https://doi.org/10.15407/microbiolj85.06.003

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CHARACTERISTICS OF *STENOTROPHOMONAS RHIZOPHILA* LIPOPOLYSACCHARIDE, A REPRESENTATIVE OF ANTARCTICA

In 2002, Wolf described a new species of Stenotrophomonas, Stenotrophomonas rhizophila, which is a non-pathogenic plant inhabitant. The defining characteristics of the new species, in contrast to S. maltophilia, were the following: growth at 4 °C, but its absence at 37 °C; the use of xylose as a carbon source; low osmolytic tolerance. S. rhizophila is isolated exclusively from the rhizosphere or from the internal tissues of plants, in particular from the vascular tissues of the root and stem. In 2014, a number of authors, studying cultivated heterotrophic bacteria and yeasts, which dominate in plant samples collected from various terrestrial biotopes near the Ukrainian Antarctic station on Galindez Island in Marine Antarctica, isolated a number of bacterial isolates. The authors' phylogenetic analysis using only BLAST analysis made it possible to determine the approximate phylogenetic affiliation of the bacterial isolate to the family Gammaproteobacteria (genus Stenotrophomonas, species S. rhizophila). It is known that many processes that determine the biological features of microorganisms, the nature of their relationships between them, as well as micro- and macroorganisms in biocenoses, are carried out with the participation of the surface structures of the bacterial cell, which are in direct contact with the environment. Among them, of particular interest are lipopolysaccharides (LPS), components of the outer membrane of Gram-negative bacteria. However, to date, we have not found any works devoted to the study of S. rhizophila lipopolysaccharides in the literature available to us. The ***aim*** *of the work was to clarify the phylogenetic position of the 6p5m bacterial strain isolated from the Antarctic region by constructing dendrograms, to study some of its phenotypic properties, to isolate lipopolysaccharides and study chemical and component composition, serological, and biological properties.* ***Methods.*** *Identification was carried out via ribosomal phylogeny. A fragment of the 16S rRNA gene of bacterial strain 6p5m was amplified by PCR using primers 8F and 1492R. The phylogenetic position of the strains was determined by construction of dendrograms, which show the position of the investigated strains among closely related and typical species and type species (programs ClustalX 2.1, Mega v. 6.00). LPS was obtained from cells by water-phenol extraction, electrophoresis was carried out in polyacrylamide gel, monosaccharide and*

Citation: Brovarska O.S., Varbanets L.D., Gladka G.V., Tashyrev O.B. Characteristics of *Stenotrophomonas rhizophila* Lipopolysaccharide, a Representative of Antarctica. *Microbiological journal.* 2023 (6). P. 3—14. https://doi.org/10.15407/microbiolj85.06.003

fatty acid composition was determined by chromato-mass spectrometry, antigenic activity was studied by immunodiffusion in agar. ***Results.*** *Phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene revealed a high level of homology (99.8%) of the bacterial strain 6p5m with typical strain S. rhizophila DSM 14405^T from the GenBank database, which allows us to classify it. Comparing phylogenetic analysis with phenotypic data, we can assert that strain 6p5m belongs to the S. rhizophila species. When studying the biopolymer composition of LPS by specific reactions to each component, it was found that the carbohydrate content was relatively low and amounted to 27.42% and 13.34% for LPS1 and LPS2, respectively. The content of characteristic LPS components: heptose and 2-keto-3-deoxyoctonic (KDO) acid was 1.78% and 0.034%, as well as 5.38% and 0.09%, respectively, for LPS1 and LPS2. Analysis of the monosaccharide composition of LPS preparations showed that fucose (72.57%) is present as a dominant monosaccharide in LPS1 and rhamnose (65.04%) in LPS2. The main monosaccharides of the extracellular component were galactose (39.92%) and rhamnose (24.36%). Analysis of the lipid part of the studied LPS indicates significant differences between them. In the composition of lipid A LPS1 and LPS2 of S. rhizophila 6p5m, anti-iso-pentadecanoic acid (a-i-C15:0) was predominant (25.58% and 39.24%, respectively), and 3-hydroxydodecanoic acid (3-OH-C12:0) in LPS2 (26.21%), hexadecanoic acid (C16:0) (27.06% and 10.51%, respectively) for LPS1 and LPS2. Cyclic acids were present only in LPS1, while i-C15:0 and 9-C16:1 only in LPS2. Electrophoretic analysis showed heterogeneity characteristic of an LPS molecule, manifested by a plurality of bands in the form of a «ladder» when the gel was stained with silver ions, which is associated with the presence in the composition of LPS preparations of O-specific polysaccharide structures with different lengths of oligosaccharide chains, which determine their different molecular weight. On the electropherogram of LPS1 and LPS2, bands were not visualized in the upper part of the electrophoretic track, which indicates the absence of S-forms of LPS with long O-specific chains, but a significant predominance of SR-form molecules in the membrane LPS pool. S. rhizophila 6p5m LPS showed significantly higher pyrogenic activity compared to pyrogenal, a pharmaceutical preparation, an active ingredient of which is Shigella typhi LPS. The extracellular polymer did not exhibit pyrogenic activity. In the double immunodiffusion reaction in agar according to Ouchterlony, it was found that the studied LPSs in the homologous system exhibit antigen activity. The antigens present in both LPS1and LPS2 and the extracellular component are identical: the antigen present in each of the wells binds to all antibodies that can interact with the antigen from the other well. It was shown that pre-sowing treatment of seeds with LPS preparations of S. rhizophila 6p5m stimulates the energy of seed germination. LPS1 (+2.82 cm) showed the greatest impact in comparison with the control. A significant stimulatory effect of LPS1, LPS2, and extracellular polymer was observed when studying their effects on mustard seedlings.* ***Conclusions.*** *The taxonomic position of bacterial strain 6r5m of the ecosystems of the polar region (Antarctica) was determined via the phylogenetic analysis taking into account the phenotypic features of the strain. The bacterial strain is represented in the phylum Proteobacteria, class Gammaproteobacteria, family Xanthomonadaceae, genus Stenotrophomonas, species S. rhizophila. From S. rhizophila cells, LPS preparations were obtained, which differed in monosaccharide and fatty acid composition, but showed high pyrogenicity. S. rhizophila represents a promising alternative to S. maltophilia for applications in agricultural biotechnology and biological control due to its ability to both stimulate plant growth and protect roots from biotic and abiotic stresses.*

