ЕКСПЕРИМЕНТАЛЬНІ ПРАЦІ

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ISOLATION OF BACTERIA FROM THE SITES OF FEED AND NESTING ACTIVITY OF LARUS DOMINICANUS (GALINDEZ ISLAND, THE MARITIME ANTARCTIC) AND THEIR CHARACTERISTICS

The aim of the study was to investigate the number of different groups of microorganisms in soil samples from sites of feed and nesting activity of Larus dominicanus (Galindez Island, the maritime Antarctic) and to characterize the physiological and biochemical properties of isolated microorganisms. Methods. In the work standard microbiological and biochemical research methods were used (cultural, microscopy methods, determination of enzymatic activity). Genomic DNA was isolated by soft lysis. The 16 S rRNA gene was amplified using universal primers 27F and 1492R. Identification of isolates was performed based on the determination of the 16 S rRNA gene sequence, physiological and biochemical properties. **Results.** The number of microorganisms of different groups in soil samples from sites of food and nesting activity of Larus dominicanus (Galindez Island, the maritime Antarctic) was established. 74 isolates of bacteria were isolated. Isolate 2U-K-37, that was isolated from upper layer of soil, and isolate 2B-K-54, that was isolated from a depth of 2-5 cm, were characterized by amylase, lipase, phospholipase, protease activities, the ability to form exopolysaccharides. They were identified by sequencing of the 16 S rRNA gene, physiological and biochemical properties as Pedobacter sp. 2U-K-37 and Pseudarthrobacter sp. 2B-K-54. In the soil samples from sites of feed and nesting activity of Larus dominicanus microorganisms that metabolize nitrogen of organic compounds were the most numerous. Oligotrophic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds were less numerous. The number of microorganisms of groups in the samples from the soil surface and lower layers differed statistically significant. Isolated and identified obligate psychrophilic strain Pedobacter sp. 2U-K-37 and psychrotolerant strain Pseudarthrobacter sp. 2B-K-54 are moderate halophiles that are able to hydrolyze starch, gelatin, tween-20 and synthesize exopolysaccharides.

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Key words: Antarctic microorganisms, Pedobacter sp., Pseudarthrobacter sp., exopolysachharides, halophiles, enzymatic activity

Despite the fact that Antarctica is the largest desert on Earth, which is almost completely covered with ice, the various biotopes of this continent are characterized by a significant species diversity of microorganisms [11, 12, 24]. In areas with extreme conditions of existence, the microorganisms that are resistant to unfavourable factors of environment are often found, and understanding of their biology has significant fundamental importance because it expands the knowledge about the properties of microorganisms of extreme environments and mechanisms of their adaptation at these conditions, and is also important for the creation of biotechnologies that have applications in industry, medicine, etc. [18]. Enzymes of psychrophilic microorganisms are of practical importance because they are active at low temperatures. Resistant to factors of environment psychrophilic microorganisms can be used for the synthesis of nanoparticles, in particular, silver, tellurium-containing nanostructures and semiconductor fluorescent nanoparticles, biofuels, biocontrol of phytopathogens, bioremediation of contaminated environments, etc. [24]. Among Antarctic microorganisms a number of producers of antimicrobial compounds [12] and antiproliferative molecules [15], which can be used as anticancer drugs [21], have been detected. Antarctic microbial populations have been intensively studied in recent decades [11, 12, 24]. The use of deep sequencing techniques has significantly expanded knowledge about the diversity of prokaryotes and the composition of microbial populations of different Antarctic biotopes [10]. It is established that the main representative groups of Antarctic microorganisms are phyla Actinobacteria, Bacteroidetes, Proteobacteria, and Firmicutes [21]. Culturally independent methods make it possible to obtain a large number of sequences and determine the genera of microorganisms that are present in the sample, but do not provide information about the physiological and biochemical characteristics of these microorganisms.

It is shown that one of the carriers of various components of vegetation may be a common in the region flying bird – the kelp gull (*Larus dominicanus*) [17], it also forms a somewhat specific biotope that has not been studied from the view point of microbiology.

The aim of the study was to investigate the number of major groups of microorganisms in soil samples from nesting sites of *Larus dominicanus* (Galindez Island, the maritime Antarctic) and to characterize the physiological and biochemical properties of isolated microorganisms.

Materials and methods

The material for the study were samples from the soil surface and from a depth of 2–5 cm from the site of food and nesting activity of *Larus dominicanus* in the area of the Moss Valley oasis on Galindez Island (-65.247453, -064.249915), 15 m asl. selected 04.03.2021 (Fig. 1). Gulls have here a site of their activity and nesting on the edge of the rocky slope covered by the grouping of *Short moss turf and chusion subformation* and *Bryophyte mat and carpet subformation* with the participation of *Deschampsia antarctica*. There are fragments of *Tall moss turf*



subformation. Directly in the area of gulls activity, a specific grouping was formed on the placers of martin food waste – limpets and plant materials uprooted and applied by Larus dominicanus- moss mats Sanionia sp., Pohlia cruda (Hedw.) Lindb, Usnea antarctica Du Rietz. Here is the nest of these birds, which is used for many years and consists mainly of plant materials. Mainly on these area there are leptozole soils. The depth of the section of the soil, formed mainly by organic residues, is 3-4 cm. Samples were taken in four replicates and the average sample was prepared by mixing equal masses of each sample. The obtained average samples were used to determine the number of microorganisms by cultural method.



