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POLYPHASE TAXONOMY OF ANTARCTIC BACTERIA

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The phylogenetic structure of bacteria is not always consistent with the traditional classification scheme based on the phenotypic properties of bacteria. That is one of the problems of modern bacterial taxonomy. In addition, traditional methods to identify bacteria using phenotypic properties have a number of disadvantages. In recent decades, significant progress was achieved in the investigation of microbial world using molecular methods for fast identification. ***The aim*** *of the study was to clarify the species status of four strains of bacteria isolated from black lichens of the cliffs of Galindez Island in the Antarctic on the basis of phenotypic and genetic analysis.* ***Methods.*** *Morphological and cultural properties of bacteria were studied according to generally accepted microbiological methods. Physiological and biochemical ones were investigated using test systems API Coryne and API 20E (bioMerieux SA, France), according to the manufacturer's instructions. Phylogenetic analysis was performed based on the nucleotide sequences of the 16S rRNA gene. To identify closely related species, a comparative analysis of the nucleotide sequences of 16S rRNA genes was performed using the BLAST software package. The phylogenetic position was determined by constructing trees (dendrograms) to show the position of the studied strains among closely related and typical species (programs ClustalX 2.1, Mega 6.06). The tree was constructed applying ClustalX 2.1 by comparing the nearest neighbors with bootstrap analysis (bootstrap NJ tree) using 1000 bootstrap tests (1000 alternative trees). Then the phylogenetic tree was edited by the program Mega v. 6.00.* ***Results.*** *Based on the results of comparative, phylogenetic and phenotypic analysis, the studied Antarctic strains 180n1, 181n2, 188n2, 190n2 were identified as Pseudomonas fluorescens, Microbacterium foliorum, Sporosarcina aquimarina and Rothia sp., respectively. The coefficient of similarity of 16S rRNA genes of strain 180n1 with such a closely related species from the database P. fluorescens NBRC 14160 was 99.5%; 181n2 with M. foliorum P 333/02 – 99.4%; 188n2 with S. aquimarina SW28 – 99.7%. These strains form common clusters with closely related species on phylogenetic dendrograms. The strain 190n2 can be considered as Rothia sp., since has the remote position from closely related strains in the cluster Rothia and a low percentage of similarity (97.3%) with the species Rothia endophytica YIM 67072. These strains belong to the phyla: Firmicutes, Actinobacteria, Proteobacteria.* ***Conclusions.*** *Phylogenetic and phenotypic analyzes allowed determining the taxonomic position of isolated aerobic chemoorganotrophic microbial strains of the Antarctic. Nucleotide sequences of 16S rRNA genes are deposited in the International GenBank database under numbers HG518622, HG518623, HG518625, HG518626.*

Keywords: Antarctic, identification, phylogenetic analysis, comparative analysis, phenotypic analysis, 16S rRNA, closely related species.

The phenotypic systematics of bacteria based on the similarity of bacteria on the set of their morphological and physiological properties was formed in microbiology for a considerable period of time [1, 2]. A genesystematics of bacteria is the fundamentally new approach [3]. It is based on the similarity of bacterial genetic material and can reflect not only the similarity, but also their relationships. The modern classification of bacteria is now increasingly recognized among scientists to be based on a polyphase approach taking into account phenotypic and genotypic traits, including as many different techniques as possible. Thus, the polyphase approach has led to the changes in the taxonomy of actinobacteria, for which the application of traditional methods did not allow selecting the strains for industrial screening. Using the results of polyphase analysis it was possible to reclassify such genera as: *Rhodococcus*, *Streptomyces*, *Mycobacterium*, *Nocardia* etc. [3]. The studies of the primary structure of DNA and, in particular, the analysis of nucleotide gene

sequences began in the early 1960s. Using DNA-RNA hybridization methods and direct analysis of nucleotide sequences, the 16S rRNA gene was shown to be the most conservative molecule, i.e. it has undergone the least changes in the process of biological evolution [4]. That is why the analysis of nucleotide sequences (sequence analysis) of 16S rRNA genes of bacteria became the basis for the development of phylogenetic taxonomy [5, 6].

Methods of classical microbiology were used for microbiological analysis of Antarctic soils, resulting in the study of a variety of soil bacterial communities [7, 8, 9, 10]. Bacteria were identified as the representatives of the following genera: *Micrococcus*, *Pseudomonas*, *Brevibacterium*, *Corynebacterium*, *Arthrobacter*, *Flavobacterium*, *Streptomyces*, *Aeromonas*, *Chromobacterium*, *Bacillus* and other. The studied bacterial strains were morphologically diverse: rods or chains of rods, cocci and individual long filamentous cells [10].

