Maslovska, O., Komplikevych, S., Danylo, I., Parnikoza, I., & Hnatush, S. (2024). Plant growth-promoting potential of bacterial isolates from the rhizosphere of *Deschampsia antarctica*. *Ukrainian Antarctic Journal*, *22*(1), 63–81.
https://doi.org/10.33275/1727-7485.1.2024.728

Olga Maslovska [1], Solomiia Komplikevych [1], Iryna Danylo [1], Ivan Parnikoza [2, 3], Svitlana Hnatush [1, *]

[1] Ivan Franko National University of Lviv, Lviv, 79005, Ukraine
[2] State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, Kyiv, 01601, Ukraine
[3] Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, 03143, Ukraine

*** Corresponding author:** svitlana.hnatush@lnu.edu.ua

Plant growth-promoting potential of bacterial isolates from the rhizosphere of *Deschampsia antarctica*

Abstract. Plants' adaptations, in combination with the rhizosphere and endosphere microbiome, ensure their survival in the extreme conditions of the Antarctic. The work aimed to quantify the culturable microorganisms of different groups and establish the plant growth-promoting potential of bacterial isolates from the rhizosphere of *Deschampsia antarctica*. Standard microbiological methods (enumeration of microorganisms and study of the properties of isolates, in particular, the cell wall organization by Gram staining, motility, endospore formation, growth at different temperatures, halotolerance, need for oxygen, ability to assimilate some organic carbon sources) and biochemical methods to determine the properties of isolates (oxidase, catalase activities, tests for the ability to reduce nitrate, produce amylases, lipases, proteases). The ability of the isolates to produce siderophores was determined using a medium with chromazurol S and hexadecyltrimethylammonium bromide, to solubilize insoluble phosphate-containing compounds using Pikowska's medium with $Ca_3(PO_4)_2$ and Menkina's medium with egg lecithin. The content of auxin-like substances in the medium was determined by the Salkowski method. The effect of the isolates on wheat growth was determined by the germination of wheat seeds treated with the isolates, the length of shoots and roots of plants, and chlorophyll content in leaves. The isolates were identified using phylogenetic analysis of the 16S rRNA gene. The most abundant groups of microorganisms in the rhizosphere of *D. antarctica* were oligotrophic, oligonitrophilic, and cellulose-degrading microorganisms. Among 120 isolates of the rhizosphere zone of *D. antarctica*, 7 oligonitrophilic isolates (RE1, RE3, RE4, RE8, RP8, RO4, RT1) were selected, which solubilized insoluble phosphate-containing compounds, synthesized lipases, amylases, auxin-like substances, and siderophores. Isolate RT1 (*Bacillus* sp. RT1 by physiological and biochemical properties and the 16S rRNA gene) showed the best plant growth-promoting properties. Treatment of wheat seeds with this isolate increased germination by 25%, shoot and root length by 15%, leaf area 3 times, and chlorophyll content 1.6 times.

Keywords: auxin-like substances, *Bacillus*, microbiota of the rhizosphere, phosphorus solubilization, plant growth-promoting bacteria, siderophores

1 Introduction

Extreme conditions affect the terrestrial biota of Antarctica, which is reflected in its low diversity. In particular, the vascular plants are represented by only two species: Antarctic hairgrass *Deschampsia antarctica* È. Desv. 1854 and Antarctic pearlwort *Colobanthus quitensis* (Kunth) Bartl. 1931

(Yerkhova et al., 2022). The ecological success of these two plants in Antarctica is associated with their adaptation to many factors, in particular, low temperatures (Ozheredova et al., 2015; Znój et al., 2021), freeze-thaw cycles (Znój et al., 2021), drought (Ozheredova et al., 2015), flooding (Znój et al., 2021), UV radiation (Ozheredova et al., 2015; Znój et al., 2021), high concentrations of trace elements, sodium, etc. (Znój et al., 2021). However, in addition to the adaptations of Antarctic plants to environmental factors, the microbiome of the rhizosphere and endosphere plays a significant role in the plants' survival (Ozheredova et al., 2015; Znój et al., 2021; 2022; Bertini et al., 2022).

The microbiota of the rhizosphere of *D. antarctica* has been studied in different regions of the maritime Antarctica (Teixeira et al., 2013; Znój et al., 2021; Prekrasna et al., 2022). However, these studies examined all potential microbiota using the metagenomic method. At the same time, the study of the overall potential of culturable microorganisms is of great promise, as they can be of practical use. Rhizosphere bacteria solubilize mineral compounds by secreting organic acids (Znój et al., 2021), siderophores, or cyanides (Glick & Pasternak, 2003; Hayat et al., 2010), providing the plant with bioavailable phosphorus and metal compounds (Hayat et al., 2010; Znój et al., 2021). The rhizosphere microbiota is also involved in the biocontrol of soil-borne pathogens by competing for nutrients and synthesizing antimicrobial compounds (such as bacteriocin-like peptides), various classes of antibiotics and lytic enzymes (in particular chitinases or β-1,3-glucanases) (Persello-Cartieaux et al., 2003; Hayat et al., 2010; Znój et al., 2021). Bacteria directly stimulate root growth by secreting phytohormones, in particular indole-3-acetic acid (Hayat et al., 2010; Znój et al., 2021). The endosphere microbiota is involved in the induction of the plant's stress response, mainly by reducing the ethylene content in the plant due to its hydrolysis by 1-aminocyclopropane-1-carboxylate deaminase (Znój et al., 2021).

The use of microorganisms for the prevention and treatment of infectious plant diseases and increasing yields is of increasing interest to mankind as an alternative to chemical crop protection. In most experiments, after the treatment of agricultural plants with endophytic microorganisms, growth and resistance to environmental factors improved. Symbionts of Antarctic plants improve the physiological parameters of agricultural plants under abiotic stress (Acuña-Rodríguez et al., 2019). *Arthrobacter* sp., rhizospheric microorganisms of *C. quitensis,* have a positive effect on the physiological properties of this and the other Antarctic vascular plant *D. antarctica* under osmotic stress (Gallardo-Cerda et al., 2018). It has been experimentally established that root endophytes and rhizosphere microorganisms improve the physiological properties of not only Antarctic plants but also other plants. *Penicillium brevicompactum* and *P. chrysogenum* isolated from the endosphere of the root of *C. quitensis* promoted growth and protected lettuce (*Lactuca sativa* L., 1753) and tomato (*Solanum lycopersicum* L., 1753) against osmotic stress (Molina-Montenegro et al., 2020). *Amycolatopsis* sp. Cq 72-27, isolated from the rhizosphere of *C. quitensis*, is able to improve the mineral nutrition of wheat seeds and inhibit the growth of phytopathogenic microorganisms (Roman & Gromyko, 2023). *Pseudomonas* sp. ATCC PTA-122608 from the rhizosphere of *D. antarctica* improved the growth of soybean (*Glycine max* (L.) Merr., 1917) roots (Garcia et al., 2021).

Crop growing cycles in the temperate climate zone usually begin in early spring or at the end of winter. This period is characterized by low temperatures and periodic frosts, which negatively affect the growth of mesophilic soil bacteria or biofertilizers, as their activity is inhibited or slowed down under these conditions, so the application of psychrophilic Antarctic microorganisms with plant growth-promoting properties can have a positive effect on biocenoses in the temperate climate zone (Styczynski et al., 2022).

The work aimed to quantify the culturable microorganisms of different groups and establish the

plant growth-promoting potential of bacterial isolates from the rhizosphere of *D. antarctica*.

2 Materials and methods

2.1 Study location

Samples of *D. antarctica* were collected in the Point Thomas Oasis (King George Island, South Shetland Islands), near W. Puchalski's grave (-62.163491, -58.468756, 90 masl), during the 27th Ukrainian Antarctic Expedition (April 2022). The study plot is a typical for this area patch of Antarctic herb tundra formation with two aboriginal Antarctic vascular plants, *D. antarctica* and *C. quitensis*, accompanied by mosses: *Pohlia nutans* (Hedw.) Lindb. 1879, *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb. 1802, *Polytrichastrum alpinum* (Hedw.) G. L. Sm. 1971, and *Sanionia georgicouncinata* (Müll. Hal.) Ochyra & Hedenäs 1998 and liverworts: *Cephaloziella varians*

