BGRS/SB-2024

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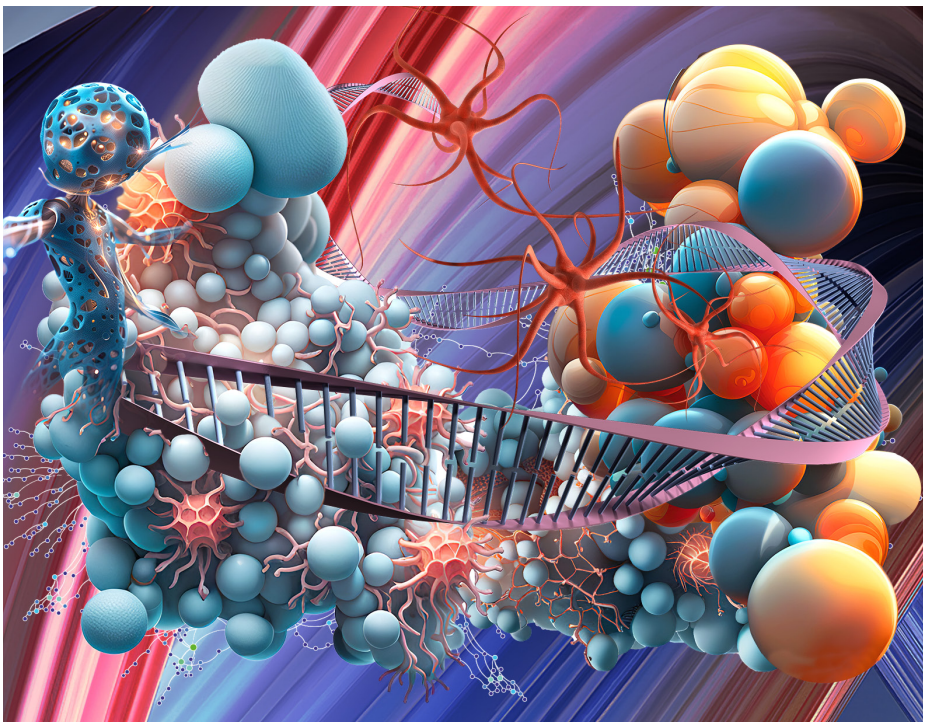
5–10 августа 2024, Новосибирск, Россия

BIOINFORMATICS OF GENOME REGULATION AND STRUCTURE / SYSTEMS BIOLOGY

14th International Multiconference

August 5–10, 2024, Novosibirsk, Russia



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Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences
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Abstracts

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Isolation and characterization of endolithic strain *Pseudomonas chlororaphis* S15: a study on heavy metal resistance and siderophore production

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Key words: endolithic bacteria; siderophores; *Pseudomonas chlororaphis*; heavy metals

Motivation and Aim: Serpentinite is a metamorphic rock resulting in the formation of serpentine minerals [1]. These minerals contain high levels of heavy metals, which provides a distinctive ecological niche for endolithic microbial communities. The endolithic bacteria found in serpentinite demonstrate a high resistance to heavy metals, the capacity to produce siderophores, and represent a valuable resource for understanding extremophile biology and for developing innovative biotechnological applications.

Endolithic bacteria have evolved unique adaptations to survive under extreme conditions, such as desiccation and nutrient limitation. The utilization of these bacteria for the improvement of soil in arid regions represents a promising strategy for the improvement of soil fertility and crop productivity. *Pseudomonas chlororaphis* is well known for its potential application in bioremediation, agriculture, and industrial biotechnology [2]. This bacterium produces different secondary metabolites, including pigments, antibiotics, and siderophores.

Thus, this study outlines the isolation and comprehensive characterization of serpentinite-derived strain *Pseudomonas chlororaphis* S15.

Methods and Algorithms:

Isolation and Identification: The strain S15 was isolated from a colony on Luria Agar (LA) agar that had been plated with aqueous rinsate of crushed mineral. Genomic DNA from strain S15 was extracted from an overnight LB-grown culture using the phenol-chloroform method. The 16S rRNA gene (1,500 bp) was amplified with polymerase chain reaction, then then sequenced using instrument ABI 3730 DNA Analyzer (Life Technologies, USA) following Sanger's method. The bacterial sequences were analyzed using the Basic Local Alignment Search Tool (BLASTn).

Heavy Metal Resistance: P. chlororaphis S15 was exposed to different concentrations of heavy metals (Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, $Cr_2O_7^{2-}$) to determine their minimum inhibitory concentrations (MICs).

Siderophore Production Screening: The Chrome Azurol S (CAS) agar assay was used for screening *P. chlororaphis* S15 capacity for siderophore production [3]. Additionally, a replacement of Fe^{+3} with heavy-metal ions (Al^{+3}, Cu^{+2}, Ga^{+3}) in the standard CAS agar method was tested [4].

Growth Dynamics and Siderophore Production: The influence of different concentrations of heavy metals on *P. chlororaphis* S15 growth and siderophore production was studied in M9 minimal medium at 30 °C, 250 rpm. The Arnow and Atkin assay were used to detect catecholate- and hydroxamate-types of siderophores [5].

