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Isolation and characterization of culturable actinobacteria associated with *Polytrichum strictum* (Galindez Island, the maritime Antarctic)

Abstract. The main objective of the study is the evaluation of the diversity of actinobacteria associated with *Polytrichum strictum* — dominant species of widespread Antarctic Tall moss turf subformation and their characteristics as the producers of biologically active compounds. The actinobacterial isolates were isolated by direct inoculation, phenol pretreatment, and heated treatment. The cultural properties of the isolates were evaluated using diagnostic media. The antimicrobial activity of the isolates was determined by the point inoculations method. The phylogenetic analysis was based on sequence analysis of the 16S rRNA gene. The biosynthetic genes screening was performed using polymerase chain reaction. A total of 23 actinobacterial isolates associated with *P. strictum* were isolated, the four identified genera being *Streptomyces* (7 isolates), *Micromonospora* (14 isolates), *Kribbella* (1 isolate), and *Micrococcus* (1 isolate). Eight psychrotrophic strains of all identified genera were identified. The optimal pH values for all isolates were in the range 6–10. Four isolates grew on the medium with 7.5% NaCl. A significant number of the isolates showed a wide range of enzymatic activities. Antagonists of a wide range of pathogenic microorganisms were found, including against multidrug-resistant strain of *Candida albicans* and Methicillin-resistant *Staphylococcus aureus*. Some strains were active against phytopathogenic bacteria, namely three strains against *Erwinia amylovora*, one strain against *Agrobacterium tumefaciens*, and one strain against *Pectobacterium carotovorum*. More than half of the isolates showed antifungal activity against *Fusarium oxysporum* and *Aspergillus niger*. The biosynthetic genes involved in synthesizing a wide range of bioactive compounds were found in more than 80% of isolates. Antarctic actinobacteria isolated in this study demonstrate potential as the producers of a wide range of biologically active compounds. Further studies of these actinobacteria may lead to the identification of previously unknown biologically active compounds.

Keywords: Antarctic actinobacteria, *Polytrichum strictum*, antimicrobial activity, biosynthetic genes

1 Introduction

Galindez Island belongs to the Argentine Islands (the maritime Antarctic), which are composed of different rocks favorable for the development of vegetation. Meanwhile, the vegetation of these islands is considerably limited in diversity. In summer, it occupies most of the ice-free areas. It includes Tall moss turf subformation, Bryophyte mat and carpet subformation, Fruticose lichen and moss cushion subformation, etc. The first community type forms the most extensive continuous stands, the moss banks. They are dominated by the moss *Polytrichum strictum* Menzies ex. Brid. 1801 (Polytrichaceae) (Parnikoza et al., 2018). The Antarctic microbiota, on the other hand, is quite diverse (Molina-Montenegro et al., 2019).

Among the bacteria, one of the most common is the phylum *Actinobacteria*, in particular, the order *Actinomycetales* (Pudasaini et al., 2017). The phylum *Actinobacteria* includes gram-positive bacteria with high G+C content. The individual genera (*Streptomyces, Streptoverticillium*, etc.) form branched mycelium (Petrus & Claessen, 2014). These microorganisms play an important environmental role as soil-forming bacteria, and also they are a part of the plants' rhizosphere (Sathya et al., 2017). The rhizosphere microorganisms of the Antarctic, including actinobacteria, can produce cold-shock proteins, cold-active enzymes, antifreeze enzymes, and polysaccharides, providing tolerance to temperature changes but also indirectly protecting plants from the extremely cold climate (Raymond, 2016; Muñoz et al., 2017).

Actinobacteria are well known as producers of a wide range of bioactive compounds (Ding et al., 2019). These bacteria, especially the genus *Streptomyces*, are major targets of microbial biotechnology as producers of most currently known antibiotics (Liu et al., 2018). Antimicrobial, antitumour, antiparasitic, and other drugs based on natural actinomycete products are widely used in medicine, including veterinary (Baltz, 2019). Therefore, the isolation and study of actinobacteria from natural habitats are relevant.