Intensive research on the taxonomic diversity of microorganisms using modern methods of molecular biology is currently being conducted in Antarctic soils. It provides more accurate assessment of the composition of the bacterial community [11, 12]. Based on the analysis of sequences of 16S rDNA genes isolates from the soil of the Barrientos Island in the Antarctic were determined as eight different genera, namely *Sphingomonas, Bradyrhizobium, Methylobacterium, Caulobacter, Paracoccus, Ralstonia, Rhizobium* and *Staphylococcus* [13]. Recently, new species of bacteria have been described in Antarctic ecosystems belonging to *Proteobacteria* [14], *Acidobacteria* [15], *Firmicutes* [16, 17], etc. According to the literature, the studies focused on the microbial diversity of Antarctic soils revealed the main bacterial phyla *Proteobacteria* and *Actinobacteria*. *Firmicutes* and *Bacteroidetes* were much less spread [18, 19].

Thus, the modern approach for the classification of bacteria is based on molecular biological studies. They establish the affinity of taxa and the phylogenetic position of individual strains (species), and are supplemented by phenotypic characteristics of bacteria. **The aim** of this work was to clarify the species status of four strains of bacteria isolated from black lichens of the cliffs of Galindez Island in the Antarctic on the basis of phenotypic and genetic analysis.

Materials and methods. The aerobic chemo-organotrophic bacteria (180n1, 181n2, 188n2 and 190n2) isolated from black lichens of the climbing wall 2 (vertical rock) from the different observation points Tsk-1, Tsk-2, Tsk-9, Ts-11 at the temperature of 30 ºC were the objects of the study.

Cell morphology was studied by microscopy of live and Gram-stained preparations by standard methods. Cells and the purity of the strains control were conducted by light microscopy via the microscope Mikmed-2 (LOMO, Russia) (x1500). The shape, size, mobility, the presence of spores were determined. Cultural properties of bacteria (pigmentation, consistency and size of colonies, release of water-soluble pigment, formation of extracellular mucus, the presence of air and substrate mycelium and other characteristics) were determined via the cultivation on agar nutrient medium (NA) of HiMedia Laboratories Pvt. Ltd., India. To suppress the growth of micromycetes, 50 mg of nystatin per 1 L of the medium was added.

Physiological and biochemical properties of bacteria were studied using bacterial identification kits (test systems) API Coryne (catalog number 20900) and ARI 20E (catalog number 20100) (bioMerieux SA, France), according to the manufacturer's instructions. The systems are based on standard biochemical microassays containing dehydrated substrates for the study of enzymatic activities and fermentation of sugars. The catalase test was performed by adding 1 drop of hydrogen peroxide (3 %) to the esculin or gelatin test. The appearance of bubbles after one minute evidenced a positive reaction.

Isolation of DNA from cell suspensions of microorganisms was performed using a kit GenElute Bacterial Genomic DNA (Sigma-Aldrich, USA) described in the papers [20, 21]. Bacterial genomic DNA was extracted using a kit GenElute Bacterial Genomic DNA (Sigma-Aldrich, USA) according to the manufacturer's instructions. Universal bacterial primers specific for the 16S rRNA gene were used in this work 8F (5′–3′: AGAGTTTGATCCTGGCTCAG) and 1492R (5′–3′: GGTTACCTTGTTACGACTT [20] as described in the paper [22]. The reaction mixture of polymerase chain reaction (PCR) (20 µl) consisted of: 1–10 ng of template, Prime Taq Premix 2x (GENET BIO, South Korea) containing 1 U/10 µl DNA Polymerase, sample, 20 mM Tris-HCl, 80 mM KCl, 4 mM $MgCl_{2,}$ enzyme stabilizer, loading dye, pH 9.0, 0.5 mM of each dATP, dCTP, dGTP, dTTP, and 400 nM of each primer. Amplification was performed on T100 Thermal Cycler (BIO-RAD, USA). The following reaction mode was used: in the first cycle denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C

for 30 s, and 72 °C for 60 s, then a final extension step at 72 °C for 5 min. The full sequence (8-1,492 nt; *Escherichia coli* numbering) of the 16S rRNA gene of the strains was determined with Applied Biosystems model 373A DNA sequencer by using the ABI PRISM cycle sequencing kit (Macrogen, South Korea).