Metabolite Analysis: The solid phase extraction (SPE) of metabolites produced by *P. chlororaphis* S15 in the presence of heavy metal and BIP (2.2-bipiridyl) as a control was used. HPLC was performed on an Acclaim® PolarAdvantage II (PA2) C18 reverse-phase column (5 μm, 250 × 4.6 mm) using an UltiMate 3000 UHPLC system (Thermo Fisher Scientific, United States). Gradient elution was achieved using two mobile phases containing water with 0.01 % (vol/vol) trifluoroacetic acid (TFA) and 100 % (vol/vol) acetonitrile with 0.01 % (vol/vol) TFA at a flow rate of 1 mL/min. The elution of the siderophores was monitored at 220, 260, 285 nm and with a fluorescence detector (excitation at 470 nm and emission at 530 nm wavelength).

Results: The endolithic strain S15 was isolated from serpentinite sampled from the Khalilovsky massif, Russia [6]. The 16S rRNA sequencing was performed for identification of the strain. The BLAST results demonstrated that S15 exhibited a high degree of similarity to *Pseudomonas chlororaphis*, with a similarity score of 99 %. The resistance of *P. chlororaphis* S15 to various heavy metals was quantified by determining their minimum inhibitory concentrations (MICs). The tolerance of the strain to heavy metals was tested on LA supplemented with salts of heavy metals, and the results are presented in the Table 1.

Table 1. Growth of endolithic *P. chlororaphis* S15 in the presence of different concentrations (mM) of heavy metals

Bacterial strain	Ni^{2+}	Co^{2+}	$Cr_2O_7^{2-}$	Cu^{2+}	Zn^{2+}	Fe^{3+}
P. chlororaphis S15	3	1	0.25	4	4	5

The results demonstrated that *P. chlororaphis* S15 is capable of producing siderophores on CAS agar plates. The medium color changed from blue to yellow, indicating the presence of siderophores. The replacement of Fe^{+3} with Al^{+3}, Cu^{+2}, Ga^{+3} ions revealed the formation of increased halo zones formed around the *P. chlororaphis* S15. The analysis of siderophore accumulation in the culture supernatant over 120 hours of growth revealed that the presence of heavy metals in concentrations of 100 μM $CoCl_2 \times 6H_2O$, $AlCl_3$, $GaBr_3$ or $CuSO_4$ and 400 μM $NiSO_4 \times 7H_2O$ and $ZnSO_4$ inhibited the production of catecholate- and hydroxamate-types of siderophores. Starting form 24 hours of *P. chlororaphis* S15 growth, the M9 medium supplemented with 10 μM $GaBr_3$ stimulated the production of hydroxamate-type (600 ± 25 μM) and catechol-type (40 ± 5 μM) siderophores compared to the Fe-limited medium. The HPLC analysis of extracted metabolites produced by *P. chlororaphis* S15 revealed the presence of a metabolite with a retention time of 13.0 min exclusively in the supernatant, in the absence of any heavy metals. The 10 μM $GaBr_3$ concentration significantly stimulated the production of metabolites with RT = 16.3 and 17.7 min (Fig. 1).

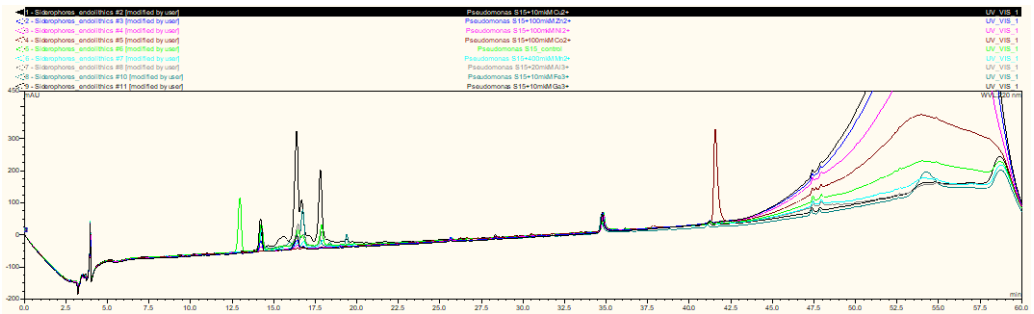

Fig. 1. HPLC analysis of extracted metabolites from the supernatant of *P. chlororaphis* S15 in the M9 medium without (green line) or supplemented with 10 μM $CuSO_4$ (brown line), 100 μM $ZnSO_4$ (blue line), 100 μM $NiSO_4 \times 7H_2O$ (pink line), 20 μM $CoCl_2 \times 6H_2O$ (red line), 400 μM $MnSO_4$ (turquoise line), 20 μM $AlCl_3$ (grey line), 10 μM FeCl3×6H2O (emerald line), 10 μM $GaBr_3$ (black line)

Conclusion: *Pseudomonas chlororaphis* is a promising bacterium with diverse applications in bioremediation, agriculture, and biotechnology. The capacity of *P. chlororaphis* S15 to synthesize siderophores and resist heavy metals renders it an optimal candidate for environmental remediation, enhancing plant health and suppressing pathogens. This underscores its potential for sustainable agriculture.

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