



BOOK of ABSTRACTS

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Min-Tze Liong
Short Bio – 2021

MinTze Liong obtained her PhD from Victoria University, Melbourne, Australia (2006) at the age of 28. She joined Universiti Sains Malaysia in Dec 2006, promoted to Senior Lecturer in Aug 2008, Associate Professor in July 2010 and Professor in October 2015.

She has actively published research papers, serves as Editorial Board members of reputable journals, and delivered keynote and plenary lectures both nationally and internationally. Prof. Liong has also commercialized her research to industries from Asia and Europe. Prof. Liong was the recipient of the Best Research Student Award (Victoria University, Australia; 2006), finalists for the Malcolm Bird Award (Australian Institute of Food Science and Technology; 2006), and named one of the three top young women scientists in Malaysia (2007) by FWIS L’Oreal-UNESCO. She was also awarded the IAP Young Scientist, to represent Malaysia at the World Science Forum in Budapest to pitch for changes in science policies in the Hungarian Parliament (2015). In 2016, Prof. Liong was named as one of the Top Research Scientists of Malaysia, awarded by Academy of Sciences Malaysia (ASM). In 2020, she is listed among the top 2% of global scientists as compiled by Stanford University.

Probiotic: A Next Generation Agent for Healthier Gut & Brain

Min-Tze Liong

Professor (Ph.D.), School of Industrial Technology, Universiti Sains Malaysia (USM), 11800 Penang, Malaysia.

Probiotic microorganisms have a long history of safe use and have been documented for their various health benefits. Many strains of lactic acid bacteria (LAB) from the genera of *Lactobacillus* have been identified to possess probiotic properties. They have been much emphasized on their roles to regulate gut well-being, including the alleviation of lactose intolerance, improvement of diarrhoea and inhibition towards pathogenic bacteria in the gut. Recent evidences have illustrated the potential of LAB for brain health, ranging from neurodegenerative diseases to stress, memory and cognition. *Lactobacillus plantarum* DR7, a cow's milk isolate from Penang, Malaysia has shown a reversal of symptoms associated with Alzheimer's Disease in a fruit fly model, accompanied by distinct changes of gut microbiota profiles. In aging and hyperlipidemia rats, DR7 has shown improved stress and anxiety symptoms, accompanied by improved memory. The administration of DR7 for 12-weeks in stressed adults has contributed to improve stress and anxiety scores, in addition to memory and cognitive abilities, primarily via enhancement along the serotonin pathway and maintenance of the dopamine pathway. My talk will highlight the evidence of a probiotic strain, primarily targeting brain and gut health, via models ranging from insects to human.



Vyacheslav K. Ilyin
Short Bio – 2021

Ilyin Vyacheslav Konstantinovich, Doctor of Medical Sciences, Professor, was born in 1959, has been working at the State Research Center of the Russian Federation at the Institute of Biomedical Problems of the Russian Academy of Sciences since 1982 after graduating from the 1st Moscow I.M. Sechenov Medical Institute, Faculty of Sanitary and Hygiene. V.K. Ilyin heads the Department of sanitary and hygienic safety of humans in an artificial environment, and the Laboratory of human microbial ecology.

V.K. Ilyin's scientific interests are mainly related to the study of the influence of extreme environmental factors (space flight factors, hyperbaric environment) on natural colonization barriers of humans in an artificially modified environment (space flights, long stay in ground-based hermetic facilities), as well as methods for correcting their condition, based on autoprotobiotics. The obtained results are reflected in the monograph "Colonization resistance of the organism in changed habitat conditions." With the active participation of V.K. Ilyin, new probiotics are being developed, including autoprotobiotics. Another area of scientific activity of V.K. Ilyin is the study of prokaryotic and eukaryotic cells: cultural characteristics, the possibility of horizontal exchange of genetic material during conjugation and transduction, the ability to withstand high temperatures in astrobiological experiments, in particular, in experiments to study the possibility of penetration of exogenous unicellular organisms into composition of the meteorite mass to the Earth. This work is carried out in the interests of lunar program implementation.

VK Ilyin is a full member of the International Academy of Astronautics (Department of Life Sciences). V.K. Ilyin is a member of the editorial board of the journal Ecological Engineering and Environment Protection.

Autoprotobiotics as a promising mean for colonization resistance strengthening in interplanetary flights

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At present, as the program of interplanetary flights is developing, the problem of ensuring the infectious safety of the crew members of long-term orbital stations is of particular importance, which was pointed out by researchers back in the days when space flights were not so long.

Over the years, a number of researchers have demonstrated the activation of opportunistic microflora of a person during his stay in sealed compartments for various purposes. Distinctive feature of these microorganisms is their ability to cause infectious diseases under certain conditions, the main of which is a decrease of immunological reactivity and increasing of pathogenicity potential of microbial community during long-term presence in confined habitat.

In space orbital station, the composition of the normal microflora significantly changes in cosmonauts, as a result resistance of human body to penetration of infectious agents is suppressed. In medical and technical experiment, a significant decrease, up to complete disappearance, the number of Bifidobacteria and Lactobacilli, a decrease in enzymatic and antagonistic activity in *E. coli* strains, an exchange of *E. coli* strains, an increase in the toxicity of a number of organisms, as well as increase of antibiotic resistant strains were registered in the intestine. The results of microbiological studies in simulation experiments were confirmed in real space flights.

In long-term space flights that are not accompanied by visiting crews, for example, flights on orbital stations Salyut, there is a decrease in the number of opportunistic pathogens, their species, and in their population the share, formed by their representatives, having expressed ability to produce enzymes of aggression and defense, decreases. At the ISS and Mir stations, this process takes, in fact, the entire period of time until crew return, or until next microecological perturbation caused by newly arrived crew members, or as part of the cargo traffic. Between the newly arrived and the main crew members, there is an active and rapid spread of not only strains of opportunistic microorganisms persisting in the upper respiratory tract, but also representatives of the intestinal microflora. They are very easily transmitted from members of the visiting group to members of the main group, and some of them are able to persist there until the end of the flight.

Therefore, given the very high recipient ability of the crews, we can assume a rather problematic scenario for Martian and lunar expeditions when landing on planets in which there is a microbial consortium, capable of integrating into crew members microbiocenosis, or when returning to Earth.

Therefore it is necessary to develop effective preventive measures to strengthen colonial resistance of crew members to meet these projected challenges. Today, there are plenty probiotic agents capable to strengthen colonial resistance, including probiotics, based on lactobacilli strains, isolated from astronauts. The latter circumstance makes possible to conduct series of studies of the of probiotics effectiveness, based on autologous strains of protective microorganisms. The studies made possible to using cultures of bifidobacteria, lactobacilli, enterococci, corynebacteria, salivary streptococci, both in combination and separately, as autoprotobiotics, depending on the biotope, requiring sanitation. Probiotic preparations based on lactobacilli strains have good activity. This activity is comparable to the activity of a commercial drug, and in some cases exceeds it.

To optimize the intestinal microflora, bifidobacteria, lactobacilli and enterococci should be used. An example of a positive result from the use of enterococcal-based autoprotobiotics is the data of the Mars-500 experiment, in which the administration of autoprotobiotics in the first half of the experiment led to the eradication of opportunistic microorganisms in the intestine. It is reasonable to conclude that autoprotobiotics should be used to achieve unwanted bacteria eradication, while using commercial drugs it is possible only to stabilize the microflora at an acceptable level. For scenarios of interplanetary flights and lunar bases, both options seem acceptable.

For of high respiratory tract sanitation, it is necessary to use corynebacteria, lactobacilli and salivary streptococcus, for the sanitation of the periodontium - salivary streptococcus and veillonella. Preliminary results of autoprotobiotics consumption in experiments, simulating altered living conditions, as well as in clinical studies, indicate their straight effectiveness. The absence of complications, biological incompatibility and engraftment suggests the effectiveness of autoprotobiotics in the treatment of newborns, the elderly and immunosuppressed patients. Currently, the concept of creating cryobanks of human microbiocenoses and autoprotobiotics can be considered as a separate part of personalized medicine. The individualized approach in choice of drugs will increase the effectiveness of prevention and treatment, as well as reduce the risk of adverse effects in each individual person.



Alexander N. Suvorov
Short Bio – 2021

Professor, MD, Corresponding member of Russian academy of sciences. He is head of the Department of Molecular Microbiology in the Institute for Experimental Medicine, Saint Petersburg, Russia. At the same time, he is head of the Department of Fundamental medicine in Saint Petersburg state University, and the Head of the Department of microbial therapy in the National Center of the world level. His main scientific interest is molecular microbiology of pathogenic and health beneficial bacteria and vaccine development. During his scientific career, he obtained several awards and grants from several Russian and international funding agencies. In 2020 he was appointed as the Honorary director of the Institute of Agro-food Science and technology Shandong Academy of Agricultural Sciences. He co-authored more than 20 patents, more than 200 papers and several books .

Autoprotobiotics: from the concept to the clinical evidence

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Introduction

Presently, it is well established that the human immune system is developing during the first years of life and this development is shaping and personifying the host microbiota. This process results in the stable microbial consortium (eubiosis), which accompanies each human being during his entire life. Content of personal bacteria is involved in the complex process of intermicrobial and host-microbial communications and metabolic activities and signaling which result in the normal functioning of the organism. In case of distortion of microbiota composition, an individual falls into the dysbiotic condition, which is a trigger for the vast majority of pathologies of infectious and non-infectious nature. The key to effective therapy is to eliminate dysbiosis as quickly as possible. The effective treatment of dysbiosis which is often associated with the overgrowth of pathogenic bacteria can be obtained either by the introduction of antimicrobial agents or probiotic organisms. Another option is the usage of indigenous bacteria isolated as individual genetically characterized clones or consortium of indigenous bacteria which are grown on artificial media. This strategy called autoprotobiotic therapy was well studied on the animal model by Suvorov A, Karaseva A, Kotyleva M, et al. (doi:10.3389/fmicb.2018.01869) and later was implemented for human patients. The study of autoprotobiotics as a means of improving human health in several clinical conditions was performed by the scientists at the Institute of Experimental Medicine as a project sponsored by the Ministry of Science and Higher Education. The study, which included patients with gastrointestinal, oncological, dental, and endocrinal diseases, revealed that autoprotobiotic therapy is well tolerated and the health improvement correlated with an improvement of microecology of the patients under the trial. Examples of different clinical applications of autoprotobiotics are provided.

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Koen Venema
Short Bio – 2021

Prof. Koen Venema is i) Associate Professor at University Maastricht - campus Venlo, ii) project leader within the Top Institute Food & Nutrition, iii) founder and CEO of the company Beneficial Microbes® Consultancy, iv) the organizer of the Beneficial Microbe Conference-series, and v) editor-in-chief of Beneficial Microbes.

Prof. Venema studied Chemistry at the University of Groningen, The Netherlands in 1990. He received his PhD in Natural Sciences from the same university, on the antimicrobial activity of bacteriocins produced by lactic acid bacteria (LAB) in 1995. He pursued the health-beneficial activity of these microbes as a Post-Doc at North Carolina State University, Raleigh, USA, where he studied the potential of LAB to function as carrier for vaccines. Thereafter, in 1998 he was employed by TNO for > 15 years, where he used the sophisticated, dynamic, computer-controlled in vitro models of the gastro-intestinal tract developed by TNO (nick-named TIM) to study the effect of functional foods and drugs on bioavailability and effects on the colonic microbiota.

Within TNO he has led many multi-disciplinary projects on health of the host (e.g. gut health, inflammatory disease, allergy, obesity) and the role of probiotics and the gut microbiota in these diseases and disorders. In the period 2004-2009 he has been project leader within the Public Private Partnership TI Food & Nutrition, of which TNO is a partner. Within this multidisciplinary project his research was devoted to the effect of the activity of the microbiota on gut health and obesity using, amongst others, the TNO in vitro models. Since January 2011 he is project leader within TIFN of a new project “Interaction of probiotics and prebiotics with the host”, which looks at the interaction of fibers and probiotics with the immune system of the host.

Since Sept. 01, 2014 he runs a newly established research group at the University Maastricht, where the focus is on the health beneficial effects of the gut microbiota on the host, and where he continues to use the TNO in vitro models. He studies the role of beneficial microbes in health and disease.

Towards personalized prebiotics: opportunities and challenges

Koen Venema

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Over the past three decades it has become clear that the gut microbiota is heavily involved in health and disease. Not only for the gut itself, but also for the rest of the body. Correlations have been found between the presence/absence of certain bugs and many diseases and disorders. One of the major activities of the gut microbiota is the fermentation of carbohydrates, including dietary fibre and prebiotics. Prebiotics are defined as non-digestible carbohydrates that specifically stimulate one or a few members of the gut microbiota, leading to improved health. However, it is the phrase ‘specifically’ in that definition that usually is not met. Although previously e.g., inulin and fructooligosaccharides were thought to specifically increase bifidobacteria, with the advent of new and better molecular tools to study the gut microbiota, this bifidogenic concept has been challenged. Besides, other members of the microbiota have been identified as beneficial, such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*. Moreover, even other bugs may need to be stimulated when dysbiosis occurs, such as in inflammatory bowel disease, obesity, etc. In addition, every individual has his/her own microbiota composition, and hence other species and strains, each with their own (carbohydrate degrading) enzyme machinery. Due to next generation sequencing methods we have access to a tremendous amount of information, either from single strain sequencing, or from metagenomic samples. Genes encoding glycosylhydrolases and other carbohydrate active enzymes (CAZy) can be identified in these sequences. Attempts and challenges to use this information for the development of strain-specific prebiotics will be highlighted. Moreover, the concept of personalized prebiotics will be discussed.



Wilhelm Heinrich Holzapfel
Short Bio – 2021

Dr. rer. nat. Wilhelm H. Holzapfel is Chair Professor in the Department of Advanced Convergence, Handong Global University, South Korea; from 2007 to 2013 he has been full Professor in the School of Life Sciences, Handong Global University. Formerly he was Head (Director and Professor) of the Federal Institute of Hygiene and Toxicology, Karlsruhe, Germany, and also served as Hon. Professor for Industrial Microbiology at Karlsruhe Technical University (KIT) (1996-2007), Germany. He doctorated (Dr.rer. nat.) in 1969 at the Technical University of München, Germany. He has > 45 years' experience in teaching and research in various fields of basic and applied Microbiology, also including Industrial Microbiology, Food Microbiology and Food Hygiene. His major research activities focus on the lactic acid bacteria (physiology, taxonomy and beneficial applications), probiotics, food biotechnology and gut microbiota. He is President of the ICFMH of the IUMS (International Committee on Food Microbiology and Hygiene of the International Union of Microbiological Societies) since 1996, and is member of the IUMS (International Union of Microbiological Societies) Subcommittee on the Taxonomy of Bifidobacterium and Lactobacillus and related organisms since 1986. He is an Honorary member of the Hungarian Society of Microbiology since 2003, and has been member of the Advisory Board of the Federation of Food Legislation and Food Science of the German Food Industry from 1989 to 2015. He co-authored > 350 scientific papers, 70 chapters in scientific textbooks and is editor/co-editor of 8 scientific textbooks.

Prospecting for beneficial (lactic acid) bacteria with focus on fermented foods

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Introduction

Current developments in biotherapy are driven and supported by an increase in understanding of the beneficial role of probiotics in the human host and their impact on the gut microbiome. Dysbiosis of the gastro-intestinal tract (GIT) is associated with several adverse health conditions; however, functional microbial strains may modulate the microbiome towards the restoration of gut homeostasis. The lactic acid bacteria (LAB) still represent the major candidate group when searching for beneficial strains; diverse LAB groups are distributed throughout a wide range of ecosystems. Only some LAB representatives are associated with traditional food fermentations. These still comprise a highly promising “prospecting” field for novel beneficial strains. Suitable strains have been isolated and selected for application in industrial food fermentations, bio-preservation, and also find their way into the rapidly growing probiotic market. Several factors will determine the future success in application of beneficial LAB strains. It is imperative to clarify the basic mechanisms underlying postulated beneficial functions, e.g., by using both validated *in vitro* and *in vivo* models and involving meta-omics approaches. The efficacy of a probiotic is not only strain-specific but may differ from person to person, based on the unique gut microbiota specific for each person. The composition of the gut microbiome may also reflect underlying diseases and/or can be disrupted by particular infections. Individual differences in intestinal microbiota may explain the unpredictable activity of a probiotic strain.