Keywords: *phylogenetic analysis, Stenotrophomonas rhizophila, 16S rRNA, lipopolysaccharides, monosaccharide, fatty acid composition, heterogeneity of lipopolysaccharide molecule, serological activity, germination energy and growth of seedlings.*

In 2002, Wolf described a new species of *Stenotrophomonas*, *Stenotrophomonas rhizophila*, which is a nonpathogenic plant inhabitant [1]. A polyphasic taxonomic study conducted on 16 *Stenotrophomonas* strains from environmental and clinical sources showed that a group of three plant isolates was phenotypically distinct from other strains. This group formed a separate physiological cluster (B1) with 42% heterogeneity relative to other isolates. The defining characteristics of the new species, in contrast to *S. maltophilia*, were the following: growth at 4 °C, but its absence at 37 °C; the use of xylose as a carbon source; low osmolytic tolerance. *S. rhizophila* is isolated exclusively from the rhizosphere or from the internal tissues of plants, in particular from the vascular tissues of the root and stem. To date, they have been found in associations with various plants. Thus, it was shown [2] that they constitute the main microbiome of tomato roots, where they

coexist with representatives of the genera *Pseudomonas*, *Bacillus*, and *Rhizobium*. Moreover, *S. rhizophila* was identified as one of the species colonizing decaying alder nodules [3]. The ability of *S. rhizophila* to grow at 4 °C and the inability to reproduce at 37 °C reduce the likelihood of it infecting humans, and no pathogenic strains of *S. rhizophila* have been reported to date.

In 2014, the authors of [4] studied cultivated heterotrophic bacteria and yeasts that dominate in plant samples collected from various terrestrial biotopes near the Ukrainian Antarctic station on Galindez Island in marine Antarctica. The authors' phylogenetic analysis using only BLAST analysis made it possible to determine the approximate phylogenetic affiliation of the bacterial isolate to the family Gammaproteobacteria (genus *Stenotrophomonas*, species *S. rhizophila*). *S. rhizophila* synthesizes a number of metabolites involved in the processes of symbiosis with plants such as spermidine, which stimulates plant growth and is also a precursor for other polyamines involved in protecting plants from drought and salinity. It is also known [5] that during growth, *S. rhizophila* produces glucosylglycerol and trehalose in the soil, which have a high water-retaining capacity and increase the resistance of plants to salinity. In addition, *S. rhizophila* synthesizes dodecanal, which inhibits the growth of fungal mycelium, and chitinases, which destroy its cell walls. It is known that a lot of processes that determine the biological characteristics of microorganisms, the nature of their interrelationships, as well as microbe and macroorganisms in biocenoses, are carried out with the participation of the surface structures of the bacterial cell, which are in direct contact with the environment. Among them, of particular interest are lipopolysaccharides (LPS), components of the outer membrane of Gram-negative bacteria. However, so far, in the literature available to us, we have not found any works devoted to the study of *S. rhizophila* lipopolysaccharides. This determined the purpose of our work to clarify the phylogenetic position of the 6p5m bacterial strain isolated from the Antarctic region by constructing dendrograms, to study some of its phenotypic properties, and to isolate lipopolysaccharides to study their chemical and component composition, serological and biological properties.

Materials and methods. The object of the study was the bacterial strain *Stenotrophomonas rhizophila* 6p5m, isolated from a sample of moss sampled at the biogeographical polygon (Antarctica, Galindez Island) at 5 °C. The phylogenetic affiliation of bacterial isolates was determined by the analysis of 16S rRNA gene sequences. Purification and PCR sequencing of rRNA products were performed by Macrogen Inc. (South Korea). The rRNA small subunit gene was amplified from the extracted DNA using 16S rRNA gene-specific oligonucleotide primers 8F (5′— 3′: AGAGTTTGATCCTGGCTCAG) and 1492R — (5′—3′: GGTTACCTTGTTACGACTT) according to [6]. The obtained nucleotide sequences of the bacterial isolate were compared to the homologous sequences deposited in the GenBank database using the BLAST program to determine their closely related species and phylogenetic affiliation. The correction of the 16S rRNA gene sequences was carried out using the BioEdit editor. The phylogenetic position of the strains was determined by constructing trees (dendrograms) showing the position of the studied strain among related and typical species using ClustalX 2.1, Mega v. 6.00 programs. The tree was built by the nearest neighbor comparison method with bootstrap analysis (bootstrap NJ tree) using 1000 bootstrap trials (1000 alternative trees).