The study of the diversity of actinobacteria from poorly explored biotopes is a promising strategy for identifying new biologically active compounds or their producers (Núñez-Montero & Barrientos, 2018). This is especially relevant today because the ever-increasing number of multidrug-resistant (MDR) pathogenic microorganisms requires regular screening of new antibiotics.The analysis of the biotechnological potential of actinomycetes from the Crimean Peninsula (Ukraine), still poorly explored, allowed identifying more than ten new biologically active compounds (Raju et al., 2013; Raju et al., 2015). One of them is leopolic acid (Raju et al., 2012), which is considered today a potential antiviral drug against SARS-CoV-2 (Mazzini et al., 2020). This renders the perspective of screening studies in poorly studied biotopes, as the Antarctic.

This study is a continuation of our previous studies on the diversity of Antarctic actinobacteria. It focuses on the isolation and characterization of actinobacteria associated with *P. strictum*, the dominant moss of the Tall moss turf subformation from Galindez Island.

2 Materials and methods

2.1 Isolation of actinobacteria

The rhizoid samples of the P. strictum from moss bank were collected on the Galindez Island, the Argentine Islands, the maritime Antarctic (–65.24615°, –64.24969°) during the 21st Ukrainian Antarctic Expedition in 2017. The plants of the *P. strictum* were without signs of disease, and the pH of the soil — 5.1. The samples were transported into sterile containers to the laboratory of the Microbial Culture Collection of Antibiotic Producers of the Ivan Franko National University of Lviv (MCCAP LNU). The actinobacteria were isolated using three different methods: I — rhizoid samples (2 grams) were placed in flasks with 100 ml of sterile tap water and shaken for 15 min; II — rhizoid samples (2 grams) were placed in flasks with 1.5% aqueous phenol solution and shaken for 30 min; III — rhizoid samples (2 grams) were heated for 60 min at 100 °C and then treated the same as method I. Methods II and III were applied to inhibit fast-growing microorganisms, including streptomycetes, to isolate slow-growing actinomycetes, such as *Micromonospora* spp. Further, the ten-fold serial dilutions of the samples were plated (10^{-1}—10^{-5}) on the OM (Gromyko, 2012), ISP4 (Kieser et al., 2000), SA (HiMedia), and HVA (Zhang & Zhang, 2011) media. Nalidixic acid (25 μg/ml) and nystatin (50 μg/ml) were added to isolation media to inhibit the growth of other bacteria and fungi. The plates were incubated at 28 °C for 30 days. The colonies with actinobacteria-like morphology were selected. The pure cultures of the isolates were stored in TSB medium (Kieser et al., 2000) at –80 °C in 25% (v/v) glycerol. These isolates were deposited in the MCCAP LNU.

2.2 16S rRNA gene sequencing and analysis

The isolates were cultured in Tryptic Soy Broth (TSB) (HiMedia Laboratories Pvt Ltd., India) at a temperature of 28 °C for 3–5 days and a shaking rate of 180 rpm.

The total DNA was isolated as described by Kieser et al. (2000). The amplification of the 16S rRNA gene was done using primers (Table 1). The polymerase chain reaction (PCR) was carried out in a total volume of 50 µl containing 2.0 µl of genomic DNA (~50 ng), 1.0 µl of each primer (10 pmol), 2.5 µl of dimethyl sulfoxide, 2.0 µl of deoxynucleotide triphosphates (10.0 mM each), 5.0 µl of 10 × PCR buffer, 0.5 µl of DNA polymerase (5 U/µl) and 36.0 µl MilliQ grade water. The parameters of the PCR were as follows: the initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 90 s. A final extension was at 72 °C for 10 min. The amplified PCR products of the 16S rRNA were separated by electrophoresis in 1% agarose gel, purified using the QIAquick Gel Extraction Kit (Qiagen, Venlo, Netherlands), and sequenced with the amplification primers by Eurofins Genomics (GATC Services). The forward and reverse sequences were assembled with Geneious software version 9.1.3 (Kearse et al., 2012).