Fig. 1. Place of soil sampling for research

The content of hygroscopic water in the samples was determined by the weight method. The soil was dried for 5 hours in drying cabinet at 105 °C to receive a constant mass. The content of hygroscopic water in the soil was determined by the formula: $\frac{a \times 100}{b} = \%$ hygroscopic water, where a is the weight loss after drying, b is the mass of dry soil.

To study the number of microorganisms of different groups, a soil suspension was prepared. To do this, 1 g of soil was added to 9 ml of 0.9% NaCl solution, mixed thoroughly and left for one hour, then mixed again. Serial dilutions of soil suspension were sown on tryptone soy agar to isolate the microorganisms that metabolize nitrogen of organic compounds, diluted tryptone soy agar (1/10) – oligotrophic microorganisms, Ashby's medium – oligonitrophilic and nitrogen-fixing microorganisms, starch-ammonia agar – microorganisms that metabolize inorganic



nitrogen compounds, Hutchinson's medium – cellulolytic microorganisms, Vynogradsky medium – microorganisms that carry out the I and II phases of nitrification, Menkina medium – microorganisms that metabolize organic phosphate-containing compounds. To obtain the isolates, single colonies of microorganisms were reseeded into appropriate media, received a pure culture and investigated their properties.

Genomic DNA was isolated using the soft lysis method [8]. Purification from proteins was carried out by salting with potassium acetate. DNA was precipitated with isopropanol and washed with 70% ethanol. DNA was dissolved in deionized water.

The 16S rRNA gene was amplified using universal primers 27F and 1492R [23]. PCR reaction was performed in a volume of 50 µl using Taq polymerase (NEB M0273X) on a Mastercycler pro thermal cycler (Eppendorf, Germany). Genomic DNA of strains was used as a template for the PCR reaction. The reaction mixture typically contained 1.0 U of Taq Polymerase and 10× PCR buffer (ThermoFischer Scientific, USA), 0.04 mM of each deoxynucleotide, 600 nM of each amplification primer, ca. 50 ng of genomic template DNA, and purified water to volume. The PCR reaction products were analyzed by electrophoresis of DNA in agarose gel and visualized by staining with ethidium bromide. PCR products about 1.5 kbp were purified from the gel using silica columns "QiaQuick" ("Qiagen", USA), analyzed for DNA concentration and purification quality 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 GenBank database by BLAST search.

The morphology of cells (cell shape, size, ability to form spores, determination of the composition and structural organization of the cell wall after Gram staining) was investigated using light (Carl Zeiss Axio Lab.A1 binocular microscope, an Olympus IX73 inverted microscope with a DP-74 digital camera) and transmission electron microscopy [19]. Gram staining was performed using a dye kit (Merck, USA). Also, the type of bacterial cell wall was determined using a 3% solution of KOH. A culture was added to a drop of KOH solution and mixed. Gram-negative bacteria form strands that extend 0.5-2 cm. Bacterial ability to spore formation was determined both microscopically (Peshkov-Trujillo method) and by culturing a pre-pasteurized cell suspension. Catalase activity was detected by the apparent release of O, after application to the colony of the isolate a few drops of 10% H₂O₂[1,2]. Oxidase activity was detected using strips with N,N-dimethyl-p-phenylenediamine oxalate, and α-naphthol (Millipore, USA). Relation to oxygen was determined by the nature of growth in fluid thioglycollate medium (Merck, USA). Bacterial motility was detected by the nature of growth in the TSA column with 0.2% agar. The optimum growth temperature was determined after 5 days of growing at 4, 16, 20, 25, or 37 °C. Halotolerance of isolates was established after 5 days of cultivation on starch ammonia agar with 0.9-15% NaCl. ID 32 GN kit (bioMérieux, France) was used to detect the ability of isolates to metabolize different carbon sources. Different carbon sources fermentation was detected during growth in Hiss media with arabinose, glucose, dulcite, inositol, xylose, lactose,

maltose, mannitol, mannose, rhamnose, sucrose, sorbitol. Peculiarities of metabolism of nitrogen-containing compounds were determined after growth in TSB with cysteine (0.01%) by a color change of litmus (ammonia release) and lead acetate (hydrogen sulfide, mercaptan release) indicator papers. For detection the ability to fix nitrogen bacteria were grown on Ashby medium. The proteolytic activity of the investigated isolates was evaluated by their ability to liquefaction of gelatin after growth in a column of TSB-gelatin [1, 14]. Amylase activity was evaluated by the growth on SAA and the formation of visible zones of starch hydrolysis around the colonies after the application of Lugol's solution on the colony [1, 22]. Lipase activity was evaluated by the ability of isolates to form crystals of calcium salts of fatty acids around the colonies after growth on medium with Tween-20 [13]. Exopolysaccharides were extracted from EDTA (2%) for 3 hours at 4 °C, then centrifuged at 15000 g for 20 min at 4 °C [16]. The content of exopolysaccharides in the obtained supernatant was determined using anthrone [6].