Methods

After isolation, potentially beneficial strains have been selected by *in vitro* and *in vivo* procedures, including the determination of their safety and ability to survive passage of the upper intestinal tract, and performance in a murine model. Specified knowledge of a person’s gut microbiota and its potential beneficial modulation can be determined by a simple and rapid *in-vitro* screening system called PMAS (Pharmaceutical Meta-Analysis Screening) (patented). In this system the live microbial communities in fecal samples are “challenged” with probiotic candidate strains during anaerobic incubation, and the modulated microbiota and metabolites analyzed by next-generation sequencing and bioanalytical methods. By comparing the results of the experimental groups, treated with the specific candidate probiotic strains, with the control (without any treatment), the most ideal probiotic strain can be selected on the basis of a person’s fecal sample and its microbiota. In addition to the *in-vitro* system, the impacts of candidate probiotic strains on the disease-specific microbiome were investigated as well as their functional efficacy through various animal disease models.

Results and Discussion

A dietary induced obese (DIO) murine model was applied for studying the impact of selected LAB strains on conditions related to metabolic diseases, and significant beneficial effects could be recorded for selected strains isolated from different fermented foods. Moreover, a strain (*Lacticaseibacillus rhamnosus* BFE5264) isolated from traditional Maasai fermented milk significantly reduced hypercholesterolemia symptoms and related biomarkers in a murine model receiving a high cholesterol diet. This strain was subjected to the PMAS screening test on 20 clinical samples; the results correlated well with the outcome of preliminary clinical trial. The results suggest the potential of the PMAS as an *in-vitro* screening system for selecting functional (probiotic) strains for the development of personalized biotherapeutic approaches.



Georgy Yu. Laptev
Short Bio – 2021

Dr. Georgy Yu. Laptev is f Head of the Biotroph Ltd. In 1999 he fouded Biotroph Ltd. Now it is one of the leader Russian market of the microbial feed additives. He serves as f Head and a professor of the Departament of Forage Biotechnology in Sankt-Petersburg Agrarian University (Sankt-Petersburg, Russia). Dr. Laptev is an applied molecular microbiologist studying microorganisms in silage and gastrointestinal microbiota of the farm animals.

He holds the M.S. summa cum laude degree in Microbial Genetics from the Sankt-Petersburg State University (Russia) and the Ph.D. degree from the Institute of Agricultural Microbiology (Sankt-Petersburg , Russia). He is Doctor of Science (2009, Animal Husbandry Institute, Moscow region).

Dr. Laptev is the laureate of prise of the Russian Federation Government (Agricultural Science, 2017).

He co-authored several patents, more than 60 papers and 4 book chapters. Dr. Laptev serves as a Member of the Editorial Boards of Agricultural Biology, Journal of Dairy and Meat Husbandry, Husbandry of Russia, Proc. Sankt-Petersburg Agrarian State University.

Transcription of bacterial genes in the rumen of dairy cows.

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Few studies are devoted to the study of the rumen microbiome of cows using transcriptomics methods. Despite the high relevance of studying the metabolic activity of the bovine rumen microbiome, its functional potential is studied mostly using metagenomics methods, which are unable to assess the actual levels of gene expression. The aim of this study was to investigate the expression of bacterial genes associated with the main metabolic enzymes in the rumen of dairy cows in relation to different physiological periods.

Materials and methods

The experiment was carried out in JSC “Agrofirm Dmitrova Gora” in the Tver region in 2020 on fifteen dairy cows. The study of gene expression of the rumen microbiome was carried out on animals from five groups: group I – dry period, II - post-partum period, III - early lactation period, IV - mid-lactation period, V - late lactation period. For the analysis of gene expression (phosphofructokinase (pfk), phosphoenolpyruvate carboxykinase (pepk), linoleic acid reductase (cla-r), L-lactate dehydrogenase (ldh-L), D-lactate dehydrogenase (ldb 0813), methylmalonyl CoA- guanine amino hydrolases (gah1)), chyme samples from cows’ rumen were collected using a sterile probe. RNA was isolated from the samples. Analysis of relative gene expression was performed by real-time PCR with preliminary reverse transcription of RNA into cDNA.

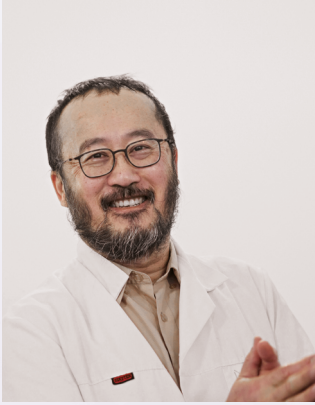
Results and discussion

As a result of the analysis, it was shown that in cows at different physiological periods was a change in the expression levels of some bacterial genes associated with the main enzymes involved in the processes of rumen metabolism. Similar changes were observed in the expression levels of the pfk, pepk, and cla-r genes of the cow rumen community. In the rumen of cows during the post-partum period (group II), the level of expression of these genes increased up to 2.6 times, and during the early lactation period (group III) up to 3.3 times compared with dry animals (group I) ($p \leq 0.05$). An increase in the expression level of genes ldh-L ($p \leq 0.01$) and ldb 0813 ($p \leq 0.05$) was found in the rumen of post-partum period animals (group II) compared to dry animals (group I). On the other hand, a reverse trend was observed in cows from groups III and IV (compared with dry animals of group I) (at $p \leq 0.05$) the level of expression of the scpA gene associated with the synthesis of methylmalonyl CoA mutase, which catalyzes the conversion of methylmalonyl- CoA in succinyl-CoA. The level of expression of the GAH1 gene associated with the synthesis of the guanine aminohydrolase enzyme decreased in the rumen of cows during milk production ($p \leq 0.05$) and stabilization of lactation ($p \leq 0.01$) compared with dry animals. Thus, an increase in the level of expression of genes associated with glucose utilization (pfk) and lactate synthesis (ldh-L, ldb0813) was observed against the background of a decrease in the expression of genes associated with energy (scpA) and protein metabolism (gah1) during lactation. This may indicate that the metabolism of the rumen microbiome in dairy cows is subject to more negative changes than during the dry period.

Conclusions

Probably, changes in the level of transcription of genes of microorganisms of the rumen of cows are associated with the characteristics of the metabolism of animals, feeding, stress (for example, due to calving and the onset of lactation), immune and hormonal status, due to the specificity of various physiological periods of animals. Changes in the level of gene expression could contribute to the composition of intermediates or final metabolites in the rumen of animals at different physiological periods.

The study was supported by the RFBR grant No. 20-016-00168.



Dzung B. Diep
Short Bio – 2021

Dr. Dzung B. Diep is a group leader of the research group Laboratory of Microbial Gene Technology (Norway), and a professor at the Faculty of Chemistry, Biotechnology and Food Science (Norwegian University of Life Sciences). He has a background in molecular microbiology and has a keen interest in bacteriocin research, both fundamental and applied. This includes bacteriocin screening, purification, functional genetics, quorum sensing/gene regulation, receptor identification and mode of action studies. His recent focus is to understand how bacteriocins interact with their receptors on target cells and how these interactions eventually lead to the destruction of target cells. Knowledge from these studies is important to develop bacteriocins into safe and efficient applications, both as food preservatives and as novel drugs. He has an increasing interest in applied research, especially to develop bacteriocins into drugs to fight antibiotic-resistant pathogens. He holds a Master of Science from the University of Oslo and a Dr. science degree from the Agricultural university of Norway. His academic training includes a postdoc at University of Victoria (Canada) and a postdoc at the National hospital (Norway). He serves as PI, co-PI or partner in several national projects and one EU project. He coauthored several patents, more than 100 peer-reviewed papers and book chapters. His current H-index at Web of Science is 37.

Bacteriocins, an Important Source of Antimicrobials to Deal with Antibiotic Resistance

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The emergence of antibiotic-resistant pathogens has caused a serious worldwide problem in infection treatment in recent years. One of the pathogens is methicillin-resistant *Staphylococcus aureus* (MRSA), which is a major cause of skin and soft tissue infections. Alternative strategies and novel sources of antimicrobials to solve antibiotic resistance problems are urgently needed. In this study, we have explored the synergistic effect of two broad-spectrum bacteriocins, garvicin KS and micrococcin P1, and penicillin G, in their efficacy against MRSA. The three antimicrobials displayed poor or no activity when assessed individually as bacterial cells easily became resistant due to adaptation or mutations. However, when combined in a three-component formulation, the bioactivity was able not only to efficiently kill the pathogen but also to prevent it from resistance development. The synergistic effect was validated in different settings: in planktonic cells, in biofilms and in murine skin infection model.



Santosh Kumar Tiwari
Short Bio – 2021

Dr. Santosh Kumar Tiwari is a Head of the Microbial Genetics Laboratory and Associate Professor at Department of Genetics, Maharshi Dayanand University, Rohtak, Haryana, India. Presently, he is Deputy Coordinator of Government of India sponsored Capacity Building Programme, “Bioperspecting natural products for socioeconomic developments”. He was Indo-US Research Fellow at Rutgers University, USA and India- Australia Research Fellow at Monash University, Australia. Dr. Tiwari works in the area of microbial technology on different aspects of bacteriocins of probiotic lactic acid bacteria for their applications in food and medicine. He holds PhD degree in Genetics from Delhi University, India. Dr. Tiwari’s external funding stems from mostly government organizations such as Indian Council of Medical Research, University Grant Commission, Council of Scientific and Industrial Research, Indian National Science

Academy, Department of Science and Technology, Department of Biotechnology, Ministry of Science and Technology, Government of India etc. He has completed 9 research projects with total funding of Rs. 3 crores. He co-authored one book, seven book chapters and more than 40 research papers. Dr. Tiwari serves as a member of Editorial Board of Journal

Health benefits of bacteriocin-producing lactic acid bacteria isolated from indigenous fermented foods

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Fermented foods are chief source of probiotic bacteria especially several species of lactic acid bacteria. Looking at their importance for health benefits, 127 isolates of lactic acid bacteria were isolated from most commonly consumed fermented foods such as curd, milk, Dosa, Sambar, Idle, Jalebi etc. These isolates were identified using biochemical tests and based on higher antimicrobial activity; two isolates from Dosa batter were selected for further studies. Isolate LD1 was identified as *Lactobacillus plantarum* LD1 and LD3 as *Enterococcus hirae* LD3. They showed probiotic properties such as resistance to host gastrointestinal conditions such as tolerance to acids, bile salts and hydrolytic enzymes, and auto-aggregation (Gupta and Tiwari, 2014; 2015). Plantaricin LD1 was detected in the cell-free supernatant of *L. plantarum* LD1 was found to be cationic, heat stable bacteriocin with molecular weight 6.5 kDa. It shows antimicrobial activity against broad range of Gram-positive and Gram-negative bacteria (Gupta and Tiwari 2014b; Kumar et al., 2016). *E. hirae* LD3 was characterized to produce bacteriocin (enterocin LD3) inhibiting broad-range of related and pathogenic bacteria such as *Lactobacillus curvatus*, *L. acidophilus*, *L. plantarum*, *Lactococcus lactis*, *E. faecium*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Salmonella typhi*, and *Listeria monocytogenes* (Gupta et al., 2016). Enterocin LD3 exhibits novel fascinating attributes involving unique m/z ratio (4114.62 Da), unique N-terminal sequence (H2NQQGGQANQ-COOH), and heat stability up to 100°C. The mode of action of enterocin LD3 was studied against *Micrococcus luteus* in terms of efflux of intracellular ions, loss of cell viability and disruption of membrane potential (Tiwari et al., 2015; Sheoran and Tiwari, 2019a). The efficacy of enterocin LD3 was also studied in milk for the inhibition of *Staphylococcus aureus* (Sheoran and Tiwari, 2019b). Thus, present study has demonstrated the isolation, identification and characterization of beneficial bacteriocin-producing lactic acid bacteria for application in food safety and therapeutics.

Role of PFNA Operon of Bifidobacteria in Species-specific Adaptation to Host's Immune System

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Bifidobacteria are anaerobic bacteria that inhabited Earth long before the oxygenation of the atmosphere. As oxygen levels rose, bifidobacteria receded to favorable anaerobic environments, notably the intestines of animals. Bifidobacteria possibly shaped the immune system of animals ranging from insects to humans. The genus *Bifidobacterium* comprises 51 species ten of which are common inhabitants of animals. Some species of bifidobacteria, namely *Bifidobacterium longum infantis*, are the first to colonize the intestines of newborn children and to shape their immune system. This is evidenced by their ability to induce cytokines through Toll-like and other receptors. Concurrently, little if anything is known about the pathways mediating the interaction between the host immune system and symbiotic intestinal microbes, namely bifidobacteria. Our study was grounded in the premise that bifidobacteria possess genes involved in host-bacteria interaction. We hypothesized that such genes are species-specific and that they would comprise molecular machinery of signal transduction. Serine-threonine protein kinase STPK-2 fit our description as they were found across all bifidobacteria species [Nezametdinova et al., 2018 ; Zakharevich et al., 2015]. Next we established that STPK-2 are part of the PFNA gene cluster, which consists of five to seven genes that were found to be divergent between bifidobacteria due to positive selection [Nezametdinova et al., 2014; Dyachkova et al., 2019]. One of the key genes of PFNA is FNIII, which encodes a protein containing two type III fibronectin domains, including motifs of cytokine receptors. Following the incubation of the FNIII fragment with the cytokines TNF α , IL-6, IL-1 β , IL-10, we determined its ability to bind specifically to TNF α [Dyakov et al., 2020]. In a follow-up study, we demonstrated that coincubation of *B. longum* GT15 with TNF α alters the expression of 176 operons, among which are those involved in antioxidative properties and genes making up the PFNA operon [Veselovsky et al., 2020]. Currently, studies are ongoing to understand the molecular mechanisms underlying the FNIII-TNF α interaction. Our data indicate that the PFNA operon is a species-specific feature of bifidobacteria that serves the purpose of communication with components of the human immune system and perhaps among bifidobacteria. The discovered phenomenon will pave the way for future studies of the pathways of speciation, adaptation and biodiversity in bacteria. Naturally, this begs the question of what other mechanisms of bacteria-host communication were developed by other genera and families of commensal inhabitants of the human microbiota.

Low carb diet and microbiome

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Low carb diets, understood in a broad sense, refers to any dietary strategy on restriction carbohydrates below 100g. The diets, which can induce physiological ketosis (increase of ketone bodies), are called ketogenic. There are several approaches for the development of ketonemia: classic ketogenic diets, periods of fasting, time-limited feeding, calorie restriction, or even vigorous exercise. Ketone bodies are energetic substrates, which can be oxidized in heart, brain, and muscle, therefore, participating in bioenergetics homeostasis; control mitochondrial metabolism and energetics; reduce oxidative stress by inhibiting ROS/superoxide production, reverting lipid peroxidation and protein oxidation and increasing antioxidant protein levels; act as signaling intermediators; modulate inflammation and immune cell function.

Keto diet has a double effect on the microbiota, directly by modifying substrate availability and indirectly modifying several functions which also impacts on microbiota. In the same line, epigenetic changes can be influenced by microbiota, but also by ketone bodies. Ketone bodies (β -hydroxybutyrate and acetate) have been confirmed to affect epigenetic mark by inhibiting histone deacetylase class, modifying proteins at the post-translational level by butyrylation, affecting DNA methylation and acetylating histone and non-histone proteins. But, metabolites produced by microbiota are also substrates or cofactors of enzymes involved in epigenetic process.