To isolate LPS, the culture was grown on meat-peptone agar (mattresses) at 28 °C for 24 h. LPS was obtained by the classical water-phenol method [7]. Determination of the chemical composition of LPS was carried out by conventional methods: total carbohydrates — according to Dubois [8], proteins — according to Lowry [9], nucleic acids — according to Spirin [10]. The presence of 2-keto-3-deoxyoctonic acid

(KDO) in LPS was determined by the reaction with thiobarbituric acid [11].

The monosaccharide composition [12] was analyzed as polyol acetates on an Agilent 6890N/5973 inert chromatography-mass spectrometry system equipped with a DB225mS column (30 m × × 0.25 mm × 0.25 μm); the carrier gas was helium at a flow rate of 1 mL/min. The identification of monosaccharides was performed by comparison of the retention times with the authentic samples.

The fatty acid composition was determined by methanolysis of LPS with 1.5% acetyl chloride in methanol (100 °C, 4 h). The identification of fatty acids was performed as methyl esters by GLC-MS on Agilent 6890N/5973 inert chromatography-mass spectrometry system equipped with an HP 5ms column (30 m × 0.25 mm × 0.25 nm) using the temperature program from 150 to 250 °C at 4 °C/min; helium was used as a carrier gas at a flow rate of 1.2 mL/min. The evaporator temperature was 250 °C, and the flow distribution was 1:100. To identify fatty acids, the standard mixture of fatty acid methyl esters and the available databases were used [11].

The quantitative content of individual monosaccharides and fatty acids was expressed as% to the total sum of peak areas.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAAG electrophoresis) was performed according to Laemmli [13] (4% concentrating and 12% separating gel, current 30 mA). The load on the gel lane was 20 μg. To visualize LPS, the gels were stained with silver salts according to Tsai's recommendations [14] as modified by Kulikov [15].

In the study of biological and functional activity, the following LPS concentrations were used: the minimal pyrogenic dose was 7.5 × 10—3 μg/mL per 1 kg of animal weight (pyrogenicity); 1 mg/mL (serological studies). The work was conducted in accordance with the «General Ethical Principles of Animal Experiments».

The LPS pyrogenicity was determined in rabbits (breed Chinchilla, age of 1.0—1.5 years) weighing 2.0—3.5 kg. Animals with an initial temperature within a range of 0.2 °C were used. The temperature was measured in 30 min intervals before the injection and in 1 hr intervals after the injection. The LPS solutions under test were considered apyrogenic if the temperature did not increase more than by1.4 °C for 3 hr.

For the O-antiserum preparation, heated cells were used (2.5 h, boiling water bath); the cell concentration was 2×10^9 CFU/mL. Rabbits were immunized intravenously five times at 4-day intervals (from 0.1 to 1 mL). The antigenic activity of LPS was studied by the method of double immunodiffusion in agar according to Ouchterlony [16].

Statistical analysis of the data obtained was carried out using statistical methods, as well as the Excel 2000 computer program.

The phytotoxicity of LPS preparations was tested by immersing young yellow mustard seedlings cut underwater in a solution of LPS and extracellular component (1 mg/mL). The experiments were repeated 3—4 times. For two days, the appearance of any signs of preparation toxicity was monitored [17]. To detect the effect of LPS preparations on the germination energy and germination of agricultural plants, yellow mustard seeds were soaked for 2 hr in preparation solutions with a concentration of 1 mg/mL, then the seeds were dried on filter paper at room temperature. Seeds soaked in sterile water served as a control. The seeds were germinated in Petri dishes on a wet bed of cotton wool and filter paper first (before the first recording) at 27 °C, then at room temperature [17]. 25 seeds were placed in each cup. The number of seeds that germinated on the 3—5^{th} day was considered as laboratory germination energy, and those that germinated after 10—14 days (second count) — as germination. The arithmetic mean of all repetitions was taken as an accounting result.

Results. In order to determine the taxonomic position, morphological-cultural and molecular-biological analyses of the bacterial strain 6r5m isolated from the extreme region of Antarctica were carried out. Primary identification

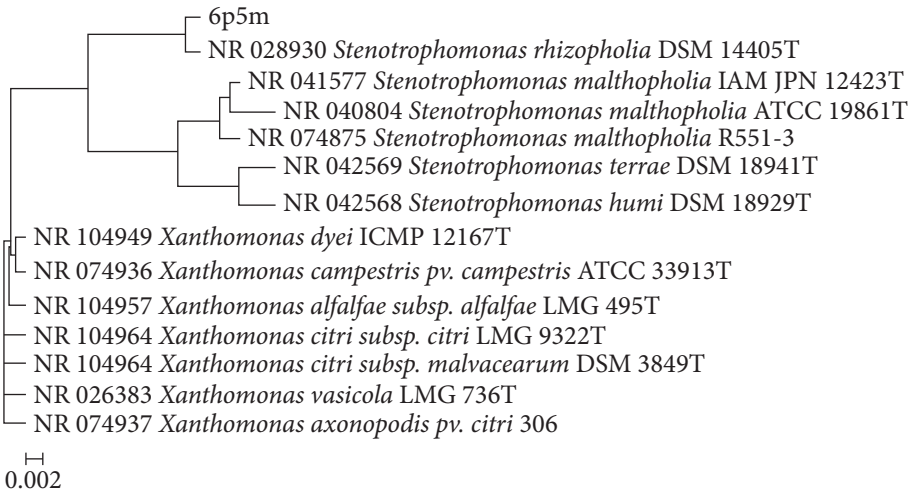


Fig 1. Phylogenetic position of strain 6r5m among closely related representatives of the genera *Stenotrophomonas* and *Xanthomonas* (family *Xanthomonadaceae*, phylum *Proteobacteria*). A scale of 0.002 corresponds to 2 substitutions per 1000 bp.