The results are presented as the arithmetic mean (M) taking into account the standard error (m). The significance of the difference between the mean values was determined by Student's criterion ($p \le 0.05$; $p \le 0.01$). Statistical analysis of the data was performed in "Microsoft Office Excel 2016" and OriginPro 8.5.

Results and discussion

In soil samples from the nesting site of *Larus dominicanus* (Galindez Island, the maritimeAntarctic), the number of microorganisms of different groups was in the range of 10⁴–10⁶ CFU/g of dry soil and depended on the depth of sampling (Fig. 2). Oligotrophic microorganisms were the most numerous group of microorganisms in the sample taken from the soil surface, and in the sample from a depth of 2-5 cm were dominated the microorganisms that metabolize nitrogen of organic compounds, which is probably due to higher content of organic compounds in deeper soil layers. The number of microorganisms that metabolize nitrogen of inorganic compounds in both samples was lower by 70–95%, compared with the number of microorganisms that metabolize nitrogen of organic compounds and oligotrophic microorganisms in the upper and lower layers. The difference in the number of microorganisms in the samples from the soil surface and from a depth of 2–5 cm was statistically significant. In the samples taken from the soil surface, the number of microorganisms that metabolize inorganic nitrogen compounds was 10 times higher, the number of nitrifying microorganisms was 33 times higher, and the number of microorganisms that metabolized phosphate-containing organic compounds was 12 times higher compared to samples taken from a depth of 2-5 cm. In the studied samples the number of cellulolytic microorganisms was insignificant, and the colonies that grew had the morphology of microscopic fungi.

The ability of bacteria to grow on starch-ammonia medium, Hutchinson's medium, Menkina's medium, etc. involves the presence in the cells of these bacteria the necessary enzymes for the destruction of substances that are in composition of the media. 74 isolates of bacteria were obtained from the studied Antarctic soil samples. Of these, 21 metabolized twin-20 (lipase activity), 7 – egg lecithin (phospholipase activity), 6 – starch (amylase activity). 3 isolates were characterized by lipase and phospholipase activities, 4 isolates – by lipase and amylase activities,



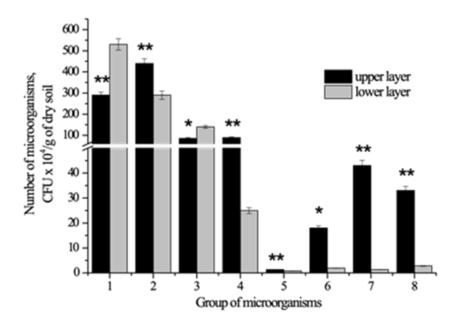


Fig. 2. The number of microorganisms of different groups from the soil from the feed and nesting site of Larus dominicanus (Galindez Island, the maritime Antarctic): - microorganisms that metabolize nitrogen of organic compounds; 2 - oligotrophic microorganisms; 3 - oligonitrophilic microorganisms; 4 - microorganisms that metabolize inorganic nitrogen compounds; 5 – cellulolytic microorganisms; 6 – microorganisms that carry out the I phase of nitrification; 7 – microorganisms that carry out the II phase of nitrification; 8 – microorganisms that metabolize phosphate-containing organic compounds ($p \le 0.05$; $p \le 0.01$ – significant changes in the number of microorganisms in the upper and lower soil layers)

and 3 isolates – by phospholipase and amylase activities. Isolates 2U-K-37 and 2B-K-54 were characterized by well-expressed amylase, lipase, phospholipase, protease activities, ability to form exopolysaccharides. The 16 S rRNA gene sequence of these isolates was established. According to the results of pairwise alignment performed in the BLASTN NCBI service, the sequence of the 16 S rRNA gene of isolate 2U-K-37 was 98.37% identical to the sequence of the 16 S rRNA gene of strain *Pedobacter humicola* R135, 97.84% identical to the sequence of the 16 S rRNA gene of strain *Pedobacter borealis* G-1; the sequence of the 16 S rRNA gene of isolate 2B-K-54 was 99.24% identical to the sequence of the 16 S rRNA gene of strain Pseudarthrobacter sulfonivorans ALL.

Cells of bacteria *Pedobacter* sp. 2U-K-37 are rod-shaped, cells are single or form chains. Spores don't form. Immovable. Gram-negative. On starch-ammonia agar, surface round shiny colonies of pink color with a smooth edge are formed, which darken as the colonies age (Fig. 3, A). Colonies are small, 1–3 mm in diameter. Catalase-positive, oxidase-negative. Grow at temperatures of +4...+25 °C. The optimum temperature for growth is $+20\pm1$ °C. The optimum pH is 6.8–7.3. Microaerophiles. Halotolerant, grow on starch-ammonia agar with 15% NaCl. On



Fig. 3. Colonies of Pedobacter sp. 2U-K-37 (A) and Pseudarthrobacter sp. 2B-K-54 (B) on starch-ammonia agar

tryptone soy broth, the nature of growth is steady, which is accompanied by the production of ammonia. Hydrogen sulfide during growth on tryptone soy broth is not produced. Indole is not formed from tryptophan. When used as carbon sources lactose, maltose and glucose form an acid. During growth in Hiss medium with lactose, xylose, mannitol and glucose cause liquefaction of the medium. Characterized by amylolytic, lipolytic (hydrolysis of tween-20) and proteolytic (gelatin dilution) activities. They are capable to nitrification. Grow on agar water and on diluted tryptone soy agar.