It is known that intestinal epithelial cells feed exclusively on butyrate, which is produced by our resident microbiota after consuming dietary fiber or other prebiotics. At the same time, the body metabolic flexibility lies in the fact that one of the ketones, B-hydroxybutyrate, is also able to support the energy needs of intestinal cells. In fact, there are at least four molecules that can replace butyrate: isobutyrate, acetoacetate, B-hydroxybutyrate, and acylcarnitines. Isobutyrate is a protein metabolite. When butyrate is deficient, isobutyrate can be absorbed from the intestinal lumen by intestinal epithelial cells and metabolized for energy. Fecal isobutyrate is known to be elevated in people on keto diet. Isobutyrate can stimulate the same receptors in the gut as butyrate to influence mucus secretion, antimicrobial peptide release, and immune regulation. Despite the fact that the concentration of isobutyrate is much lower than that of butyrate, the metabolic effect of isobutyrate is much higher.

Keto diet reduced microbial diversity but increased the abundance of *Akkermansia muciniphila*; *Methanobrevibacter* spp.; *Roseburia* spp. The presence of *Methanobrevibacter* (Archae) and *Akkermansia* are associated with increased expression of innate and adaptive immune response genes.

Reduction of conditionally pathogenic bacteria in gut microbiota in children with functional digestive disorders after treatment with autoprobiotic and industrial enterococcus strain

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Relevance of the study

Personalized symbiont therapy based on autoprobiotic technology, which involves isolating individual microbiota members as pure cultures, their genetic analysis, and returning bacteria back to the gastrointestinal tract after propagating them outside the body. The possibility of prolonged persistence of autoprobiotic strains in the body and prolonged action is an advantage that distinguishes them from industrial probiotic strains. In pediatric practice, the therapeutic effect of autoprobiotic strains has not been studied.

Research objective – to detect the effect on clinical symptoms of functional digestive disorders in children and to establish the frequency of reduction of opportunistic bacteria in the gut microbiota with after treatment with autoprobiotic and industrial enterococcus strain.

Patients and methods

35 children over 3 years of age with functional digestive disorders were observed on an outpatient basis and randomly prescribed an autoprobiotic or industrial probiotic strain of *E. faecium*. Examination of the gut microbiota was carried out by the PCR-RV method in faeces using the Colonophlor primer set twice: before and after treatment. Patients formed groups: group 1 (n = 16) - treated autoprobiotic strain and group 2 (n = 18) - treated industrial probiotic strain *E. faecium* L3. The autoprobiotic and probiotic were used as a liquid form (soybean protein isolate starter containing 109 CFU in 1 ml) at a dose of 25 ml 2 times a day for 10 days. Statistical processing of the results was carried out using Student's t-test, they were considered valid at a significance level of $p < 0.05$.

Results

The use of an industrial probiotic strain of *E. faecium* and an autoprobiotic strain revealed a positive effect on the clinical symptoms of the disease in group 1 and group 2 (in 29.4% and in 25%, respectively) in the absence of adverse side events. Study of the frequency of significant increase in the content of conditionally pathogenic bacteria in the gut microbiota (CFU not less than 105 in 1 g feces) in children of group 1 and group 2 in the observation dynamics found a more frequent initial total increase in the conditionally pathogenic bacteria content in group 1 ($p < 0.05$), the dynamics in group 1 showed a significantly higher frequency of their “radiation” compared to group 2 ($p < 0.001$) (Figures 1 and 2).

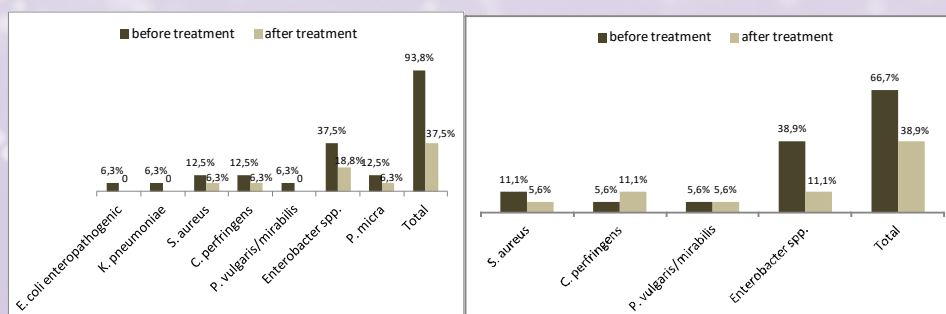


Figure 1.

Figure 2.

Conclusion.

The course use of autoprobiotic strains in children with functional digestive disorders contributed to a reliable decrease in the rate of release of conditionally pathogenic bacteria, this allows us to suggest sanitizing effects of autoprobiotics in “persistent” intestinal dysbacteriosis. The obtained data indicate the feasibility of using autoprobiotic enterococcus strains with increased content of conditionally pathogenic bacteria in children with functional digestive disorders.



Sae Hun Kim
Short Bio – 2021

Dr. Sae hun Kim is a Professor at Korea University (Department of Food Bioscience and Technology) and serves as the head of the department of food bioscience and technology (Korea University). Dr. Kim is a microbiologist studying probiotics and probiotic-derived metabolites for metabolic disorders and age-related diseases. He holds the M.S. degree in Animal Science from Korea University (Seoul, Korea) and the Ph.D. degree in Food Science from Oklahoma State University (Oklahoma, USA). Dr. Kim's professional background includes positions at industry (Culture Systems Inc.) and academia (Oklahoma State University). Dr. Kim's external funding stems from private industries such as Lotte Food, Cellbiotech, Chong Kun Dang Pharm, and from governmental agencies such as Korea Institute of Planning and Evaluation for Technology in food, agriculture, and

Forestry (IPET), National Research Foundation of Korea (NRF), Korea Testing Laboratory (KTL) etc., summing to 134 funded projects he served. He co-authored in 22 patents and has published more than 104 journals. Dr. Kim serves as a member of the editorial board of the International Journal of Clinical Nutrition and Dietetics, and the Current Topics in Lactic Acid Bacteria and Probiotics. He also served as the chairman of the Korean Society for Food Science of Animal Resources.

1 Health benefits of probiotics and postbiotics on gastrointestinal health

Sae Hun Kim

Colorectal diseases

The gastrointestinal (GI) tract is a set of organs responsible for food digestion and waste processing. Among these organs, the small intestine absorbs nutrients and water, and the large intestine forms waste into stool. Stress and inflammation are major causes of colorectal diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and colorectal cancer (CRC). IBS is a large intestine disorder with symptoms such as abdominal pain, cramping, and bloating. IBD is a chronic inflammatory disease in the digestive tract with symptoms such as diarrhea, rectal bleeding, abdominal pain, fatigue, and weight loss. CRC is cancer that occurs in the colon or rectum. Major symptoms are rectal bleeding, abdominal discomfort, fatigue, and weight loss. Recent research suggests probiotics and postbiotics as prophylactic and therapeutic agents for IBS, IBD, and CRC. Probiotics are living microorganisms that benefit the host's health. Postbiotics are non-viable bacterial products or metabolic products from microorganisms that have beneficial biological activities on the host.

Treatment of probiotics, postbiotics for colorectal diseases

Current studies reveal that probiotics and postbiotics have potential as novel prophylactic and therapeutic agents for IBS, IBD, and CRC. A clinical study giving IBS patients yogurt containing probiotics for 6 weeks alleviated IBS symptoms such as abdominal pain and constipation. In a different study, probiotic containing chocolate consumption for 4 weeks improved stool consistency in IBS patients. Moreover, in a study on the effects of *Lactobacillus* 2-week treatment in a DSS-induced colitis murine model, *Lactobacillus* treatment reduced colon damage and inflammation in the colon. Also, 10-week treatment of *Lactobacillus gasseri* fermented with *Cudrania tricuspidata* leaf extract attenuated colon damage and regulated apoptosis in an AOM/DSS induced colon carcinoma murine model. Overall, these studies suggest that probiotics and postbiotics have potential to improve colorectal structure and metabolism. Therefore, probiotics and postbiotics may be novel therapies for colorectal diseases.

Effects of *E.faecium* probiotic intake in patients with type 2 diabetes

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Introduction

Type 2 diabetes is a highly prevalent metabolic disorder characterized by an imbalance in blood glucose level, altered lipid profile and high blood pressure. Gut microbiome dysbiosis influences the intestinal barrier functions and metabolic pathways which leads to rapid progression of insulin resistance. Many studies have shown that probiotic intake in patients with type 2 diabetes might influence restoration of microbiota composition, improvements in body mass index, blood pressure, lipid profile and glucose metabolism. However, these changes are strain dependant. In our work we have studied the effects of the *E. faecium* L3 probiotic intake on microbiota composition, blood parameters and psychological state for the patients with type 2 diabetes.

Materials and methods

The study included 23 patients with type 2 diabetes. Patients received *E. faecium* L3 containing probiotic for 20 days in addition to their main type 2 diabetes therapy. Before and after probiotic treatment blood and fecal samples were taken. During the study patients filled in gastrointestinal and psychological diary. Feces were tested for microbiota status using quantitative PCR using test system “Colonoflor-16” (Alfalab, Russia) capable to detect main gut bacteria - *Lactobacillus* spp., *Bifidobacterium* spp., *Escherichia coli*, *Bacteroides* spp., *Bacteroides thetaiotaomicron*, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Clostridium difficile*, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli* enteropathogenic, *Enterococcus* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Fusobacterium nucleatum*, *Parvimonas micra*, *Staphylococcus aureus* and *Candida* spp. Several fecal samples were tested using metagenome analysis on Illumina platform. Blood was tested for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamin transpeptidase, bilirubin, albumin, glucose, insulin, lactat dehydrogenase, triglycerides, cholesterol, low density lipoprotein, high density lipoprotein and C-reactive protein change levels.

Results

After the probiotic treatment most of the patients experienced an improvement of their gastrointestinal condition with no side effects. Patients reported on disappearance of dyspeptic symptoms and increase in activity level. A significant decrease of C-reactive protein level was detected while other changes in blood parameters remained statistically unreliable. Microbiota study has shown the decrease in the abundance of *Escherichia coli* and *Bacteroides fragilis* which indicated gut microbial balance restoration. Other microbial parameters stayed constant.

Conclusion

Based on a preliminary statistical assessment of clinical and laboratory data we detected positive effect of probiotic therapy on the severity of gastroenterological complaints in patients with type 2 diabetes. The lack of significant positive dynamics in most laboratory parameters may be associated with a small sample size and a relatively short treatment period. At the same time, the revealed significant decrease in the level of C-reactive protein in the blood of patients revealed reducing the severity of systemic low-grade inflammation. Several positive, but insignificant changes in most laboratory parameters, and a significant decrease in the titer of opportunistic bacteria in the intestinal microbiota may indicate a favorable effect of probiotic therapy on the course of diabetes due to correction of dysbiotic disorders.

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Talk of A. Suvorov

Autoprobiotics from the concept to the clinical evidence

Genes-bacteria metagenomic signature of human's gut microbiota as a biomarker of depressive condition

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Introduction

In recent research great attention is being paid at the role of gut microbiota (GM) on human's health. It is currently viewed as an important independent virtual organ, which interacts with its host's organs and systems. The microbiota-gut-brain axis, in particular, provides a bidirectional communication between GM and human's nervous system, including the brain. Certain intestinal bacteria have shown the ability to produce various neurotransmitters and other molecules with neuroactive, immunomodulatory and antioxidant functions, etc.

GM studies conducted by various researchers have shown significant decline of richness and diversity in the GM of patients with depression compared to the healthy controls, as well as changes in abundances of certain taxa (Averina et al., 2020). Altered taxonomic composition may lead to changes of functionality of GM due to different abundances of metabolic pathways and, thus, may affect host's organism. In this study, we identify functional changes in GM of patients with depression using the approach of metagenomic signatures – a set of pairs gene-species that allows the complete description of the microbiota.

Methods

Patients with depression were selected among those admitted for inpatient treatment at N.A. Alexeev Psychiatric Hospital of Moscow during the first 6 days of their stay in the clinic. Fecal samples from 14 patients with depression and 15 healthy volunteers as controls were collected using standardized approaches. Total metagenomic DNA was isolated from the samples and sequenced on Illumina HiSeq X Ten. Then, the obtained metagenomic reads were assembled using MetaSPAdes (Nurk et al., 2017) and analyzed using the pipeline developed and described in our previous works (Kovtun et al., 2018). The reference catalog used in the pipeline was also significantly updated for current research. Statistically significant changes in relative abundances of the detected signature pairs were obtained by Wilcoxon rank test and correction for multiple comparison by Benjamini-Hochberg test ($P < 0.01$).

Results

The sequenced metagenomic samples of GM of patients with depression and healthy controls were assembled into contigs and analyzed with the pipeline for detection of metagenomic signatures. For that purpose, we used the revised reference catalog of bacterial genes that encode enzymes involved in synthesis and metabolism of key neurotransmitters, short-chain fatty acids and other compounds with neuroactive potential and antioxidants. For this research, the catalog was also broadened with the compounds, which abundance had been shown to change in patients with depression (Averina et al., 2020). Overall, the catalog included 1031 amino acid sequences for 101 enzymes.

As a result of the metagenomic analysis, we managed to identify 15 signature pairs gene-species with statistically significant ($P < 0.01$) decrease of relative abundance in microbiota of patients with depression. They include genes that encode the enzymes involved in formation of spermidine, acetic acid, glutamate, conjugated linoleic acid, indole-3-propionic acid (IPA), asparagine, arginine, and degradation of estradiol in such bacterial species, as *Anaerostipes hadrus*, *Eubacterium eligens*, *Faecalibacterium prausnitzii*, and *Roseburia intestinalis*.

This is an ongoing research, and the cohorts will be significantly broadened. But these first findings show that some enzymes involved in the bacterial metabolic pathways of important active compounds differ in abundance significantly in the microbiota of depressed and healthy patients. These results can further be used as biomarkers for the development of methods for correction of the condition of patients with depression, including в том числе пребиотиков и постбиотиков (Yunes et al., 2021).

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Changes in the intestinal microbiome of patients with inflammatory bowel diseases after transplantation of normal intestinal microbiota

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Background & aims

It is believed that, on average, the microbiota biodiversity in healthy people is greater than in various pathologies, in particular ulcerative colitis (UC). Therefore, with successful treatment, bacterial biodiversity in samples after fecal microbiota transplant (FMT) should increase. In our study, we evaluated how the intestinal microbiome changed in patients with ulcerative colitis after a single FMT during colonoscopy.

Methods

We used fecal samples obtained from 20 patients (27–57 years old) with ulcerative colitis. The diagnosis was confirmed on the basis of the clinic, the results of the study of the level of fecal calprotectin, fibrocolonoscopy data and histological examination of biopsies taken from different parts of the colon and ileum. FMT was performed once with fibrocolonoscopy under sedation. The donors were young healthy volunteers (20-39 years old) without chronic diseases, who had not survived infections in the last two months. All donors were examined according to the protocol of clinical trials, which included a blood test for the presence of parasites, an analysis of feces for dysbiosis and the absence of pathogenic microflora, helminths and their eggs. Fecal samples from patients were collected one to two days before FMT and 7-12 days after manipulation. Comparative analysis of libraries of 16S ribosomal RNA sequences created on the basis of samples obtained from patients with ulcerative colitis before and after FMT and sequenced on the Illumina MiSeq platform was performed.

Results

Our results indicate a reduced biodiversity of the intestinal microbiota under UC. In the studied samples from patients, significantly fewer Firmicutes sequences were present; after FMT, the number of Firmicutes did not increase. It is important that the species composition of lactobacilli increased significantly after FMT. The proportion of sequences belonging to the Bacteroidetes type in our patients with UC was not reduced, which differs from the observations of other researchers. And after FMT, the share of Bacteroidetes, like Firmicutes, remained practically unchanged. In our patients, both before and after FMT, *Prevotella* spp. prevailed, as well as in the inhabitants of Southeast Asia.

