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Morphological, physiological and biochemical properties of heavy metal resistant isolates of bacteria obtained from different Antarctic substrates

Abstract. Aim. To investigate the cultural, morphological, physiological and biochemical properties of the isolated chemoorganotrophic heavy metal resistant isolates of bacteria from different samples obtained during the Ukrainian Antarctic expedition in 2019 for further selection of the most resistant to heavy metal compounds and biochemically active substances. Methods. Pure cultures of bacteria were isolated using tryptone soy agar and nutrient agar. Obtained isolates were sown on agar media containing Cu(II) (0.3, 0.1, 1.5, 8, 78 mM), Pb(II) (0.00009, 0.0005, 0.005, 0.05, 0.5 mM), Cr(VI) (0.00096, 0.0096, 0.96, 9.6 mM). The morphological properties of the bacteria were studied using an Axio Lab.A1 Carl Zeiss binocular microscope, an Olympus IX73 inverted microscope with DP-74 digital camera and transmission electron microscopy. Endospores were detected using the Peshkov-Trujillo method. Catalase, oxidase, amylase, lipolytic, protease activities, ability to fix nitrogen were determined. The ability of microorganisms to metabolize organic carbon sources was determined during growth on Hiss medium with different carbohydrates and alcohols. The determination of the physiological properties of obtained isolates was performed using the Remel RapID™ ANA II system. Results. 92 isolates of psychrophilic microorganisms that grew at temperatures 2 °C, 6 °C and 20 °C were isolated from investigated samples. Among the isolated microorganisms, 64 grew on media containing 0.3–1.6 mM Cu(II), or 0.00009–0.004 mM Pb(II) or 0.01–0.9 mM Cr(VI). 9 isolates of psychrophilic bacteria were resistant to Cu(II) (1.5–78 mM), Pb(II) (0.5 mM), Cr(VI) (0.96–9.6 mM). Morphological, physiological and biochemical properties of 9 multiresistant isolates were described. Conclusions. The physiological and biochemical properties of obtained isolates of Antarctic microorganisms that are resistant to Cu(II) (1.5–78 mM), Pb(II) (0.5 mM), Cr(VI) (0.96–9.6 mM) are determined. Selected isolates of microorganisms are able to use monosaccharides, disaccharides, alcohols as carbon sources; possess urease, protease, lipase, aminopeptidase activities. Selected heavy metal resistant isolates can be used for further investigation and development of technologies for bioremediation of environment.

Keywords: psychrophilic microorganisms, metal resistance, copper, lead, chromium

1 Introduction

Antarctic ecosystems are unique in their physical and geographical location and climatic conditions, so it can be assumed that they contain microorganisms with specific and sometimes unique properties. The Antarctic microbiota is affected by a complex of extreme factors: UV radiation, low concentration of organic matter, high salinity, sharp temperature changes which cause frequent changes of freezing-thawing modes,

etc. Considering the extreme conditions of existence, it can be assumed that effective mechanisms of adaptation to adverse environmental conditions have emerged in the cells of Antarctic microorganisms (Sioma et al., 2018). The main representative groups of Antarctic microorganisms are the phyla *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* (Silva et al., 2018). Survival of Antarctic microorganisms is provided by synthesis of secondary metabolites, cold shock proteins, antifreeze-nucleating proteins, exopolysaccharides, systems of counteraction to osmotic and oxidative stress, biofilm formation, spore formation, motility, etc. (Núñez-Montero, Barrientos, 2018; Silva et al., 2018). These microbial survival strategies are known to be regulated by a quorum-sensing mechanism (Núñez-Montero, Barrientos, 2018; Silva et al., 2018). Resistant to environmental factors microorganisms are characterized by significant biotechnological potential. The genome analysis of rhizosphere microorganisms revealed the presence of genes involved in the metabolism of amino acids, carbohydrates and xenobiotics (Da Silva et al., 2017). Enzymes synthesized by psychrophilic microorganisms may have significant applications in industrial processes, molecular biology, medicine, since they are active at low temperatures. Adapted to influence of different environmental factors psychrophilic microorganisms can be used for the synthesis of nanoparticles, including silver, tellurium-containing nanostructures and semiconductor fluorescent nanoparticles, biofuels production, biocontrol of phytopathogens, bioremediation, etc. (Yarzábal, 2016; Borzova et al., 2019). There was isolated bacterium *Pseudomonas extremaustralis*, able to use some types of diesel fuel as the sole carbon source (Tribelli et al., 2018). A number of producers of antimicrobial compounds (Li et al., 2013) and antiproliferative molecules (Mojib et al., 2011), which can be used as anticancer drugs (Silva et al., 2018) have been identified among Antarctic microorganisms. An important factor affecting the metabolism of Antarctic bacteria are heavy metal compounds, such as cadmium, zinc, vanadium, arsenic, etc., whether leached from volcanic rocks over millennia or of anthropogenic origin in the last decades (Núñez-Montero, Barrientos, 2018; Silva et al., 2018; Romaniuk et al., 2018).