of the studied strain was carried out by generally accepted methods. The strain is a psychrotolerant chemoorganotroph that grows in aerobic conditions in a wide temperature range from 5 to 30 °C. Colonies are light brown, convex, smooth, and up to 3 mm in diameter. Cells are gram-negative rod-shaped bacteria, the size of which varies within 1.5—3 × 0.8—1 μm. Comparative and phylogenetic analyses of the aerobic chemoorganotrophic strain 6r5m based on the nucleotide sequences of the 16S rRNA gene revealed closely related species (Table 1), which were further used for phylogenetic analysis. The phylogenetic position of the strains was determined by constructing trees (dendrograms) which show the position of the studied strain among closely related and typical species.

On the phylogenetic tree (Fig. 1), the nucleotide sequence of the bacterial strain 6p5m is clustered together with typical strain *S. rhizophila* DSM 14405^T (homology 99.8%), which makes it possible to attribute the strain 6p5m to this species. Considering the low percentage of homology with *S. maltophilia* 551-3 and *S. malto-*

Table 1. **Comparative analysis of pairwise similarity of 16S rRNA genes of bacterial strain 6p5m with 16S rRNA genes of bacteria in the GenBank database**

Bacteria species that are closest to the studied strain according to the program BLASTN 2.2.28+		
GenBank accession. Species No., strain No.	Similarity, %	Compared gene fragments 16S rRNA
NR_028930 *Stenotrophomonas rhizophila* DSM 14405^T	99.8	1048/1050
NR_074875 *Stenotrophomonas maltophilia* R551-3	97.6	1025/1050
NR_041577 *Stenotrophomonas maltophilia* IAM JPN 12423^T	97.3	1022/1050
NR_074937 *Xanthomonas axonopodis* pv. *citri* 306	97.1	1020/1050
NR_104964 *Xanthomonas citri* subsp. *citri* LMG:9322T	97.1	1020/1050
NR_026383 *Xanthomonas vasicola* LMG 736T	97.1	1020/1050

philia IAM JPN 12423T strains and with representatives of the similar genus *Xanthomonas*, as well as the great depth of branching, the bacterial strain 6r5m cannot be attributed to these species. Comparing the obtained results with the data of phenotypic studies, we can state that the strain 6r5m belongs to the *S. rhizophila* species. Taxonomic position: *Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas*.

LPS was isolated from cells of *S. rhizophila* 6p5m grown at 28 °C by the water-phenol method [8]. The supernatant of the culture liquid, obtained after the sedimentation of the cells, represented an extracellular component that may contain LPS or polysaccharides. In the process of isolating LPS from the cells, three fractions were obtained, which differed visually: two aqueous fractions: the upper (milky) and the middle (transparent) and the lower - phenolic. It is known that LPS passes into the aqueous phase during extraction, therefore, in further work, the aqueous fraction (LPS1) and the milky fraction (LPS2) were studied. The phenolic fraction was discarded. Since the studied LPS preparations contained a significant amount of nucleic acids (22.2%), they were additionally purified by ultracentrifugation (LPS1) or trichloroacetic acid precipitation (LPS2) with further dialysis. The preparations were lyophilized. The yield of LPS1 was 0.49% of the dry cells and LPS2 — 18.2%.

When studying the biopolymer composition of LPS by specific reactions for each component, it was established that the carbohydrate content was relatively low and amounted to 27.42% and 13.34% for LPS1 and LPS2, respectively. A small amount of carbohydrates may be due to the presence of hexosamines, which in the reaction with phenol and sulfuric acid, used to determine the total amount of carbohydrates, do not give a positive reaction. The content of the characteristic LPS components: heptose and 2-keto-3-deoxyoctonic (KDO) acid made up 1.78% and 0.034%, as well as 5.38% and 0.09%, respectively, for LPS1 and LPS2 (Table 2).

Thus, it is shown that the cell membrane of the studied *S. rhizophila* 6p5m contains LPS, which includes all the components characteristic of these biopolymers.

Analysis of the monosaccharide composition of LPS preparations showed that fucose (72.57%) is present as a dominant monosaccharide in LPS1 and rhamnose (65.04%) in LPS2. The main monosaccharides of the extracellular component were galactose (39.92%) and rhamnose (24.36%) (Table 3).

Analysis of methyl esters of fatty acids of studied LPS lipids A by the method of chromato-mass spectrometry indicates significant differences between them (Table 3). In the composition of lipids A LPS1 and LPS2 of *S. rhizophila* 6p5m, anti-iso-pentadecanoic acid (a-i-C15:0) was predominant (25.58% and 39.24%, respectively), and 3-hydroxydodecanoic acid (3-OH-C12:0) in LPS2 (26.21%), hexadecanoic acid (C16:0) (27.06% and 10.51%, respectively) for LPS1 and LPS2. Cyclic acids were present only in LPS1, while i-C15:0 and 9-C16:1 only in LPS2.