Conclusions

A single FMT procedure during colonoscopy leads to an increase in the average biodiversity of sequences in samples obtained after FMT, compared to samples collected before FMT: the proportion of lactobacilli increases, their species composition expands; the proportion of sequences of pathogenic and opportunistic representatives of the types Firmicutes and Proteobacteria is sharply reduced. The results can be taken into account in the development of new treatments for UC. The work was carried out under the Grant of the Russian Science Foundation No. 21-14-00360 «Changes in intestinal viromes in chronic intestinal diseases as a key to microbiota normalization».

Immunomodulatory activity of *Limosilactobacillus fermentum* U-21 - genes and proteins that potentially determine it.

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The study of the immunomodulatory properties of individual strains of the human intestinal microbiota tract is a topical area of researches in world science over the past decade. It has become obvious that the microbiome of a healthy person can serve as a source of obtaining probiotics for the prevention and treatment of diseases of the immune system. In this work, we analyzed the immunomodulatory activity of the *Limosilactobacillus fermentum* U-21 [Patent RU 2705250] strain isolated from an adult resident of the Central European part of the Russian Federation, characterized by the presence of probiotic properties, synthesizing a number of biologically active substances with immunomodulatory activity. The high antioxidant and protective potential of *L. fermentum* U-21 against dopaminergic neurons in the brain under oxidative stress (OS) was shown in a paraquat-induced Parkinsonism's model in mice and rats [Marsova et al., 2020] and by a bioluminescence test systems based on transgenic strains of *E. coli* [Marsova et al., 2018]. Full genome sequencing (PNBB00000000) and comparative genomic analysis made it possible to find in this strain genes, the products of which may have immunomodulatory properties. We were identified genes encoding peptidoglycan and a cluster of genes encoding a high-molecular polysaccharide fragment of the cell wall. Proteomic analysis of the extracellular culture fluid of the *L. fermentum* U-21 strain revealed a number of proteins secreted from the cell, including the unique protein LysM [Fillipe L.R. do Carmo et al. 2018]. This protein had a higher molecular weight, in contrast to the homologous protein of the control strain - it contains the incorporated serine-threonine peptide (STpLfU21). We hypothesize that the unique proteins LysM may be responsible for the immunomodulatory activity of the strain and activate the human immune systems. To test the potential immunomodulatory activity, we analyzed the expression of genes for pro- and anti-inflammatory cytokines of the human acute monocytic leukemia cell line THP-1 in the presence of *L. fermentum* U-21 strain (live and dead cells) and culture fluid. A significant change in the expression of the pro-inflammatory cytokines IL-6, TNF α and anti-inflammatory IL-10 was found. Thus, the molecular targets through which the unique *L. fermentum* U21 strain affects the host organism can be a number of signaling pathways of the human immune system. This paper has been supported by the RUDN University Strategic Academic Leadership Program

Probiotics and their Influence on Human Hygiene: A Literature Review

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Probiotics are living microorganisms that are of great importance in restoring the composition and functional activity of the human microbiota. The microbiota plays a fundamental role in shaping the health of the entire body. There are about 40 trillion bacteria in the human body that are interconnected. These microorganisms live in different loci of our body. Probiotics regulate the activity of the microbiota. When used in adequate amounts, probiotics have a positive effect on the health of the host. Probiotics can be found in a variety of foods, medications, and supplements.

Purpose of the study

To study the effect of probiotics on human health based on the analysis of literature sources.

Research results

From a hygienic standpoint, probiotics are used to maintain the physical health of various body systems. Hygienists highlight the hygiene of the gastrointestinal tract, oral cavity, genitourinary system, skin, namely the arms and armpits. Probiotics are essential in restoring the composition and functional activity of the microbiota. The results of clinical studies confirm the positive effect of probiotics on gastrointestinal, allergic and skin diseases, on strengthening the immune system. The use of probiotics was effective in the treatment of gastrointestinal diseases (gastrointestinal disorders, irritable bowel syndrome, cancers, etc.). Their use as an alternative to antibacterial drugs for oral hygiene also had a positive trend in the treatment of caries, periodontal disease, halitosis and bad breath. The results of clinical studies have shown the benefits of probiotics for reducing the colonization of candidiasis by the mucous membranes of the vagina and gastrointestinal tract, reducing the incidence and severity of clinical symptoms of various forms of candidiasis. However, data on the effect of probiotics on the urinary system are very contradictory, which indicates the need for further study of this problem. The immunomodulatory effect of probiotics promotes epidermal health and helps fight pathogens. There are currently no specific studies on the effects of probiotics on underarm hygiene, although hygiene products containing probiotic elements are becoming very popular.

Output

Our review of the literature revealed a positive effect of probiotics on human health, however, the insufficiency and, in some cases, contradictory research results indicate the relevance and need for further research on probiotics.



Hiroshi Ohno
Short Bio – 2021

Dr. Hiroshi Ohno is a Team Leader of the Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences, and a Professor at the Graduate School of Medical Life Science, Yokohama City University and at the Graduate School of Medicine, Chiba University. Dr. Ohno is an immunologist studying intestinal immunology, especially the function and differentiation of M cells, a unique intestinal epithelial cell subset specialized for uptake of luminal bacteria for initiation of intestinal immune responses toward them. He is also studying the host-gut microbiota interaction to understand the impact of gut microbiota on the host physiology and pathology. Dr. Ohno co-authored nearly 200 original articles in English, including highly cited papers published in Nature and other high profile journals.

The role of small intestinal microbes for central nervous system inflammation

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Enormous numbers of commensal bacteria, the gut microbiota, reside in our intestines. Accumulating evidence in the past decade has suggested that gut microbiota deeply impacts the pathogenesis of various diseases including autoimmune diseases including multiple sclerosis (MS); nevertheless, the underlying mechanisms are still largely unclear. MS is an autoimmune demyelinating disease in the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is an mouse model of MS, where mice are immunized with a self antigen, myelin oligodendrocyte glycoprotein (MOG) peptide to induce autoimmune CNS inflammation. Employing EAE, we have revealed the role of small intestinal microbiota in the pathogenesis of autoimmune CNS inflammation. One strain, *Lactobacillus reuteri* possesses a MOG mimicry peptide to promote proliferation of MOG-specific T cells migrating into the small intestine from periphery. The other strain, belonging to *Erysipelotrichaceae*, acts as an adjuvant to promote differentiation of the MOG self antigen-reactive T cells into inflammatory Th17 cells. These two small intestinal commensal bacteria act together to exacerbate EAE pathogenesis.

Fasting and fasting mimetics address Sirtuin pathways and microbiota structure

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Background

Fasting and fasting mimetics - bioactive compounds mimicking fasting effects, are of growing interest as potential means to slow down the aging process and increase health span. Sirtuins are known as enzymes that interfere with mitochondrial energy metabolism and molecular pathways involved in longevity. Although their activation is determined as a response to stress i.e. caloric restriction, Sirtuin- activating nutraceuticals are believed to mimic the effects of nutrient deprivation, thus activating signaling pathways correlated to an improved health span. Additionally, microbiota structure and especially metabolites can be involved in beneficial effects of CR, fasting and fasting mimetics. In this study, we compared 7 days periodic Buchinger fasting intervention with 3 months fasting mimetic supplementation, a drink formula, containing secondary plant ingredients considered to activate Sirtuins.

Methods

We analyzed pathways in response to fasting and a Sirtuin activating drink (Sirt food shot). Genetic and epigenetic biomarkers including telomere length, LINE1 methylation, and a set of mRNAs and miRNAs were assessed using qPCR analysis. Gut composition and metabolites were compared using Illumina sequencing and mass spectrometry.

Results Fasting, but also the fasting mimetic could increase expression of FoxO1, SIRT1, and MLH1 mRNA, all genes discussed in aspects of longevity. A positive correlation between telomere length and both SIRT1, and SIRT6 was observed. Following fasting ketogenic BHB, the GI microbiota diversity, Proteobacteria but also the abundance of longevity related *Christensenella sp.* increased. Interestingly a significant correlation was seen between Sirt 1 expression and the abundance of *Prevotella sp.* and *Lactobacilli sp.* In the Sirt mimetic group diversity and actinobacteria increased. Firmicutes/Bacteroidetes ratio decreased and correlated with the body mass index (BMI).

Conclusions

Our results confirm the effects of fasting on longevity associated mechanisms but also suggest that fasting mimetic intervention addresses some of these effects. Whereas longevity, caloric restriction and fasting was mostly discussed with a focus on signaling pathways and epigenetics, microbiota distribution and metabolites certainly have an additional effect on healthy aging and longevity. Furthermore, personal characteristics of microbiota may explain personal different metabolic responses to nutrition and foods as well as personal different metabolization and bioavailability of nutraceuticals.

Impact of *Bacillus subtilis* Probiotic Cultivated on Local Agro-Industrial Waste on Broiler Productivity

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The problem of avian dysbacteriosis is a challenge in the poultry industry. To solve the problem, appropriate antibiotics are used. However, the widespread concern is the appearance of pathogenic microorganisms that are resistant to modern antibiotics. The growing demand to improve the quality of poultry products has put on the agenda the search for alternative methods to replace antibiotics in poultry products. To achieve this goal, the strain of *Bacillus subtilis* Katmira 1933 exhibiting exceptional probiotic potential has been tested in broilers as a feed additive. *B. subtilis* was cultivated in solid-state fermentation of local agro-industrial raw materials (wheat bran and vinasse), then dried fermented biomass containing 1×10^{12} CFU/g was used at a concentration of 0.03%, 0.04%, and 0.05% as a feed additive in the broiler farm "Roster". In parallel with three above-mentioned test groups of birds, the control group was treated with an antibiotic commonly used on a poultry farm. Both control and experimental groups of broilers were fed by a complete combined feed, which met the broiler's demand for nutrients, minerals, and biologically active substances, according to the phases of broiler development.

Based on the experiments, it was found that the optimal dose of probiotic *B. subtilis*, cultivated on plant raw materials, used as a feed additive in broiler is 0.04%. Under these conditions, feed conversion ratio was almost the same in both groups. In the experimental groups, the average daily weight gain during the rearing period was 3.5-3.7 g higher than in the control groups (on average, 53.0-53.2 g/day), the absolute gain in live weight of the experimental broilers increased by 7.3%. Survival rate of experimental groups was 96-98% which is 2-4% higher than in the control group. The productivity index for the experimental groups turned out to be higher by 24-34 points in comparison with the control group.



Jin-Seng Lin
Short Bio – 2021

Mr. Jin-Seng Lin is the Director of the Culture Collection & Research Institute of SYN-BIO TECH INC. He got his M.S. degree in Applied Microbiology from Taiwan University. He worked as senior scientist in Central Research Institute of Uni-President Enterprises and was in charge of the development of fermented dairy product for 12 years. He was awarded the Outstanding Food Technician from Taiwan Association for Food Science and Technology in 2006. He co-authored several papers, and more than 20 patents. He served as a Council member of Taiwan Association for Lactic Acid Bacteria from 2013.

***Lactobacillus plantarum* TWK10 attenuated age-related muscle weakness, bone loss, and cognitive function decline via modulation of gut microbiota in mice**

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Aging is the major risk factor for developing chronic diseases. Hence, developing strategies for promoting healthy aging is a crucial issue. As previous study indicated, *Lactobacillus plantarum* TWK10 has been demonstrated to show benefits on improving muscle strength, exercise endurance, and on regulating body composition in healthy humans. In this study, effects of TWK10 on attenuating the progression of age-related impairments were investigated in mice. Male ICR mice were first grouped by age, namely young (4-month-old), and aged groups (19-22-month-old), and then assigned to Y-Control (young mice administered PBS), Y-TWK10 (young mice administered 1x10⁹ CFU/day TWK10), A-Control (aged mice administered PBS), and A-TWK10 (aged mice administered 1x10⁹ CFU/day TWK10) groups. During the experimental trial, body weight, food intake and water intake were recorded every week. Freshly voided fecal sample was also collected at 8 weeks after administration for microbiome analysis. Forelimb grip strength was monitored at baseline, 4 weeks, and 8 weeks after administration. After 8 weeks of administration, all animals were subjected to the evaluations of body composition, and cognitive function. The forelimb grip strength in the aged mice groups was significantly decreased than in young mice groups. After eight-week administration of TWK10 in the A-TWK10 group, age-related weakness of grip strength was significantly restored compared with that in the A-TWK10 at week 0. Further, the improvement of muscle strength was accompanied with elevated levels of muscle glycogen. Age-related bone loss was demonstrated by remarkable reduction of trabecular number (Tb.N) and a trend of enlarged trabecular spacing (Tb.Sp) compared with Y-Control. By administration of TWK10, Tb.N in A-TWK10 group was significantly increased than those in the control mice. Amelioration of age-related learning and memory decline was demonstrated by shortened escape latency in A-TWK10 group. Further analysis of gut microbiota by NGS of the 16S rRNA gene showed the pattern of gut microbial composition was clearly altered by TWK10 administration. The abundance of short-chain fatty acid (SCFA)-producing bacteria was enriched by TWK10 and was also evidenced by the levels of gut SCFA. Furthermore, a decreasing trend on age-related changes in the bacterial taxa was observed in TWK10-administration group. In conclusion, *Lactobacillus plantarum* TWK10 could be a potential therapeutic agent for promoting healthy aging via attenuating age-related disorders and modulating the imbalance of gut microbiota.

A new systematic approach to the creation of probiotic preparations based on innovative engineering solutions.

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At the moment, there are no general approaches to the search for probiotics, their testing in vitro and in vivo, the selection of strains and their comparison with each other. This makes difficult selecting strains for the creation of new drugs for medicine and agriculture.

We offer our own approach to the searching for new probiotic bacteria.

1. Search for strains of probiotic bacteria in organisms of the same genus and species for which the probiotic preparation will be created. In our studies, we selected a litter of chickens of various types of housing (cage, outdoor, free-range), from which we obtained potential probiotic strains of *Bacillus* and *Lactobacillus*.
2. Security check. In this study, we tested the strains for mutagenicity, hemolytic activity, and antibiotic resistance profile.
3. Investigation of potential probiotic properties in vitro. We investigated the antioxidant and genoprotective properties of probiotic strains using lux-biosensors.
4. We have developed a model of artificial intestine of birds, which allows us to simulate the conditions of the cecum of chickens. We also developed an synthetic intestinal media that simulates the intestinal contents of chickens. This makes it possible to study the properties of probiotic bacteria under conditions similar to those of the intestine.
5. Use of control strains with known probiotic activity.

Thus, we have obtained potential probiotic strains that are active in the gut of birds. The effect of probiotic strains in vivo has been studied. It has been shown that probiotic preparations in broiler feed increase live weight and growth rate, reduce feed conversion, stimulate the immune system, but at the same time do not have a negative effect on intestinal microbiota and blood biochemical parameters.

Systemic effects of probiotics

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The main mechanisms that ensure the positive effect of probiotics on the host organism are considered to be as follows:

- antagonism with pathogenic microflora
- stimulation of specific and non-specific immunity
- stimulation of the growth of normal microflora
- release of digestive enzymes
- production of amino acids and vitamins
- destruction of xenobiotics (allergens, mutagens), as well as substances that impede the absorption of food

However, recent studies increasingly show that not all of the effects of probiotics fit into this pattern. Apparently, the effect of probiotics is systemic, affecting not only individual organs and organ systems but also the whole organism. This work examines the effects of probiotics, which may be associated with subtle regulatory influences, their mechanisms, and their relationship with DNA protection (antimutagenic activity). Our research also shows that the antimutagenic and regulatory properties of probiotics can be the basis of their systemic effects.

Thus, in a study of the biological activity of two *Bacillus* strains carried out on chickens, it was found that a preparation based on the *Bacillus subtilis* KATMIRA1933 strain caused a decrease in the number of mitochondrial DNA damage (by 34% compared to the control). The probiotic supplement was found to increase the transcriptional activity of vitellogenin synthesis genes. In addition, the rate of reproductive aging of chickens compared with the control slowed down by 2.1%. There was also an improvement in several physiological and biochemical parameters.

The systemic principle assumes action on critical points, regulators, and this action should be transmitted inside the cell according to the principle of cascade amplification of the signal, which would explain the effects of minimal doses. Probiotic bacteria can implement this principle in symbiotic effects with the host and antagonism with other bacteria.