There are publications about the pollution of the Antarctic by organic and inorganic compounds. Volatile halogenated aromatic compounds and pesticides, including dichlorodiphenyltrichloroethane and polychlorinated biphenyls, have been identified among organic pollutants. There were detected increased concentrations of heavy metals, including copper, lead, mercury, chromium, cadmium and arsenic (Romaniuk et al., 2018; Núñez-Montero, Barrientos, 2018; Silva et al., 2018; Chu et al., 2019). Cu(II) and Cr(VI) ions are strong oxidizing agents that inhibit the growth of microorganisms at 10 ppm (Tashyrev, 2009). Pb(II) is also toxic to microorganisms' cells (Jarosławiecka, Piotrowska-Seget, 2014). Besides, the ions of copper, chromium and lead are contained in high concentrations in industrial wastewaters (Jarosławiecka, Piotrowska-Seget, 2014; Elabbas et al., 2016; Un et al., 2017; Wahaab, Alseroury, 2019; Da Silva et al., 2020). We assume that Antarctic chemoorganotrophic microorganisms that are resistant to heavy metals can be used in the field of bioremediation of wastewater with a high content of organic substances and heavy metal compounds.

The aim of the study was to investigate the cultural, morphological, physiological and biochemical properties of the isolated chemoorganotrophic heavy metal resistant isolates of microorganisms from different samples obtained during the Ukrainian Antarctic expedition in 2019 for further selection of the most resistant to heavy metal compounds and biochemically active. Such studies are important to evaluate the feasibility of usage of isolates in industry and environmental technologies, in particular for soil bioremediation, treatment of domestic or industrial effluents contaminated with organic compounds and toxic heavy metals. Unlocking the powerful biotechnological potential of isolates of Antarctic microorganisms adapted to stress factors, capable of transforming a wide range of substances, is valuable for solving the problem of contamination applying effective, profitable and environmentally friendly biological methods.

2 Materials and methods

The materials of research were 21 samples of soil and mossy soil, obtained during the Ukrainian Antarctic expedition in February–April 2019 by Dr. Pavlo Khoetski (Table 1). 92 isolates were isolated from these samples.

To obtain cultures of microorganisms, 1 g of the sample was added to 10 mL of 0.9% NaCl. The resulting suspension was thoroughly shaken and let to settle for 20 min then shaken again and 0.1 mL of it was sown on tryptone-soy agar (TSA, Merck, USA) and nutrient agar (NA, Merck, USA), pH 7.4 ± 0.2 (Hudz' et al., 2014). The bacteria were grown at 2 °C, 6 °C and 20 °C during 7 days. The obtained isolates were sown on TSA and NA media that contained Cu(II) (0.3, 0.1, 1.5, 8, 78 mM), Pb(II) (0.00009, 0.0005, 0.005, 0.05, 0.5 mM), Cr(VI) (0.00096, 0.0096, 0.96, 9.6 mM). Metal ions were added as salts: $CuCl_2 \cdot 2H_2O$, $Pb(NO_3)_2$, $K_2Cr_2O_7$. Pure cultures of isolates, resistant to the Cu(II), Pb(II) and Cr(VI) were obtained by multiple sowings using streak plate method on TSA and NA media.