Table 1. Composition of media used in the study

Medium, application	Components, g·L^{-1}	Reference
Starch-ammonia agar, for bacteria that metabolize mineral forms of nitrogen	starch – 10.0; $(NH_4)_2SO_4$ – 2.0; K_2HPO_4 – 1.0; $MgSO_4$ – 1.0; $CaCO_3$ – 3.0; agar – 20; distilled water; pH 7.0	Komplikevych et al., 2023
Ashby's mannitol agar, for oligonitrophilic, including nitrogen-fixing bacteria	mannitol – 20.0; K_2HPO_4 – 0.2; NaCl – 0.2; $MgSO_4 \cdot 7H_2O$ – 0.4; K_2SO_4 – 0.1; $CaCO_3$ – 5.0; agar – 20.0; distilled water; pH 7.0. To the sterilized medium, a solution of trace elements according to Fedorov was added – 1 ml	Komplikevych et al., 2023
Vynohradsky medium (for bacteria that provide the first phase of nitrification)	$(NH_4)_2SO_4$ – 2.0; K_2HPO_4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.5; NaCl – 2.0; $FeSO_4 \cdot 7H_2O$ – 0.05; $CaCO_3$ – 5.0; agar – 20.0; distilled water; pH 7.0–7.2. To the sterilized medium, a solution of trace elements according to Pfennig was added (1 ml)	Hudz' et al., 2014
Vynohradsky medium (for bacteria that provide the second phase of nitrification)	$NaNO_2$ – 1.0; K_2HPO_4 – 0.5; $MgSO_4 \cdot 7H_2O$ – 0.5; NaCl – 0.5; $FeSO_4 \cdot 7H_2O$ – 0.4; Na_2CO_3 – 1.0; agar – 20.0; distilled water; pH 7.0–7.2. To the sterilized medium, a solution of trace elements according to Pfennig was added (1 ml)	Hudz' et al., 2014
Menkina's medium, for microorganisms that metabolize phosphate-containing organic compounds	glucose – 10.0; $(NH_4)_2SO_4$ – 0.5; $MgSO_4 \cdot 7H_2O$ – 0.3; KCl – 0.3; $MnSO_4$ – 0.001; $FeSO_4$ – 0.001; $CaCO_3$ – 5.0; egg lecithin (Merck, Millipore) – 3.5; agar – 20.0; distilled water; pH 7.0–7.2	Komplikevych et al., 2023
Pikovska's medium, for microorganisms that metabolize inorganic phosphate-containing compounds	Glucose – 10.0; $Ca_3(PO_4)_2$ – 5.0; $(NH_4)_2SO_4$ – 0.5; NaCl – 0.2; $MgSO_4 \cdot 7H_2O$ – 0.1; KCl – 0.2; yeast extract – 0.5; $MnSO_4 \times \times H_2O$ – 0.002; $FeSO_4 \cdot 7H_2O$ – 0.002; agar – 20.0; distilled water; pH 7.0–7.2	Nautiyal, 1999
Medium with carboxymethyl cellulose, for cellulose-degrading microorganisms	NaCl – 6.0; $(NH_4)_2SO_4$ – 1.0; KH_2PO_4 – 0.5; K_2HPO_4 – 0.5; $MgSO_4$ – 0.1; $CaCl_2 \cdot 2H_2O$ – 0.1; carboxymethyl cellulose – 1.0; agar – 20.0; distilled water; pH 7.0–7.2	Ahmed et al., 2018
Medium with Tween-20, for detection of lipolytic activity	Peptone – 10; NaCl – 5; $CaCl_2 \cdot 6H_2O$ – 0.1; agar – 20.0; distilled water; pH 7.0–7.2. Tween-20 – 10, sterilize separately, and add to the sterilized medium	Hudz' et al., 2014
Solution of trace elements according to Fedorov	H_3BO_3 – 5.0; $(NH_4)_2MoO_4 \cdot 2H_2O$ – 5.0; $ZnSO_4 \cdot 7H_2O$ – 0.2; KI and NaBr – 0.5; $Al_2(SO_4)_3 \cdot 18H_2O$ – 0.3; distilled water	Hudz' et al., 2014
Solution of trace elements according to Pfennig	EDTA, $Na_2MoO_4 \cdot 2H_2O$ – 0.5; $FeSO_4 \cdot 7H_2O$ – 0.2; $ZnSO_4 \times \times 7H_2O$ – 0.01; $MnCl_2 \cdot 4H_2O$ – 0.003; H_3BO_3 – 0.03; $CoCl_2 \times \times 6H_2O$ – 0.02; $CuCl_2 \cdot 2H_2O$ – 0.001; $NiCl_2 \cdot 6H_2O$ – 0.002; distilled water	Hudz' et al., 2014

(Gottsche) Steph. 1901 and *Barbilophozia hatcheri* (A. Evans) Loeske 1907 (Kozeretska et al., 2010).

2.2 Isolation and cultivation of culturable microorganisms

To 1.0 g of soil from the rhizosphere zone, 9.0 ml of 0.9% NaCl solution was added. The resulting suspension was thoroughly mixed and incubated for 20 min, after which it was shaken again, diluted, and 0.1 ml of the obtained suspension was sown onto the culture media. For anaerobes, the cultures on tryptic soy agar (TSA, Merck, Millipore) and Reasoner's 2nd Agar (R2A, Merck, USA) were placed in a GENbox with Anaerocult A (Merck, Millipore) as the reagent for the generation of an anaerobic medium and Anaerotest (Merck, Millipore) to indicate an anaerobic atmosphere. The cultures were grown for 5–10 days at 14 to 16 °C, at which both psychrophilic and mesophilic microorganisms can grow. Isolation of microorganisms was carried out using conventional microbiological methods of cultivation on agar and liquid nutrient media (Hudz' et al., 2014). The media (Merck, Millipore) were used: TSA – to quantify culturable microorganisms that metabolize organic nitrogen-containing compounds, R2A – to quantify culturable oligotrophic microorganisms, Sabouraud 4% dextrose agar – to quantify culturable microscopic fungi. The media listed in Table 1 were used for the evaluation and isolation of culturable microorganisms of other groups.

2.3 Properties of the isolates

The amylase activity of bacteria was assessed by the ability to grow on starch-ammonia agar and the formation of visible zones of starch hydrolysis around the colonies after the application of Lugol's solution to the colonies (Vijayalakshmi et al., 2012; Komplikevych et al., 2023). Lipase activity was assessed by the ability of the isolates to form calcium salt crystals of fatty acids around the colonies after growth in Tween-20 medium (Lo Giudice et al., 2006; Komplikevych et al., 2023). The proteolytic activity of the investigated isolates was evaluated by their ability to liquefy gelatin after growth in a column of tryptic soy gelatin (Loperena et al., 2012; Komplikevych et al., 2023). To detect the ability to synthesize siderophores, a medium with chromazurol S and hexadecyltrimethylammonium bromide was used (Louden et al., 2011). To examine the ability of microorganisms to synthesize auxin-like substances, we used the method with the addition of Salkowski's reagent during 5 day of growth (Gang et al., 2019).

To establish the optimal growth temperature for the isolates, each of them was inoculated onto an optimal medium. The cultures were grown for 6–10 days at temperatures of 4; 8; 16; 20; 28; 35; 40 °C.

To determine cell motility, the test bacteria were inoculated into a column of medium containing 0.2–0.6% agar and grown for 3–7 days at appropriate temperatures. The bacteria *Proteus vulgaris* (motile) and *Staphylococcus albus* (non-motile) were used as test cultures (Hudz' et al., 2014).

Resistance to NaCl for each isolate was determined on the optimal medium containing different concentrations of NaCl (in the range of 1.0 to 30.0%). The bacteria were grown for 5–10 days at the optimal temperature for each isolate.

The need for oxygen was assessed by the growth pattern after injecting the bacteria into a column of thioglycolate medium (Merck, Millipore).

The ability to reduce nitrate ions was determined in a TSB medium with 0.2% KNO_3. The prepared medium was poured into tubes with floats and sterilized by autoclaving at 1 atm for 20 min. The medium was inoculated with the bacterial culture and grown for seven to ten days, then the qualitative reactions to nitrate and nitrite ions were observed. To detect nitrite ions, a drop of Griess reagent was added to a drop of culture on a slide. In the presence of nitrite ions, a red-pink nitrogen compound is formed (Hudz' et al., 2014).

Peculiarities of metabolism of nitrogen-containing compounds were determined after growth in tryptic soy broth (TSB, Merck, USA) with cysteine (0.01%) by litmus (ammonia release) and

lead acetate (hydrogen sulfide, mercaptan release) indicator papers color change (Hudz' et al., 2014).

To determine the catalase activity, a drop of 10% H_2O_2 was applied to a colony of the bacteria. The release of bubbles of O_2 indicates catalase activity in the cells (Hudz' et al., 2014).

To detect oxidase activity, strips containing N,N-dimethyl-p-phenylenediamine oxalate and β-naphthol (Merck, Millipore) were used. The result was considered positive if the strip turned blue. The oxidase-positive bacteria *S. albus* and oxidase-negative *Escherichia coli* were used as test cultures.

The ability of microorganisms to assimilate organic carbon sources was determined by growth in Hiss medium (Farmaktiv, Ukraine) with glucose, lactose, rhamnose, galactose, sorbitol, raffinose, fructose, mannose, or mannitol.

2.4 Microscopic studies

Morphological features of microorganisms (cell shape, size, the ability to form spores, organization of the cell wall after Gram staining) were studied using a Carl Zeiss Axio Lab.A1 binocular microscope, an Olympus IX73 inverted microscope with a DP-74 digital camera, and transmission electron microscopy (Reynolds, 1963).

Gram staining of bacteria was performed using a kit of dyes produced by Merck (Millipore, USA).

Endospores in cells were detected by the Peshkov-Trujillo method (Hudz' et al., 2014). To detect endospores by the cultural method, the bacterial suspension in the medium was heated in a water bath at 80 ± 1 °C for 10 min, cooled, and cultured for 7–10 days at 20 ± 1 °C. *E. coli* and *Bacillus subtilis* were used as test cultures.

2.5 Wheat seed germination

Wheat seeds (*Triticum aestivum* L. 1753) of the cultivar Tybalt were washed twice with sterile distilled water, surface sterilized with a 5.6% sodium hypochlorite solution for 3 min, and then washed three times in sterile water. To determine germination "on paper", 100 wheat seeds per replica pre-soaked for 12 hours in a bacterial suspension (density 5.0 according to McFarland Equivalence Turbidity Standards (Remel™) were spread between two layers of moistened paper. The seeds were germinated on filter paper in containers. Each layer of filter paper was moistened with water. Seeds were placed in rows to facilitate visual assessment of seedlings (DSTU 4138-2002[1]).

To study the influence of Antarctic microorganisms on wheat germination rates in soil, the modified method (Khan et al., 2019) was used. Bacterial isolates were grown on TSA for 3 days. After the cultivation, a suspension of bacterial cells with a density of 5.0 according to McFarland was prepared in 0.9% NaCl solution. 50 wheat seeds per replica were soaked in a bacterial suspension for 12 hours and sown into moist soil (soil weight for each sample was 250 ± 20 g). The control was wheat seeds soaked for 12 hours in a 0.9% NaCl solution without bacteria. The seeds were placed in rows for easier counting. The seeds were planted into the soil and sprayed with water. Every day, 50 ml of tap water was added to each pot of the soil. Wheat was grown for 14 days at 16–18 °C. Seedlings were first counted on the third day of growth. The final count of wheat seed germination was determined on the 8th day of growth (DSTU 4138-2002).

The study of seed germination was conducted in two independent experiments in March 2023. Each experiment was conducted in triplicate.