The following can be distinguished as target processes for systemic influences:

1) In prokaryotes - SOS-repair and expression of genes for response to stress reactions; quorum-sensing; horizontal gene transfer. This process, being associated with the SOS response at the level of regulation (via the RecA protein), is also an essential mechanism for adaptation to antimicrobial agents.

2) In eukaryotic cells - processes in mitochondria and expression of nuclear regulator genes that trigger such cascades as, for example, the p38 MAP kinase pathway.

The existing paradigm of probiotic perception, in our opinion, overlooks significant systemic effects that occur in the host's body. The new concept of interaction of probiotics with eukaryotic hosts will allow revising approaches to the practical application of probiotics in medicine and veterinary medicine and creating new strategies for the search and development of new strains and probiotic preparations.

Using natural aquatic organisms as taste attractants for aquaculture of salmon

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Farming salmonids is considered one of the most promising areas of aquaculture. However, there is a problem of creating attractive feed mixtures that would not only satisfy the biological needs of fish, but also serve as gustatory stimuli capable of increasing the feed intake. The development of a technology for the use of wastes obtained during cutting of marine hydrobionts for the production of feed additives, based on the study of the taste preferences of fish, is extremely relevant for modern aquaculture.

Purpose: development of additives and technology for the use of waste obtained during cutting of marine aquatic organisms for the production of feed additives.

Material and research methods. The fish were caught with net fishing gear in the upper reaches of the Paratunka River (char - *Salvelinus malma*) and the Karymayskaya channel of the Bolshaya-Bystraya River (coho salmon - *Oncorhynchus kisutch*). On April 2, 2021, 200 juveniles of chum salmon (*Oncorhynchus keta*) were caught at the salmon farm in the village of Ketkino and delivered to the laboratory under similar conditions. Experimental setup for aquariums with a volume of 5 liters. Experiments were performed using methods behavioral test reactions to artificial fish feed pellets soaked in the aqueous extract of hydrobionts, *Salvelinus* - 20 copies, 20 copies with coho salmon and 60 specimens. with chum salmon. The number of experiments carried out on char was 840, and on coho salmon - 325, chum salmon - 200. As aquatic extracts of invertebrates, hydrobionts were used (northern chillim shrimp *Pandalus borealis*, larvae of Chironomidae, snow crab - *Chionoecetes opilio*, kelp (stem and thallus) - *Saccharina bongardiana*) and artificial feed (company "Agro Server", starting) in a concentration of 300 g / l. These aqueous extracts were added to the granules as flavoring agents for dry food granules. The dry feed pellets were used as a control. First, the aquatic organisms were weighed, then kneaded in a mortar and gradually mixed with settled water until homogeneous. The resulting extract was stored in a refrigerator for no more than 1 day. Dry granulated food was soaked in an aqueous extract of the attractant for 1 hour, before feeding the fish.

Results. Among the granules with extracts of marine organisms and algae tested (crab, shrimp, bloodworm larvae, kelp), most of the tested objects had an attractive taste, one extract had an indifferent property, and no deterrent properties were found for juvenile coho salmon. Granules containing shrimp extract at a concentration of 300 g / l had the maximum palatability for juvenile coho salmon. The most attractive for char were the granules containing the water extract of shrimp, their consumption was 78.9% in comparison with the control granules, the granules containing the bloodworm extract were slightly less attractive - 65.6%. Consumption of granules with artificial feed extract (29.7%) was at the level of consumption of control granules (28%) and did not differ statistically. The shrimp pellets were most readily consumed immediately after a single setting, in contrast to other pellets, where several grasps were noted in the experiment. The most attractive stimulus for chum salmon is snow crab extract (500 g / l). Chum salmon reacts to feed with additives more actively than to dry granulated compound feed, the reaction of chum salmon to granules with extract manifests itself instantly, as soon as the granule enters the water. Young chum salmon feed more actively in the morning and before lunch time.

Conclusion. As a result of the experiments, it was found that the used extracts of aquatic organisms have a highly attractive taste for coho salmon, chum salmon and char, except for granules with bloodworm extract (an indifferent stimulus, that is, their consumption did not differ significantly from the consumption of dry food granules). The most effective appealing flavoring stimulus was the shrimp and snow crab extract pellets at all concentrations used, which significantly increased pellet consumption by up to 98%. The results obtained showed that natural food items (marine aquatic organisms and algae) can be used as attractants that enhance the consumption and irrigation attractiveness of artificial pelleted food.

The work was carried out in the laboratory of the Department of Aquatic Bioresources, Fisheries and Aquaculture, with the financial support of the Russian Fisheries Agency.

The use of Mundtucin E28 (KS) for decontamination of *Listeria monocytogenes* from poultry products.

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Objective(s)

The food safety requires reliable instruments for foodborne pathogens presence control. The human society are becoming increasingly aware of the human health risk posed by the use of chemical preservatives and antimicrobials in foods. Active use of antibiotics cause the forming of wide antibiotic resistance in the human microbiota so there is now a need in the new alternative. Numerous bacteria produce small peptides with antimicrobial activities called bacteriocins. Mundtucin E28 belongs to class IIa bacteriocins and produced by strain *Enterococcus mundtii* E28 isolated from sour milk and deposited under the catalog number B-8398 (here and after *E. mundtii* B-8398) into the GCPM-Obolensk collection. It is known that mundtucins inhibits the growth of most Gram-positive foodborne pathogens including *Listeria monocytogenes* – the cause of listeriosis. Raw poultry meat and semi-products considered as risk of *Listeria monocytogenes* infection, due to both the prevalence and growth potential on these products. Biopreservation may be a mild and natural effective strategy for minimizing this risk. This study aimed to evaluate the suitability of mundtucin E28 (KS) for listerial decontamination of poultry products.

Results

A fraction of mundtucin E28 with a purification quality of about 70% was obtained by one-step elution from the CM Sephadex C-25 sorbent added to the culture medium before cultivation. Mass spectral analysis, electrophoresis, and DNA sequencing showed the high range similarity of mundtucin E28 to mundtucin KS. The activity of mundtucin E28 (KS) remained stable over a wide range of pH, temperatures and enzymes except proteinase K and α -chymotrypsin. The antibacterial activity was established for all tested strains of *Listeria* spp., *Enterococcus* spp., *Clostridium perfringens* and other foodborne pathogens in nanomolar concentrations. To study the potential of its practical application, a several models of listerial contamination and decontamination of poultry products were performed such as alginate food wrap, spraying, dipping. The strain *Listeria monocytogenes* ATCC 19111 at the concentration 1×10^6 CFU was used. It was found that the listerial contamination decreased five orders of magnitude at 4°C for 40 minutes and remained at this level for three days at a mundtucin E28 (KS) concentration of 40 ng/ml. At the same concentration but at 25°C the listerial contamination decreased three orders of magnitude. At a concentration of 160 ng/ml, complete elimination of *Listeria monocytogenes* ATCC 19111 from the test samples was observed within 40 min at both temperature conditions.

Conclusion

According to the obtained results, mundtucin E28 (KS) may potentially be used as a bioprotector to improve the food safety of poultry products.



Svetoslav Dimitrov Todorov
Short Bio – 2021

Svetoslav Todorov has completed his Ph.D at ENITIAA, Nantes, France and Sofia University, Sofia, Bulgaria and postdoctoral studies from Stellenbosch University, Matieland, South Africa. From 2008 till 2019 he was visiting researcher and invited lecture at University of Sao Paulo, Sao Paulo (SP), Brazil and Federal University of Vicosa, Vicosa (MG), Brazil and served as consultant on several project for EMBRAPA Sobral (CE) and EMBRAPA Rio de Janeiro (RJ), Brazil. Since May 2019 he is a research professor at Handong Global University, Pohang, Republic of Korea. He has published more than 190 papers, in reputed journals (h-factor 44) and serving as a member of the editorial board. He reviewed more than 120 international journals and serves as associated editor for Probiotics and Antimicrobial Proteins, a journal published by Springer-Nature.

Angels and demons in our GIT: The dark side of LAB

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Good or bad, red or blue, north or south, east or west, angels or demons: the philosophy always tries to frame everything in the bipolar models and to explain processes framing them in the convenience life ideas. Are microorganisms read the philosophy books? Are they following our ideas for good or bad or they are just behaving according to the natural physiological life processes? Conveniences and natural processes of biochemical reactions and basics of life processes are more relevant in microbial lifecycles compared with human's society philosophical theories.

The genus *Streptococcus* includes species remarkably differing in their behavior, applications, virulence and safety. *Streptococcus infantarius* subsp. *infantarius* has been found as predominant in some African dairy products that are widely consumed and considered to be safe. However, its safety may be questioned due to the association of this species with clinical cases. We have evaluated isolates from dairy origin, selected based on their bacteriocinogenic potential, differentiated by RAPD-PCR, identified by 16S rRNA sequencing as *St. infantarius* subsp. *infantarius* and investigated regarding their potential beneficial properties and factors related to virulence and safety. A series of *in vitro* tests included properties related to survival in the GIT and beneficial intestinal activities; production of bacteriocin/s, detection of related genes and partial characterization of expressed antimicrobial protein were evaluated. Genes related to folate biosynthesis were detected in both studied strains. Evaluation of physiological tests related to strains virulence, adhesion, resistance to antibiotics and detections of virulence and biogenic amines production related genes were also investigated. Taking in consideration beneficial properties and virulence characteristics, both strains cannot be considered safe for human or other animals application, even though they have been isolated from dairy products.

Our results reinforce that in order to confirm that one culture as beneficial its properties need to be analyzed in a greater detail, taking into account not only the high survival under GIT conditions, but also adhesion properties, production of specific enzymes and antimicrobials, interaction with other bacterial species in the same environment. It is important to know the potential "dark side" of the investigated strain(s). Questions should consider whether such strains carry virulence factors, facilitate colonization of non-desirable species/strains in the GIT, produce antimicrobials active other inhabitants of the GIT, exclude or sustain pathogens by their aggregation properties, etc. Moreover, just because a strain was isolated from food and no one reported sick after consuming the product, does not automatically confirm strains safety. Analyzing and setting the entire picture, it may be concluded that a potentially pathogenic bacteria has developed strong mechanisms for survival.



Gwénaél Jan
Short Bio – 2021

Gwénaél Jan is a senior research scientist working at the French National Institute for Agricultural Research (INRAE). He dedicates his scientific carrier to the microbiology of fermented foods, with a particular interest in “2-in-1” bacteria, which exhibit both technological and probiotic abilities. He originally studied Microbiology, as well as Molecular and Cell Biology, up to a PhD in science, in The French University of Rennes1. He then moved to the Institute of Medical Sciences in Aberdeen, Scotland, for a Post-Doc in Human Cell Biology. Being interested in both Microbiology and Human Cell Biology, he joined INRA to work on interaction between food beneficial bacteria and human cells.

His research activity, in collaboration with French and international academics, stakeholders, and technical centres, focuses on both probiotic and technological abilities of beneficial bacteria found in fermented food products. Mining this biodiversity revealed the ability, in selected strains of such bacteria, to modulate key parameters of human physiology such as proliferation, differentiation, apoptosis, inflammation and mucosal immunity. This exciting field of research opens new perspectives for the development of functional fermented foods for target populations.

This research activity is within the frame of a strong collaboration with Pr Benoit Foligné (Institut Pasteur, Lille University, France), with Pr Vasco Azevedo (UFMG, Belo Horizonte, Brazil), and with Pr Xiao Dong Chen (Dept Chemical Engineering, Soochow University, China).

Expertise field (keywords)

Food Microbiology, Probiotics, Proteomics, Surface Proteome, Cheese, Propionic Acid Bacteria, Lactic Acid Bacteria, Dairy products, Bacterial Interactions, Host Adaptation, Immunomodulation, Apoptosis.

Bibliometrics (2021)

H-Index: 32 - 102 publications, average citation per item: 32.72 - 3 international patents - 6 book chapters.

The key role of surface layer protein SlpB in the probiotic properties of *Propionibacterium freudenreichii*.

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Scope

Inflammatory bowel disease (IBD) constitute a growing public health concern in western countries. Bacteria with anti-inflammatory properties are lacking in the dysbiosis accompanying IBD. Selected strains of probiotic bacteria with anti-inflammatory properties accordingly alleviate symptoms and enhance treatment of ulcerative colitis in clinical trials. We found promising immunomodulatory properties in selected strains of *Propionibacterium freudenreichii*, a beneficial bacterium consumed as a cheese-ripening starter and as a probiotic. In the work presented here, we investigated the specific role of bacterial surface layer associated proteins in this modulatory effect exerted by *P. freudenreichii*, both in vitro and in vivo.

Methods and results

A preliminary study, based on immunologic, genomic, proteomic and transcriptomic comparison of 20 strains of *P. freudenreichii*, indicated that surface layer proteins (Slp) were involved in immunomodulation. To confirm this, a series of mutants was constructed, deleted in each of *slpA*, *slpB* and *slpE* genes. The *slpB* mutation caused dramatic suppression of *P. freudenreichii* probiotic properties. The mutant exhibited reduced adhesion to cultured human intestinal epithelial cells, compared to the wild-type. It moreover failed to induce the release of regulatory IL-10 in human peripheral blood leucocytes. In a murine model of mucositis, wild-type *P. freudenreichii* protected gut mucosa from inflammatory injury, while the mutant failed to do so. Accordingly, extracted SlpB protein was shown to exert anti-inflammatory effects in vitro.

Conclusion

This work enhanced our understanding of the immunomodulatory effects exerted by dairy propionibacteria. It evidenced the key role played by the SlpB protein in the anti-inflammatory effect of *P. freudenreichii*. It should lead to the development of functional fermented products for target populations. It opens new avenues for the use of SlpB protein as a bioactive compound.

FISH-FLOW: A non PCR-based technique for detection of vancomycin resistant enterococci

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The indiscriminate use of antibiotics over several decades has led to the emerging of different «super bacteria», adapted with defense mechanisms to counteract the effects of these antimicrobial agents. This is well documented and exemplified in the case of vancomycin resistant enterococci (VRE). As opportunistic pathogenic bacteria they pose an increasing health risk to the human population and in particular in hospitalized situations. A high morbidity and a mortality close to 10% has been reported for these nosocomial infections. The German national nosocomial infection surveillance system (KISS) determined that the number of cases between 2007 and 2016 increased by approximately 54%. To date, 8 different genes for resistance to vancomycin were described; however, *vanA* and *vanB* represent a latent risk, since these genes are plasmid associated and a horizontal gene/s transfer to another pathogen/s or beneficial microorganism/s is a possible scenario. Moreover, a direct correlation was reported between the time elapsed in the detection / treatment of bacteremia and mortality in patients with VRE, reaffirming the importance of a prompt diagnosis as a critical factor to select an appropriate antibiotic treatment and to reduce the number of deaths from this kind of infection. However, even when the “gold standard” of the currently applied methods for diagnosis has been proven to be efficient, we need to realize that they are time consuming and generally the time lapse until the final result can be more than 24 hours.

In our research, we have explored the appropriateness of Fluorescent In Situ Hybridization with the Flow Cytometry (FISH-FLOW) technique as an alternative to current methods, taking in consideration the analytical time and sensitivity of the method, down to 10⁴ bacteria, and the possibility of identifying different target genes, directly from the sample, without the need for DNA extraction or amplification.

In this study, 40 fecal samples were obtained from patients with bacterial infections from different hospital in the Republic of Korea. Putative VRE isolates were differentiated by RAPD-PCR, and 6 VRE strains were identified by 16S rRNA partial sequencing. Moreover, vancomycin resistant genes *vanA* and *vanB* and virulence genes associated with enterococci were detected by PCR in all 6 selected strains. For identification of VRE by FISH-FLOW we designed PNA (peptide nucleic acid) probes specific for the genus *Enterococcus* and for the *vanA* resistance gene. Results confirmed that the designed PNA specific probes can be used for detection and quantification of VRE strains in single and mixed bacterial populations, even directly from fecal samples. Previous results were validated by standard microbiological techniques growing VRE in media supplemented with vancomycin. Interestingly, the obtained results suggest that not all VRE in the population of a resistant strain contain or express the resistance gene under antibiotic free conditions, and only 20-50% of the population appear to carry these antibiotic resistance determinants. In conclusion, these results suggest that FISH-FLOW can be recommended as a rapid and reliable approach for the detection of VRE strains. This may be further developed as a future diagnostic tool for prompt diagnosis of VRE and other multidrug resistant nosocomial bacterial infections.

