencing with the Illumina Novaseq 6000 platform was performed by Hong Kong-based Novogene. Then, the genetic content of the biomass collected on 22.0 micrometer filters was extracted using a standard phenol-chloroform method.

RESULTS

According to the results of SrRNA16 gene sequencing, the cyanobacteria of the lake were members of the Phormidiaceae, Leptolyngbyaceae and Cyanobiaceae families. In all three wetlands, the genera Leptolyngbya_VRUC, Cyanobium_PCC-6307 and ynechococcus_CC9902 were found in different abundances. Leptolyngbya_VRUC had the highest frequency in oil-contaminated wetlands with salinity of 6%, while this species was less in lakes with salinity of 3% and 1%, indicating hydrocarbon decomposition pathways and high salinity tolerance. This hints at cyanobacterial diversity in the hypersaline system.

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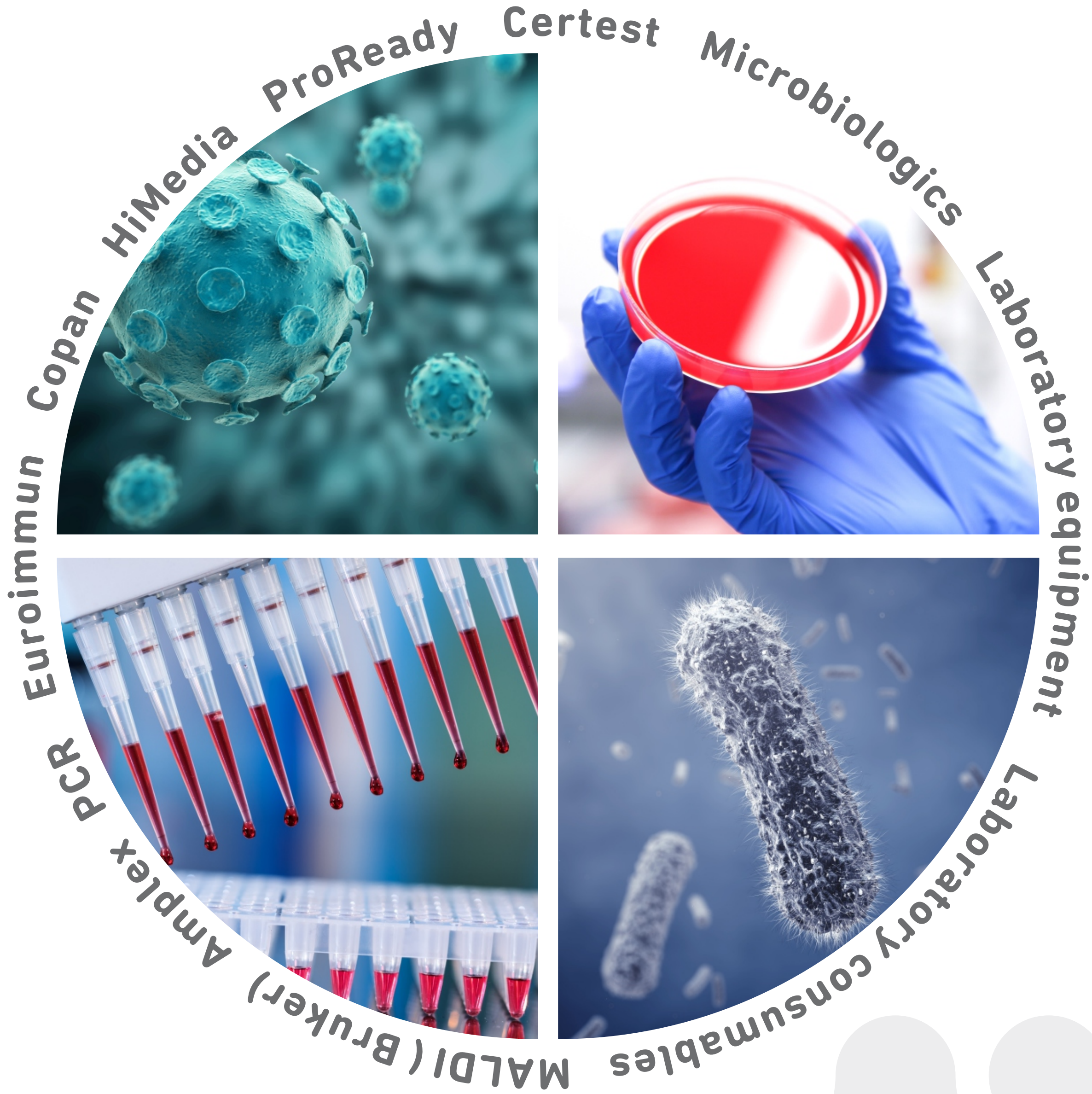

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