Bacteria *Pedobacter* sp. 2U-K-37 are obligate psychrophiles that differ from closely related strains of P. borealis G-1^T, P. humicola R135^T, Pedobacter kyonggii K-4-11-1^T, *Pedobacter yonginense* HMD1002^T in tolerance to high concentrations of NaCl (table 1). A common feature of *Pedobacter* sp. 2U-K-37 and *P. borealis* G-1^T is the ability to hydrolyze starch. The assimilation of carbon sources differs in different strains of *Pedobacter*, so it is obviously a strain-specific feature.

Cells of bacteria *Pseudarthrobacter* sp. 2B-K-54 are wrong shape, cells are single. Spores don't form. Immovable. Gram-positive. On starch-ammonia agar form surface round shiny colonies of white color with a smooth edge, 1-3 mm in diameter (Fig. 3, B). Catalase-positive, oxidase-negative. Grow at temperatures of +4...+28 °C. The optimum temperature for growth is +20±1 °C. The optimum pH is 6.8–7.3. Aerobes. Halotolerant, grow on starch-ammonia agar with 15% NaCl. In tryptone soy broth the nature of growth is steady, which is accompanied by the hydrogen sulfide production. Ammonia is not produced during growth on tryptone soy broth. Indole is not formed from tryptophan. Assimilate L-rhamnose, N-acetylglucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconic acid, lactic acid, L-alanine, potassium 5-ketogluconate, potassium 2-ketogluconate, glycogen, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicine, D-melibiose, L-fucose, D-sorbitol, L-arabinose, 3-hydroxybutyrate, L-proline. When used rhamnose, arabinose, glucose, dulcite, inositol, xylose, lactose, maltose, manitol, manose, sucrose and sorbitol an acid and gas are not formed.



Table 1
Differential characteristics of strain 2U-K-37 and phylogenetically related species of the genus *Pedobacter*

Properties	Pedobacter sp. 2U-K-37	Pedobacter borealis G-1 ^T [5]	Pedobacter humicola R135 ^T [7]	Pedobacter kyonggii K-4-11-1 ^T [4]	Pedobacter yonginense HMD1002 ^T [9]
Isolation source	Soil	Soil	Soil	Forest soil	Fresh water
Colony colour	Light pink	Reddish- pink	Light pink	Salmon- coloured	Red
Maximum growth temperature (°C)	25	30	42	32	37
NaCl tolerance (%, w/v)	15.0	nd	6.0	1.5	0.5
Catalase	+	+	+	+	+
Oxidase	_	+	_	+	+
Hydrolysis of: gelatin starch	+ +	+ +	nd –	+ -	
Assimilation of: L-Rhamnose Sodium acetate Lactic acid Melibiose Glycogen 3-Hydroxybenzoic acid	+ - - - -	+ nd nd nd - nd	+ + W nd - W	W	- nd - + + nd
L-Arabinose Propionic acid Valeric acid 4-Hydroxybenzoic acid	_ _ _ _	+ nd nd nd	+ + + W	+ - - -	– nd nd
L-Proline	_	nd	_	+	+

Note: "+" – positive; "w" – weak reaction; "–" – negative; "nd" – data not available.

Do not assimilate suberic acid, sodium malonate, sodium acetate, propionic acid, capric acid, valeric acid, sodium citrate, L-histidine. They are characterized by amylolytic, lipolytic (hydrolysis of tween-20) and proteolytic (gelatin dilution) activities. They are capable to nitrogen fixation and nitrification. Grow on agar water and on starvation agar.

Bacteria *Pseudarthrobacter* sp. 2B-K-54 differ from closely related species *Pseudarthrobacter psychrotolerans* YJ56^T, *P. sulfonivorans* ALL^T, *Pseudarthrobacter oxydans* DSM 20119^T, *Pseudarthrobacter polychromogenes* DSM 20136^T in resistance to NaCl and ability to hydrolyze starch and gelatin (table 2).

During the growth of bacteria *Pedobacter* sp. 2U-K-37 and *Pseudarthro-bacter* sp. 2B-K-54 on starch-ammonia agar for 14 days, they produced 10 times more expolysaccharides at a temperature of 4 °C than of 18 °C (Table 3).