To determine the phylogenetic position of bacteria, the obtained sequences of 16S rRNA genes of bacterial strains were compared with the sequences of 16S rRNA genes of bacteria deposited in the GenBank database, using the software BLAST (http://blast.ncbi.nlm.nih. gov/Blast.cgi). The adjustment of 16S rRNA gene sequences was performed using an editor BioEdit (http://bioedit.software.informer.com/). The phylogenetic position was determined by constructing of the dendrograms (trees) showing the position of the studied strain among the closely related and typical species, using 1000 bootstrap tests (programs ClustalX 2.1 (http://www.clustal.org), Mega v. 6.00 (http://www.megasoftware.net/mega.php). The nucleotide sequences of the studied strains were deposited in the international database GenBank.

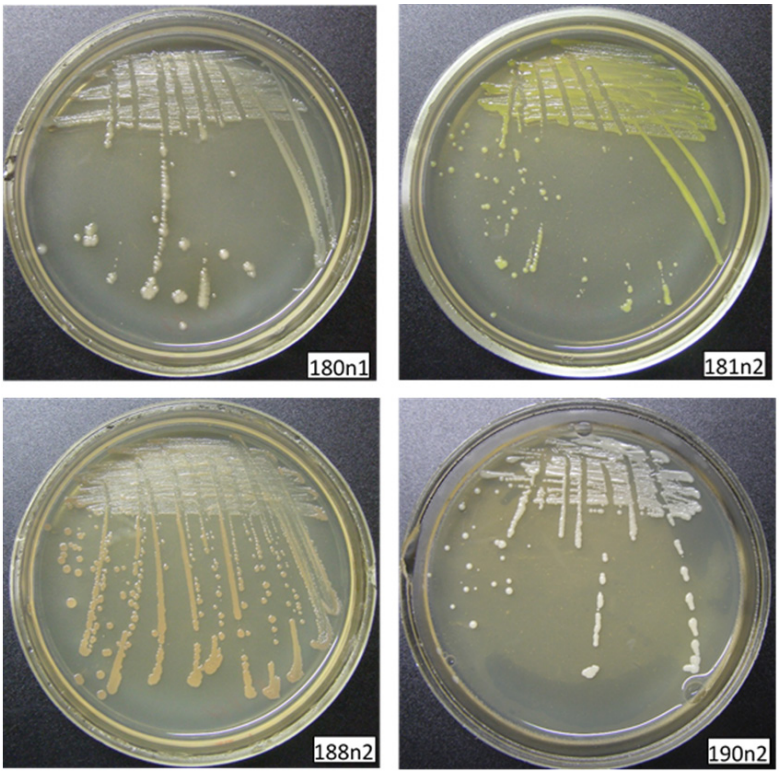
Experiments were replicated 3–5 times. Analysis of the data was conducted using Student's t-criterion. Values were considered significant at $P < 0.05$.

Results. The morphological, cultural, physiological and biochemical properties of Antarctic bacteria were used to identify them.

The strain 180n1 formed unpigmented, round, semi-convex, smooth and translucent colonies with the diameter of 1–3 mm during surface growth on the agarized medium (Nutrient agar, NA) (Fig. 1).

Cells were the gram-negative rods, 1.5–2× ×0.5–0.8 μm, motile, did not form spores and mycelium. This strain was psychotolerant, aerobic, chemoorganotrophic. The metabolism was oxidative. The acid was formed from a limited number of carbohydrates. The tests were positive for the arginine dihydrolase, the tryptophan deaminase and weakly positive for the urease, the lysine decarbixylase, the ornithine decarboxylase. It hydrolyzed gelatin, catalase-positive (Table 1). The strain 180n1 grew in the temperature range of 5–30 °C and in the presence of 2.5 % NaCl. Basic morphological and physiological characteristics make the strain 180n1 similar to the members of the genus *Pseudomonas* (class *Gammaproteobacteria*) [1, 23].

The strain 181n2 formed round, glossy, slightly convex colonies of yellow color, 1–2 mm in diameter growing on the agar medium NA (Fig. 1). Cells were gram-positive rods 1.2–1.6×0.4–0.6 μm, single or in pairs, immobile, non-spore-forming, did not form mycelium. The metabolism was



F i g. 1. General view of the Antarctic bacteria colonies

respiratory, aerobic, chemoorganotrophic. Strain was catalase-positive, hydrolyzed esculin and was weakly positive relatively for pyrazinamidase and gelatin hydrolysis (Table 1). The strain 181n2 grew in the temperature range of 1–30 °C and in the presence of 4 % NaCl. A number of characteristics make the strain 181n2 close to the genus *Microbacterium*, as well as to the species *Microbacterium foliorum* described in the paper [1, 24].