LPS is a heterogeneous population of molecules, which is clearly seen on the PAAG elec-

Table 2. **Characteristics of LPS of *Stenotrophomonas rhizophila* 6p5m**

Preparations	Mass	Carbohydrates	Protein	KDO	Heptoses	Nucleic acids
	mg	% of the dry weight of the preparation				
Exracellular component	560.0	12.08	13.12	—	—	3.48
LPS1	473	27.42	2.5	0.034	1.78	4.34
LPS2	58.0	13.34	3.33	0.09	5.38	6.23

trophoresis (Fig. 2). Electrophoretic analysis showed heterogeneity of LPS, manifested by a plurality of bands in the form of a «ladder» when the gel was stained with silver ions, which is associated with the presence of preparations of O-specific polysaccharide structures with different lengths of oligosaccharide chains, which determine their different molecular weights. On the electrophoregram of LPS1 and LPS2, bands were not visualized in the upper part of the electrophoretic track, which indicates the absence of S-forms of LPS with long O-specific chains, but a significant predominance of SR-form molecules in the membrane LPS pool.

For a comparative assessment of the pyrogenic properties of *S. rhizophila* 6p5m LPS, the minimum pyrogenic dose was established, which was 7.5×10^{-3} μg/mL of a pyrogen-free isotonic solution. The results of thermometry showed that LPS solutions caused an increase in temperature in experimental animals by more than 0.45 °C, which is beyond the physiological norm of healthy animals (Fig. 3). An hour after the injection of LPS1 and LPS2, a sharp increase in the temperature of the experimental animals was observed and the temperature increased for two hours. At the third hour, there was a slight decrease in temperature with a tendency to its normalization. As evidenced by the results, the LPS of *S. rhizophila* 6p5m showed significantly higher pyrogenic activity than pyrogenal, a pharmaceutical preparation whose active ingredient is LPS of *Shigella typhi*. The extracellular polymer did not exhibit pyrogenic activity.

LPS is the main thermostable antigen of a microbial cell, the serological activity of which is determined by its composition and structure. Immunization with LPS or gram-negative bacteria induces the formation of antibodies only to the O-specific chain of LPS, which has antigenic properties. When conducting serological studies, rabbit polyclonal antisera obtained to the heated culture of *S. rhizophila* 6p5m were used as antibodies, and as antigens — LPS1, LPS2,

Fig. 2. Electropherogram of LPS preparations in 14% SDS-PAGE: *1* — LPS1, *2* — LPS2

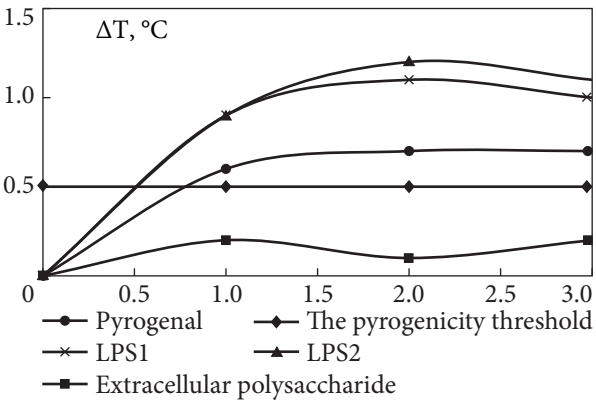


Fig. 3. Pyrogenic activity upon administration of LPS preparations of *S. rhizophila* 6p5m to experimental animals

Table 3. **Monosaccharides and fatty acid composition of LPS of *S. rhizophila* 6p5m**

Monosaccharides	LPS1	LPS2	Extracellular component
	% of the sum of the area of peaks		
Rha	6.76	65.04	24.36
Fuc	72.57	—	—
Man	—	18.01	17.99
Gal	20.67	3.98	39.92
Glu	—	12.97	15.36
Fatty acids			
3-OH-$C_{12:0}$	—	26.21	—
i-$C_{15:0}$	—	11.15	—
a-i-$C_{15:0}$	25.58	39.24	—
9-$C_{16:1}$	—	12.83	—
$C_{16:0}$	27.06	10.57	—
cyclic acids	19.29	«— «	—
	28.07	«— «	—

«—» — not detected

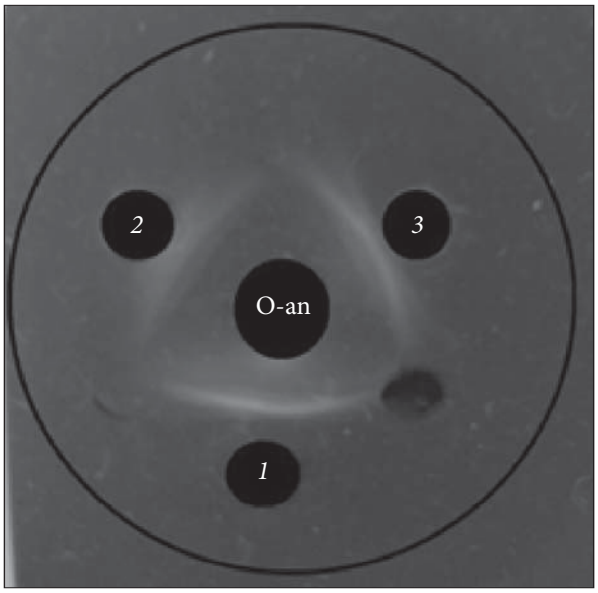


Fig. 4. Double immunodiffusion reaction in Ouchterlony agar O-antiserum *S. rhizophila* 6p5м (central well) to LPS1 (*1*), LPS2 (*2*), and extracellular polysaccharide (*3*)

and an extracellular component. In the ring precipitation reaction, the antiserum titer was determined, which was 1:160 000. In the double immunodiffusion reaction in agar according to Ouchterlony, it was found that the studied LPS in the homologous system exhibit antigen activity (Fig. 4). An identity reaction is observed: the precipitation bands completely merge, which indicates the identity of the antigens present both in LPS1, LPS2, and in the extracellular component. The line of identity indicates that the antigen in each well binds to all antibodies that can interact with the antigen in the other well.