Table 2

Differential characteristics of strain 2B-K-54 and phylogenetically related species

of the genus Pseudarthrobacter

Pseudarthrobacter polychromogenes DSM 20136 ^T [20]	Air	10–37	7.5	+	≱+
Pseudarthrobacter oxydans DSM 20119 ^T [20]	Air	28–37	10.0	+	» +
Pseudarthrobacter sulfonivorans ALL'[20]	Soil from the root balls of Allium aflatunense	4-30	2.5	+	1 1
Pseudarthrobacter psychrotolerans YJ56 ^T [20]	Antarctic soil	4-28	6.0	I	
Pseudarthrobacter sp. 2B-K-54	Antarctic soil	4–28	15.0	1	+ +
Properties	Isolation source	Growth temperature,	NaCl tolerance (%, w/v)	Oxidase activity	Hydrolysis of: starch gelatin

Note: "+" - positive; "w" - weak reaction; "-" - negative; "nd" - data not available.



Table 3 Influence of temperature on synthesis of exopolysaccharides by bacteria *Pedobacter* sp. 2U-K-37 and Pseudarthrobacter sp. 2B-K-54

Bacteria	Concentration of exopolysaccharides, mg/g bacteria biomass Temperature of cultivation			
	18 °C	4 °C		
Pedobacter sp. 2U-K-37	10.86±1.29	108.86±1.84**		
Pseudarthrobacter sp. 2B-K-54	11.45±0.59	117.73±15.96**		

Note: "**" $-p \le 0.01$ - significant changes in the concentration of exopolysaccharides of bacteria cultivated at 4 °C compared with the concentration of exopolysaccharides of bacteria cultivated at 18 °C.

We assume that the higher content of exopolysaccharides synthesized by the bacteria Pedobacter sp. 2U-K-37 and Pseudarthrobacter sp. 2B-K-54 at 4 °C is associated with their cryoprotective function, as described in bacteria Marinobacter W1–16 from the maritime Antarctic [3].

Conclusion

In the soil samples from sites of feed and nesting activity of Larus dominicanus microorganisms that metabolize nitrogen of organic compounds were the most numerous. Oligotrophic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds were less numerous. The number of microorganisms of groups in the samples from the soil surface and lower layers differed statistically significant. Isolated and identified obligate psychrophilic strain *Pedobacter* sp. 2U-K-37 and psychrotolerant strain Pseudarthrobacter sp. 2B-K-54 are moderate halophiles that are able to hydrolyze starch, gelatin, tween-20 and synthesize exopolysaccharides.

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ВИДІЛЕННЯ БАКТЕРІЙ З МІСЦЯ ХАРЧОВОЇ І ГНІЗДОВОЇ АКТИВНОСТІ LARUS DOMINICANUS (О. ГАЛІНДЕЗ, МОРСЬКА АНТАРКТИКА) ТА ЇХНЯ ХАРАКТЕРИСТИКА

Реферат

Метою роботи було дослідити чисельність різних груп мікроорганізмів у зразках грунту з місця харчової і гніздової активності Larus dominicanus (острів Галіндез, Морська Антарктика) та охарактеризувати фізіолого-біохімічні властивості виділених мікроорганізмів. Методи. У роботі використовували стандартні мікробіологічні і біохімічні методи досліджень (культуральний, методи мікроскопування, визначення ензиматичної активності). Хромосомну ДНК виділяли методом м'якого лізису. Ген 16~SрРНК ампліфікували із використанням універсальних праймерів 27F і 1492R. Ідентифікацію ізолятів проводили на основі визначення послідовності гена 16 S рРНК і фізіолого-біохімічних властивостей. Результати. Встановлено чисельність різних груп мікроорганізмів у зразках ґрунту з місця харчової і гніздової активності Larus dominicanus (острів Галіндез, Морська Антарктика). Виділено 74 ізоляти бактерій. Ізолят 2U-К-37, виділений з верхнього шару трунту, та ізолят 2В-К-54, виділений з глибини 2–5 см, характеризувались амілазною, ліпазною, фосфоліпазною, протеазною активностями, здатністю утворювати екзополісахариди. Їх ідентифікували за результатами секвенування гена 16 S рРНК та фізіолого-біохімічними властивостями як Pedobacter sp. 2U-K-37 ma Pseudarthrobacter sp. 2B-K-54. Висновки. У зразках трунту з місця харчової і гніздової активності Larus dominicanus найбільш чисельними були мікроорганізми, які метаболізують нітроген органічних сполук. Менше було оліготрофів і мікроорганізмів, які метаболізують нітроген неорганічних сполук. Чисельність мікроорганізмів у зразках з поверхні трунту і нижчих шарів статистично відрізнялась. Виділені й ідентифіковані облігатно психрофільний штам Pedobacter sp. 2U-K-37 і психротолерантний штам Pseudarthrobacter sp. 2B-K-54 ϵ помірними галофілами, які здатні гідролізувати крохмаль, желатин, твін-20 і синтезувати екзополісахариди.