The strain 188n2 formed round, glossy, pale pink colonies, 1–3 mm in diameter on the agar medium NA (Fig. 1). Cells were gram-positive, oval rods 2–4×1–1.3 μm, which were found singly or in pairs, and sometimes in short chains, mobile. The strain was psychrotolerant, aerobic, chemoorganotrophic, grew in the temperature range of 1–30 °C and in the presence of 5 % NaCl. The strain 188n2 can be attributed to the genus *Sporosarcina* (phylum *Firmicutes*) [1, 25]. The cells were gram-positive, motile, and catalase-positive. The strain hydrolyzed gelatin, fermented only ribose among the studied carbohydrates, did not hydrolyze esculin, urease tests was negative, the enzymes were absent (Table 1). According to these properties, the strain

Table 1

Physiological and biochemical properties of the Antarctic bacteria (results obtained using API 20E and API Coryne test systems)

Signs	API 20E	API Coryne		
	Strains No.			
	180n1	181n2	188n2	190n2
Indole Products	–	ni	ni	ni
Production of H_2S	–	ni	ni	ni
Hydrolysis of gelatin	+	(+)	+	+
Urease	(+)	–	–	–
β- galactosidase	–	–	–	–
Arginine dihydrolase	+	ni	ni	ni
Lysine decarbixylase	(+)	ni	ni	ni
Ornithine decarboxylase	(+)	ni	ni	ni
Tryptophan deaminase	+	ni	ni	ni
Citrate disposal	+	ni	ni	ni
Pyrazinamidase (PYZ)	ni	(+)	–	(+)
Pyrrolidoninarylamidase (PYRA)	ni	–	–	–
Alkaline phosphatase (PAL)	ni	–	–	–
β-glucuronidase (ßGUR)	ni	–	–	–
α-glucosidase (αGLU)	ni	–	–	–
N-acetyl-β-glucosaminidase(ßNAG)	ni	–	–	–
β-glucosidase (esculin) (ESC)	ni	+	–	+
The presence of catalase (CAT)	+	+	+	+
Nitrate recovery	–	–	–	+
Fermentation:				
Glucose (GLU)	+	(+)	–	+
Mannitol (MAN)	–	(+)	–	+
Saccharose (SAC)	–	(+)	–	+
Arabinose (ARA)	+	ni	ni	ni
Inositol (INO)	–	ni	ni	ni
Sorbitol (SOR)	–	ni	ni	ni
Rhamnose (RHA)	–	ni	ni	ni
Melibiose (MEL)	+	ni	ni	ni
Amygdalin (AMY)	–	ni	ni	ni
Ribose (RIB)	ni	(+)	+	(+)
Xylose (XYL)	ni	(+)	–	–
Maltose (MAL)	ni	(+)	–	+
Lactose (LAC)	ni	–	–	–
Glycogen (GLYG)	ni	(+)	–	–

Legend: + – positive result; – – negative result; (+) – weakly positive result; ni – not investigated.

188n2 is close to the members of *Planococcaceae* family.

The strain 190n2 formed convex, glossy, white colonies with smooth edges with a diameter of 2–4 mm during surface growth on the agarized medium NA (Fig. 1). The cells of the strain were gram-positive cocci, 2.0–2.5 μm in diameter, motionless, endospores were absent. It was facultative anaerobic; chemorganotrophic. The strain 190n2 was characterized by a predominantly fermentation type of metabolism; acid was actively formed from many carbohydrates. Strain was catalase-positive, reduced nitrate, hydrolyzed esculin, gelatin and was weakly positive relatively to the pyrazinamidase (Table 1). The strain 190n2 grew in the temperature range of 5–30 °C and in the presence of 10 % NaCl. Based on a set of diagnostic features, the studied strain 190n2 belongs to the family *Micrococcaceae*, genus *Rothia* [1, 26, 27].

Thus, the analysis of morphological, cultural, physiological and biochemical properties of bacteria showed them to be psychrotolerant, aerobic, chemoorganotrophic, phenotypically close to genera *Pseudomonas, Microbacterium, Sporosarcina* and *Rothia.* The further identification of the studied strains on the base of phenotypic traits is difficult. Therefore, we used phylogenetic analysis based on the analysis of 16S rRNA genes sequences.

To identify closely related species, a comparative analysis of the nucleotide sequences of 16S rRNA genes with the ones deposited in the GenBank database was performed. Based on the obtained data closely related species were identified for the studied bacteria (Table 2).