Bacterial lipopolysaccharides have the recognized status of pathogenicity factors. However, a more detailed analysis of the data on the effect of these biopolymers on plants indicates that they can exhibit both toxic activity and growth stimulators for plants. Thus, Table 4 and Fig. 5 show that pre-sowing treatment of seeds with preparations of *S. rhizophila* 6p5m stimulates the energy of seed germination. LPS1 showed the greatest influence in comparison to the control (+2.82 cm).

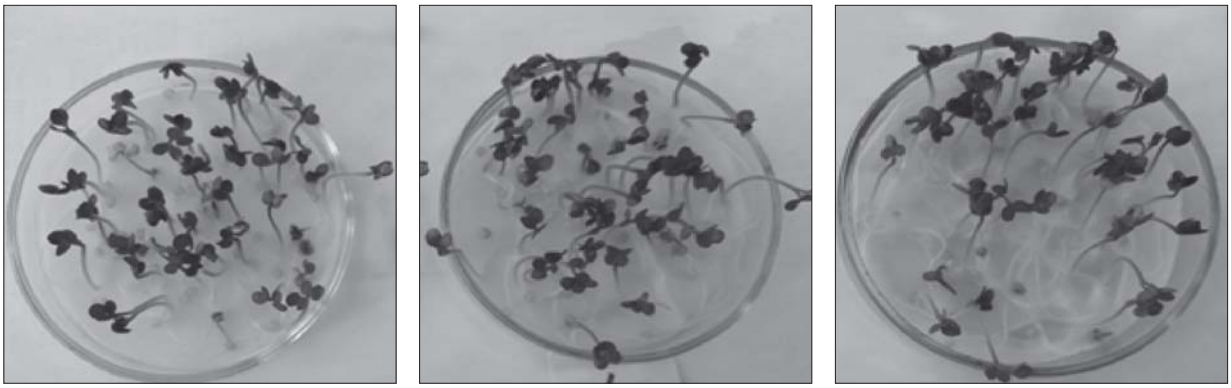
Table 4. **Effect of *S. rhizophila* 6p5m preparations on root growth (cm)**

Effect	Control	Extracellular component	LPS2	LPS1
Δ	2.51	2.67	3.82	5.33
max	4.90	5.90	10.90	12.50
min	0.10	0.10	0.10	0.30

A significant stimulating effect of LPS1, LPS2, and extracellular polymer was observed when studying mustard seedlings (Fig. 6).

Discussion. In their natural habitat, bacteria face both seasonal and sudden changes in many environmental conditions. Therefore, they have developed very sophisticated acclimatization strategies to deal with such situations. Representatives of Gram-negative bacteria of the genus *Stenotrophomonas* have been found in a wide variety of environments and geographic regions, and are also associated with humans as a nosocomial pathogen [2, 6]. Although *Stenotrophomonas* strains are effective biocontrol agents, their use in the field raises doubts because some of them, in particular *S. maltophilia*, are also associated with humans as nosocomial pathogens. Recently, a new non-pathogenic species *S. rhizophila* has been described, which includes strains isolated from the plant rhizosphere. Representatives of this species have great potential for applications in biotechnology and biological control due to their ability to both stimulate plant growth and protect roots from biotic and abiotic stresses, but little is known about the role of LPS in these processes. Since the strain we studied was isolated from Antarctic mosses, it is necessary to clarify its taxonomic position, as well as to characterize the main component of the outer membrane of gram-negative bacteria, in particular lipopolysaccharide, since it plays a significant role in plant-microorganism interactions.

Phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene revealed a high level of homology (99.8%) of the studied bacterial strain 6r5m with the typical strain from the GenBank



Control Extracellular polysaccharide LPS-1

Fig. 5. Effect of LPS1 and the extracellular component on seed germination (day 6)

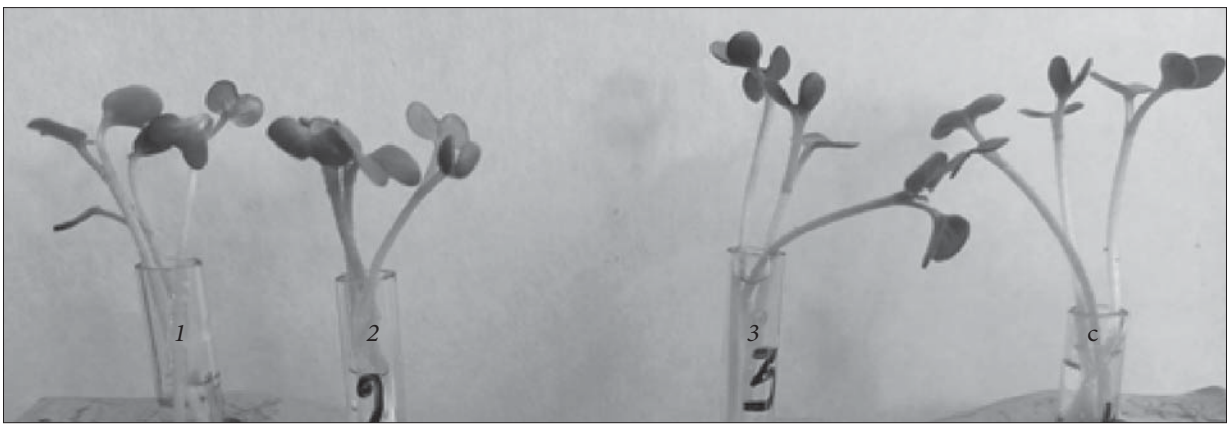


Fig. 6. The effect of the extracellular component LPS1 (1), LPS2 (2), and LPS1 (3) on the growth of seedlings (day 4)

database *S. rhizophila* DSM 14405T, which allows it to be assigned to this species. Having compared the phylogenetic analysis with the data of phenotypic studies, we can state that the studied strain 6r5m belongs to the species *S. rhizophila*.