The role of bacteria in the plant rhizosphere and the perspectives of PGPR application as plant probiotics

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Soil bacteria play a crucial role in maintaining the terrestrial ecosystems stability and are particularly important for plants. The rhizosphere, a thin soil layer adjacent to the root surface, acts as an interface through which the plant interacts with the soil environment, and there are many ways the rhizosphere microbial communities are involved in this interaction. The idea that microbes are directly influencing the plant performance has attracted attention since XIXth century. For example, in the epigraph of his article “On the microbial activity of soil” (1894) D. I. Ivanovsky quoted A.S. Famintsin: “It is reasonable to pose a question – will the successful crop cultivation in the future be reduced to the adaptation of the soil for flourishing development of microorganisms in it?”. This idea was far ahead of its time. Throughout the twentieth century, the advancement of agricultural science was marked by the rapid development of the chemical industry, the intensification of agricultural production, the introduction of new types of fertilizers and pesticides into practice. Only in the last decades the spotlight of the research has shifted to sustainable agriculture, soil microbial augmentation, and plant probiotics. The bacteria can be beneficial for their plant hosts in a plenty of ways. Firstly, they are involved in mineral nutrients supply to the plants. Nitrogen fixing bacteria are widely used as plant inoculants, both for legumes and non-legumes. Nitrogen-fixing bacteria such as *Azospirillum*, *Paenibacillus*, *Azotobacter* and others have been successfully used to improve the nitrogen nutrition of a large variety of crops. Phosphorus mobilizing bacteria are also commonly used in bacterial fertilizers, with *Bacillus megatherium* as of the most commonly used species. Much less is known about potassium solubilization by silicate bacteria; however, there are commercial products containing these microorganisms. Secondly, bacteria can be used as eco-friendly plant protection products: biofungicides or bioinsecticides. Biofungicides can elicit prolonged action against fungal pathogens, by colonizing root surface and preventing invasion. Bioinsecticides are based on bacteria, producing toxic proteins that interfere with digestive system of pests (i.e. *Bacillus thuringiensis*). Thirdly, certain species of act like phyto-stimulating agents via several mechanisms: bacteria produce hormone-like substances, decrease ethylene level, etc. Indirect mechanisms include the production of antioxidants, osmoprotective agents, produce exopolysaccharides decreasing Na⁺ uptake under saline conditions. Thus, plant growth promoting rhizobacteria (PGPR) have a complex beneficial effect on the plants and can be considered plant probiotics.

Acknowledgements

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The microbial communities of highly polluted soils as a source of plant probiotics

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The microbial communities are characterized by a great level of adaptation to unfavourable environmental conditions, including anthropogenic pollution. The selection of resistant strains in contaminated soils is of great research interest that leads to the accumulation of bacteria capable to degrade or immobilize pollutants. These properties are beneficial not only for the bacteria themselves but also for the plants and other components of the ecosystem. The decrease of contaminants bioavailability due to the application of resistant bacterial strains reduce the stress in plants grown in contaminated soils as well as improves the total environment conditions. Thus, the beneficial effects of these microorganisms allow to consider them as a probiotics.

In this study, the microbial community structure of Spolic Technosols of dried lake Atamanskoe was studied using metagenomics approach. The lake served as a sludge reservoir for more than 40 years prior to drying. The studied Technosols contain high concentrations of heavy metals (for example, Zn content was up to 72900 mg kg⁻¹) and 16 priority polycyclic aromatic hydrocarbons (PAHs) (sum of 16 priority PAHs was up to 6670 mg kg⁻¹). 10 strains of metal-resistant strains of bacteria were isolated from the plots with the most significantly altered microbial community structure. All the strains were identified by 16S rRNA gene sequencing and MALDI TOF mass-spectrometry as species of *Bacillus* genus. The bacteria were tested for metal tolerance and plant-growth promoting properties, and three best strains were selected for further studies. The selected strains were identified as *Bacillus cereus*, *Bacillus atrophaeus* and *Bacillus pumilus*. An experiment with growing spring barley in contaminated soil has shown, that the inoculation of the consortium containing metal tolerant *Bacillus* strains improved the morpho-physiological state of the plants greatly. The root and shoot length of barley plants treated with bacteria increased compared to the control plants, grown in polluted soils. The photosynthetic performance of the plants was also improved, resulting in increased quantum yields of photosynthesis. Moreover, the concentration of heavy metals in plant tissues was reduced, suggesting the influence of the bacterial inoculant on the decreasing of heavy metals and polycyclic aromatic hydrocarbons bioavailability. Thus, the selected strains can be considered as a plant probiotics and can be applied for the remediation of polluted soils by improving the vigour of the plant cover of the affected territories.

Acknowledgements

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Prospects for The Use of Probiotic Drugs to Improve The condition of Soils and Plants

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The soil is enriched with varieties of microbes among them 95% are bacteria. Certain root colonizing soil microbes have evolved the mechanism that enhances plant growth mainly by nutrient mobilization, phyto-stimulation, modulating the abiotic stress, and protecting the plants by pathogen and pests. Plant Growth-Promoting Rhizobacteria (PGPR) are given preference for their use as plant probiotics. Probiotic bacteria exhibiting nutrient solubilizing capabilities mainly for the unavailable P and Zn in the soil as inaccessible to plants.

The group of PGPR *Pseudomonas putida* has been reported to increase the root length by 35–50% than the roots from seeds treated with the indole acetic acid. Whereas, several species of *Acinetobacter*, *Aeromonas*, *Arthrobacter*, *Azospirillum*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, and *Klebsiella*, *Serratia* have been documented in the last couple of decades for their potential to promote plant growth and health.

Probiotic bacteria also produce a variety of compounds including plant growth hormones, enzymes, antibiotics, and antimicrobial or pathogen-inhibiting volatile compounds. Siderophore-producing probiotics are helpful in Fe uptake by plants. Few commercial PGPR-based products are Bioaab (NFRDF, Pakistan), Punto, Sanremo, Italy, and Abtech PGPR India.

The sustainable agriculture practice is nowadays more focused on developing plant probiotics for bio-fertilizer applications with the commercial aspects. Studies have confirmed that 65% of worldwide nitrogen and 20-50% of soil organic phosphorus in agricultural soil are brought about by bio-fertilization. They are considered as the best alternatives to chemical fertilizers, pesticides, and artificial growth regulators which have numerous side effects.

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The postgenomic molecular picture of the aerobic fermentation phenomenon

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In the 1920s Otto Warburg covered that growing malignant cells consumed glucose more intensely than normal cells and released lactate even in the presence of oxygen, a phenomenon known as the “Warburg effect”. A few years later Herbert Grace Crabtree discovered that high glucose concentrations played a key role in causing the Warburg effect by inhibiting aerobic metabolism and stimulating the accumulation of fermentation products. The latter phenomenon, demonstrated both on animal cells and in the yeast *Saccharomyces cerevisiae*, is now known as the “Crabtree effect”. A similar effect demonstrated by facultatively anaerobic bacteria is known as “aerobic glycolysis” or “pyruvate/acetate overflow”. In particular, *Escherichia coli* can accumulate acetate and other fermentation products when growing aerobically on glucose.

From the very beginning it was clear to everyone that the discovered aerobic fermentation (AF) effects did not fit into the logic of the Pasteur effect discovered 50 years earlier, which states that fermentation is caused by a lack of oxygen. Thus, ironically, three of the most studied objects, representing three different biological kingdoms, exhibit similar properties that defy the explanation of modern science.

The day when all microorganisms of economic importance will be sequenced is not far off. It would seem that controlling such deeply studied microorganisms should not be difficult. But practice shows that between the full set of postgenomic knowledge describing molecular properties of microorganism, and ability to control its productivity lies a whole chasm of its physiological properties. The most interesting work in the coming decades will be the study of how individual molecular events add up to form a picture of a physiological phenomenon.

Using the knowledge engineering approach, data on AF was collected and analyzed. Molecular model of AF phenomena is created and discussed.

Development of Exogenous Photobiomodulation of Human Gut Microbiota

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In humans, the composition and functions of the gut microbiota (GM) reflect the host's health status. The total number of anaerobes, bifidobacteria and lactobacilli decreases in the elderly or as a result of certain diseases. In healthy people, there is also a change in the composition of GM, in particular, with a lifestyle change. Thus, noticeable changes in GM are observed already during the first 14-30 days when simulating stressful conditions of space flight on healthy volunteers. After leaving the 510 days stay in the test module, complete restoration of GM to the initial balance as before the experiment is not accomplished within the first 14 days of rehabilitation. This is even more true when people are treated with antibiotics. As shown in the experiment on mice, on the 10th day after the end of the 5-day course of taking the antibiotic gentamicin, the content of bifidobacteria in the feces was only 0.3%, lactobacilli - 0.05%, and Escherichia - 10% of the normal level. Therefore, to normalize the gut microbiota (GM), various techniques are being worked out using probiotics, prebiotics, and various options for GM transplantation. Along with these approaches, it is important to develop biophysical methods of photobiomodulation (PBM) using low-energy light radiation of the visible and / or near-infrared ranges to accelerate the restoration of the microbiota ecosystem in case of its disturbance, as well as to directed changes in its qualitative and quantitative composition to improve the physiological parameters of the organism. at increased loads and to remove age restrictions. For the direct method of light exposure to normalization of human GM, the "Photonic Tablet" was invented (<http://www.duny.ru>). As clinical studies have shown, during its monotherapy of dysbiosis, a significant increase in the total amount of *E. coli* (from 20 million / g to 300 million / g, $p < 0.05$, 15 times growth), lactobacilli (from 100 / g to 10 million / g $p < 0.05$, an increase of 100,000 times), which indicates an increase in the colonization abilities of saprophytes. The concentration of lactobacilli increased especially markedly, which was not observed in the control group of patients (30 people). However, the procedure itself, associated with swallowing a capsule, is very inconvenient for its invasiveness and is quite expensive. Therefore, as an alternative, it is possible to evaluate possible approaches for the exogenous PBM GM method when irradiated with light from the outside through the wall of the human abdomen. A direct experiment performed on a human with a fiber illuminator during the colonoscopy procedure shows that red light passes through the abdominal wall quite well and is well recorded even visually. Estimated measurements using an LED light source with maximum radiation of 660 nm on human muscle tissue give an attenuation coefficient (total absorption and scattering) of about 50 ± 20 times per 1 cm of tissue thickness, and skin with subcutaneous fatty tissue at 20 ± 5 times per 1 cm. Thus, with a thickness of the abdominal wall of 2 cm (1 cm of muscle and 1 cm of skin - adipose tissue) we obtain a weakening of the red light by 1000 times. The measured luminous flux on the skin surface, created using a LED matrix (660nm) with a power of 100W and an emitter size of 2x2 cm, at a distance of 3 cm through a contact glass lens is about 4000 W / m², which, taking into account the attenuation on the abdominal wall by 1000 times, makes it possible to create a luminous energy flux of about 4 W / m². If you do not use additional methods of skin cooling, the exposure time until unpleasant thermal pain sensations are obtained is about 30 seconds. Therefore, a single dose of local irradiation makes it possible to bring the dose of light flux at the level of the large intestine to about 120 J / m². An urgent question is, will this be enough for the PBM of bacteria in the gut ecosystem? Based on the literature data, numerous studies on the culture show that in the dose range of 10-100 W / m² (the maximum point at a dose of 50 W / m²), an increase in the rate of cell division is achieved by 50-100% more than in the control without irradiation. Moreover, for near-infrared pulsed laser radiation at a wavelength of 890 nm, the range of doses that increase the rate of cell division is 100 times less and is about 0.5 J / m². However, all of these experiments were carried out under aerobic conditions as opposed to anaerobic in the intestine and stimulation effects were observed for the *E. coli* WP2 cell strain. According to the literature, under anaerobic conditions, a significant stimulation (up to 10%) of the growth rate was observed with PBM on the bacteria *Propionibacterium acnes*; however, experiments on photodynamic therapy were carried out only for sufficiently high doses of about 100 kJ / m². Thus, the obtained estimates indicated the possibility of creating a new technology for exogenous PBM of human GM. To address this challenge, the appropriate radiation parameters have to be determined such as wavelengths and radiation doses. Also, to increase the effectiveness of the PMF effect, radiation modulation can play a large role .

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Luís Augusto Nero
Short Bio – 2021

Dr. Luís Augusto Nero is a Professor at the Department of Veterinary Medicine from Viçosa Federal University, located in Viçosa, Minas Gerais State, Brazil. He is a food microbiologist with a Doctorate degree from the Food Sciences Graduate Program (São Paulo University, São Paulo, São Paulo State, Brazil), working with foodborne pathogens and lactic acid bacteria from animal origin foods. Specifically on lactic acid bacteria, Dr. Nero focuses his studies on characterizing the bacteriocinogenic, beneficial, technological and virulent potentials of isolated strains, aiming their application in the food industry as biopreservatives, probiotics and starter cultures. Dr. Nero received several grants during his career from Brazilian governmental agencies (CAPES, CNPq and FAPEMIG) and from private companies, allowing the development of several research studies, resulting in more than 150 scientific publications in peer-reviewed journals and training of more than 50 professionals (Doctorate and Master, in Veterinary Medicine and Food Sciences). Dr. Nero is the coordinator of the Food Microbiology area in the Brazilian Society of Microbiology, and the secretary of the Brazilian Association for Food Protection, and an affiliate of the International Association for Food Protection (IAFP). He is also a researcher 1A from CNPq, and an associate editor of the Probiotics and Antimicrobial Proteins and Brazilian Journal of Microbiology, as well as a member of the editorial board of Food Microbiology.

Weissella: an Emerging Beneficial Bacterium with Promising Health Benefits

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1. Relevance of Weissella

Studies revealed that some *Weissella* have high interest for the pharmaceutical, medical and food industries, because of their capacity to produce antimicrobial agents, exopolysaccharides, and some beneficial health compounds. A biofunctional relevance of *Weissella* might be related to the control of foodborne pathogens through the production of bacteriocins, hydrogen peroxide and organic acids. Besides, this genus has presented promising results for the medical area, related to the therapeutic treatment of cancers and atopic dermatitis. *W. cibaria*, *W. confusa* and *W. paramesenteroides* strains have been characterized for their ability to survive through the gastrointestinal tract and to ferment prebiotic fibers, delineating probiotic claims. *Weissella* might be technologically relevant and can be used for its their health and probiotic features, as well as its potential to produce exopolysaccharides. Moreover, most of *Weissella* strains were described as safe because of the absence or low frequencies of virulence and antibiotic resistance related genes.

2. Biodiversity and technological features of Weissella isolated in Brazilian cheeses

Weissella spp. (n = 57) were isolated from different Brazilian food-related environments and characterized based on their genetic profiles and technological potential. PFGE and Rep-PCR revealed the high level of biodiversity among isolates: PFGE grouped the isolates in three major profiles (similarity from 60 to 80%), while rep-PCR characterized the isolates as belonging to a single profile. Based on these profiles, 26 isolates were selected and characterized based on their inhibitory and bacteriocinogenic activity against *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 6538, *Salmonella* Typhimurium ATCC 14028 and *Escherichia coli* ATCC 11229. Most isolates (n = 20) were able to inhibit the targets through organic acids. Most of the isolates (n = 12) were able to produce diacetyl, proteases, and/or coagulating milk, but none were able to produce exopolysaccharides. Isolate 16, in particular, was characterized as possessing high acidification ability, diacetyl and protease production, with promising technological potential in the dairy industry. The obtained results allowed the understanding of the *Weissella* spp. strains role as starter cultures in the dairy industry.