According to the phylogenetic analysis, the strain 180n1 can be identified as *P. fluorescens*, which is also consistent with the results of the comparative analysis (Table 2, Fig. 2a). As shown on the dendrogram, the strain 180n1 formed a separate cluster with the species *P. fluorescens* and *P. canadensis*. It was close to the cluster that unites the species *P. trivialis*, *P. poae*, *P. lurida*, *P. costantinii* where the similarity with the studied strain ranged from 99.0 % to 99.2 %. It should be noted that the strain *P. antarctica* (NR025586) with homology (99.2 %) we are interested was remote from the studied strain 180n1 according to the dendrogram.

Bacterial strain 188n2 had a high similarity (99.7 %) with *Sporosarcina aquimarina.* On the dendrogram (Fig. 2b), this strain formed a common cluster with *S. aquimarina*, which allows to reliably

Table 2
Comparative analysis of pairwise similarity of 16S rRNA genes of bacteria with the genes of 16S rRNA of bacteria in the GenBank database

Strain No. / The number of nucleotide pairs	Bacterial species being the most close to the studied strains in the program BLASTN 2.2.28+		
	GenBank accession Species No.	Similarity,%	Taxonomic position of the strain
180n1/1415	NR_113647 *Pseudomonas fluorescens* NR_156852 *Pseudomonas canadensis* NR_025586 *Pseudomonas antarctica*	99.5 99.4 99.2	***Proteobacteria***, *Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas*
181n2/1388	NR_025368 *Microbacterium foliorum* NR_025405 *Microbacterium phyllosphaerae* NR_042263 *Microbacterium hydrocarbonoxydans*	99.4 99.4 98.8	***Actinobacteria***, *Actinobacteridae, Actinomycetales, Micrococcineae, Microbacteriaceae, Microbacterium*
188n2/1101	NR_025049 *Sporosarcina aquimarina* NR_114283 *Sporosarcina luteola* NR_112844 *Sporosarcina luteola*	99.7 98.6 98.6	***Firmicutes***, *Bacilli, Bacillales, Planococcaceae, Sporosarcina*
190n2/676	NR_109752 *Rothia endophytica* NR_025310 *Rothia nasimurium* NR_044873 *Rothia mucilaginosa*	97.3 95.6 95.6	***Actinobacteria,*** *Actinobacteridae, Actinomycetales, Micrococcineae, Micrococcaceae, Rothia*

identifying the analyzed strain as *S. aquimarina* (family *Planococcaceae*). The genus *Sporosarcina* of this taxon includes a significant number of validated species: *S. newyorkensis*, *S. ureae*, *S. contaminans*, *S. soli*, *S. koreensis,* etc.

Two isolates of the *Actinobacteria* phylum were also studied: strain 181n2 belonging to the family *Microbacteriaceae,* and strain 190n2 belonging to *Micrococcaceae* (Table 2). According to the analysis of the fragment of the 16S rRNA gene the nucleotide sequence of bacterial strain 181n2 (Fig. 2c) was shown to have the highest percentage of similarity with *Microbacterium foliorum* P 333/02 (NR025368) that allows attributing the strain to this species.

The Antarctic bacterial strain 190n2 had a very low percentage of homology (97.3%) with the closest cultured relative *Rothia endophytica* (Table 2). Given this, the strain 190n2 can be considered as *Rothia* sp. (Fig. 2c).

Thus, comparative and phylogenetic analysis of the nucleotide sequences of the 16S rRNA gene fragments allowed determining the species affiliation of three isolated strains and one of the genera. The strains are defined with high homology to the most related strains from the GenBank database as: 180n1 – *P. fluorescens*, 188n2 – *S. aquimarina*, 181n2 – *M. foliorum*. These strains belong to the phyla – *Firmicutes*, *Actinobacteria*, *Proteobacteria.*

Discussion. A number of recommendations on the taxonomy of microorganisms were proposed by the International Committee for Systematic Bacteriology (ICSB) [28, 29]: 1. Taxonomy of bacteria should represent one adequate system; 2. The complete DNA sequence will be the standard for phylogeny; 3. Phylogeny must define the taxonomy and, as a consequence, the species are taxonomic units, and must be determined phylogenetically.