The morphological features of the microorganisms (cell shape, size, ability to form spores, composition and structural 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. For electron microscopic studies the cells of the bacteria, twice washed with distilled water, were precipitated by centrifugation for 15 min (4000 g), fixed in 1.5% OsO_4 solution in cacodylate buffer (pH 7.2) for 90 min at 0 °C. The fixed cells were washed, dehydrated in solutions with increasing concentrations of ethanol (50%, 70%, 90%, 99.9%) and propylene oxide. The samples were transferred to Epon 812 epoxy resin. Cell sections were obtained on a UMTP-6 ultramicrotome and contrasted with plumbum citrate according to (Reynolds, 1963). Samples were viewed and photographed using a PEM-100 transmission electron microscope at 75 kV accelerating voltage and 10000× magnification.

Endospores were detected using the Peshkov-Trujillo method (Hudz' et al., 2014). For additional detection of endospores, a suspension of cells with medium was heated in a water bath at 80 °C for 10 min and cultivated for 7–10 days (Hudz' et al., 2014).

Gram staining was performed using a dye kit (Merck, USA).

To determine motility of the cells, the investigated bacteria were injected into a column of meat-peptone agar (MPA), containing 0.2—0.5% of agar, grown for 3—7 days at 2 °C, 6 °C and 20 °C. *Proteus vulgaris* (motile) and *Staphylococcus albus* (non-motile) bacteria were used as test cultures.

The need of microorganisms for oxygen was evaluated by the nature of growth after the injection of bacteria into the MPA column (Hudz' et al., 2014).

To determine the catalase activity, a drop of 10% H_2O_2 was applied to the colony of investigated bacteria (Arenas et al., 2014; Hudz' et al., 2014). Strips with N,N-dimethyl-*p*-phenylenediamine oxalate and α-naphthol (Millipore, USA) were used to detect oxidase activity. *Staphylococcus albus* (oxidase-positive microorganisms) and *Escherichia coli* (oxidase-negative microorganisms) were used as test cultures.

0.01% cysteine was added to the meat-peptone broth (MPB) before microorganisms sowing to detect their ability to use organic nitrogen-containing compounds. Inside the test tubes were fixed moistened with distilled water litmus paper and paper, saturated with lead acetate. As test cultures we used *Proteus vulgaris* for positive control, *Escherichia coli* for negative one (Hudz' et al., 2014).

The ability of bacteria to reduce nitrate ions was detected after their cultivation in the MPB with 0.2% KNO_3 and Bubble Durham tubes. To detect nitrite ions, a drop of Griss's reagent (a mixture of sulfanilic acid and 2-naphthylamine in acidic medium) was added to the culture drop on a slide. In the presence of nitrite ions, an azo compound of red-pink colour is formed. The reduction of nitrites to N_2 was determined by gas accumulation in the Bubble Durham tube (Hudz' et al., 2014).

To detect the lipolytic activity of microorganisms we used medium of the following composition, g/L: peptone — 10; NaCl — 5; $CaCl_2 \cdot 6H_2O$ — 0,1; agar — 20; distilled water — 1 L, pH 7.4. After sterilization, aqueous solutions of tween-20 (polyethylene glycol sorbitan monolaurate) and tween-80 (polyethylene

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Table 1. Samples used for isolation of bacteria