The isolated LPS and the extracellular polymer of *S. rhizophila* differed little from the other species of this genus in terms of monosaccharide composition. The fatty acid composition of the studied LPS was unexpected for us. Thus, LPS2 contains 3-OH-C12:0 (26.21%), which was not detected in LPS1. According to the literature [18], various fatty acids are identified in most *Stenotrophomonas* species, however, the iso-branched C15:0 is present as the main fatty acid, indicating that it is a trait common to members of the *Stenotrophomonas* genus [1]. In particular, in the strain of *S. rhizophila* studied by us, anti-isopentadecanic acid predominated: 25.58 and 39.24% in LPS1 and LPS2, respectively. If for most species of gram-negative bacteria the composition of LPS fatty acids is a species property, for *Enterobacteriaceae* it is characteristic of representatives of the family-wide property, while for representatives of *Stenotrophomonas* it is characteristic for the genus. Our result on the serological activity of antiserum against a heated culture of *S. rhizophila*, with not only of both LPS but also of the extracellular component, was unexpected, which indicates the presence of common antigenic determinants in their composition. It is possible that the extracellular

component contains LPS, which is easily bound to the outer membrane of *S. rhizophila* cells.

The majority of *Stenotrophomonas* representatives are well adapted to harmful and nutrient-limited environments. Several factors are known or predicted to affect the ability of *Stenotrophomonas* to colonize and survive on plant surfaces. Interactions between plants and microorganisms in the rhizosphere are preceded by the movement of free-living microorganisms to plant roots and may involve chemotaxis to attractants present in plant root exudates. In addition to flagellar motility, other factors favoring the colonization of plant tissues by *S. rhizophila* include high bacterial growth rates, vitamin B1 synthesis, NADH dehydrogenase synthesis, the ability to produce extracellular enzymes such as proteases, lipases, nucleases, chitinases, and elastases, and also bacterial lipopolysaccharides [19]. We have shown the ability of LPS of the studied strain of *S. rhizophila* to positively influence both the energy of seed germination and the growth of mustard seedlings.

Taking into account the above information, *S. rhizophila* is a promising alternative to *S. maltophilia* for applications in agricultural biotechnology and biological control due to its ability to both stimulate plant growth and protect roots from biotic and abiotic stresses [20].

Conclusions. The taxonomic position of bacterial strain 6r5m of the ecosystems of the polar region (Antarctica) was determined via the phylogenetic analysis taking into account the phenotypic features of the strain. The bacterial strain is represented in the phylum *Proteobacteria*, class *Gammaproteobacteria*, family *Xanthomonadaceae*, genus *Stenotrophomonas*, species *S. rhizophila.* From *S. rhizophila* cells, LPS preparations were obtained, which differed in monosaccharide and fatty acid composition and showed high pyrogenicity. *S. rhizophila* represents a promising alternative to *S. maltophilia* for applications in agricultural biotechnology and biological control due to its ability to both stimulate plant growth and protect roots from biotic and abiotic stresses.

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Received 6.04.2023

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ХАРАКТЕРИСТИКА ЛІПОПОЛІСАХАРИДУ *STENOTROPHOMONAS RHIZOPHILA*, ПРЕДСТАВНИКА АНТАРКТИКИ