Andrey V. Dubrovin
Short Bio – 2021

Andrew Dubrovin is a young scientist, a biotechnologist at Biotrof Ltd. molecular-biology laboratory (St.-Petersburg, Russia). He graduated from the St. Petersburg State University of Veterinary Medicine with a degree in veterinary medicine (Russia), completed postgraduate studies at the “All-Russian Research Veterinary Institute of Poultry Science”. He observes the microbiota and transcriptome of the gastrointestinal tract of livestock animals and poultry, and search for ways to correct livestock health and productivity through the use of biological feed additives, essential oils. Andrey has more than 70 scientific publications in Russian and international journals, including 4 papers of the Web of Science 1st quartile. He participated in grants from the Russian Foundation for Basic Research, the Russian Science Foundation and others.

Expression of immunity genes in broiler chickens under *Salmonella* Enteritidis infection

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The teams of BIOTROF Ltd and ARRVIPS, have set an experiment on broiler chickens infected with the strain of *S. Enteritidis*. Also, a feed additive based on essential oils effect on chickens was analyzed. The total of 120 chicks were raised in a vivarium from 1 to 42 days divided to analogous groups: Control (C) and Experiment (E). At the age of 19 days, chicks were infected intramuscularly into the pectoral muscle with the epizootic strain *Salmonella* Enteritidis then further divided into Control (C), Infected Control (IC), Experiment (E), Infected Experiment (IE). The Intebio (BIOTROF Ltd, St. Petersburg) was added to daily feed from the first day of experiment at a dose of 90 mg / kg of compound feed. For laboratory analysis, samples of caecum tissue were taken a day and 3 weeks after infection. Laboratory research of the samples was carried out in the BIOTROF Ltd. To analyze gene expression, eukaryotic RNA was isolated from the caecum tissue, a reverse transcription reaction was performed to obtain cDNA, and then quantitative PCR was performed. The calculation of the relative expression was performed using the $2^{-\Delta\Delta Ct}$ method.

At 1 day post infection in IC the expression of cytokines genes IL6 and IL8 increased 1.6 and 1.7 times respectively. In the experiment groups, the expression of interleukin genes increased by 4-6 times in IE group and by 3.5-5 times in E group relative to the C. In IC group expression of the defensin genes AvBD-10 increased by about 2 times, and AvBD-11 decreased to 0.7 times relative to the C group. The expression level of the defensin genes AvBD-9 and AvBD-11 increased by 1.5 times in E and IE groups. The expression level of AvBD-10 increased by 17 times in the E group and by 2 times in the IE group. The expression of the cysteine protease Casp6 gene increased by 2.5 times both in IC and IE groups. In E group Casp6 expression increased by 4 times. The level of IRF7 gene expression, increased by 3 times in IC, 9.5 times in IE, 6.5 times in E groups compared to the C group.

By the 3 weeks post infection, in all groups the expression level of observed genes decreased compared to the C group with the exception of AvBD-10 expression which was still higher in IC group by 2.1 times, in IE group by 3.82 times and in E group by 2.7 times compared to the C group.

The obtained data indicate that the use of essential oils helped to enhance the immune response in infected poultry. This work was supported by the RFBR grant № 20-116-00001.

The ameliorative effects of *Lactiplantibacillus plantarum* APsulloc 331261 oral administration in an allergic asthma mouse model

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Introduction

Allergic asthma is a chronic inflammatory disease of the respiratory tract and characterized by type 2 inflammation. It has been reported that allergic asthma is associated with alteration in the gut microbiota, also referred to as dysbiosis. Probiotics are considered as alternative solutions to attenuate allergic asthma by positively influencing immunomodulation and gut microbial homeostasis. In this research, we aimed to evaluate the ameliorative effects of *L. plantarum* APsulloc 331261 (AP1) in an ovalbumin (OVA)-induced allergic asthma mouse model.

Materials and methods

To induce an asthmatic state, 6-week-old Balb/c female mice were sensitized with OVA absorbed in alum by intraperitoneal injection on days 0 and 7. Subsequently, mice were challenged by OVA via the intranasal route on days 14, 17, 21, 24, 28 and 31. *L. plantarum* AP1 was orally administered to asthmatic mice for 5 consecutive days per week for 3 weeks. After sacrifice on day 32, serum, bronchoalveolar lavage fluid (BALF) and lungs were extracted for immune marker analysis while the cecum was extracted for microbiome analysis.

Results

L. plantarum AP1 suppressed the representative type 2 inflammation markers including total immunoglobulin E (IgE), OVA-specific IgE, interleukin (IL)-4 and IL-5 and infiltration of cells around alveoli by histological analysis. Furthermore, *L. plantarum* AP1 downregulated type 2 cytokines, chemokines and mucin genes on a transcriptional level. Next, short chain fatty acids (SCFA) production and microbiota alteration were analyzed in the cecum. The *L. plantarum* AP1 group showed the increasing tendency of propionate and butyrate production. There was no significant difference between groups in alpha diversity, however, each group formed distinct clusters in beta diversity. The taxonomic composition of the different groups was analyzed from the phylum to species level. *L. plantarum* AP1 increased the relative abundance of the genus *Clostridium*. Specifically, unknown species in *Clostridium* cluster IV and the Ruminococcaceae were increased in the *L. plantarum* AP1 group suggesting that this strain may contribute to augment SCFA-producing bacteria and attenuate allergic asthma.

Conclusions

Our data demonstrated that the oral administration of *L. plantarum* AP1 suppressed type 2 inflammation and promoted the production of SCFAs with an increase in SCFA-producing bacteria. In conclusion, *L. plantarum* AP1 attenuates allergic asthma along with gut microbiota modulation.

The beneficial effect of *Bifidobacterium animalis* subsp. *lactis* HEM 20-01 on depressive-like behavior and physiology in a murine model

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Introduction

Depression is considered the leading cause of health defects globally, with high rates of suicidal mortality. Anti-depressants, such as selective serotonin uptake inhibitors (SSRIs), are currently the most widely prescribed medications, but due to their high side-effects and low efficacy rates, there is still an expanding demand for the development of other therapeutic agents. Limited but increasing evidence proposes a close relationship between composition of gut microbiota and host behavioral and mental disorders. Accordingly, probiotics have been in the focus as therapeutic agents for treating depression, since they are able to modulate gut microbial composition and induce anti-inflammatory responses. In this study, we evaluated the anti-depressive effect of *B. animalis* subsp. *lactis* HEM20-01 in BALB/c mice, an inbred, spontaneous depressive mouse model.

Methods

BALB/c mice (7-8 weeks old, 17-23 g) were administered with the strain HEM20-01 at a concentration of 1x10⁹ CFU/mouse/day for 4 weeks. Behavior tests were performed during the last week of treatment. Subsequently, blood, hippocampus, proximal colon, and colonic contents were collected from the experimental animals for qRT-PCR, ELISA, and NGS analysis.

Results and Discussion

In behavior tests, BALB/c mice supplemented with the strain HEM 20-01 showed improved grooming duration and latency to grooming in the splash test, and a shortened latency for food in the suppressed novelty feeding test. Also, the strain treated group increased sucrose consumption in the sucrose preference test, and reduced immobility time in the tail suspension test. Regarding gene modulation in the hippocampus, consumption of the strain HEM 20-01 down regulated the gene expression of pro-inflammatory cytokines such as IL-1 β , IL-6, and IFN- γ . This was confirmed at the protein level. In the proximal colon, the strain also supported the down regulation of IL-1 β , IL-6, and tight junction protein gene expressions, and it induced significantly lower endotoxin levels in the plasma. The impact of the strain on the gut microbial ecology was reflected in modulation in beta-diversity and taxonomic relative abundances compared to the control group. The relative abundance of Proteobacteria and Desulfobivrio were significantly decreased while the level of Prevotella was significantly increased after the strain treatment. Through PICRUST analysis, we could find that the predicted metagenomic functions of the gut microbiota were also affected by the supplementation of the strain HEM20-01.

Conclusions

Behavioral and physiological changes after consumption of the strain *B. animalis* subsp. *lactis* HEM 20-01 show promising potential for this strain as an anti-depressive agent. This was supported by the beneficial modulation of the gut microbial composition and function, by lowering the systemic endotoxin level, and by improving inflammatory status in the hippocampus and the proximal colon.

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Probiotic as a food additive can affect the weight gain of poultry chickens

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Relevance of the study

Poultry and primarily chicken meat is considered the main source of protein in the majority of the countries around the world. Besides the importance of growing healthy and fast weight-increasing birds, the issue of chickens' feed became especially important. The main problem currently became finding a way of growing chickens healthy without antibiotics. Antibiotics were included in animal feed as a powerful tool allowing avoiding bacterial infections caused by bacterial pathogens. However, the increasing number of the antibiotic resistant bacterial strains and the microecological consequences of antibiotics in meat products and the environment forced the farmers and manufacturers to limit antibiotic usage. Presently addition of probiotic bacteria to the animal feed is considered as a possible replacement for antibiotics. The reasons behind antibiotic usage are based on the proven ability of several probiotic strains to restore the microenvironment of the animals due to the antagonistic ability against the pathogens and immunomodulatory features. However, the specifics of probiotic strain action and their ability to compete with antibiotics in the animal growth parameters were not well studied.

Research objective

The comparison of the effect of enterococcal probiotic *Enterococcus faecium* L3 to antibiotic enramycin being added as components of chicken feed.

Materials and methods

Two hundred and sixteen chickens were divided into three groups and tested by different parameters, including weight gain, food consumption, blood biochemistry, immunology and caecal microbiome at two checkpoints, 21 and 39 days after birth. Group A was used as a control, group B included antibiotic enramycin 8 mg per gram of feed [8 g/T]), and group C contained probiotic *Enterococcus faecium* L3 (1×10^7 CFU per gram of feed). Each treatment group was divided into six repeated groups, one chicken cage for each repetition, and 12 chickens for each cage. Statistical processing of the results was carried out using Student's t-test, they were considered valid at a significance level of $p < 0.05$.

Results

Group of chickens getting probiotic by the end of the experiment demonstrated weight gain more than 100 grams at the average as compared to control group ($P < 0.05$) (Figure 1 A – control group, B- group taking antibiotics, C- group taking

Bacteriocinogenic and safety properties of *Bacillus subtilis* ST830CD isolated from *gochujang*

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Traditional fermented food products have become increasingly popular for a variety of reasons especially related to health benefits. Safety evaluation on a strain basis are considered as a key step towards the application of a putative probiotic strain. This project aims to identify and characterize bacteriocin-producing strains for biocontrol of *Listeria monocytogenes* and *Staphylococcus* spp., both clinical and food-associated pathogens, and to evaluate their safety.

In a pre-selection process, different bacterial strains with inhibitory activity against *L. monocytogenes* ATCC15313 and *Staphylococcus* spp. were isolated from *gochujang*, a traditional Korean fermented food prepared from red chili powder, glutinous rice, meju (fermented soybean) powder, yeotgireum (barley malt powder), and salt, obtained from the local market (Pohang region, Republic of Korea). Isolates were differentiated by RAPD-PCR and rep-PCR, and isolate ST830CD was selected for future studies and identified via partial 16S rRNA sequencing as *Bacillus subtilis* ST830CD.

Treatment of the cell free supernatant (CFS) from *B. subtilis* ST830CD with proteolytic enzymes (proteinase K, papain, and α -chymotrypsin) did not inactivate the produced inhibitory metabolite/s when tested against *L. monocytogenes* and selected *Staphylococcus* spp., indicating that the studied strain most probably expressed more than one type of antimicrobial metabolite. The hypothesis was confirmed from the PCR-based analysis targeting genes related to different antimicrobials, including Class IIa bacteriocins, assurfactin (*srfa*) and subtilosin (*sbo*) in the total DNA from *B. subtilis* ST830CD. Moreover, Beneficial genes related to adhesion (*map*, *mub*, *eftu*, *ef2380*, *ef2662*, *ef1249*, and *prg*), the production of folate (*folPE*, *folKQ*, *pabB*, *pabC*), and other antimicrobials (*bli*, *thu*, *coa*, *nis*, and *ped*) were absent in DNA of *B. subtilis* ST830CD. Moreover, the stability of the produced antimicrobial compound against *Staphylococcus* spp. was not affected by varying temperatures (4-100°C), chemicals (NaCl, Tween 80, SDS, and skim milk), and different pH (4-8). Bacteriocin activity of *B. subtilis* ST830CD against *L. monocytogenes* ATCC15313 was determined to be 1600 AU/mL, and 3200 AU/mL and 1600 AU/mL, respectively, against *S. simulans* KACC13241 and *S. auricularis* KACC13252. Furthermore, *B. subtilis* ST830CD was found to produce gelatinase enzyme, considered as a virulence factor, and biogenic amines.

Bacteriocin production is generally considered as a beneficial property in that producer strains in a starter culture may dominate in an ecological niche. However, if the bacteriocinogenic strains are carrier of virulence factors, the produced antimicrobial peptides may enhance potential virulence factors, thereby constituting a potential health hazard to consumers. Therefore, good manufacturing practices, including hygiene management in the preparation process, are a guarantee for safety and quality of the food products.

Evaluation of beneficial properties of *Lactobacillus sakei* ST69HK as a potential probiotic candidate for the oral cavity

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The human oral cavity provides optimal conditions for the growth and colonization of different microorganisms. In the oral ecosystem different obstacles and mechanisms contribute to the effective reduction and control of different pathogenic “intruders”. On the other side, specificity of the oral cavity conditions can be optimal for the colonization of different beneficial microorganisms including temperature, sufficient moisture, available nutrients, minerals, vitamins, and various surfaces that allow microbial attachment, and also including factors that will facilitate lactic acid bacteria (LAB) to colonize. This study aimed to select beneficial strains from the oral cavity of healthy volunteers and to evaluate these as potential oral probiotic candidates. The selection process was based on the isolation a differentiation, identification and safety assessment of LAB strains, followed by series of experiments directing to the selection of appropriate candidates with beneficial properties.

In the preliminary screening an isolate from the oral cavity of a Caucasian volunteer was identified as *Lactobacillus* (Lt.) *sakei* (*Lactobacillus sakei*) ST69HK based on 16S rRNA sequencing. Physiological and phenotypic tests showed no hemolytic, proteinase, or gelatinase activities, neither production of biogenic amines. Lt. *sakei* ST69HK can be considered safe based on negative results for the detection of *efaA*, *cyt*, *IS16*, *esp*, *asa1*, and *hyl* virulence genes, and the absence of vancomycin resistance (*vanABCDEG*) genes. This strain was resistant to vancomycin (10 µg/disk), but not to other antibiotics applied in this study. Moreover, cell-to-cell antagonism indicated that Lt. *sakei* ST69HK was able to inhibit the growth of *Bacillus cereus*, *Bacillus pumilus*, *Enterococcus avium*, and *Enterococcus faecalis*. Lt. *sakei* ST69HK does not produce β-galactosidase and positive results were found for *eftu* and *gad* genes encoding adhesion and GABA production, respectively, but not for genes related to folate production. A cell surface hydrophobicity level of 39.04% was determined. The studied strain was able to survive in a wide range of pH values ranging from 4.0 to 8.0, and ox-bile concentrations, of up to 0.1%, as a single factor. In the simulated GIT passage (SSDP) model Lt. *sakei* ST69HK showed a good survival rate after 3 hours, and also showed good survival in two artificial saliva models for 30 minutes. Interaction of Lt. *sakei* ST69HK with drugs from different generic groups and oral cavity hygiene products was evaluated and the MIC for drugs showing an inhibitory effect on bacterial growth was determined to predict possible negative consequences for the combined application of evaluated drug/hygienic products and studied the oral cavity probiotic candidate. Overall, antagonistic properties, safety assessment, and high rates of survival in the GIT and oral cavity models, and even in the presence of commonly used drugs and oral hygiene products, indicate Lt. *sakei* ST69HK to be a promising oral cavity probiotics candidate.