The analysis of the 16S rRNA gene sequence of the actinobacteria isolates was performed by RDP Release 11 (Wang et al., 2007). The closest related species to the 16S rRNA were identified by BLAST search data in the National Center for Biotechnology Information database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using two-making algorithms neighbor-joining (NJ) (Saitou & Nei, 1987) in MEGA X (Kumar et al., 2018). The evolutionary distances were computed according to the Kimura two-parameter method (Kimura, 1980), and the robustness of the tree topology was evaluated by the bootstrap test (1000 replicates) (Felsenstein, 1985).

2.3 Determination of cultural properties of the actinobacterial isolates

Growth at different temperatures. The isolates were spot-inoculated on Bennett's medium (Kieser et al., 2000) and cultivated at a temperature of 4 or 10 °C for 30 days, and 28, 37, or 45 °C for 10 days (Strickler et al., 2015). The growth at different temperatures was identified visually.

Growth at various* pH *values. The isolates were spot-inoculated on Bennett's medium with different pH

Table 1. The PCR primers used in this work

Gene	Primers	Sequence (5'-3')	References
16S rRNA	8F	AGAGTTTGATYMTGGCTCAG	Pei et al., 2010
	1510F	TACGGYTACCTTGTTACGACTT	
PKS I	K1 F	TSAAGTCSAACATCGGBCA	Ayuso-Sacido & Genilloud, 2005
	M6 R	CGCAGGTTSCSGTACCAGTA	
PKS II	KSα	TSGRCTACRTCAACGGSCACGG	Ayuso et al., 2005
	KSβ	TACSAGTCSWTCGCCTGGTTC	
NRPS	A3F	GCSTACSYSATSTACACSTCSGG	Ayuso-Sacido & Genilloud, 2005
	A7R	SASGTCVCCSGTSGCGTAS	
CYP450	cypF	TGGATCGGCGACGACCGSVYCGT	Han et al., 2010
	cypR	CCGWASAGSAYSCCGTCOTACTT	
oxyB	oxyB F	CTGGTCGGCAACCTGATGGAC	Wood et al., 2007
	oxyB R	CAGGTACCGGATCAGCTCGTC	
HMG-CoA	HMG F	GGGCATCGCCGCGACCCTCGTCGACGAGCG	Sigmund et al., 2003
	HMG R	GCGATGACGGCGAGGCGGCGGGCGTTCTC	
APH	STR-F	CGGCTGCTCGACCA CGAC	Anderson et al., 2002
	STR-R	GTCCTCGATGTCCCACAG	

values (4, 5, 6, 7, 8, 10, 12) (Strickler et al., 2015). 37% HCl and 10NNaOH were used to adjust the pH of the media. After seven days of incubation at 28 °C, the growth at various pH values was identified visually.

Growth at different NaCl concentrations. The isolates were spot-inoculated on Bennett's medium with different NaCl concentration (2.5%, 5%, 7.5% and 10%) (Moradi et al., 2011). After seven days of incubation at 28 °C, the growth at different NaCl concentrations was identified visually.

Pectinase activity. The pectinase activity was determined in a medium containing 0.5% of pectin (Sigma-Aldrich). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the pectinase activity was identified by the appearance of a clear zone around the colony after pouring plates with a 1% Hexadecytrimethyl-ammonium bromide solution for 30 min (Elbeltagy et al., 2000).

Amylase activity. The amylase activity was determined in a medium with starch (g/L: peptone — 10, KH_2PO_4 — 5, soluble starch — 2, agar — 15; pH 7.4). The isolates were spot-inoculated and after seven days of incubation at 28 °C. After culturing, the agar plates were filled with Lugol's solution and evaluated after 10 minutes. The medium containing starch was colored blue, and the hydrolysis zone remained discolored or colored reddish-brown if the starch was hydrolyzed to dextrins (Egorov, 1995).

Protease activity. The protease activity was determined in Bennett's medium containing 1% milk powder (Fluka). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the protease activity was identified by the appearance of a clear zone around the colony (Egorov, 1995).