2.6 Determination of pigment content, leaf area, and root length

Using a metal spatula, after 8 days of wheat growth, 5 plants were carefully removed from the soil, trying to capture a larger volume of soil around the plant to minimize damage to the roots. Plants were washed from soil residues with tap water, root and

[1] Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine (2002). *DSTU 4138–2002 – Seeds of agricultural crops: methods of quality determination*. Derzhspozhyvstandart Ukrainy

shoot lengths were measured with a ruler, and leaf area was determined by the formula $X = (a \cdot b)/c$ (c – mass of the filter paper square in grams; a – mass of the leaf contour on paper in grams; b – area of the paper square in mm^2; X – leaf area in mm^2) (Romaniuk et al., 2005).

The pigment content was determined on the 14th day of growth. Fresh plant material (0.3–0.5 g) was thoroughly ground in a porcelain mortar with a small amount of $CaCO_3$ in a 96% ethanol solution (2–3 ml). After infusing for 2–3 min, the extract was filtered. The extraction of pigments was continued with small portions of pure solvent on the filter until the pigments were completely isolated. The extracts were quantitatively transferred to a 25 ml volumetric flask and the volume of the extract was made up to the mark with 96% ethanol. To quantify the pigments, a portion of the extract was poured into a photocolorimeter cuvette (10 mm). Another cuvette was filled with 96% ethanol solution (control). The optical density of the extract was determined at 665 and 649 nm (absorption maxima of chlorophyll *a* and chlorophyll *b*, respectively) (Romaniuk et al., 2005).

2.7 PCR amplification of 16S rRNA gene and phylogenetic analysis

Genomic DNA was isolated using the soft lysis method (Green & Sambrook, 2012). Purification from proteins was carried out by salting out with potassium acetate. DNA was precipitated with isopropanol and washed with 70% ethanol. DNA was dissolved in sterile deionized water (Milli-Q® Millipore Water, obtained with the Barnstead™ GenPure™ Pro Water Purification System). The 16S rRNA gene was amplified using universal primers 27F and 1492R (Turner et al., 1999). PCR amplification was performed in 50-µL samples using Taq polymerase (NEB M0273X) using a Mastercycler pro thermal cycler (Eppendorf, Germany). The genomic DNA of strains was used as a template for the PCR. The reaction mixture typically contained 1.0 U of Taq Polymerase and 1OX PCR buffer (ThermoFischer Scientific, USA), 0.04 mM of each deoxynucleotide triphosphate, 600 nM of oligonucleotide primers 27F AGAGTTTGATCCTGGCTCAG and 1492R GGTTACCTTGTTACGACTT, ca. 50 ng of genomic template DNA, and purified water. The PCR products were analyzed by electrophoresis of DNA in agarose gel and visualized by staining with ethidium bromide. PCR products of about 1.5 kbp were purified from the gel using silica columns "QiaQuick" ("Qiagen", USA) and analyzed for DNA concentration (260-nm wavelength) and purification quality (ratio of 260/280 nm wavelength) using DeNovix DS-11 microvolume spectrophotometer. The products were sequenced from primers 27F and 1492R using BigDye terminators mix and fragments were analyzed on ABI Prism 3130 xl. The resulting nucleotide sequences (two for each sample corresponding to DNA readings from 27F and 1492R primers) were quality-checked, assembled, trimmed, and compared with the sequences in the GenBank database by BLAST search. Multiple alignment was performed using the program ClustalW (Thompson et al., 1994). For the aligned sequences, the search for the optimal model of nucleotide substitution (Nei & Kumar, 2000) and phylogenetic reconstruction in the program MEGA X (https://www.megasoftware.net/; Kumar et al., 2018) was performed. Phylogenetic reconstruction by the method of maximum likelihood after 1000 bootstrap replications was performed using the Tamura-3-parameter model (Tamura, 1992).

2.8 Statistical processing of data

The results are presented as the average adjusted for standard deviation ($x \pm SD$). The reliability of the data and the differences between them were assessed by the Student's ratio. The difference was considered significant at $p \leq 0.05$ (Petrovska et al., 2022). Statistical processing of research results was performed using "Microsoft Excel 2016" (Microsoft Corporation, USA) and plotted using OriginPro 8.5 (OriginLab Corporation, USA).

3 Results

3.1 Enumeration of culturable microorganisms of different groups of the rhizosphere zone of *Deschampsia antarctica*

To quantify the culturable oligotrophic and copiotrophic microorganisms from the rhizosphere zone of *D. antarctica*, TSA and R2A media were used. TSA is a nutrient-rich medium containing 2.0% of organic nitrogen-containing compounds, so copiotrophic microorganisms grow well on this medium. R2A medium contains a low content of yeast extract, casein hydrolyzate, peptone (total content of these nitrogen-containing compounds is 0.1%), and glucose, which allows the isolation of oligotrophic microorganisms (Merck, Microbiology Manual, 2005). Among the microorganisms of the rhizosphere zone of *D. antarctica*, culturable oligotrophic microorganisms significantly exceeded the number of culturable copiotrophic microorganisms. Anaerobic culturable oligotrophic microorganisms were more numerous than aerobic culturable oligotrophic microorganisms. Among the culturable copiotrophic microorganisms of the rhizosphere zone of *D. antarctica*, aerobic microorganisms were more numerous (Fig. 1, a). Using different media for cultivation, we established that the most abundant groups of microorganisms in the rhizosphere zone of *D. antarctica* were oligotrophic, oligonitrophilic, and cellulose-degrading microorganisms (Fig. 1, b). The enumeration of microorganisms of these groups was in the range of $7–8 \times 10^5$ CFU $\cdot$ g^{-1} of dry soil. The enumeration of microorganisms of other groups was in the range of $3.5–4.5 \times 10^5$ CFU $\cdot$ g^{-1} of dry soil.

3.2 Properties of bacterial isolates from the rhizosphere zone of *Deschampsia antarctica*

After the initial inoculation of samples from the rhizosphere zone of *D. antarctica* onto selective

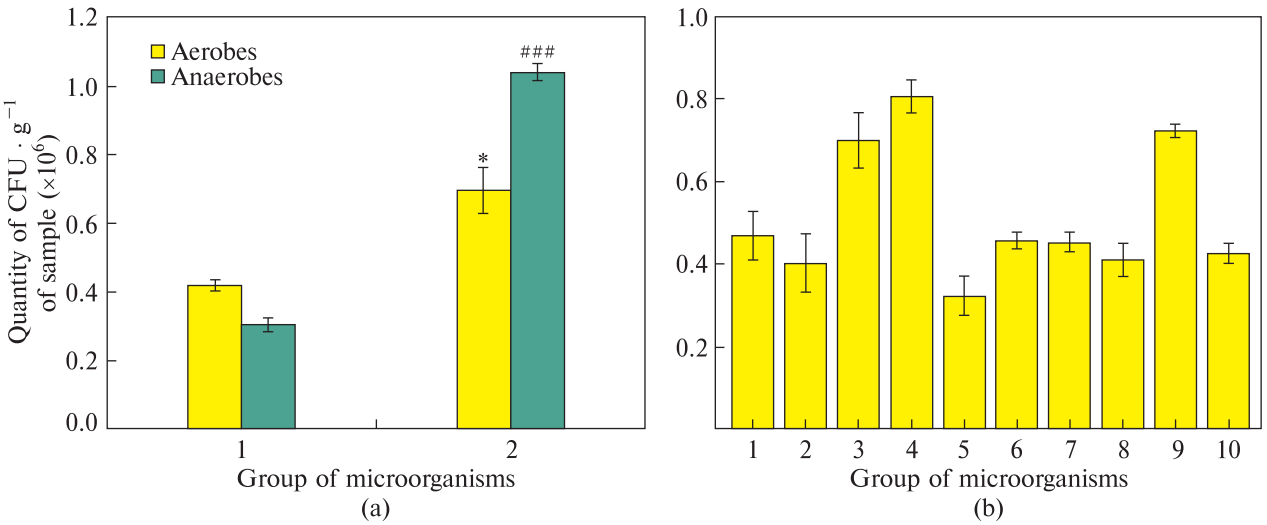

Figure 1. (a) quantification of copiotrophic (1) and oligotrophic (2) culturable microorganisms. (b) quantification of culturable microorganisms of different groups of aerobes in the rhizosphere zone of *Deschampsia antarctica* (1 – microorganisms that metabolize organic nitrogen-containing compounds; 2 – microorganisms that metabolize inorganic nitrogen-containing compounds; 3 – oligotrophic microorganisms; 4 – oligonitrophilic microorganisms; 5 – microorganisms that carry out the first phase of nitrification; 6 – microorganisms that carry out the second phase of nitrification; 7 – microorganisms that metabolize organic phosphate-containing compounds; 8 – microorganisms that metabolize inorganic phosphate-containing compounds; 9 – cellulose-degrading microorganisms; 10 – microscopic fungi) (* – $p < 0.05$; – probable difference between oligotrophic aerobic and copiotrophic aerobic microorganisms; ### – $p<0.001$ – probable difference between oligotrophic anaerobic and copiotrophic anaerobic microorganisms)

media to enumerate microorganisms of different groups, 120 isolates of microorganisms were selected and inoculated onto Ashby's, Vinogradsky's, Pikovska's, Menkina's, and media with Tween-20 to determine their properties. As a result, it was found that 15% of the isolates grew on Ashby's medium, 14% – on Vinogradsky's medium for the first and second phases of nitrification, 13% cleaved Tween-20, and 10% and 6% solubilized inorganic and organic phosphate-containing compounds, respectively.