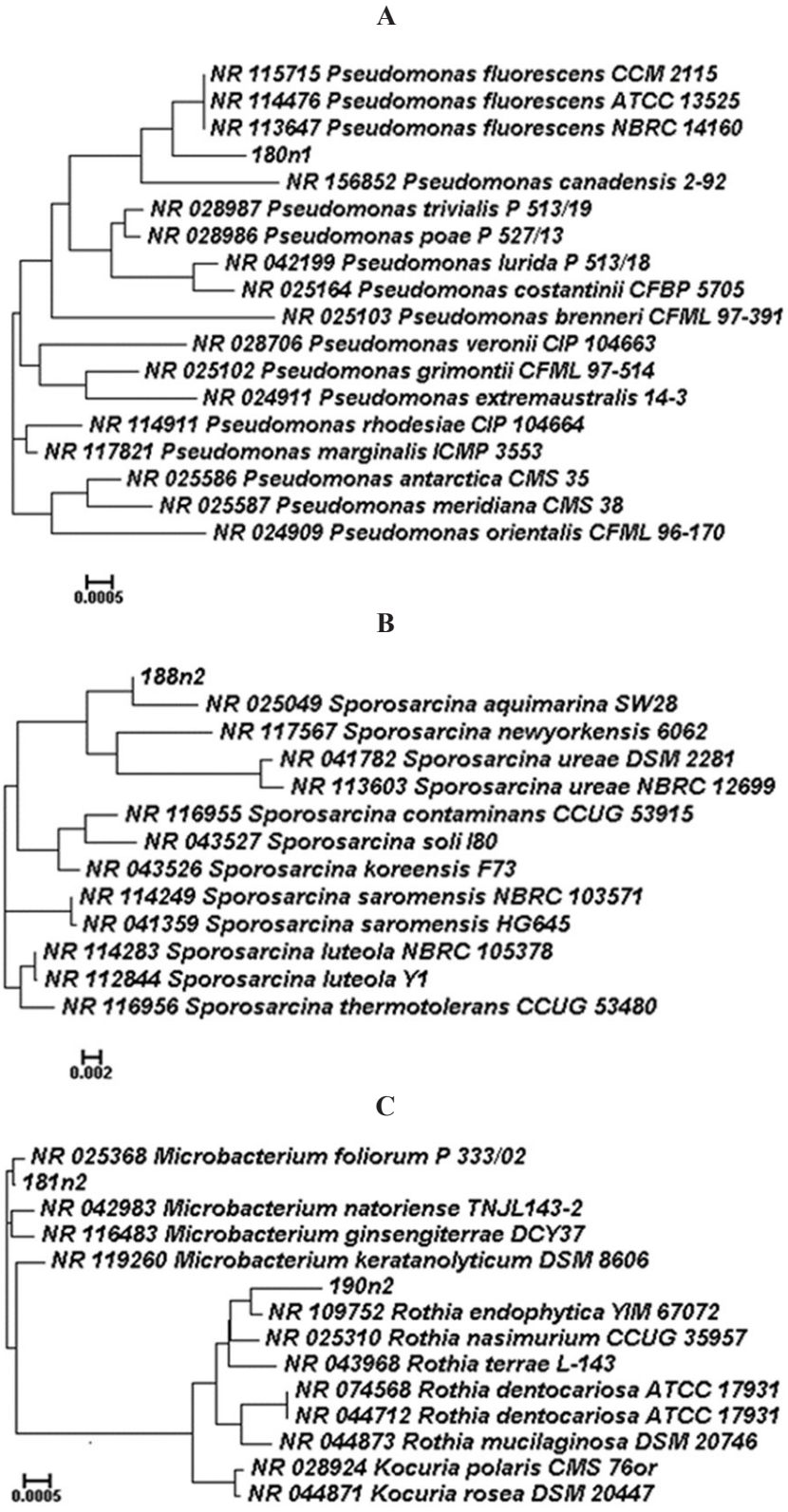
The ICSB [29] proposed to use a polyphasic approach involving genotypic methods and phenotypic analysis to determine the genus of prokaryotes. In case of contradictory results, preference is given to phenotypic analysis with subsequent detection of the consequences of the contradiction.

We tried to compare the results of phylogenetic analysis based on the sequence of 16S rRNA genes with the phenotypic characteristics of isolated strains of Antarctic bacteria in our work.

According to the literature, the main phyla of Antarctic bacteria belong to *Proteobacteria* and *Actinobacteria*, much fewer species of *Firmicutes* and *Bacteroidetes* [18, 19]. It is important to note that the diversity of *Proteobacteria* in the Antarctic phytocenoses is consistent with previous reports of *Proteobacteria* as the richest phylum among the microorganisms in the Antarctic [30, 31]. The members of the class *Gammaproteobacteria* are shown to be the most numerous cultivated group in both mineralized and ornithogenic soils of Antarctica [32]. The phylum *Proteobacteria* is known from the literature to be phenotypically universal. Its representatives can exist in a variety of habitats. They were characterized by great diversity, high number [33], and the capacity to produce hydrolytic enzymes such as amylase, chitinase, DNase, elastase, esterase, etc. The strain 180n1 studied by us belongs to the phylum *Proteobacteria*, family *Pseudomonadaceae*, whose representatives are found in wastewater, surface water, sediments and biofilms, etc. Earlier, according to preliminary data, strain 180n1 was classified as *Pseudomonas antarctica* with homology 99 % [21]. After a detailed analysis several *P. fluorescens* (NR115715, NR114476, NR113647), included in different international collections, with similarity of sequences 99.5 % (Table 2, Fig. 2a), and the strain of *P. canadensis* (NR156852) with the homology of 99.4 % appeared to be the closest homologues of this strain. As a result of phylogenetic trees construction, strain 180n1 was clustered with *P. fluorescens* species and can be assigned to this species.