Lecithinase activity. The lecithinase activity was determined in a medium containing 10% of the egg yolk emulsion (HiMedia) (g/L: peptone — 10, yeast extract — 5, NaCl — 10, glucose — 5, agar — 15, distilled water — 900 ml; pH 7.4; after autoclaving, 100 ml of egg yolk emulsion was added). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, lecithinase activity was identified by the appearance of a white zone around the colony (Bates & Liu, 1963).

Nitrate reduction. The nitrate reduction was determined in a nitrate medium (g/L: peptone — 5, beef extract — 3, KNO_3— 1, agar — 15; pH 7.2). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the nitrate reduction was identified by the appearance of a pink zone around the colony after pouring plates with a Griess solution (10 g Griess reagent in 100 ml 12% acetic acid) for 30 min (Egorov, 1995).

Cellulase activity. The cellulase activity was determined in Bennett's medium containing 1% of the carboxymethyl cellulose (Sigma-Aldrich). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the cellulase activity was identified by the appearance of a clear zone around the colony after pouring plates with a Gram's iodine solution (Iodine — 0.33 g, Potassium iodide — 0.66%, distilled water —100 ml) for 20 min (Kasana et al., 2008).

Lipase activity. The lipase activity was determined by growing the isolates on a medium containing a lipid (Tween 20, Sigma-Aldrich) as the primary carbon source. The medium was as follows: peptone — 1.0 g, yeast extract — 0.1 g, $CaCl_2 \times H_2O$ — 0.1 g, agar — 18 g, Tween 20 — 10 ml (autoclaved separately from the rest of the medium), distilled water — 990 ml. The presence of the fatty acid crystals around the colony was identified as positive result (Egorov, 1995).

Laccase activity. The laccase activity was determined in Bennett's medium containing 0.01% guaiacol (Sigma-Aldrich). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the laccase activity was identified by the appearance of a brown zone around the colony (Unuofin et al., 2019).

Oxidoreductase activity (decolorisation of Azur B). The oxidoreductase activity was determined in a medium containing Azur B (Sigma-Aldrich) (g/L: peptone — 5, glucose — 20, Azur B — 0.1, agar — 15; pH 7.4). The isolates were spot-inoculated, and after seven days of incubation at 28 °C, the oxidoreductase activity was identified by the appearance of a zone of the decolorization of Azur B around the colony (Levin et al., 2019).

2.4 Screening for antimicrobial activity

The antimicrobial activity screening was performed against *Bacillus subtilis* ATCC 31324, *Staphylococcus*

aureus ATCC 25923, *S. aureus* 120 (Methicillin-resistant *S. aureus* (MRSA)), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* ATCC 29905, *Mycobacterium smegmatis* DSM43268, *Candida albicans* ATCC 885-653, *C. albicans* 12 (MDR clinical strain), phytopathogenic bacteria *P. syringae* IMB 8511, *Pectobacterium carotovorum* IMB 8982, *Agrobacterium tumefaciens* IMB 8628, *Erwinia amylovora* Mi2, and fungi *Fusarium oxysporum* IMB 54201 and *Aspergillus niger* IMB 16706. The strains were obtained from the MCCAP LNU. The actinobacterial isolates were inoculated onto ISP2, DNPM, and NL19 media (Kieser et al., 2000; Axenov-Gribanov et al., 2016) at seven isolates per plate around the perimeter and cultured at 28 °C for 14 days. The test cultures were prepared as follows: overnight cultures of the test microorganisms were adjusted to a concentration of 10^6 cells/ml, and 100 µl of the test culture were added to 5 ml of soft agar and poured onto OM plates with actinobacterial isolates. LB soft agar (g/L: 10 tryptone, 10 NaCl, 5 yeast extract, 7 agar) and Sabouraud soft agar (g/L: 10 peptone, 40 dextrose, 7 agar) were used for bacteria and yeast, respectively. The plates were incubated at 28 °C for yeast and 37 °C for bacteria overnight. The antifungal activity against filamentous fungi was studied as follows. The isolates were inoculated on the above media in eight isolates per plate at a distance of 3–4 cm from the center. After actinobacteria incubation for 3 days at 28 °C, an agar block with a 3-day test culture of the fungus was placed in the center of the plate. The incubation resumed for 5–6 days under the same conditions. The antimicrobial activity was observed with the appearance of zones of the growth inhibition of the test cultures. All the experiments were performed in triplicate.