Among these isolates, 29 were selected, which formed morphologically distinct colonies on solid media (Table 2). All of these isolates grew on

Table 2. Properties of selected bacterial isolates from the rhizosphere zone of *Deschampsia antarctica*

Isolate	Cultural properties						Enzymatic properties			Synthesis of siderophores
	Growth on the medium				The ability to solubilize		amylase	lipase	protease	
	Ashby's	I nitrification phase	II nitrification phase	with carboxymethyl cellulose	inorganic phosphate-containing compounds	organic phosphate-containing compounds				
RC1	+	+	+	+	–	–	+	+	–	–
RC3	+	+	–	–	–	–	+	+	–	–
RC4	+	+	+	+	+	–	+	+	–	–
RC6	+	+	–	–	+	–	+	–	–	–
RC8	+	+	+	+	+	–	+	+	–	–
RI1	+	+	+	+	–	–	+	+	–	–
RI2	+	+	+	+	–	–	+	+	–	–
RI3	+	+	+	+	–	–	+	+	–	–
RI4	+	+	–	+	–	–	+	+	–	–
RII1	+	+	+	+	–	–	+	+	–	–
RII4	+	+	+	+	+	–	+	+	–	–
RP2	+	+	+	+	+	+	+	+	–	–
RP3	+	+	+	–	+	–	–	+	–	–
RP4	+	+	–	+	+	–	+	+	–	–
RP5	+	+	+	+	–	–	+	+	–	–
RP6	+	+	+	+	–	–	+	+	–	–
RP7	+	+	+	+	–	–	+	+	–	–
RP8	+	+	+	+	+	+	+	+	–	+
RO1	+	+	+	+	–	–	+	+	–	–
RO4	+	+	+	+	–	+	+	+	–	+
RT1	+	+	+	–	+	+	–	–	+	+
RE1	+	+	+	–	+	+	+	+	–	+
RE2	+	+	+	+	+	–	+	+	–	–
RE3	+	+	+	–	+	–	–	–	+	+
RE4	+	+	+	+	+	–	+	+	–	+
RE5	+	+	+	+	+	+	+	+	–	–
RE6	+	+	+	–	+	+	+	–	–	–
RE7	+	+	+	+	+	+	+	+	–	–
RE8	+	+	+	+	+	–	+	+	–	+

Notes: "+" – the feature is detected; "–" – the feature is not detected.

Ashby's and Vinogradsky's media. 17 isolates solubilized inorganic phosphate-containing compounds, and 8 isolates solubilized organic phosphate-containing compounds. 26 isolates from the rhizosphere of *D. antarctica* cleaved starch. 25 isolates synthesized lipases to break down Tween-20. Only two isolates, RE3 and RT1, synthesized proteases for gelatin degradation. 22 isolates grew in a medium containing carboxymethyl cellulose as the only carbon source. Isolates RE1, RE3, RE4, RE8, RP8, PO4, RT1 synthesized siderophores.

For further studies, seven isolates of bacteria (RE1, RE3, RE4, RE8, RP8, RO4, RT1) were selected that grew on Ashby's and Vinogradsky's medium, solubilized insoluble phosphate-containing compounds, synthesized lipases, amylases, and siderophores.

Cells of RE1, RE3, RE4, RE8, RP8, RO4, and RT1 were rod-shaped or irregular, single or formed chains. Non-motile, did not form spores, except for isolate RT1. All studied isolates were Gram-negative, except for RT1. RT1 was able to grow at 4–40 °C, and all other isolates grew at 4–25 °C. When lactose, maltose, and glucose were used as carbon sources, isolates RE4 and RT1 produced acid. All isolates, except RO4, were moderately halophilic. Isolates RE1, RE3, RE4, RE8, RP8 grew at 5% NaCl, RT1 – at 7.5% NaCl.

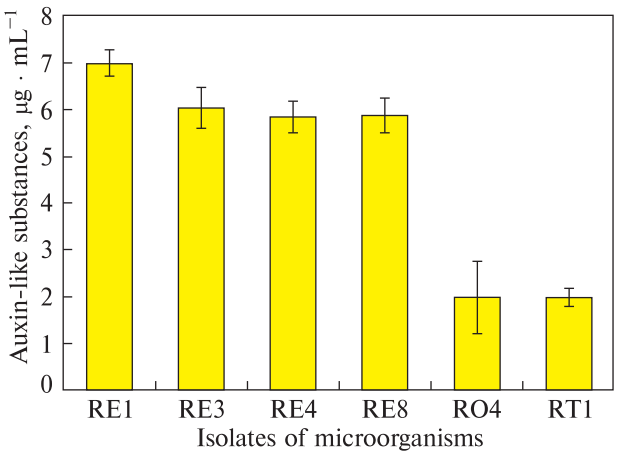

Figure 2. Content of auxin-like substances in the growth medium of isolates from the rhizosphere of *Deschampsia antarctica*

Isolates RE1, RE3, RE4, RE8, RP8, RO4, RT1 are capable of synthesizing auxin-like substances (Fig. 2). Isolate RP8 did not produce auxin-like substances. The highest content of auxin-like substances was found in the growth medium of isolate RE1 (6.99 ± 0.27 μg · mL^{-1}). Isolates RE3, RE4, and RE8 produced auxin-like substances in the range of 5.8–6.0 μg · mL^{-1}. The content of auxin-like substances in the medium during growth of RO4 and RT1 did not exceed 2.0 μg · mL^{-1}.

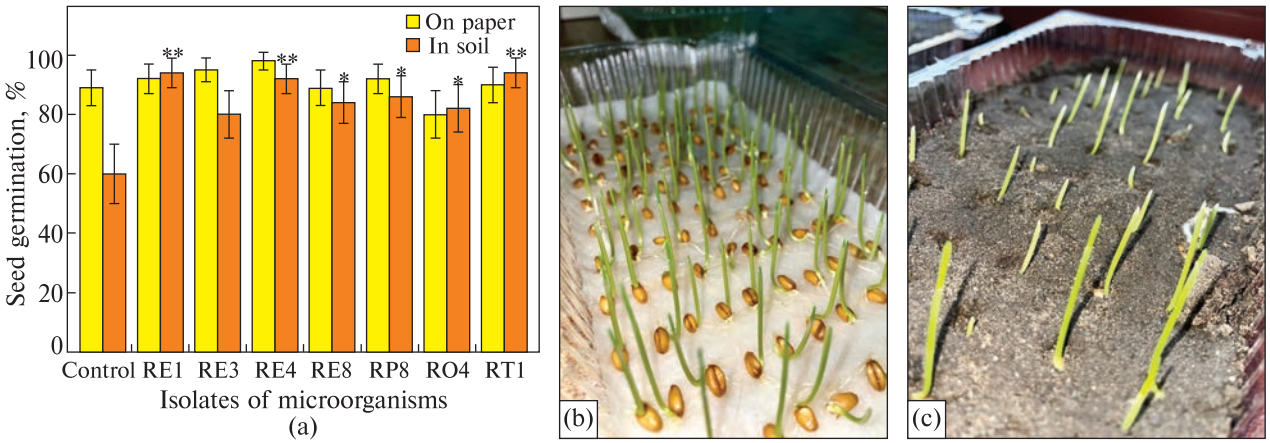

Figure 3. Germination of wheat seeds treated with bacterial isolates from the rhizosphere zone of *Deschampsia antarctica* (a) (* – $p < 0.05$, ** – $p < 0.01$ – probable difference in seed germination compared to the control). Wheat seeds treated with RE1 isolate that germinated on filter paper (b) and in the soil (c)

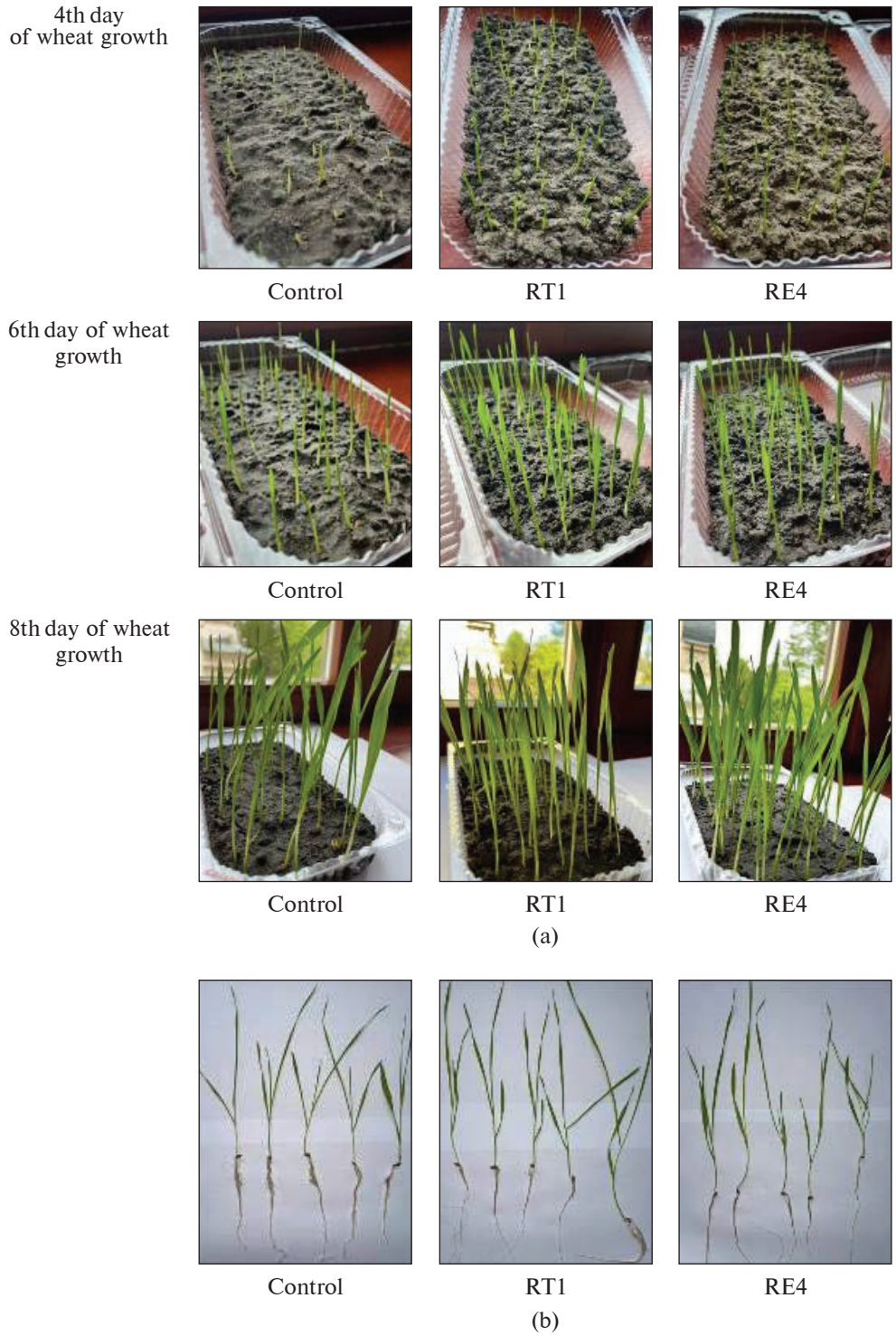

Figure 4. Growth (a) and morphology (b) of wheat from seeds treated with isolates of bacteria from the rhizosphere zone of *Deschampsia antarctica* in soil during growth for eight days

3.3 Growth parameters of wheat under the influence of bacterial isolates from the rhizosphere zone of *Deschampsia antarctica*

The germination of wheat seeds pre-treated with isolates of bacteria from the rhizosphere zone of *D. antarctica* was studied. In the experiment using filter paper (DSTU 4138-2002), the percentage of germination of wheat seeds treated with isolates and with 0.9% NaCl solution did not differ significantly (Fig. 3).