Comparison of the phenotypic characteristics of strain 180n1 with the strains of closely related species in the literature showed the morphology of colonies of the studied strain to be typical for representatives of *P. fluorescens* species. They also have similar optimal growth parameters [23]. The obtained results agreed with the data from literature on positive test results for catalase, urease, gelatin hydrolysis, arginine dehydrolase, citrate utilization [23]. The studied strain wasn't capable to produce H_2S and reduce nitrate, while the literature data [23] shows variation of the response to these tests.

The phylum *Actinobacteria* is also known to be widely represented in samples of phytocenoses and soils of the Antarctic. *Actinobacteria* usually predominate in the soils of the Antarctic Dry Valleys, as the predominant type in cold arid soils [12, 34]. This is quite natural, since the destroyers of organic compounds being hardly decomposed



F i g. 2. Phylogenetic dendrogram based on the analysis of nucleotide sequences of 16S rRNA genes: a) phylogenetic position of the strain 180n1 among closely related members of the genus *Pseudomonas*. The scale corresponds to 5 substitutions per 10,000 bp; b) phylogenetic position of the strain 188n2 among closely related members of the genus *Sporosarcina* (phylum *Firmicutes*). The scale corresponds to 2 replacements per 1000 bp; c) Phylogenetic position of the strains 181n2 and 190n2 among closely related genera *Microbacterium* and *Rothia* (phylum *Actinobacteria*). The scale corresponds to 5 substitutions per 10,000 bp.

are the dominant groups of *Actinobacteria*. They actively function and are stable in extreme environmental conditions. In addition, as noted [35] an increased amount of *Actinobacteria* is found in the permafrost. In our study, two strains belonging to the phylum *Actinobacteria* and represented by two families *Microbacteriaceae* (strain 181n2) and *Micrococcaceae* (strain 190n2) were isolated from one sample. Considering the high percentage of similarity (99.4%) with *M. foliorum* P 333/02 (NR025368) from the GenBank database phylogenetic analysis showed thatthe strain 181n2 could be attributed to the species *M. foliorum*. In the study of morphological and cultural characteristics the strain 181n2 was shown to have the following characteristics. The cells of the strain are gram-positive rods, non-spore-forming. The colonies are yellow. They are aerobic, chemoorganotrophic. The data correspond to the description of actinobacteria (order *Actinomycetales*), genus *Microbacterium* and species *M. foliorum* [1, 24]. The main physiological and biochemical properties of the strain are shown in Table 1. The results for strain 181n2 were shown to be almost completely agreed with the literature data on the positive results of tests for catalase, hydrolysis of gelatin, esculin and carbohydrate fermentation (glucose, sucrose, maltose) [24] (ribose, arabinose) [36]. However, there is the difference in the negative β-galactosidase test [24]. According to these properties, the strain 181n2 is similar to *M. foliorum*.

Strain 190n2. Cells are gram-positive cocci, immobile, without endospores. It produces acid from sugars (Table 1), reduces nitrate and hydrolyzes esculin. Facultative anaerobes. Chemoorganotrophic. The listed features of the strain 190n2 (Table 1) are similar to those described in the literature [1, 26, 27] and determine the possibility to classify it to the genus *Rothia*. Phylogenetic analysis showed its remote position from closely related strains in the cluster *Rothia* (Fig. 2 c), and a low percentage of similarity (97.3 %) with the species *R. endophytica* (Table 2). In this regard, the strain 190n2 can be considered as *Rothia* sp.

Bacteria of *Firmicutes* require available nutrients. Therefore, they are rare in mineral soils, but they can live in rich ornithogenic soils or phytocenoses of the Antarctic [37]. We also have identified the species of this phylum conducting our experiments in the Antarctic based on the morphological and cultural properties as well as sequence of 16S rRNA genes. Comparative analysis of the sequence of 16S rRNA gene fragment of the studied strain 188n2 showed 99.7 % similarity with *S. aquimarina* strain from the GenBank database. According to the phylogenetic dendrogram (Fig. 2 b) this strain forms a common cluster of *S. aquimarina*, which allows attributing the analyzed strain to this species. Table 1 contains the description of the main characteristics of the strain as well as its attribution to this species. The results also do not differ (except for urease) from the literature data. Therefore, obtained data confirms the classification of this strain as *S. aquimarina* [25].