Sample	Substrate	Sampling location	Coordinates	Isolates
№ 1 Ant 2019	mossy soil	Booth Island	65°04.043′ S, 064°01.427′ W	1 Ant_1T_2, 1 Ant_1T, 1 Ant_2T_20
№ 2 A 2019	mossy soil	Cape Tuxen, Antarctic Peninsula	65°16.184′ S, 064°07.141′ W	2A_1N, 2A_1T_20
4 A 2019	soil, *Deschampsia antarctica* É. Desv., 1854, moss	Darboux Island	65°23.711′ S, 064°12.912′ W	4A_1N, 4A_1N, 4A_1T_20
5 A 2019	soil, *D. antarctica*, moss	Berthelot Islands	65°19.705′ S, 064°08.060′ W	5A_1T_20, 5A_2T_20, 5A_1N_20
6 A 2019	soil, *D. antarctica*, moss	Berthelot Islands	65°19.701′ S, 064°08.603′ W	6_1T, 6A_1T_20, 6A_2T_20
8 A 2019	soil, *D. antarctica*, moss	Yalour Islands	65°14.020′ S, 064°09.666′ W	8_1T, 8_2N, 8A_1T_20, 8A_2T_20, 8A_1N_20, 8A_2N_20
9.9 A 2019	soil, moss, mushrooms	Rasmussen Point, Antarctic Peninsula	65°14.848′ S, 064°05.080′ W	9.9A_1T_2, 9.9_1N, 9.9A_1T_20, 9.9A_1N_20, 9.9A_2N_20
10 A 2019	mossy soil	Roca Islands	65°10.730′ S, 064°29.534′ W	10A_1T_20, 10A_2T_20, 10A_3T_20, 10A_4T_20, 10A_1N_20, 10A_2N_20, 10A_3N_20
11 A 2019	mossy soil	Skua Island	65°15.287′ S, 064°16.417′ W	11_1T, 11_2N, 11A_1T_20, 11A_1N_20
12 A 2019	mossy soil	Weller Island	65°26.947′ S 065°24.103′ W	12A_1T_2, 12A_1N_2, 12A_1T_20, 12A_2T_20
14 A 2019	mossy soil	Weller Island	65°26.947′ S, 065°24.103′ W	14A_1T_2, 14A_1N_2, 14_1T, 14_2N, 14A_1T_20, 14A_2T_20, 14A_3T_20, 14A_4T_20, 14A_1N_20, 14A_2N_20
64 A 2019	benthos	Roca Islands	65°10.443′ S, 064°29.313′ W	64A_1T_20, 64A_1T, 64A_1T_2
79 A 2019	soil, *D. antarctica*, moss	Petermann Island	65°10.342′ S, 064°08.317′ W	79A_1N_20, 79A_1T, 79A_1N_2
88 A 2019	*D. antarctica*	Galindez Island	65°14.704′ S, 064°15.160′ W	88A_1T_20, 88A_1T, 88A_1T_2
89 A 2019	*D. antarctica*	Galindez Island	65°14.783′ S, 064°14.788′ W	89A_1T_2, 89A_1N_2, 89_1T, 89_2N, 89A_1T_20, 89A_2T_20, 89A_1N_20, 89A_2N_20
95 A 2019	mossy soil	Irizar Island	65°13.183′ S, 064°11.695′ W	95A_1T_20, 95A_1T, 95A_1T_2
96 A 2019	moss	Forge Islands	65°13.790′ S, 064°6.700′ W	96A_1T_2, 96A_1N_2, 96_1T, 96_2N, 96A_1T_20, 96A_1N_20, 96A_2N_20, 96A_3N_20
№ 3	lichen	Cape Tuxen, Antarctic Peninsula	65°16.441′ S, 064°06.816′ W	3_1N_2, 3_1T_20, 3_2T_20
67 A 2019	benthos	Reservoir on Galindez Island	65°14.910′ S, 064°14.707′ W	67A_1N_2, 67A_1T_20, 67A_2T_20, 67A_1N_20, 67A_2N_20
68 A 2019	benthos	Reservoir on Galindez Island	65°14.945′ S, 064°14.785′ W	68A_1N_2, 68A_1T_20, 68A_2T
75 A 2019	mossy soil	Galindez Island	65°14.798′ S, 064°14.930′ W	75A_1T_2, 75A_1N_20, 75A_2T

glycol sorbitan monooleate) were added to the medium at a concentration of 10 g/L. In the presence of lipases, around the strokes of the studied microorganisms on this medium there formed an opaque zone of calcium salts of fatty acids released from the tween (Lo Giudice et al., 2006). The proteolytic activity of the investigated isolates was evaluated by their ability to grow in milk and to liquefy gelatine (Loperena et al., 2012; Hudz' et al., 2014).

To detect the ability of bacteria to degrade casein, milk was degreased (centrifuged at 4000 g for 20 min and the fat film removed from the surface). The skimmed milk was diluted with water at a ratio of 1 : 4, to it was added litmus indicator (10 mL of 4% solution for 1 L of milk), poured into tubes of 8–10 mL and sterilized at 0.5 atm for 20 min. The experiment was analysed seven days after seeding. We recorded changes in pH, color of the medium, a clot of casein formation (coagulation) (Loperena et al., 2012; Hudz' et al., 2014). To detect the ability of microorganisms to break down starch and therefore to form amylase, the method of sowing by a stroke on agar medium supplemented with 1–2% starch was used. To determine the degree of starch hydrolysis by amylolytic enzymes of the culture of microorganisms, its colonies were flooded with a solution of Lugol (Sushma, et al., 2012; Hudz' et al., 2014).