In the soil, the percentage of germinated seeds treated with the studied isolates significantly exceeded the percentage of germinated seeds in the control. Germination of wheat seeds pretreated with 0.9% NaCl solution did not exceed 70%. Germination of wheat seeds treated with isolates RE1, RE4, and RT1 was 95%. About 80% of seeds treated with isolates RE3, RE8, and RO4 germinated (Fig. 3).

The treatment of wheat seeds with the studied isolates of Antarctic microorganisms had a positive effect on both seed germination and wheat growth within 8 days (Fig. 4).

The root length of wheat treated with the RE3 isolate exceeded the root length of wheat treated with 0.9% NaCl solution by 53 %. However, the shoot height of these plants was not significantly different from the control (Fig. 5, a). The roots of wheat treated with isolates RE8, RP8, RT1 were longer by 15–16 %, compared to the control. The shoots of plants treated with RE4 and RT1 isolates were higher compared to the control by 16–17 %.

The leaf area of wheat seedlings treated with isolates of the rhizosphere zone of *D. antarctica* was larger compared to the control (Fig. 5, b). The largest leaf area was recorded for plants treated with isolate RT1 (it was 3 times larger than in control). The leaf area for plants treated with isolates RE1, RE3, RE4, and RP8 was in the range of 400–450 mm^2, and the area of wheat leaves treated with isolates RE8 and RO4 did not exceed 300 mm^2. Isolates RP8 and RT1 caused a statistically significant increase in leaf area compared to the control.

The total chlorophyll content in leaves of plants pre-treated with 0.9% NaCl solution was 1250 ± 25 μg · g^{-1} of raw plant weight. The content of total chlorophyll in the leaves of wheat pretreated with the RE3 isolate was slightly lower. All other isolates of Antarctic microorganisms, except for RP8, caused a statistically significant increase in the total chlorophyll content in wheat leaves (Fig. 6).

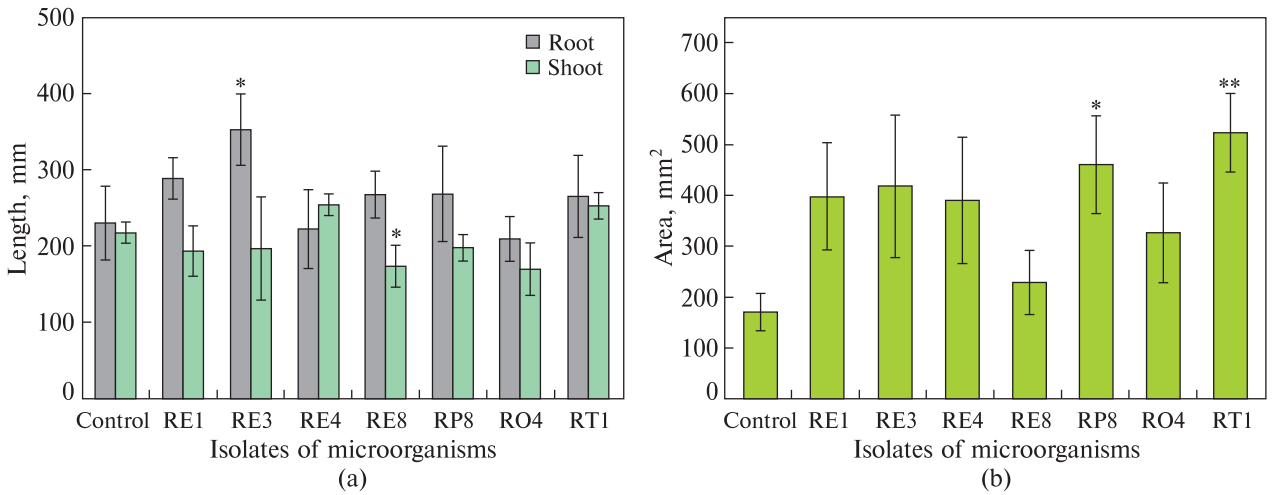

Figure 5. Morphometric parameters (a) and leaf area (b) of seedlings from the seeds pre-treated with bacterial isolates from the rhizosphere zone of *Deschampsia antarctica* (* – $p < 0.05$, ** – $p < 0.01$ – a significant difference compared to the control)

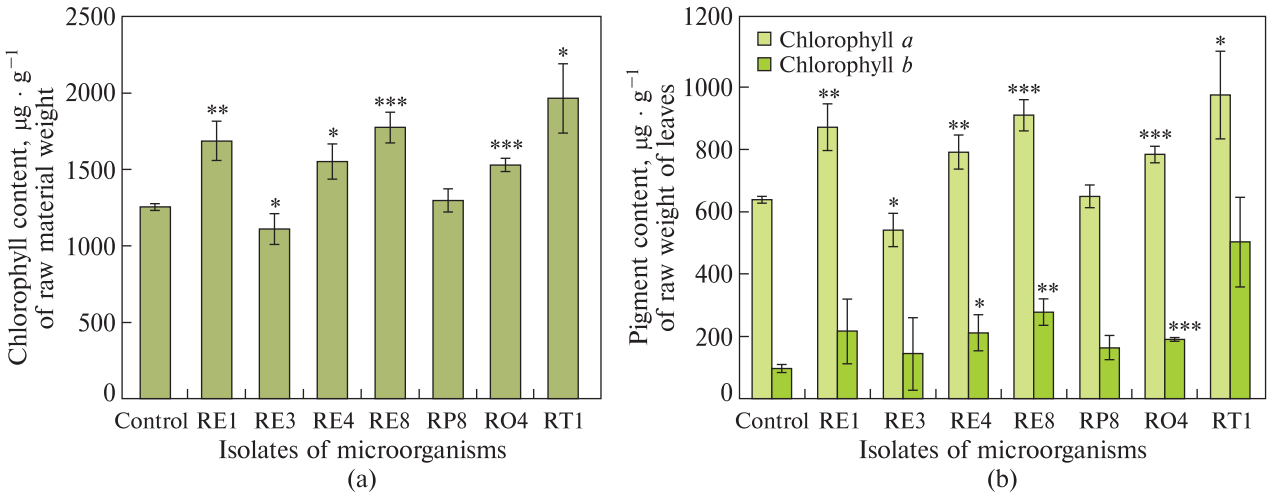

Figure 6. Total chlorophyll content (a) and chlorophyll *a* and *b* content (b) in leaves of wheat pre-treated with bacterial isolates from the rhizosphere zone of *Deschampsia antarctica* (* – $p < 0.05$, ** – $p < 0.01$ – *** – $p < 0.001$ – probable differences in chlorophyll content compared to the control)

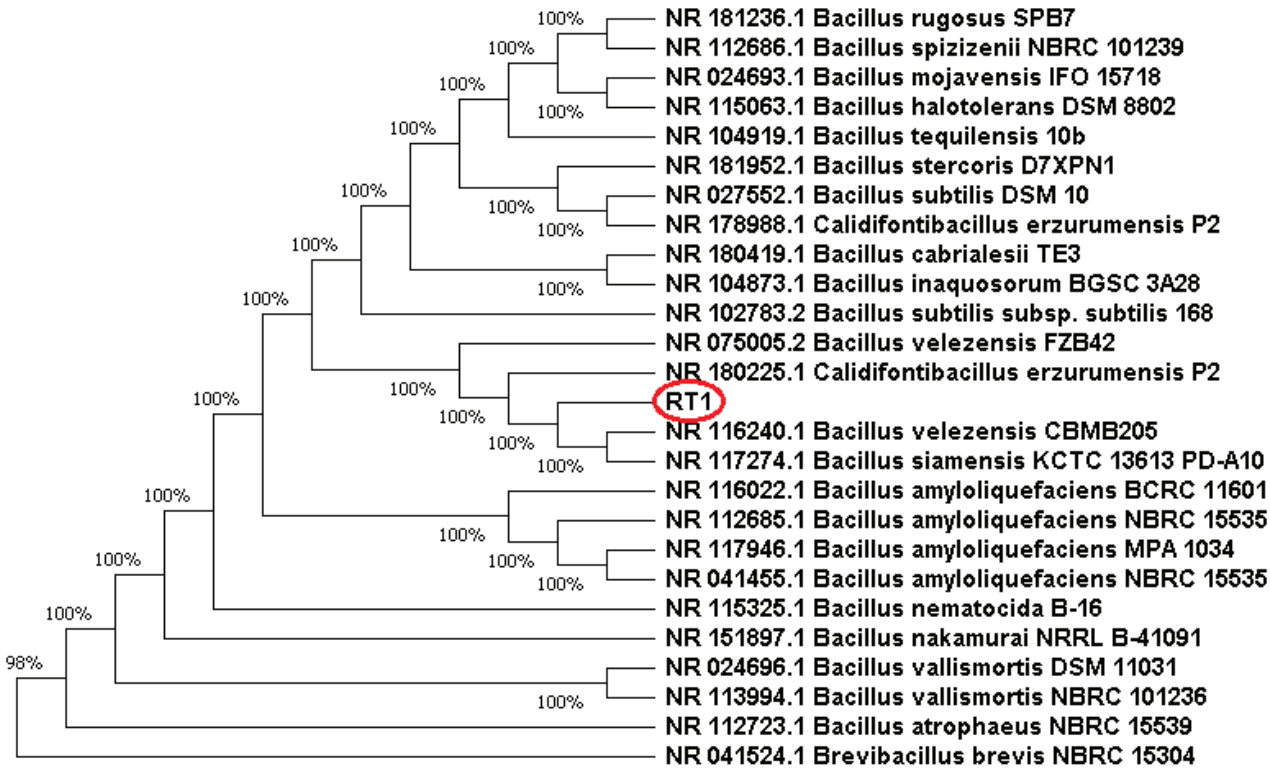

Figure 7. Phylogenetic reconstruction of the 16S rRNA gene of the RT1 and its next relatives with the highest log likelihood (−2812.01) by the maximum likelihood method according to the Tamura 3-parameter model. Near the branches are the values of the percentage of 1000 bootstrap replications. Phylogenetic analysis was conducted using ClustalW and MEGA X