2.5 Screening of biosynthetic genes

The total DNA of isolates was screened for the presence of biosynthetic genes responsible for the synthesis of type I polyketide synthases (PKS I), type II polyketide synthases (PKS II), nonribosomal peptide synthetases (NRPS), polyene-specific P450 hydroxylase (CYP450), aminoglycoside phosphotransferase (APH), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), and glycopeptide monooxygenase B gene (oxyB). The PCR amplification of these genes was performed using the primers listed in Table 1. The PCR reaction was performed in a total volume of 50 µl, as mentioned above. The amplification of the biosynthetic genes was carried out using the following reaction conditions:

PKS I: the template denaturation at 95 °C (5 min), followed by 40 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 10 min.

PKS II: the template denaturation at 95 °C (5 min), followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 10 min.

NRPS: the template denaturation at 95 °C (5 min), followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 59 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 5 min.

CYP450: the template denaturation at 95 °C (5 min), followed by 30 cycles of denaturing at 95 °C for 40 s, annealing at 65 °C for 60 s, and extension at 72 °C for 30 s. Final extension step at 72 °C for 10 min.

APH: the template denaturation at 95 °C (5 min), followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 10 min.

HMG-CoA: the template denaturation at 95 °C (5 min), followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 62 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 7 min.

OxyB: the template denaturation at 95 °C (5 min), followed by 35 cycles of denaturing at 95 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 2 min. Final extension step at 72 °C for 5 min.

The presence of biosynthetic genes was determined based on the appearance of amplicons of appropriate sizes following the visualization of the PCR products in agarose gel. The PCR products were visualized as described above.

3 Results

3.1 Isolation and phylogenetic characterization of actinobacteria

In this study, 23 actinobacteria-like isolates selected by morphological characteristics (the shape and color

of colonies, the formation of substrate and aerial mycelium, and the formation of soluble pigments) were isolated (Fig. 1). The largest number of actinobacteria-like colonies was isolated after treatment of the sample with 1.5% phenol solution — 17 (74%). Only three isolates of the actinobacteria were isolated after direct inoculation, and another three from the sample were kept at 100 °C for one hour (Table 2).

The 16S rRNA gene sequence-based phylogenetic analysis showed that all the isolated bacteria belong to the class *Actinobacteria*. Four genera: *Streptomyces* (7 isolates), *Micromonospora* (14 isolates), *Kribbella* (1 isolate), and *Micrococcus* (1 isolate) were identified. The analysis of the phylogenetic trees demonstrates that isolates form tight monophyletic groups with representatives of the respective genera (Fig. 2).

3.2 Determination of cultural properties of the actinobacterial isolates

The growth of isolates was estimated in a wide range of temperatures. All the actinobacterial isolates grew well in the temperature range of 28–37 °C. Among the studied isolates, eight psychrotrophic grew well in the range of 4–37 °C, and seven grew at 45 °C. The actinobacterial isolates grew well on media with pH from 6 to 10. Two isolates, namely *Streptomyces* sp. Psp 67-13 and Psp 67-15, grew in a wide range of pH values (4–12) and temperatures (4–45 °C). Most isolates were sensitive to increased concentrations of NaCl. Half of them did not grow in the presence of 2.5% NaCl. Only four isolates grew at 7.5% NaCl.

The actinobacterial isolates produced a wide range of hydrolytic enzymes (Table 3). All isolates hydrolyzed carboxymethylcellulose, and most of them, except four isolates of the genus *Micromonospora*, produced amylase. More than half of the isolates hydrolyzed casein, and almost half of the isolates reduced nitrate. Only five streptomycetes showed lecithinase activity. All isolates of the genus *Streptomyces*, half isolates of the genus *Misromonospora*, and *Kribbella* sp. Psp 67-2 produced lipase. Five of the 23 isolates produced pectinase. The *Streptomyces* sp. Psp 67-03 showed all these enzymatic activities. The actinobacterial isolates positive for laccase and oxidoreductase activity screenings were not detected.