Ashby's agar medium was used to determine the ability of bacteria to fix nitrogen (Hudz' et al., 2014). To detect hydrolysis of esculin, the bacteria were grown on bile-esculin agar with sodium azide (Bile Aesculin Azid Agar, Merck, USA). The ability of microorganisms to assimilate organic carbon sources was determined by growth on Hiss medium with arabinose, glucose, dulcite, inositol, xylose, lactose, maltose, mannitol, mannose, rhamnose, sucrose, sorbitol and by changes in the color of the medium.

The study of the physiological features of the isolates was performed using the Remel RapID™ ANA II system (http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=R8311002).

The identity of isolates to pathogenic microorganisms was verified by seeding on Endo agar, Kligler's agar, bile-esculin agar, Tergitol-7 agar and selective medium for *Bacillus cereus* (Merk, USA).

All research results are presented as average with mean error (M ± m). The significance of the obtained results was calculated using the Student's *t*-test in Microsoft Excel 2003 (Humets'kyy et al., 2004).

Table 2. Resistance of isolates, obtained from different Antarctic substrates, to heavy metal ions

Isolate	Sample substrate	The optimum growth temperature, °C	Resistance to heavy metal ions, mM*		
			Cu(II)	Pb(II)	Cr(VI)
12A_1N_2	mossy soil, Weller Island	2 ± 1	7.8	0.5	9.6
9.9A_1T_2	soil, moss, mushrooms, Rasmussen Point, Antarctic Peninsula	2 ± 1	7.8	0.5	9.6
8_1T	soil, *D. antarctica*, moss, Yalour Islands	8 ± 1	78.0	0.5	9.6
14_2N	mossy soil, Weller Island	8 ± 1	78.0	0.5	9.6
11_1T	mossy soil, Skua Island	8 ± 1	78.0	0.5	9.6
9.9A_1T_20	soil, moss, mushrooms, Rasmussen Point, Antarctic Peninsula	20 ± 1	1.5	0.5	9.6
14A_4T_20	mossy soil, Weller Island	20 ± 1	1.5	0.5	0.96
5A_1N_20	soil, *D. antarctica*, moss, Berthelot Islands	20 ± 1	7.8	0.5	9.6
10A_3T_20	mossy soil, Roca Islands	20 ± 1	78.0	0.5	9.6

Note: * – The concentrations of used metals exceed the MPC for industrial water by 2–100 times (Hygienic requirements for drinking water intended for human consumption)

3 Results and discussion

As a result of seeding aqueous suspensions from the Antarctic samples, 92 isolates, which grew at temperatures of 2 °C (18 isolates), 6 °C (22 isolates), and 20 °C (52 isolates), were obtained on TSA and NA (Table 1). The resulting isolates differed in shape, size, colony morphology and optimal growth temperature.

Among the isolated microorganisms, 64 isolates were characterized by intensive growth on TSA and NA medium containing 0.3–1.6 mM Cu(II), or 0.00009–0.004 mM Pb(II) or 0.01–0.9 mM Cr(VI). Among these isolates, 10 grew at 2 °C, 13 grew at 6 °C, 41 isolates grew at 20 °C. Thus, among the obtained isolates, mesophilic bacteria were characterized by higher resistance to copper, lead, and chromium ions. Selected bacteria