Anti-Biofilm properties of bacteriocinogenic *Enterococcus faecium* ST7319ea

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The silent war against the continuous emergence of antibiotic-resistant pathogens has been going on for decades. The next problem has been predicted to include the high prevalence of infections associated with the emerging and re-emerging antimicrobial-resistant pathogens. Thus, the current focus is on the search for naturally occurring and mechanistically stable antimicrobials from various sources. In this study, *Enterococcus faecium* ST7319ea, isolated from Korean traditional fermented soybean paste, was characterized for its ability to produce bacteriocins active against the food-borne pathogen *Listeria monocytogenes* ATCC15313 and a clinical vancomycin-resistant *E. faecium* strain (VRE19), an emerging opportunistic pathogen.

The bacteriocin of strain ST7319ea showed an activity of 25600 AU/mL against *L. monocytogenes* ATCC15313. Molecular-based screening for bacteriocin-associated genes showed that *E. faecium* ST7319ea harbors *entA* and *entB*. The estimated size of the expressed bacteriocins was confirmed through tricine-SDS-PAGE as 4.5 kDa. The expressed bacteriocin was found to be effective against most *Listeria* spp. and *Enterococcus* spp., but not against *Staphylococcus*, *Streptococcus*, and the majority of the LAB strains included in the test panel, indicating a narrow spectrum of activity. The partially purified (60% 2-propanol) bacteriocin ST7319ea was further characterized in a challenge test against non-metabolically and metabolically active cells of *L. monocytogenes* ATCC15313 and vancomycin-resistant *Enterococcus* VRE19 indicating significant reduction in both set-ups.

To further characterize the antibacterial activity of the bacteriocins expressed by *E. faecium* ST7319ea, biofilms formed by *L. monocytogenes* ATCC15313 and *E. faecium* VRE19 were challenged using CFS containing the bacteriocin. The synergistic activity of the CFS and semi-purified bacteriocin (60% 2-propanol) was also tested with ciprofloxacin and vancomycin, known as broad-spectrum and last-resort antibiotics for systemic infection, respectively. Quantification of biofilm reduction was significantly reduced using a minimum concentration of 1600 AU/mL of crude bacteriocin. Synergistic activity of crude CFS against *L. monocytogenes* ATCC15313 showed significant reduction at 800 AU/mL of bacteriocin supplemented with 0.031 mg/L ciprofloxacin and 400 AU/mL of bacteriocin supplemented with 0.5 mg/L vancomycin. Additionally, synergistic inhibitory properties against *E. faecium* VRE19 have been observed at levels 800 AU/mL with 0.031 mg/L ciprofloxacin and 50 AU/mL with 1 mg/L of vancomycin. Observations were further confirmed by determining the bacterial metabolic activity after the biofilm-oriented antimicrobial assay by allowing residual cells to grow in BHI supplemented with 0.01% triphenyl-tetrazolium chloride; colorimetric interpretations were used to identify metabolically active cells. Phenotypic safety assessment was confirmed; yet, molecular-based screening for putative virulence factors detected the presence of genes for *IS16*, *esp*, and *hyl*, but not *efaA*, *cyt*, *asa1*, and *vanABCDEG*. Based on these results, it can be concluded that *E. faecium* ST7319ea is a promising source of potent antimicrobial peptides; however, its would not be employed as live microorganism for human and animal consumption due to the presence of various putative virulence factors.

Fruit pulps can increase the nutritional and functional features of fermented dairy products

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Passion fruit (*Passiflora edulis* f. *Flavicarpa* Deg) and buriti (*Mauritia flexuosa*), tropical fruits widely produced and consumed in Brazil, were recognized for their beneficial properties and bioactive compounds which identified by their high total solids, proteins, ash, lipids of good profiles, and presence of soluble and insoluble fibers. Good antioxidant activity has also been identified, enabling these fruits as a promising raw ingredient as a supplement that can exacerbate beneficial properties of existing fermented food products. The supplementation of these food products with fruit pulps (FP) of tropical fruits contributes to an additional health appeal aside from the increase on the nutritional value of the product itself due to the bioactive compounds that are naturally present in these matrices. This study aimed to evaluate the beneficial properties and bio-viability of fermented dairy products supplemented with buriti pulp (BP) and passion fruit pulp (PFP) with selected probiotic candidates *Lacticaseibacillus casei* SJRP38, *Lactiplantibacillus plantarum* ST8Sh, *Streptococcus thermophilus* TA080 and *Lacticaseibacillus rhamnosus* ATCC53103 (as a reference strain).

The selected LAB for this study were profiled for their carbohydrate metabolic properties, (API50CHL), enzyme production profiles (APIZym), and the effect of FP on the growth of the studied strains as observed on modified MRS or M17 agar, wherein glucose was substituted by 1% FP, separately. The chemical composition of BP stood out for its unique characteristics compared to PFP due to its higher protein content, total fatty acids (FA) and total fiber (TF). Additionally, BP had higher FA variety, compared to PFP demonstrated by the 81% and 44% of monounsaturated FA (ω -3, 6, 7, 9, and 11), and oleic acid (ω -9) with 77% and 38%, accordingly. The antioxidant activity which was higher in the BP compared to the PFP at 5.23 ± 0.37 and 0.87 ± 0.08 $\mu\text{mol Trolox/g}$ of pulp, respectively, was associated with the bioactive compounds found in the FP.

Studied LAB were able to metabolize erythritol, D-arabinose, L-rhamnose, dulcitol, D-sorbitol, methyl- α -D-mannopyranoside, D-melibiose, D-sucrose, D-turanoside, D-tagatose, and L-fucose. Among the enzymes tested in the APIZym panel, only 9 enzymes were found to be not expressed by the selected strains. On the other hand, the bacterial growth of the strains *Lb. plantarum* ST8Sh and *Str. thermophilus* TA080 in the presence of FP showed only one log reduction on the viable cells after 24 h incubation compared to glucose; other strains evaluated had the same bacterial growth with the control, indicating that variability in the carbohydrate sources, particularly with the substitution of glucose with the FP, does not alter the bacterial growth dynamics. Therefore, it can be suggested that FP can be used in fermented dairy products, and contribute to beneficial health qualities and nutritional values of the products.

Current Developments in Physiology and Application of Bacillus Probiotics

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The use of bacilli as probiotics is rapidly expanding due to their inherent ability to form endospores with unique survival and resistance to extreme environmental conditions, and they meet all the requirements for good probiotic microorganisms. *Bacillus* spp. are characterized by high antagonistic activity owing to the secretion of antimicrobials, possess anti-oxidant and immuno-modulatory abilities, in their vegetative form, bacilli produce extracellular amylases, proteases, cellulases, xylanases, pectinases, and lipases contributing in digestibility and absorption of nutrients. The spectrum of beneficial characteristics, ability to grow in the form of a biofilm, ease of *Bacillus* spp. production and stability during processing and storage make them a suitable candidate for the commercial manufacture of novel foods or dietary supplements for human and animal feeds. Therefore, mass production is one of the important aspects of the commercial use of spore-forming probiotics. Naturally, the development of new approaches and competitive technologies for the production of *Bacillus* probiotic requires an understanding of physiological peculiarities and mechanisms determining both the growth and spore production by bacilli. In this presentation, the recent literature and our data on the current state of knowledge about the physiology of bacilli growth and spore production in the submerged and solid-state fermentation conditions will be reported, focusing on the common characteristics and unique properties of individual bacteria as well as on several approaches providing increased spore formation.

Polyamines produced by probiotics necessary for the correction of cognitive impairment

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With aging, cognitive impairment is an increasingly pressing problem in society. Currently, the role of polyamines (putrescine, spermidine, and spermine) in the pathogenesis of cognitive impairments of various origins is being actively discussed. It has been shown that the content of polyamines in the brain tissue decreases with age, which plays a leading role in age-related memory impairments (Liu et al., 2008). A clear correlation has been established between spermidine level in the blood and cognitive indicators in diseases such as Alzheimer's and Parkinson's (Paker et al., 2020, Makletsova et al., 2018). The introduction of polyamines allows to avoid cognitive impairment and influence the pathogenetic processes associated with the progression of pathologies. The neuroprotective effect of exogenously administered spermidine has been shown in animal experiments when modeling such pathologies as Huntington's, Alzheimer's, Parkinson's, traumatic brain injury, inflammatory diseases associated with memory loss (Ramos-Molina B., Quipo-Ortuno M.I, 2019). One of the molecular mechanisms of this positive effect of spermidine on the functional activity of the brain is the activation of autophagy, the so-called process of "cleaning" from deformed proteins and organelles. Due to this, orally administered spermidine prolongs life expectancy, reduces cardiac hypertrophy, and has cardioprotective and neuroprotective properties (Eisenberg et al., 2016, Schroeder et al., 2021).

It is important to note that Wirth et al. (2021) showed the ability of spermidine to maintain telomere length was shown, thereby influencing the mechanisms of aging. Thus, it is obvious that at present, exogenous administration of polyamines can be considered as an effective way of correcting age-related cognitive impairments and as a "protection" against aging.

There are three ways of getting polyamines into the human body: from food, synthesis by bacteria in the intestine, and its biosynthesis in the body. One of the most promising approaches to preventing cognitive impairment and maintaining the quality of life during aging is consuming foods high in polyamines and the possibility of using various probiotics that synthesize polyamines.

Since 2018, pilot projects have been started in many European countries to assess the impact of a diet high in polyamines on cognitive processes (Hirano et al., 2021). The first results showed the high efficiency of polyamines. It should be noted that clinical trials of the effect of dietary polyamines on cognitive processes and blood biochemical parameters in patients with oncological diseases were carried out, and the absence of a stimulating effect of polyamines on oncology was shown (Hirano et al., 2021).

Age-related Changes in Intestinal Microflora and Anti-aging Effects of Bifidobacteria

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Today, Japan is facing a super-aging society unparalleled in the world, with the proportion of people aged 65 and over exceeding 30% by 2025, and total medical costs estimated to exceed 50 trillion yen. In the human intestine, there are hundreds of species of intestinal bacteria numbering up to 40 trillion, and they form a complex intestinal environment by interacting with each other, viruses, fungi, and phages as well as with host cells. Recent analyses have shown that changes in the gut microbiota play a major role in host health and disease induction, and are considered to be one of the most important targets for disease prevention. Various factors that cause changes in the intestinal microbiota have been identified, including the host's age and health status, lifestyle habits such as diet and exercise, and medication, but several cohort studies have shown that aging has a particularly large impact on the balance of the intestinal microbiota. In fact, we have also analyzed the intestinal microflora of 453 healthy Japanese subjects aged 0 to 104 years and demonstrated a decrease in obligate anaerobes such as Bifidobacterium but an increase in facultative anaerobes such as Escherichia coli in the elderly. In this study, we found that the intestinal microflora could be classified into age-dependent clusters but there also exist some age-mismatched ones, however, the impact of age-related changes in the intestinal microbiota on host health remains largely unexplored.

In this presentation, I will introduce our new studies in the analysis of fecal metabolites measured by CE-TOF-MS between age-matched and age-mismatched elderly subjects to examine the roles of the gut microbiota in the health of the elderly. Our findings suggest that metabolites produced by the aged gut microbiota could contribute to intestinal and systemic homeostasis and could be targets for preventing aging-associated diseases. In addition, I will introduce some results of the latest clinical trials on the effect in improving cognitive function of a probiotic B. breve strain on elderly with mild cognitive impairment (MCI).

Genomics of Soil Probiotic Bacteria

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Probiotic bacteria of the genera *Bacillus* and *Lactobacillus* are widespread soil organisms. They are found even in soils and ground ecosystems contaminated with polycyclic aromatic hydrocarbons and heavy metals. Metagenomic sequencing of soil DNA in impact zones with a significant level of technogenic load showed that *Bacillus* and *Lactobacillus* are included in the list of dominant bacterial genera in total DNA samples of 4 soil samples, represented by Haplic Chernozem and Spolic Technosols, taken from the territory subject to long-term technogenic pollution.

Soils were sampled from the territory adjoined to the Persianosvskaya preserve steppe, Novocherkassk Power Station, and Atamanskoye dried lake territories to compare the contaminated territory and uncontaminated preserve Haplic Chernozem soil. DNA was isolated with a commercial FastDNA™ Spin Kit for soil. Libraries were created using the NEBNext Ultra II DNA library prep kit (NEB) according to the manufacturer’s instructions (the protocol is publicly available on the NEB website). Ultrasonic fragmentation of genomic DNA was performed on a Covaris S220 device. The quality of the obtained libraries was checked on a 2100 Bioanalyzer (Agilent) using the DNA High Sensitivity kit (Agilent). The amount was estimated using a Qubit 2.0 instrument. Sequenced on NextSeq 500.

Many key enzymes of the biosynthesis of vitamins and cofactors were not found in *Lactobacillus*. Most *Lactobacillus* do not have the ability to synthesize various amino acids *de novo*. According to the literature, the exception is *L. plantarum*, which have complete biosynthetic pathways of all amino acids, with the exception of branched-chain amino acids (Kleerebezem et al., 2003). In the genomes of *Lactobacillus* of the studied samples, a wide range of uptake systems of amino acids/peptides, as well as peptidases, necessary for the assimilation of exogenous nitrogen, was found.

Presumably responsible for the biosynthesis of polysaccharides, including highly conserved gene clusters of cell surface exopolysaccharide biosynthesis (EPS). Also, these bacteria have genes for stress response (acidic, osmotic, oxidative) and a number of genes responsible for cell surface factors.

The results obtained suggest that probiotic bacteria found in soils and grounds contaminated with PAHs and HM possess all the genes necessary for their autonomous existence in such conditions.

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Dynamic changes in *Lactobacillus* and *Bifidobacterium* abundance in the intestinal microbiota of patients with moderate COVID-19 and healthy individuals

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COVID-19 is a pandemic disease of the severe acute respiratory syndrome caused by the novel SARS-CoV-2 coronavirus. According to latest reports, patients with COVID-19 show a gut microbial dysbiosis characterized by low levels of various probiotic species such as *Bifidobacterium* and *Lactobacillus*. In addition, probiotics can act as potential blockers of the ACE receptor, which works as a gateway for SARS-CoV-2. The aim of this study was to determine the stability of *Lactobacillus* and *Bifidobacterium* in the gut microbiota of patients with COVID-19 and healthy individuals.

For the analysis, we took stool samples from 16 healthy people twice, at two week interval, and from 10 patients with moderate COVID-19, before and after hospital treatment. DNA was isolated using DNA-express kit (Lytech, Russia). Metagenomic sequencing of 16S rRNA gene amplicons was performed on the Illumina MiSeq platform (Illumina, USA), followed by analysis with the QIIME v1.9 package. Statistical differences in the relative abundance of taxa were calculated using the Mann-Whitney test.

At the initial stage of the COVID-19, the proportion of *Bifidobacterium* in the intestinal microbiota of patients was significantly reduced compared to healthy controls ($0.14 \pm 0.22\%$ for COVID-19, $3.71 \pm 4.96\%$ for controls, $P=0.019$). However there were no statistically significant differences in the *Lactobacillus* content ($0.02 \pm 0.04\%$ for COVID-19, $0.12 \pm 0.27\%$ for controls, $P=0.827$). No significant changes were found in the content of *Bifidobacterium* and *Lactobacillus* in the COVID-19 cohort before and after treatment, as well. At the same time, control group demonstrated wide dynamic variation in proportion of *Lactobacillus* (from 0.12% to 0.04%, $P=0.053$) and no changes in the content of *Bifidobacterium* (from 3.71% to 3.16%, $P=0.222$). Among the COVID-19 cohort, 1 patient (10%) had no *Lactobacillus* in the intestinal microbiota before and after treatment and 1 patient had no *Bifidobacterium*. In the control group, *Lactobacillus* was absent in 6 volunteers (38%) and *Bifidobacterium* was absent in 1 individual.

The data found on the relative stability of the content of *Bifidobacterium* and dynamic changes in *Lactobacillus* in healthy people and during illness can be taken into account to maintain immunity through probiotics.

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