Thus, according to the complex of morphological-cultural, physiological-biochemical properties and on the basis of the results of phylogenetic analysis aerobic chemoorganotrophic bacteria isolated from black lichens Galindez Island (the Antarctic) are identified as: *Pseudomonas fluorescens*, *Microbacterium foliorum*, *Sporosarcina aquimarina* and *Rothia* sp.

Conclusions. Phylogenetic and phenotypic analyzes allowed determining the taxonomic position of isolated aerobic chemoorganotrophic microbial strains of the Antarctic. Nucleotide sequences of 16S rRNA genes are deposited in the International GenBank database under numbers HG518622, HG518623, HG518625, HG518626.

ПОЛІФАЗНА ТАКСОНОМІЯ АНТАРКТИЧНИХ БАКТЕРІЙ

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Резюме

Однією з проблем сучасної систематики бактерій є те, що філогенетична структура бактерій не завжди узгоджується з традиційною класифікаційною схемою, яка базується на фенотипових властивостях бактерій. Крім того, традиційні методи ідентифікації бактерій з використанням фенотипових властивостей мають ряд недоліків. В останні десятиліття досягнуто значних успіхів у вивченні світу мікробів, використовуючи саме молекулярно-генетичні методи, які дозволяють в короткі терміни провести ідентифікацію. **Метою роботи** було уточнити видовий статус чотирьох штамів бактерій, ізольованих з чорних лишай-

ників кліфів острова Галіндез в Антарктиці, на основі фенотипових та молекулярно-генетичних властивостей. **Методи.** Морфологічні та культуральні властивості бактерій вивчали згідно із загальноприйнятими мікробіологічними методами; фізіологічні та біохімічні – використовуючи тест-системи для ідентифікації бактерій API Coryne та API 20E bioMerieux SA (Франція) відповідно до інструкцій виробника. Філогенетичний аналіз проводили на основі нуклеотидних послідовностей гена 16S рРНК. Для визначення близькоспоріднених видів був проведений порівняльний аналіз нуклеотидних послідовностей генів 16S рРНК, використовуючи програмний пакет BLAST. Філогенетичне положення визначали побудовою дерев (дендрограм), які показують положення досліджуваних штамів серед близькоспоріднених і типових видів (програми ClustalX 2.1, Mega 6.06). Дерево будували за допомогою програми ClustalX 2.1 методом порівняння найближчих сусідів з бутстреп аналізом (bootstrap NJ tree) з використанням 1000 бутстреп випробувань (1000 альтернативних дерев). Далі філогенетичне дерево відкривали програмою Mega v. 6.00 і коректували. **Результати.** Зважаючи на результати порівняльного, філогенетичного та фенотипового аналізів, досліджені антарктичні штами 180n1, 181n2, 188n2, 190n2, ідентифіковані як *Pseudomonas fluorescens, Microbacterium foliorum, Sporosarcina aquimarina* та *Rothia* sp. відповідно. Коефіцієнт подібності генів 16S рРНК штаму 180n1 з таким близькоспорідненим видом з бази даних *P. fluorescens* NBRC 14160 становив 99.5 %; 181n2 з *M. foliorum* P 333/02 – 99.4 %; 188n2 з *S. aquimarina* SW28 – 99.7 %. На філогенетичних дендрограмах ці штами утворють загальні кластери з близькоспорідненими видами. Враховуючи віддалене положення від близькоспоріднених штамів в кластері *Rothia* та невисокий відсоток подібності (97.3 %) з видом *Rothia endophytica* YIM 67072, штам 190n2 можна розглядати як *Rothia* sp. Дані штами належать до філумів: *Firmicutes*, *Actinobacteria*, *Proteobacteria.* **Висновки.** Філогенетичний і фенотиповий аналізи дозволили визначити у аеробних хемоорганотрофних мікроорганізмів Антарктики видову приналежність трьох ізольованих культур та одну родову. Нуклеотидні послідовності генів 16S rRNA депоновано в Міжнародній базі даних GenBank під номерами HG518622, HG518623, HG518625, HG518626.

Ключові слова: Антарктика, ідентифікація, філогенетичний аналіз, порівняльний аналіз, фенотиповий аналіз, 16S рРНК, близькоспоріднені види.

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