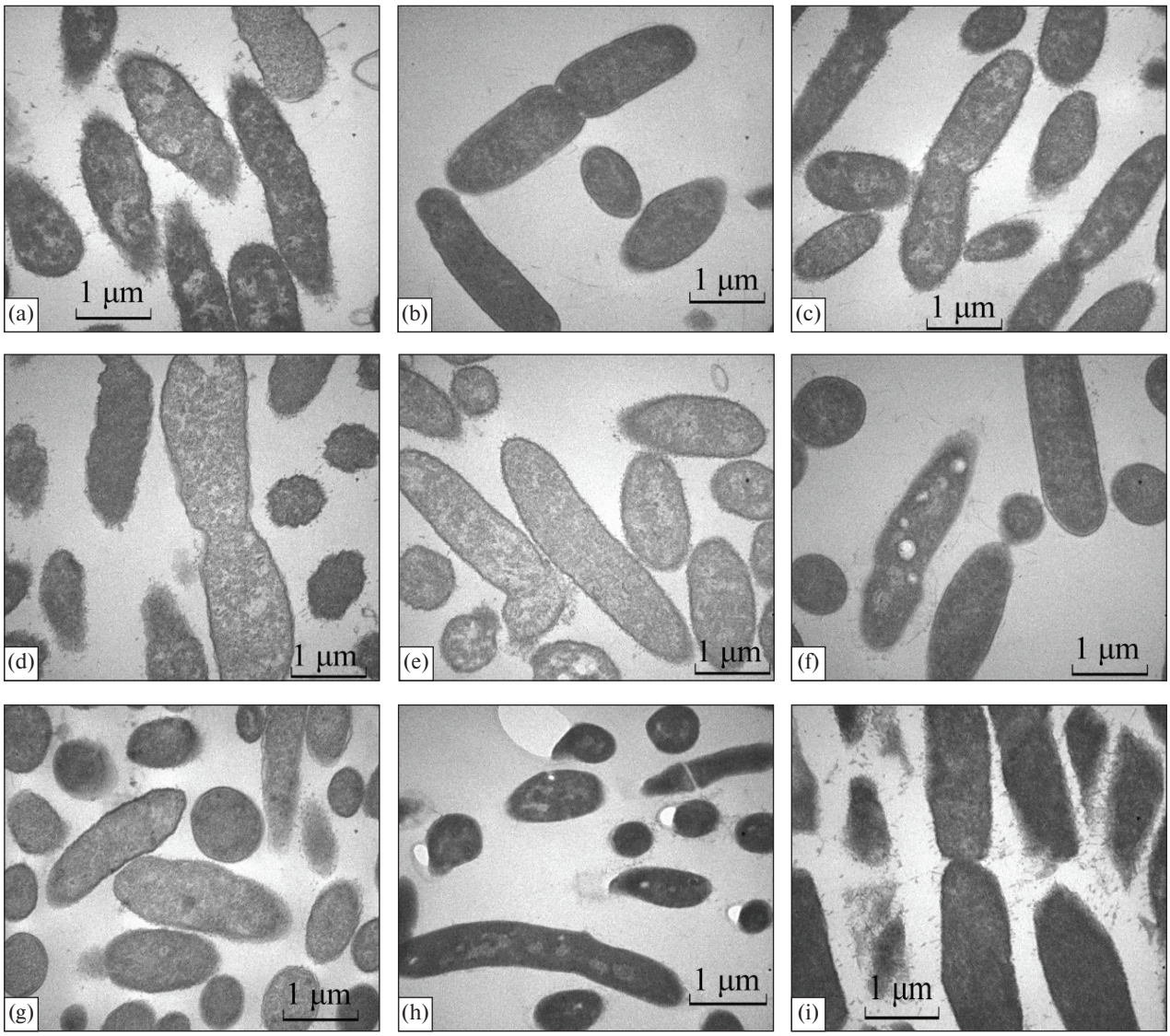

Figure. Cells of isolates of bacteria from Antarctic samples: (a) — 17A_1N_2; (b) — 9.9A_1T_2; (c) — 11_1T_1; (d) — 8_1T; (e) — 14_2N; (f) — 14A_4T_20; (g) — 9.9A_1T_20; (h) — 5A_1N_20; (i) — 10A_3T_20 (transmission electron microscopy, ×10000)

were plated on medium with a higher concentration of metal ions. All 64 isolates were resistant to the influence of Pb(II) ions at a concentration of 0.5 mM. 25 isolates were resistant to 1.5–78 mM Cu(II). The most toxic to the isolates were chromium ions — 15 isolates grew under the influence of 9.6 mM Cr(VI).

For further work, there were selected 9 multimetal resistant isolates which were resistant to 1.5–78 mM Cu(II), 0.05–0.5 mM Pb(II), 0.96–9.6 mM Cr(VI) (Table 2) and did not grow at Endo agar, Kligler's agar, bile-esculin agar, Tergitol-7 agar, and a selective medium for *Bacillus cereus*.