3.3 Antimicrobial activity

All the actinobacterial isolates were screened for their ability to produce antimicrobial compounds against gram-positive, gram-negative bacteria and fungi, including MDR clinical strains, and also against phytopathogenic microorganisms. The isolates were cultured on three different media (NL19, DNPM, and ISP2) to detect antibiotic activity. Almost 80% of actinobacterial isolates demonstrated antagonistic ac-

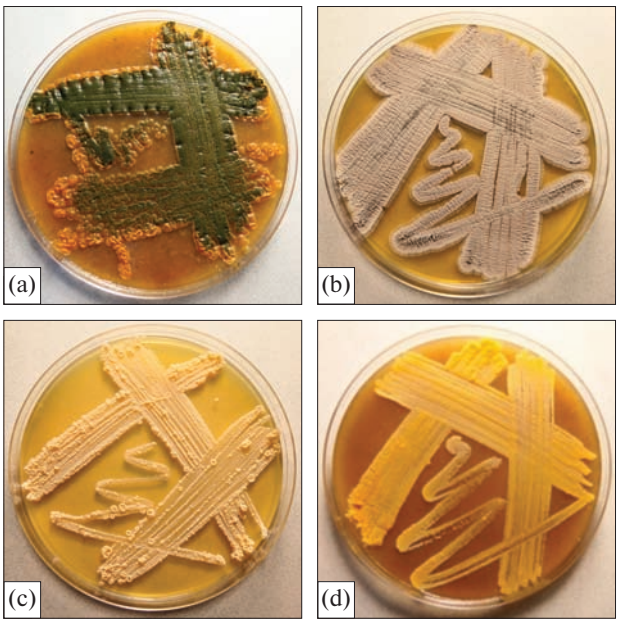

Figure 1. Selected actinobacteria isolates associated with *Polytrichum strictum*: (a) — *Micromonospora* sp. Psp 67-23, (b) — *Streptomyces* sp. Psp 67-03, (c) — *Kribbella* sp. Psp 67-02, (d) — *Micrococcus* sp. Psp 67-29

Table 2. The number of actinobacteria-like isolates associated with *Polytrichum strictum*

Medium	Pretreatment		
	Direct inoculation	Phenol 1.5%	100 °C, 1h
OM	3	8	1
ISP4	0	3	1
HVA	0	4	1
SA	0	2	0
Total	**3**	**17**	**3**