Ключові слова: антарктичні мікроорганізми, Pedobacter Pseudarthrobacter sp., екзополісахариди, галофіли, ензиматична активність



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ВИДЕЛЕНИЕ БАКТЕРИЙ ИЗ МЕСТА ПИЩЕВОЙ И ГНЕЗДОВОЙ АКТИВНОСТИ *LARUS DOMINICANUS* (О. ГАЛИНДЕЗ, МОРСКАЯ АНТАРКТИКА) И ИХ ХАРАКТЕРИСТИКА

Реферат

Целью работы было исследовать численность различных групп микроорганизмов в образцах почвы с места пищевой и гнездовой активности Larus dominicanus (остров Галиндез, Морская Антарктика) и определить физиолого-биохимические свойства выделенных микроорганизмов. Методы. В работе использовали стандартные микробиологические и биохимические методы исследований (культуральный, методы микроскопирования, определение энзиматической активности). Общую ДНК выделяли методом мягкого лизиса. Ген 16 S рРНК амплифицировали с использованием универсальных праймеров 27F и 1492R. Изоляты идентифицировали на основании определения последовательности гена 16 S pPHK и физиолого-биохимических свойств. Результаты. Установлена численность различных групп микроорганизмов в образцах почвы с места пищевой и гнездовой активности Larus dominicanus (остров Галиндез, Морская Антарктика). Выделены 74 изолята бактерий. Изолят 2U-К-37, выделенный из верхнего слоя почвы, и изолят 2В-K-54, выделенный из глубины 2-5 см, характеризовались амилазной, липазной, фосфолипазной, протеазной активностью, способностью синтезировать экзополисахариды. Их идентифицировали по результатам секвенирования гена 16 S pPHK и физиолого-биохимическим свойствам как Pedobacter sp. 2U-K-37 и Pseudarthrobacter sp. 2B-K-54. Выводы. В образцах почвы с места пищевой и гнездовой активности Larus dominicanus наиболее многочисленными были микроорганизмы, метаболизирующие нитроген органических соединений. Меньше было олиготрофов и микроорганизмов, метаболизирующих нитроген неорганических соединений. Количество микроорганизмов в образцах с поверхности почвы и низших слоёв статистически отличалось. Выделенные и идентифицированные облигатно-психрофильный штамм Pedobacter sp. 2U-K-37 и психротолерантный штамм Pseudarthrobacter sp. 2B-K-54 являются умеренными галофилами, способными гидролизовать крахмал, желатин, твин-20 и синтезировать экзополисахариды.

Ключевые слова: антарктические микроорганизмы, Pedobacter sp., Pseudarthrobacter sp., экзополисахариды, галофилы, энзиматическая активность



СПИСОК ВИКОРИСТАНОЇ ЛІТЕРАТУРИ

- 1. *Гудзь С.П., Гнатуш С.О., Яворська Г.В., Білінська І.С., Борсукевич Б.М.* Практикум з мікробіології. Львів: Видавничий центр ЛНУ імені Івана Франка, 2014. 436 с.
- 2. Arenas F.A., PuginB., Henríquez N.A., Arenas-Salinas M.A., Díaz-Vásquez W.A., Pozo M.F., Muñoz C.M., Chasteen T.G., Pérez-Donoso J.M., Vásquez C.C. Isolation, identification and characterization of highly tellurite-resistant, tellurite-reducing bacteria from Antarctica // PolarScience. − 2014. − Vol. 8, № 1. − P. 40–52.
- 3. Caruso C., Rizzo C., Mangano S., Poli A. Isolation, characterization and optimization of EPSs produced by a cold-adapted Marinobacter isolate from Antarctic seawater // Antarctic science. − 2019. − Vol. 31, № 2. − P. 69–79.
- 4. Chaudhary D.K., Lee S.D., Kim J. Pedobacter kyonggii sp. nov., a psychrotolerant bacterium isolated from forest soil // International journal of systematic and evolutionary microbiology. −2017. −Vol. 67, № 12. −P. 5120−5127.
- 5. *Dahal R.H., Kim J. Pedobacter humicola* sp. nov., a member of the genus *Pedobacter* isolated from soil // International journal of systematic and evolutionary microbiology. 2016. Vol. 66, № 6. P. 2205–2211.
- 6. Frølund B., Palmgren R., Keiding K., Nielsen P.H. Extraction of extracellular polymers from activated sludge using a cation exchange resin // Water research. 1996. Vol. 30, № 8. P. 1749–1758.
- 7. Gordon N.S., Valenzuela A., Adams S.M., Ramsey P.W., Pollock J.L., Holben W.E., Gannon J.E. Pedobacter nyackensis sp. nov., Pedobacter alluvionis sp. nov. and Pedobacter borealis sp. nov., isolated from Montana flood-plain sediment and forest soil // International journal of systematic and evolutionary microbiology. − 2009. − Vol. 59, № 7. − P. 1720–1726.
- 8. *Green M.R.*, *Sambrook J.* Molecular Cloning: Laboratory Manual. 4th ed. Cold Spring Harbor; New York, 2012.
- 9. *Joung Y., Kim H., Joh K. Pedobacter yonginense* sp. nov., isolated from a mesotrophic artificial lake in Korea // The Journal of Microbiology. 2010. Vol. 48, № 4. P. 536–540.
- 10. *Lambrechts S., Willems A., Tahon G.* Uncovering the uncultivated majority in Antarctic soils: toward a synergistic approach // Frontiers in microbiology. 2019. Vol. 10. article 242.
- 11. *Lamilla C., Pavez M., Santos A., Hermosilla A., Llanquinao V., Barrientos L.* Bioprospecting for extracellular enzymes from culturable *Actinobacteria* from the South Shetland Islands, Antarctica // Polar Biol. 2017. Vol. 40. P. 719–726.
- 12. *Lee J., Cho Y.J., Yang J.Y., Jung Y.J., Hong S.G., Kim O.S.* Complete genome sequence of *Pseudomonas antarctica* PAMC 27494, abacteriocin-producing psychrophilei solated from Antarctica // Journal of biotechnology. 2017. Vol. 259. P. 15–18.
- 13. Lo Giudice A., Michaud L., De Pascale D., De Domenico M., Di Prisco G., Fani R., Bruni V. Lipolytic activity of Antarctic cold-adapted marine bacteria (Terra Nova Bay, Ross Sea) // Journal of applied microbiology. − 2006. − Vol. 101, № 5. − P. 1039–1048.