Table 3. Properties of isolates of bacteria

Property	Isolate								
	14A_1N_2	9.9A_1T_2	11_1T	8_1T	14_2N	14A_4T_20	9.9A_1T_20	5A_1N_20	10A_3T_20
Gram straining	–	–	–	–	–	+	+	–	+
Colonies on TSA	smooth, shiny, beige	shiny, milky	smooth, shiny, cream	smooth, shiny, beige	smooth, shiny, beige	smooth, shiny, milky	smooth, shiny, milky	smooth, shiny, beige	smooth, milky
Oxidase activity	+	+	+	–	+	–	+	+	+
Catalase activity	–	+	+	–	–	+	–	+	+
Nitrogen fixation	+	+	+	+	+	–	+	+	+
Proteolytic activity	+	+	+	+	+	+	+	+	–
Production of H_2S	–	–	–	–	–	–	–	–	+
Amylolytic activity	–	–	–	–	–	–	–	–	+
Reduction of NO_3^- to NO_2^-	–	–	+	–	–	–	–	–	+

Table 4. Use of carbon sources by heavy metal resistant isolates

Carbon source	Isolate								
	17A_1N_2	9.9A_1T_2	11_1T	8_1T	14_2N	14A_4T_20	9.9A_1T_20	5A_1N_20	10A_3T_20
Arabinose	+*	+	+	+*	+*	–	–	+	–
Glucose	+*	+	+*	+	+	+	+	+	+
Dulcite	+	–	+	+	+	–	–	+	–
Inositol	–	–	+	+	+	–	–	+	–
Xylose	–	+	+	+	+	–	–	+	–
Lactose	+	+	+*	+	+	+	+	+	+
Maltose	+*	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	–
Rhamnose	+	–	+	+	+	+	+	+	–
Sucrose	–	+	+	+	+	–	–	+	–
Sorbitol	–	–	+	+	+	+	+	+	+

Note: * – acid production.

The investigated heavy metal resistant isolates of bacteria are aerobic, non-motile, non-spore-forming rods (Figure, Table 3). Among the isolates, three (14A_4T_20, 9.9A_1T_20 and 10A_3T_20) are Gram-positive, all others are Gram-negative.

The morphological, physiological and biochemical properties of nine bacterial isolates resistant to Cu(II), Pb(II) and Cr(VI) were investigated.

All of the investigated isolates, except isolate 10A_3T_20, were characterized by proteolytic activity. All of the investigated isolates are characterized by the release of NH_3 into the medium during amino acid metabolism. Release of H_2S into the medium during amino acid metabolism is typical only for 10A_3T_20 isolate. Lipolytic activity is characteristic of all investigated isolates. Only 10A_3T_20 isolate was characterized by the amylolytic activity. All investigated isolates, except 14A_4T_20, are capable of fixing N_2. None of the isolates hydrolyzed casein and formed indole from tryptophan. 10A_3T_20 and 5A_1N_20 isolates were characterized by urease, α-glucosidase, β-glucosidase, α-galactosidase, glycinaminopeptidase, prolineaminopeptidase, leucinglicinaminopeptidase, phosphatase activities.

Heavy metal resistant psychrophilic and mesophilic isolates differed in metabolism of carbon sources. All mesophilic isolates use the investigated carbon sources without the formation of acids. Psychrophilic isolates form acid in the case of arabinose (isolates 17A_1N_2, 8_1T, 14_2N), glucose (isolates 17A_1N_2, 11_1T), lactose (isolate 11_1T), maltose (isolate 17A_1N_2) metabolism.

All the investigated isolates use glucose, maltose and mannitol as carbon sources. Three psychrophilic isolates 11_1T, 8_1T, 14_2N and one mesophilic isolate 5A_1N_20 use all of the investigated carbon sources. In general, the investigated mesophilic isolates metabolize fewer carbon sources. The least amount of investigated carbon sources is metabolized by the mesophilic isolate 10A_3T_20 (Table 4).

The Antarctic microbiota has been studied extensively in recent decades (Romaniuk et al., 2018; Núñez-Montero, Barrientos, 2018; Borzova et al., 2019). Resistant to heavy metal ions microorganisms have been isolated from different Antarctic biotopes at different times. Strains of bacteria that were resistant to 1.6–8 mM Cu(II) ions; 1.4–3.4 mM Cr(VI), and to 5.3–15.8 mM Pb(II) were isolated by authors (Tomova et al., 2014). Compared to these strains, the ones obtained by us have higher resistance to copper ions (78 mM), but their resistance to chromium and lead compounds is lower. However, we suppose that the isolates that we obtained may be promising for further development of wastewater bioremediation technologies, since it is known that, depending on the origin, industrial wastewater may contain from 2 to 900 mg/L of copper ions (García-Díaz, 2018), 6.4–1600 mg/L of chromium ions (Elabbas et al., 2016; Un et al., 2017; Wahaab, Alseroury, 2019; Da Silva et al., 2020), 200–250 mg/L of lead ions (Jarosławiecka, Piotrowska-Seget, 2014). The biotechnological potential of the obtained isolates remains to be discovered, but we assume that they may be producers of proteases and lipases as they are characterized by these activities. Isolate 10A_3T_20 may be a producer of amylolytic enzymes.