У 2002 році Вольф описав новий вид *Stenotrophomonas*, а саме *Stenotrophomonas rhizophila*, який є непатогенним мешканцем рослин. Визначальними характеристиками нового виду, на відміну від *S. maltophilia,* були такі: ріст при 4 °С, але його відсутність при 37 °С; використання ксилози як джерела вуглецю; низька осмолітична толерантність. *S. rhizophila* виділяють виключно з ризосфери або внутрішніх тканин рослин, зокрема із судинних тканин кореня і стебла. У 2014 році ряд авторів, досліджуючи культивування гетеротрофних бактерій та дріжджів, що домінують у зразках рослин, зібраних із різних наземних біотопів поблизу Української антарктичної станції на острові Галіндез у морській Антарктиді, виділили низку бактеріальних ізолятів. Проведення авторами філогенетичного аналізу лише за допомогою аналізу BLAST дало можливість визначити приблизну філогенетичну приналежність одного з бактеріальних ізолятів до родини *Gammaproteobacteria* (рід *Stenotrophomona*s, вид *S. rhizophila*). Відомо, що багато процесів, які визначають біологічні особливості мікроорганізмів, характер їхніх взаємовідносин, а також з мікро- та макроорганізмами в біоценозах здійснюються за участю поверхневих структур бактеріальної клітини, які безпосередньо контактують з навколишнім середовищем. Серед них особливий інтерес представляють ліпополісахариди (ЛПС), компоненти зовнішньої мембрани грамнегативних бактерій. Однак дотепер у доступній нам літературі ми не виявили робіт, присвячених вивченню ЛПС *S. rhizophila*. **Метою** роботи було уточнити філогенетичне положення бактеріального штаму 6р5m, ізольованого з району Антарктики, побудовою дендрограм, вивчити деякі його фенотипові властивості, виділити ЛПС, вивчити їх хімічний і компонентний склад, серологічні та біологічні властивості. **Методи**. Ідентифікацію проводили за рибосомною філогенією. Фрагмент гена 16S rRNA у бактеріального штаму 6р5м ПЦР-ампліфікований з використанням праймерів 8F та 1492R. Філогенетичне положення штамів визначали побудовою дерев (дендрограм), які показують положення досліджуваного штаму серед близькоспоріднених і типових видів (програми ClustalX 2.1, Mega v. 6.00). ЛПС отримували з клітин водно-фенольною екстракцією, електрофорез проводили в поліакриламідному гелі, моносахаридний і жирнокислотний склад визначали хромато-мас-спектрометричним методом, антигенну активність вивчали імунодифузією в агарі. **Результати.** Філогенетичний аналіз нуклеотидної послідовності гена 16S rRNA виявив високий рівень гомології (99.8 %) досліджуваного бактеріального штаму 6р5м з типовим штамом з бази даних GenBank *S. rhizophila* DSM 14405^{T}, що дозволяє віднести його до цього виду. Порівнюючи філогенетичний аналіз з даними фенотипових досліджень, ми можемо стверджувати, що досліджуваний штам 6р5м належить до виду *S. rhizophila*. При вивченні біополімерного складу ЛПС специфічними реакціями на кожний компонент встановлено, що вміст вуглеводів був порівняно невисоким і становив 27.42 % та 13.34 % для ЛПС1 та ЛПС2 відповідно. Вміст характерних компонентів ЛПС: гептоз і 2-кето-3-дезоксиоктонової (КДО) кислоти становили 1.78 % та 0.034 %, а також 5.38 % та 0.09 % відповідно для ЛПС1 та ЛПС2. Аналіз моносахаридного складу препаратів ЛПС показав, що як домінуючий моносахарид у ЛПС1

присутня фукоза (72.57 %), у ЛПС2 — рамноза (65.04 %). Основними моносахаридами позаклітинного компонента були галактоза (39.92 %) та рамноза (24.36). Аналіз жирних кислот ліпідів А досліджуваних ЛПС свідчить про значні відмінності між ними. У складі ліпідів А ЛПС1 і ЛПС2 *S. rhizophila* 6р5м переважною була анти-ізо-пентадеканова кислота (a-i-C15:0) (25.58 % і 39.24 %, відповідно), а також були ідентифіковані 3-гідроксидодеканова кислота (3-OH-C12:0) у ЛПС2 (26.21 %) і гексадеканова кислота (C16:0) (27.06 % та 10.51 %, відповідно) для ЛПС1 та ЛПС2. Циклічні кислоти були присутні тільки в ЛПС1, тоді як i-C15:0 та 9-C16:1 — тільки в ЛПС2. Електрофоретичний аналіз показав характерну для ЛПС гетерогенність, що проявляється множинністю смуг у вигляді «сходів» під час фарбування гелю іонами срібла, що пов'язано з присутністю у складі препаратів ЛПС структур О-специфічного полісахариду з різною довжиною олігосахаридних ланцюгів, що зумовлюють його різну молекулярну вагу. На електрофореграмі ЛПС1 і ЛПС2 у верхній частині електрофоретичного треку не візуалізувалися смуги, що свідчить про відсутність S-форм ЛПС з довгими О-специфічними ланцюгами, але вказує на значне переважання в пулі мембранного ЛПС молекул SR-форми. ЛПС *S. rhizophila* 6р5м виявили значно більшу пірогенну активність, ніж пірогенал — фармацевтичний препарат, чинним компонентом якого є ЛПС *Shigella typhi*. Позаклітинний компонент не виявляв пірогенної дії. У реакції подвійної імунодифузії в агарі за Оухтерлоні було встановлено, що досліджувані ЛПС у гомологічній системі виявляють активність антигену. Антигени, присутні як в ЛПС1, ЛПС2, так і позаклітинному компоненті, є ідентичними. Показано, що передпосівна обробка насіння препаратами ЛПС *S. rhizophila* 6р5м стимулює енергію проростання насіння. Найбільший вплив у порівнянні з контролем проявив ЛПС1 (+ 2.82 см). Істотний стимулюючий вплив ЛПС1, ЛПС2 та позаклітинного полімеру спостерігали під час вивчення їх впливу на проростки гірчиці. **Висновки.** Згідно з філогенетичним аналізом з урахуванням фенотипових властивостей визначено таксономічне положення бактеріального штаму 6р5м екосистем полярного регіону (Антарктика). Бактеріальний штам представлено у філумі *Proteobacteria,* клас *Gammaproteobacteria*, родина *Xanthomonadaceae,* рід *Stenotrophomonas,* вид *S. rhizophila*. Із клітин *S. rhizophila* отримали препарати ЛПС, які відрізнялися за моносахаридним та жирнокислотним складом, проте виявляли високу пірогенність. *S. rhizophila* є перспективною альтернативою *S. maltophilia* для застосування в сільськогосподарській біотехнології та біологічному контролі завдяки своїй здатності стимулювати зростання рослин і захищати коріння від біотичних та абіотичних стресів.

Ключові слова: *філогенетичний аналіз, Stenotrophomonas rhizophila, 16S rRNA ліпополісахариди, моносахариди, склад жирних кислот, гетерогенність молекули ліпополісахариду, серологічна активність, енергія проростання та ріст проростків.*