- 14. Loperena L., Soria V., Varela H., Lupo S., Bergalli A., Guigou M., Pellegrino A., Bernardo A., Calviño A., Rivas F., Batista S. Extracellular enzymes produced by microorganisms isolated from maritime Antarctica // World Journal of Microbiology and Biotechnology. – 2012. – Vol. 28, № 5. – P. 2249-2256.
- 15. Mojib N., Nasti T.H., Andersen D.T., Attigada V.R., Hoover R.B., Yusuf N., Bej A.K. The antiproliferative function of violacein-like purple violet pigment (PVP) from an Antarctic Janthino bacterium sp. Ant5-2 in UV-induced 2237 fibrosar coma // International journal of dermatology. – 2011. – Vol. 50, № 10. – P. 1223–1233.
- 16. Pan X., Liu J., Zhang D., Chen X., Li L., Song W., Yang J. A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three dimensional excitation-emission matrix (3DEEM) fluorescences pectroscopy // WaterSA. –2010. – Vol. 36, № 1.
- 17. Parnikoza I., Rozhok A., Convey P., Veselski M., Esefeld J., Ochyra R., Mustafa O., Braun C., Peter H.-U., Smykla J., Kunakh V., Kozeretska I. Spread of Antarctic vegetation by the kelp gull: comparison of two maritime Antarctic regions // Polar Biology. – 2018. – Vol. 41, № 6. – P. 1143–1155.
- 18. Perfumo A., Banat I.M., Marchant R. Going green and cold: biosurfactants from low-temperature environments to biotechnology applications // Trends Biotechnol. – 2018. – Vol. 36. – P. 277–289.
- 19. Reynolds E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy // Journal of cell biology. – 1963. – Vol. 17, № 1. – P. 208-212.
- 20. Shin Y., Lee B.H., Lee K.E., Park W. Pseudarthrobacter psychrotolerans sp. nov., a cold-adapted bacterium isolated from Antarctic soil // International Journal of Systematic and Evolutionary Microbiology. – 2020. – Vol. 70, № 12. – P. 6106–6114.
- 21. Silva T.R., Duarte A.W., Passarini M.R., Ruiz A.L.T., Franco C.H., Moraes C.B., de Melo I.S., RodriguesR.A., Fantinatti-Garboggini F., Oliveira V.M. Bacteria from Antarctic environments: diversity and detection of antimicrobial, antiproliferative, and antiparasitic activities // Polar Biology. – 2018. – Vol. 41, № 7. – P. 1505–1519.
- 22. Sushma V.K., Abha S., Chander P. Isolation and characterization of Bacillus subtilis KC3 for amylolytic activity // International Journal of Bioscience. Biochemistry and Bioinformatics. – 2012. – Vol. 2, № 5. – P. 336–341.
- 23. Turner S., Pryer K.M., Miao V.P., Palmer J.D. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis // Journal of Eukaryotic Microbiology. – 1999. – Vol. 46, № 4. – P. 327–338.
- 24. Yarzábal L.A. Antarctic psychrophilic microorganisms and biotechnology: history, current trends, applications, and challenges. In Microbial models: from environmental to industrial sustainability. – Singapore: Springer, 2016. - P. 83-118.