4 Conclusions

We isolated 9 pure cultures of microorganisms from Antarctic samples, obtained during Ukrainian Antarctic expedition. Several biochemical properties of isolates of Antarctic microorganisms that are resistant to Cu(II), Cr(VI), Pb(II) are determined. Selected isolates of microorganisms are able to use monosaccharides, disaccharides, alcohols as carbon sources; possess urease, protease, lipase, aminopeptidase activities. Selected heavy metal resistant isolates can be used to further develop technologies for bioremediation of environment.

Author contributions. SH: supervised the research. SH, TP, OlM, OM, SK: did experiments, data analysis, text drafting. TK: provided advice on methods. OlM, SK, OM: editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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Морфологічні та фізіолого-біохімічні характеристики металорезистентних ізолятів бактерій, виділених з різних субстратів Антарктики

Реферат. Мета. Дослідити культурально-морфологічні та фізіолого-біохімічні властивості виділених нами хемоорганотрофних металорезистентних ізолятів бактерій із різних зразків, отриманих під час Української антарктичної експедиції у 2019 році для подальшого відбору найбільш стійких до сполук важких металів та біохімічно активних. Методи. Чисті культури бактерій виділяли із використанням триптон-соєвого агару і поживного агару. Отримані ізоляти висівали на агаризовані середовища, які містили Cu(II) (0,3, 0,1, 1,5, 8, 78 мМ), Pb(II) (0,00009, 0,0005, 0,005, 0,05, 0,5 мМ), Cr(VI) (0,00096, 0,0096, 0,96, 9,6 мМ). Морфологічні властивості бактерій вивчали із застосуванням бінокулярного мікроскопа Axio Lab. A1 компанії CarlZeiss, інвертованого мікроскопа Olympus IX73 з цифровою камерою DP-74 і трансмісійної електронної мікроскопії. Ендоспори виявляли за методом Пєшкова-Трухільо. Визначали каталазну, оксидазну, амілазну, ліполітичну, протеазну активності, здатність фіксувати азот. Здатність мікроорганізмів засвоювати органічні джерела карбону визначали за ростом на середовищі Гісса з різними вуглеводами і спиртами. Визначення фізіологічних властивостей виділених ізолятів проводили із застосуванням системи Remel RapID™ ANA II. Результати. Із досліджених зразків виділили 92 психрофільні ізоляти мікроорганізмів, які росли за температур 2 °С, 6 °С та 20 °С, з них 64 росли на середовищах, які містили 0,30–1,60 мМ Cu(II), чи 0,00009–0,004 мМ Pb(II) чи 0,01–0,9 мМ Cr(VI). 9 ізолятів психрофільних бактерій були стійкими до впливу Cu(II) (1,5–78 мМ), Pb(II) (0,5 мМ), Cr(VI) (0,96–9,6 мМ). Охарактеризовано морфологічні і фізіолого-біохімічні властивості 9 металорезистентних ізолятів. Висновки. Виділено 9 чистих культур мікроорганізмів із антарктичних зразків, отриманих під час Української антарк-

тичної експедиції у 2019 році. Досліджено деякі біохімічні властивості виділених ізолятів антарктичних бактерій, стійких до Cu (II), Cr (VI), Pb (II). Відібрані ізоляти бактерій здатні використовувати моносахариди, дисахариди, спирти як джерело карбону; виявляють уреазну, протеазну, ліпазну, амінопептидазну активності. Відібрані металостійкі ізоляти можуть бути використані для подальшого дослідження і розроблення технологій біоремедіації навколишнього середовища.

Ключові слова: психрофільні мікроорганізми, металорезистентність, купрум, плюмбум, хром