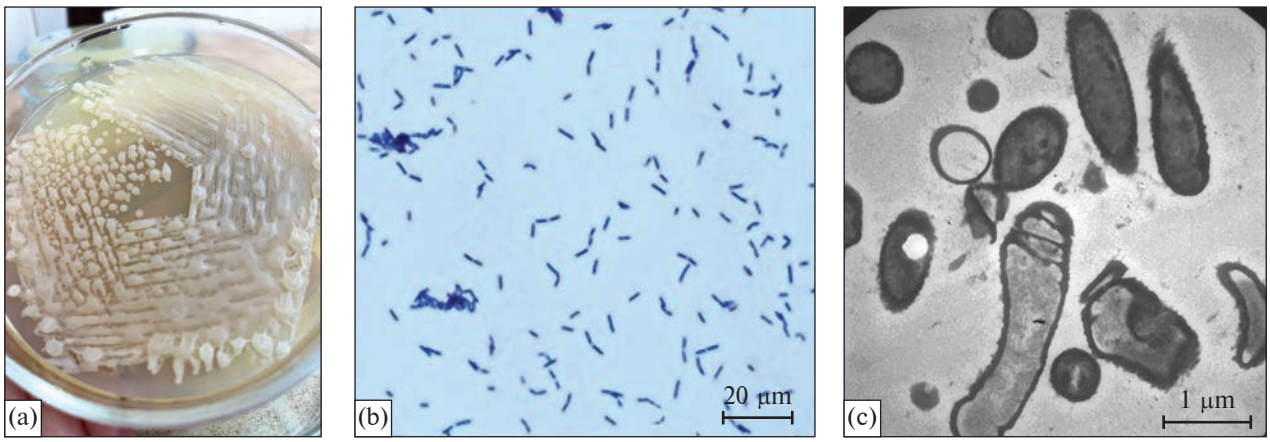

Figure 8. Isolate RT1: growth on tryptic soy agar (a), Gram stained cells (light microscopy (b), electron microscopy (c)

Thus, among the studied isolates of bacteria from the rhizosphere zone of *D. antarctica*, isolate RT1 showed the best plant growth-promoting properties, since the treatment of wheat with this isolate showed an increase in wheat seed germination, an increase in the length of the shoot and root of the plant, the leaf area, and the chlorophyll content.

Table 3. Differential diagnostic features of the RT1 isolate

Properties	RT1	*Bacillus velezensis* CBMB205*	*Calidifontibacillus erzurumensis* P2**
Source of the isolation	Rhizosphere of *D. antarctica*	Rice rhizoplane	Hot spring
Color of the colonies	Milky yellow	Creamy	Beige
Maximum growth temperature, °C	40 ± 1	45 ± 1	60 ± 1
Growth with NaCl, %	7.5	4.0	4.0
Catalase	+	+	−
Oxidase	−	+	+
Hydrolysis:			
gelatin	+	+	+
starch	+	+	−
Use of carbon sources:			
maltose	+	+	+
glucose	+	+	+
rhamnose	+	+	+
inosite	+	+	−
sucrose	+	+	+
manitol	+	+	+
dulcite	+	n.d.	−
lactose	+	n.d.	+
sorbite	+	n.d.	+

Notes. "+" – the feature was detected, "−" – the feature was not detected, "n. d." – not determined; * – data taken from (Madhaiyan et al., 2010; Hwangbo et al., 2016), ** – data taken from (Adiguzel et al., 2020)

3.4 Physiological and biochemical properties and phylogenetic characterization of the isolate RT1

The phylogenetic tree was constructed using 26 sequences where 1403 positions were analyzed. The nucleotide sequence of the 16S rRNA gene of the isolate RT1 is characterized by a high value of identity with the 16S rRNA gene sequence of the strain *Calidifontibacillus erzurumensis* P2 (99.86 %), *Bacillus velezensis* CBMB205 (99.79 %), *Bacillus amyloliquefaciens* MPA 1034 (99.79 %), *Bacillus amyloliquefaciens* NBRC 15535 (99.79 %). According to the phylogenetic analysis, the next relatives for the isolate RT1 are *Calidifontibacillus erzurumensis* P2, *Bacillus siamensis* KCTC 13613 PD-A10, and *Bacillus velezensis* strains, since the sequences of the 16S rRNA gene of these strains belonged to the same clade as the studied one (Fig. 7).

Cells of RT1 are irregularly shaped and single (Fig. 8). They form spores. Motile. Gram-positive. On the TSA, form drop-shaped matte colonies of milk-yellow color with a wavy edge, 1–3 mm in diameter, and a pitted surface. Catalase-positive, oxidase-negative. They grow in the range of 4–40 °C. The optimum temperature for growth is 20 °C. Aerobes. In TSB, the growth pattern is homogeneous, accompanied by the release of hydrogen sulfide. Ammonia is not released during growth in TSB. Reduce nitrates.

Isolate RT1 grows in a wide range of temperatures. The common feature of *Bacillus* sp. RT1 and *Bacillus velezensis* CBMB205 is the ability to hydrolyze gelatin and starch. When lactose, maltose, or glucose are used as carbon sources, RT1 produces acid (Table 3).

Based on the physiological and biochemical properties and the results of phylogenetic analysis of the 16S rRNA gene, isolate RT1 was identified as *Bacillus* sp. RT1.

4 Discussion

The rhizosphere microorganisms of Antarctic plants, like those of plants from other continents, are likely to be important in their vital processes. Representing different groups and possessing certain physiological and biochemical properties, the rhizobiome provides solubilization of mineral compounds, biocontrol of pathogens, stimulation of root growth, formation of plant response to stress, etc. (Znój et al., 2021). In recent years, the microbiome of Antarctic plants has been intensively studied. Using various approaches, including metagenomic analysis, the diversity of the microbiome of the rhizosphere, rhizoplane and endosphere of Antarctic plants (Znój et al., 2022; Yerkhova et al., 2022), ways of acquiring it by the plant (Guajardo-Leiva et al., 2022) and changes due to environmental conditions (Znój et al., 2021) have been determined. These data form an understanding of the relationships between Antarctic plants and the microbiome and their joint survival in severe Antarctic conditions. However, in order to create microbial preparations to improve the growth of agricultural plants, it is necessary to obtain pure cultures of plant growth-promoting microorganisms since a significant part of the rhizobiome and endosphere microbiome is unculturable.

Our study revealed that the most abundant microorganisms in the rhizosphere zone were oligotrophic microorganisms. In the study (Znój et al., 2022), it was found that the composition of the rhizobiome of *D. antarctica*, collected from King George Island, includes bacteria of the phylas *Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria, Sacharibacteria, Parcubacteria, Acidobacteria*, *Armatimonadetes, Gemmatimonadetes*, *Planctomycetes*, *Cyanobacteria*. Most bacteria belonging to the *Acidobacteraceae* and *Verrucomicrobiaceae* are oligotrophic. We assume that other phylogenetic groups of microorganisms also include oligotrophic bacteria since representatives of these orders are found in habitats poor in C and N compounds (Han et al., 2024).

The microbiome of the rhizosphere of Antarctic plants is not random and partly depends on the plant, the conditions that affect it, and the composition of the soil (Guajardo-Leiva et al., 2022). It is known that in order to colonize the rhizosphere or endosphere and compete for food sources, mi-

croorganisms use mechanisms to increase the availability of nutrients, including fixation of N_2, increasing the bioavailability of phosphorus, cellulolytic and ligninolytic activity, the ability to cleave complex compounds and xenobiotics, synthesis of secondary metabolites, etc. (Di Benedetto et al., 2017; Olanrewaju et al., 2017). Oligonitrophilic and cellulolytic microorganisms were also numerous in the rhizosphere of *D. antarctica*. Phosphate-solubilizing bacteria were less numerous; however, among them were those capable of dissolving organic and inorganic phosphate-containing compounds. Microorganisms that metabolize nitrogen of organic or inorganic compounds and nitrifying microorganisms are involved in the transformation of nitrogen compounds in the rhizosphere zone of *D. antarctica*.

During the screening of plant growth promoting microorganisms from the rhizosphere zone of *D. antarctica*, we obtained isolates RE1, RE3, RE4, RE8, RP8, RO4, RT1, capable of producing siderophores. Since pink zones were formed around the strokes of the isolates, we assume that these bacteria secrete organic acids – chelators of iron (Pandey et al., 2017). Iron is essential for plant growth as it is a component of many vital enzymes, is involved in the biosynthesis of chlorophyll, and is important for maintaining the structure and function of chloroplasts. Lack of bioavailable Fe limits plant growth in nature. Siderophores are secondary metabolites with high affinity and specificity for iron, functioning as organic ligands necessary for iron uptake. Plants have developed transporters that allow them to absorb bacterial siderophores. The role of the rhizosphere microbiota that produces siderophores is not fully understood, but there are hypotheses that these microorganisms improve the entry of iron compounds into the plant and, due to the intensive production of siderophores, have an antagonistic effect on phytopathogenic microorganisms, forming a locally deficient environment for available iron compounds. In addition, many bacterial siderophores, such as pyochelin, can trigger induced systemic resistance in plants (Kramer et al., 2020).