REFERENCES

- 1. Hudz' SP, Hnatush SO, Yavorska HV, Bilinska IS, Borsukevych BM Praktykum z mikrobiolohii [Guidebook on microbiology]. – Lviv: Vyd. tsentr LNU imeni Ivana Franka. – 2014. –. 436 p. (in Ukrainian)
- 2. Arenas FA, Pugin B, Henríquez NA, Arenas-Salinas MA, Díaz-Vásquez WA, Pozo MF, Muñoz CM, Chasteen TG, Pérez-Donoso JM, Vásquez CC. Isolation, identification and characterization of highly tellurite-resistant, tellurite-reducing bacteria from Antarctica. Polar Science. 2014; 8(1): 40–52.
- 3. Caruso C, Rizzo C, Mangano S, Poli A. Isolation, characterization and optimization of EPSs produced by a cold-adapted Marinobacter isolate from Antarctic seawater. Antarctic Science. 2019; 31(2): 69–79.
- 4. Chaudhary DK, Lee SD, Kim J. *Pedobacter kyonggii* sp. nov., a psychroto lerant bacterium isolated from forest soil. International journal of systematic and evolutionary microbiology. 2017; 67(12): 5120–5127.
- 5. Dahal RH, Kim J. *Pedobacter humicola* sp. nov., a member of the genus Pedobacter isolated from soil. International Journal of Systematic and Evolutionary Microbiology. 2016; 66(6): 2205–2211.
- 6. Frølund B, Palmgren R, Keiding K, Nielsen PH. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Research. 1996; 30(8): 1749-1758.
- 7. Gordon NS, Valenzuela A, Adams SM, Ramsey PW, Pollock JL, Holben WE, Gannon JE. Pedobacter nyackensis sp. nov., Pedobacter alluvionis sp. nov. and Pedobacter borealis sp. nov., isolated from Montana flood-plain sediment and forest soil. International Journal of Systematic and Evolutionary Microbiology. 2009; 59(7): 1720-1726.
- 8. Green MR, Sambrook J. Molecular Cloning: Laboratory Manual. (4th ed.). Cold Spring Harbor; New York, 2012.
- Joung Y, Kim H, Joh K. *Pedobacter yonginense* sp. nov., isolated from a mesotrophic artificial lake in Korea. The Journal of Microbiology. 2010; 48(4): 536-540.
- 10. Lambrechts S, Willems A, Tahon G. Uncovering the uncultivated majority in Antarctic soils: toward a synergistic approach. Frontiers in Microbiology. 2019; 10: article 242.
- 11. Lamilla C, Pavez M, Santos A, Hermosilla A, Llanguinao V, Barrientos L. Bioprospecting for extracellular enzymes from culturable Actinobacteria from the South Shetland Islands, Antarctica. Polar Biol. 2017; 40: 719–726.
- 12. LeeJ, ChoYJ, YangJY, JungYJ, HongSG, KimOS. Complete genome sequence of Pseudomonas antarctica PAMC 27494, a bacteriocin-producing psychrophile isolated from Antarctica. Journal of Biotechnology. 2017; 259: 15-18.
- 13. Lo Giudice A, Michaud L, De Pascale D, De Domenico M, Di Prisco G, Fani R, Bruni V. Lipolytic activity of Antarctic cold-adapted marine bacteria (Terra Nova Bay, Ross Sea). Journal of Applied Microbiology. 2006; 101(5): 1039-1048.
- 14. Loperena L, Soria V, Varela H, Lupo S, Bergalli A, Guigou M, Pellegrino A, Bernardo A, Calviño A, Rivas F, Batista S. Extracellular enzymes produced



- by microorganisms isolated from maritime Antarctica. World Journal of Microbiology and Biotechnology. 2012; 28(5): 2249–2256.
- 15. Mojib N, Nasti TH, Andersen DT, Attigada VR, Hoover RB, Yusuf N, Bej AK. The antiproliferative function of violacein-like purple violet pigment (PVP) from an Antarctic Janthinobacterium sp. Ant5-2 in UV-induced 2237 fibrosarcoma. International Journal of Dermatology. 2011; 50(10): 1223-
- 16. Pan X, Liu J, Zhang D, Chen X, Li L, Song W, Yang J. A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy. Water SA. 2010; 36(1).
- 17. Parnikoza I, Rozhok A, Convey P, Veselski M, Esefeld J,Ochyra R, Mustafa O, Braun C, Peter H-U, Smykla J, Kunakh V, Kozeretska I. Spread of Antarctic vegetation by the kelp gull: comparison of two maritime Antarctic regions. Polar Biology. 2018; 41(6): 1143–1155.
- 18. Perfumo A, Banat IM, Marchant R. Going green and cold: biosurfactants from low-temperature environments to biotechnology applications. Trends Biotechnol. 2018; 36: 277-289.
- 19. Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal of Cell Biology. 1963; 17(1): 208–212.
- 20. Shin Y, Lee BH, Lee KE, Park W. Pseudarthrobacter psychrotolerans sp. nov., a cold-adapted bacterium isolated from Antarctic soil. International Journal of Systematic and Evolutionary Microbiology. 2020; 70(12): 6106– 6114.
- 21. Silva TR, Duarte AW, Passarini MR, Ruiz ALT, Franco CH, Moraes CB, de Melo IS, Rodrigues RA, Fantinatti-Garboggini F, Oliveira VM. Bacteria from Antarctic environments: diversity and detection of antimicrobial, antiproliferative, and antiparasitic activities. Polar Biology. 2018; 41(7): 1505–1519.
- 22. Sushma VK, Abha S, Chander P. Isolation and characterization of *Bacillus* subtilis KC3 for amylolytic activity. International Journal of Bioscience, Biochemistry and Bioinformatics. 2012; 2(5): 336–341.
- 23. Turner S, Pryer KM, Miao VP, Palmer JD. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. Journal of Eukaryotic Microbiology. 1999; 46(4): 327–338.
- 24. Yarzábal LA. Antarctic psychrophilic microorganisms and biotechnology: history, current trends, applications, and challenges. In Microbial models: from environmental to industrial sustainability. Singapore: Springer, 2016. 83–118.

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