The isolates, except for RP8, were capable of synthesizing auxin-like substances. These compounds are formed in plants and affect growth processes and are involved in the interaction between the host plant and rhizosphere microorganisms (Molina-Montenegro et al., 2020). All of them were able to grow on Ashby's medium, solubilized insoluble phosphates, and exhibited some enzymatic activities. The isolates showed plant growth-promoting properties. Germination of wheat seeds treated with 0.9% NaCl solution and bacterial isolates was not significantly different in the experiment using filter paper as specified by DSTU 4138-2002. We assume that under conditions of this experiment, endogenous nutrients present in the seed in approximately equal amounts were used for seed germination. Also, under these conditions, there was no influence of soil microbiota. In the case of wheat seeds that were treated with bacterial isolates, germination of wheat seeds in soil improved, positive changes in plant morphology were observed, and the pigment content increased. It is assumed that during the growth of wheat seeds in the soil, isolates from the rhizosphere of *D. antarctica* improved the availability of nitrogen, phosphorus, iron, etc. The best changes were revealed in the case of treatment of wheat seeds with isolate RT1, which was identified as *Bacillus* sp. RT1 by the combination of physiological properties and phylogenetic analysis of the 16S rRNA gene. The metagenomic analysis of the rhizobiome of *D. antarctica* from King George Island (Znój et al., 2022) did not reveal bacteria of the phylum *Firmicutes*. However, it has been identified by (Teixeira et al., 2010; Kim et al., 2012; Grzesiak et al., 2020) in the microbiome of *D. antarctica* sampled from different locations at the Admiralty Bay shore. Probably, environmental conditions influence the composition of the rhizobium of *D. antarctica*.

5 Conclusions

Among different groups of microorganisms of the rhizosphere of *D. antarctica*, bacteria with plant

growth-promoting properties were isolated. The isolated bacteria can solubilize insoluble phosphate-containing compounds and synthesize lipases, amylases, auxin-like substances, and siderophores. Isolate RT1 (*Bacillus* sp. RT1 by physiological and biochemical properties and phylogenetic analysis of the 16S rRNA gene) showed the best growth-regulating properties. Determining the ability of the studied bacteria to penetrate the endosphere or rhizosphere of plants after seed treatment and identifying compounds that stimulate plant growth is important for further work on the development of preparations based on psychrophilic Antarctic microorganisms.

Author contributions: field material collection: I.P.; conceptualization: O.M., S.K, S.H.; investigation: O.M., S.K, I.D., S.H.; statistics: S.K, I.D.; manuscript writing: O.M., S.K, S.H., I.P. Authors have read and agreed to the final version of the manuscript.

Acknowledgments. The authors are grateful to Oles Kulachkovskyi from the Ivan Franko National University of Lviv for performing electron microscopy and to the Explogen employee for sequencing the 16S rRNA. We also thank for assistance in material collection the personnel of the Arctowski Polish Antarctic Station and Institute of Biochemistry and Biophysics of the Polish Academy of Sciences.

Funding. This study was performed and partially funded under the State Special-Purpose Research Program in Antarctica for 2011–2025.

Conflict of Interest. The authors declare that they have no conflict of interest.

References

Acuña-Rodríguez, I. S., Hansen, H., Gallardo-Cerda, J., Atala, C., & Molina-Montenegro, M. A. (2019). Antarctic extremophiles: biotechnological alternative to crop productivity in saline soils. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00022

Adiguzel, A., Ay, H., Baltaci, M. O., Akbulut, S., Albayrak, S., & Omeroglu, M. A. (2020). Genome-based classification of *Calidifontibacillus erzurumensis* gen. nov., sp. nov., isolated from a hot spring in Turkey, with reclassification of *Bacillus azotoformans* as *Calidifontibacillus azotoformans* comb. nov. and *Bacillus oryziterrae* as *Calidifontibacillus oryziterrae* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *70*(12), 6418–6427. https://doi.org/10.1099/ijsem.0.004549

Ahmed, S., Rahman, M. S., Hasan, M. M., Paul, N., & Sajib, A. A. (2018). Microbial degradation of lignocellulosic biomass: discovery of novel natural lignocellulolytic bacteria. *BioTechnologia*, *99*(2), 137–146. https://doi.org/10.5114/bta.2018.75657

Bertini, L., Proietti, S., Fongaro, B., Holfeld, A., Picotti, P., Falconieri, G. S., Bizzarri, E., Capaldi, G., Polverino de Laureto, P., & Caruso, C. (2022). Environmental signals act as a driving force for metabolic and defense responses in the Antarctic plant *Colobanthus quitensis*. *Plants*, *11*(22), 3176. https://doi.org/10.3390/plants11223176

Di Benedetto, N. A, Corbo, M. R., Campaniello, D., Cataldi, M. P., Bevilacqua, A., Sinigaglia, M., & Flagella, Z. (2017). The role of Plant Growth Promoting Bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. *AIMS Microbiology*, *3*(3), 413–434. https://doi.org/10.3934/microbiol.2017.3.413

Gallardo-Cerda, J., Levihuan, J., Lavín, P., Oses, R., Atala, C., Torres-Díaz, C., Cuba-Díaz, M., Barrera, A., & Molina-Montenegro, M. A. (2018). Antarctic rhizobacteria improve salt tolerance and physiological performance of the Antarctic vascular plants. *Polar Biology*, *41*(10), 1973–1982. https://doi.org/10.1007/s00300-018-2336-z

Gang, S., Sharma, S., Saraf, M., Buck, M., & Schumacher, J. (2019). Analysis of indole-3-acetic acid (IAA) production in *Klebsiella* by LC-MS/MS and the Salkowski method. *BIO-PROTOCOL*, *9*(9), e3230. https://doi.org/10.21769/bioprotoc.3230

Garcia, J., Schmidt, J. E., Gidekel, M., & Gaudin, A. C. M. (2021). Impact of an antarctic rhizobacterium on root traits and productivity of soybean (*Glycine max* L.). *Journal of Plant Nutrition*, *44*(12), 1818–1825. https://doi.org/10.1080/01904167.2021.1884704

Glick, B. R., & Pasternak, J. J. (2003). Plant growth promoting bacteria. *Molecular biotechnology: principles and applications of recombinant DNA* (p. 436–454). Washington DC, ASM Press.

Green, M. R., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor Laboratory Press.

Grzesiak, J., Kaczyńska, A., Gawor, J., Żuchniewicz, K., Aleksandrzak-Piekarczyk, T., Gromadka, R., & Zdanowski, M. K. (2020). A smelly business: Microbiology of Adélie penguin guano (Point Thomas rookery, Antarctica). *Science of The Total Environment*, *714*, 136714. https://doi.org/10.1016/j.scitotenv.2020.136714

Guajardo-Leiva, S., Alarcyn, J., Gutzwiller, F., Gallardo-Cerda, J., Acuña-Rodríguez, I. S., Molina-Montenegro, M., Crandall, K. A., Pérez-Losada, M., & Castro-Nallar, E. (2022). Source and acquisition of rhizosphere microbes in Antarctic vascular plants. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.916210

Han, P., Tang, X., Koch, H., Dong, X., Hou, L., Wang, D., Zhao, Q., Li, Z., Liu, M., Lücker, S., & Shi, G. (2024). Unveiling unique microbial nitrogen cycling and nitrification driver in coastal Antarctica. *Nature Communications*, *15*(1), 3143. https://doi.org/10.1038/s41467-024-47392-4

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, *60*, 579–598. https://doi.org/10.1007/s13213-010-0117-1

Hudz', S. P., Hnatush, S. O., Yavorska, H. V., Bilinska, I. S., & Borsukevych, B. M. (2014). *Guidebook on microbiology*. Publishing centre at Ivan Franko Lviv National University, Lviv. (In Ukrainian)

Hwangbo, K., Um, Y., Kim, K. Y., Madhaiyan, M., Sa, T. M., & Lee, Y. (2016). Complete genome sequence of *Bacillus velezensis* CBMB205, a phosphate-solubilizing bacterium isolated from the rhizoplane of rice in the Republic of Korea. *Genome Announcements*, *4*(4). https://doi.org/10.1128/genomea.00654-16

Kim, O.-S., Chae, N., Lim, H. S., Cho, A., Kim, J. H., Hong, S. G., & Oh, J. (2012). Bacterial diversity in ornithogenic soils compared to mineral soils on King George Island, Antarctica. *Journal of Microbiology*, *50*(6), 1081–1085. https://doi.org/10.1007/s12275-012-2655-7

Khan, N., Bano, A., & Babar, M. A. (2019). Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum* L. *PLOS ONE*, *14*(3), e0213040. https://doi.org/10.1371/journal.pone.0213040

Komplikevych, S., Maslovska, O., Peretyatko, T., Moroz, O., Diakiv, S., Zaritska, Y., Parnikoza, I., & Hnatush, S. (2023). Culturable microorganisms of substrates of terrestrial plant communities of the maritime Antarctic (Galindez Island, Booth Island). *Polar Biology*, *46*(1), 1–19. https://doi.org/10.1007/s00300-022-03103-7

Kozeretska, I. A., Parnikoza, I. Yu., Mustafa, O., Tyschenko, O. V., Korsun, S. G., & Convey, P. (2010). Development of Antarctic herb tundra vegetation near Arctowski station, King George Island. *Polar Science*, *3*(4), 254–261. https://doi.org/10.1016/j.polar.2009.10.001

Kramer, J., Özkaya, Ö., & Kümmerli, R. (2020). Bacterial siderophores in community and host interactions. *Nature Reviews Microbiology*, *18*(3), 152–163. https://doi.org/10.1038/s41579-019-0284-4

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lo Giudice, A., Michaud, L., De Pascale, D., De Domenico, M., Di Prisco, G., Fani, R., & Bruni, V. (2006). Lipolytic activity of Antarctic cold-adapted marine bacteria (Terra Nova Bay, Ross Sea). *Journal of Applied Microbiology*, *101*(5), 1039–1048. https://doi.org/10.1111/j.1365-2672.2006.03006.x

Loperena, L., Soria, V., Varela, H., Lupo, S., Bergalli, A., Guigou, M., Pellegrino, A., Bernardo, A., Calvico, A., Rivas, F., & Batista, S. (2012). Extracellular enzymes produced by microorganisms isolated from maritime Antarctica. *World Journal of Microbiology and Biotechnology*, *28*(5), 2249–2256. https://doi.org/10.1007/s11274-012-1032-3

Louden, B. C., Haarmann, D., & Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. *Journal of Microbiology & Biology Education*, *12*(1), 51–53. https://doi.org/10.1128/jmbe.v12i1.249

Madhaiyan, M., Poonguzhali, S., Kwon, S.-W., & Sa, T.-M. (2010). *Bacillus methylotrophicus* sp. nov., a methanol-utilizing, plant-growth-promoting bacterium isolated from rice rhizosphere soil. *International Journal of Systematic and Evolutionary Microbiology*, *60*(10), 2490–2495. https://doi.org/10.1099/ijs.0.015487-0

Merck. (2005). *Merck Microbiology Manual*, 12th ed. Merck, Darmstadt, Germany.

Molina-Montenegro, M. A., Acuña-Rodríguez, I. S., Torres-Díaz, C., Gundel, P. E., & Dreyer, I. (2020). Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na^+ sequestration. *Scientific Reports*, *10*(1), 5819. https://doi.org/10.1038/s41598-020-62544-4

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, *170*(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press.

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, *33*(11), 197. https://doi.org/10.1007/s11274-017-2364-9

Ozheredova, I. P., Parnikoza, I. Yu., Poronnik, O. O., Kozeretska, I. A., Demidov, S. V., & Kunakh, V. A. (2015). Mechanisms of antarctic vascular plant adaptation to abiotic environmental factors. *Cytology and Genetics*, *49*(2), 139–145. https://doi.org/10.3103/s0095452715020085

Pandey, S. S., Singh, P., Samal, B., Verma, R. K., & Chatterjee, S. (2017). Xanthoferrin siderophore estimation from the cell-free culture supernatants of different *Xantho-*

monas strains by HPLC. *BIO-PROTOCOL*, *7*(14), e2410. https://doi.org/10.21769/bioprotoc.2410

Persello-Cartieaux, F., Nussaume, L., & Robaglia, C. (2003). Tales from the underground: molecular plant–rhizobacteria interactions. *Plant, Cell & Environment*, *26*(2), 189–199. https://doi.org/10.1046/j.1365-3040.2003.00956.x

Petrovska, I. R., Salyga, Y. T., & Vudmaska, I. V. (2022). *Statistical methods in biological research: a textbook*. Agrarian Science. (In Ukrainian)

Prekrasna, I., Pavlovska, M., Miryuta, N., Dzhulai, A., Dykyi, E., Convey, P., Kozeretska, I., Bedernichek, T., & Parnikoza, I. (2022). Antarctic hairgrass rhizosphere microbiomes: microscale effects shape diversity, structure, and function. *Microbes and Environments*, *37*(2), ME21069. https://doi.org/10.1264/jsme2.me21069

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of Cell Biology*, *17*(1), 208–212. https://doi.org/10.1083/jcb.17.1.208

Roman, I., & Gromyko, O. (2023). Plant growth promoting properties of an antarctic strain *Amycolatopsis* sp. Cq 72-27. *Ukrainian Antarctic Journal*, 21 (*1*(26)), 79–89. https://doi.org/10.33275/1727-7485.1.2023.708

Romaniuk, N. D., Tsvilynyuk, O. M., Mykyievych, I. M., & Terek, O. I. (2005). *Guidebook on plant physiology*. Lviv: Ivan Franko National University of Lviv. (In Ukrainian)

Styczynski, M., Biegniewski, G., Decewicz, P., Rewerski, B., Debiec-Andrzejewska, K., & Dziewit, L. (2022). Application of psychrotolerant Antarctic bacteria and their metabolites as efficient plant growth promoting agents. *Frontiers in Bioengineering and Biotechnology*, *10, 772891*. https://doi.org/10.3389/fbioe.2022.772891

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, 9, 678–687.

Teixeira, L. C. R. S., Peixoto, R. S., Cury, J. C., Sul, W. J., Pellizari, V. H., Tiedje, J., & Rosado, A. S. (2010). Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *The ISME Journal*, *4*(8), 989–1001. https://doi.org/10.1038/ismej.2010.35

Teixeira, L. C. R. S., Peixoto, R. S., & Rosado, A. S. (2013). Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay in maritime Antarctica. In de Bruijn, F. J. (Ed.), *Molecular Microbial Ecology of the Rhizosphere* (pp. 1105–1112). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118297674.ch105

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Turner, S., Pryer, K. M., Miao, V. P. W., & Palmer, J. D. (1999). Investigating deep phylogenetic relationships among *Cyanobacteria* and plastids by small subunit rRNA sequence analysis. *The Journal of Eukaryotic Microbiology*, *46*(4), 327–338. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x

Vijayalakshmi, Sushma, K., Abha, S., & Chander, P. (2012). Isolation and characterization of *Bacillus subtilis* KC3 for amylolytic activity. *International Journal of Bioscience*, *Biochemistry and Bioinformatics*, *2*(5), 336–341. https://doi.org/10.7763/ijbbb.2012.v2.128

Yerkhova, A., Parnikoza, I., Pavlovska, M., Yevchun, H., & Prekrasna-Kviatkovska, Y. (2022). Microbiomes of Antarctic pearlwort (*Colobanthus quitensis*) of the maritime Antarctic: distinct diversity and core microbes in rhizosphere and endosphere compartments of the plant. *Ukrainian Antarctic Journal*, 20(*2*(25)), 212–240. https://doi.org/10.33275/1727-7485.2.2022.701

Znój, A., Gawor, J., Gromadka, R., Chwedorzewska, K. J., & Grzesiak, J. (2021). Root-associated bacteria community characteristics of Antarctic plants: *Deschampsia antarctica* and *Colobanthus quitensis* – a comparison. *Microbial Ecology*, *84*(3), 808–820. https://doi.org/10.1007/s00248-021-01891-9

Znój, A., Grzesiak, J., Gawor, J., Gromadka, R., & Chwedorzewska, K. J. (2022). Highly specialized bacterial communities within three distinct rhizocompartments of Antarctic hairgrass (*Deschampsia antarctica* Desv.). *Polar Biology*, *45*(5), 833–844. https://doi.org/10.1007/s00300-022-03027-2

Received: 13 March 2024
Accepted: 13 July 2024

Ольга Масловська[1], Соломія Комплікевич[1],
Ірина Данило[1], Іван Парнікоза[2, 3], Світлана Гнатуш[1, *]

[1] Львівський національний університет імені Івана Франка,
м. Львів, 79005, Україна
[2] Державна установа Національний антарктичний науковий центр МОН України,
м. Київ, 01601, Україна
[3] Інститут молекулярної біології і генетики НАН України,
м. Київ, 03143, Україна

*** Автор для кореспонденції:** svitlana.hnatush@lnu.edu.ua

Фітостимулювальний потенціал ізолятів бактерій ризосфери *Deschampsia antarctica*

Реферат. Адаптації рослин у комплексі з мікробіомом ризосфери та ендосфери забезпечують їхнє виживання в екстремальних умовах Антарктики. Метою роботи було визначити чисельність різних груп культивованих мікроорганізмів та встановити фітостимулювальний потенціал ізолятів бактерій із ризосфери *Deschampsia antarctica*. У роботі використані стандартні мікробіологічні методи (визначення чисельності груп мікроорганізмів і дослідження властивостей виділених ізолятів, зокрема, визначення організації клітинної стінки за Грамом, здатності до руху, утворення ендоспор, росту за різних температур, концентрацій NaCl, потреби у кисні, здатності засвоювати органічні джерела карбону) і біохімічні методи для визначення властивостей ізолятів (оксидазна, каталазна активності, тести на здатність відновлювати нітрати, утворювати амілази, ліпази, протеази). Здатність ізолятів синтезувати сидерофори визначали, використовуючи середовище із хромазуролом S і гексадецилтриметиламоній бромідом, солюбілізувати нерозчинні фосфатовмісні сполуки – середовище Піковської з $Ca_3(PO_4)_2$ і Менкіної з яєчним лецитином. Вміст ауксиноподібних сполук у середовищі визначали методом Сальковського. Вплив ізолятів на параметри росту пшениці визначали за схожістю насіння пшениці, обробленого ізолятами, довжиною пагонів і коренів рослин і вмістом хлорофілу у листках. Ідентифікацію ізолятів проводили з використанням філогенетичного аналізу гена 16S рРНК. Найбільш чисельними групами мікроорганізмів ризосфери *D. antarctica* були оліготрофні, олігонітрофільні і целюлозоруйнівні мікроорганізми. Серед 120 ізолятів зони ризосфери *D. antarctica* відібрано 7 олігонітрофільних ізолятів (RE1, RE3, RE4, RE8, RP8, RO4, RT1), які солюбілізували нерозчинні фосфатовмісні сполуки, синтезували ліпази, амілази, ауксиноподібні сполуки і сидерофори. Ізолят RT1 (*Bacillus* sp. RT1 за фізіолого-біохімічними властивостями та результатами філогенетичного аналізу гена 16S рРНК) виявляв найкращі фітостимулювальні властивості, оскільки у разі оброблення насіння пшениці цим ізолятом виявлено зростання схожості на 25 %, збільшення довжини пагона і кореня рослини на 15 %, площі листків у 3 рази, вмісту хлорофілу в 1,6 рази.

Ключові слова: *Bacillus,* ауксиноподібні сполуки, мікробіота ризосфери, сидерофори, солюбілізація фосфору, фітостимулювальні бактерії