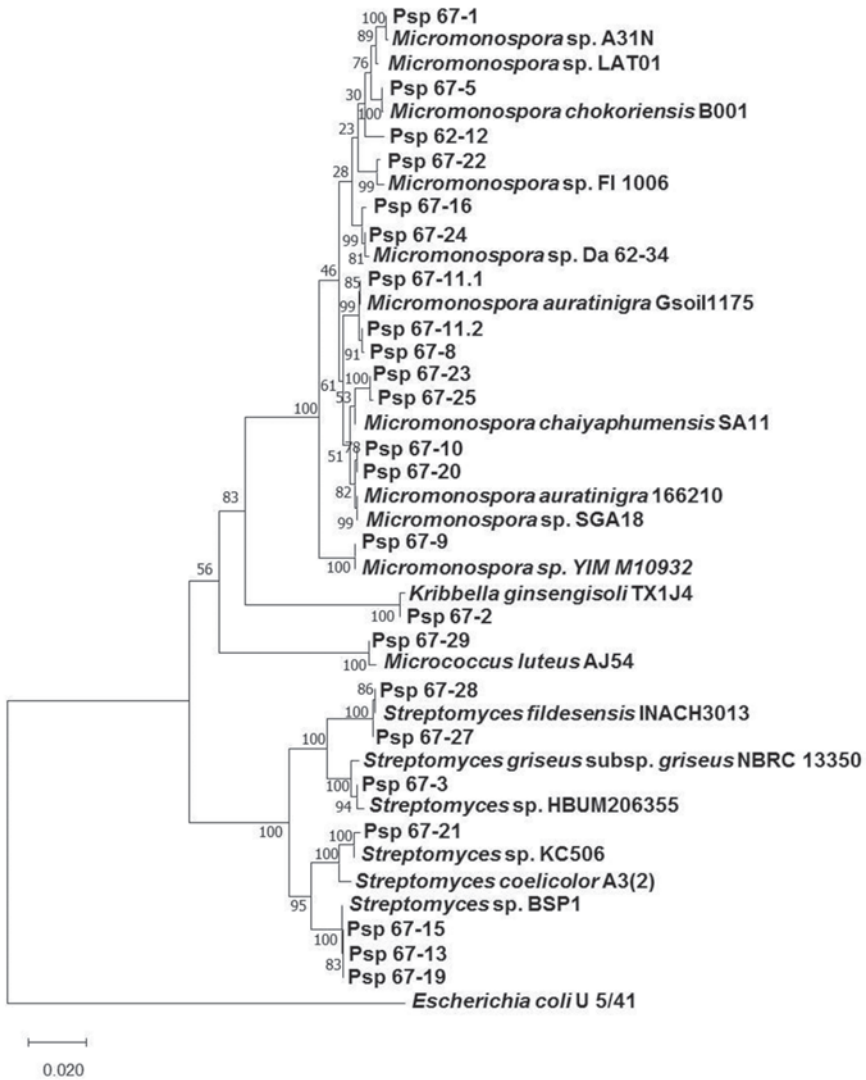

Figure 2. The 16S rRNA phylogenetic tree, showing the evolutionary relationship (the neighbor-joining algorithm) between the actinobacteria isolates associated with *Polytrichum strictum*, their closest homologs, and several typical members of the respective genera. The 16S rRNA gene sequence of *Escherichia coli* strain U5/41 was used as a root to construct a phylogenetic tree

tivity against at least one of the test cultures (Table 4). Six isolates inhibited the growth of gram-positive bacteria *B. subtilis*, *S. aureus*, and *M. smegmatis*. Only one isolate Psp 67-03, in addition to inhibiting a typical strain of the staphylococci, also inhibited the growth of MRSA. No antagonists against gram-negative clinical bacteria were detected. Three isolates of streptomycetes (Psp 67-13, Psp 67-15, Psp 67-19) demonstrated antimicrobial activity against typical and MDR strains of the *C. albicans*.

Some antagonists of phytopathogenic bacteria were also found. Among them, the isolates Psp 67-03 and Psp 67-27 inhibited the growth of *A. tumefaciens* and *P. carotovorum,* respectively. In turn, the three isolates (*Micrococcus sp*. Psp 67-29, *Streptomyces sp.* Psp 67-19, and *Micromonospora sp*. Psp 67-11.1) were antagonists of

the *E. amylovora*. More than half of the actinobacterial isolates demonstrated antifungal activity against phytopathogenic filamentous fungi, and most of them inhibited the growth of both *F. oxysporum* and *A. niger*. The isolate Psp 67-19 showed the highest spectrum of antimicrobial activity against the used pathogens.

3.4 Screening of biosynthetic genes

Twenty-three actinobacterial isolates were tested using degenerate PCR primers for the presence of PKS I, PKS II, NRPS, CYP450, APH, HMG-CoA, and oxyB genes (Table 4). PKS I biosynthetic genes were detected in more than 80% of the tested isolates. Almost 70% of the isolates were positive for PKS II and CYP 450 genes screening, while APH genes were detected in 56% of the isolates, and isoprenoid genes in about 40% of the isolates. NRPS biosynthetic genes were identified in four isolates of the genera *Streptomyces* and *Micromonospora*, and only one isolate Psp 67-02 was positive for oxyB gene screening. Most of the *Streptomyces* sp. isolates as well as *Kribbella sp*. Psp 67-02 and *Micrococcus sp*. Psp 67-29, demonstrated a wider range of the screened biosynthetic genes than members of the genus *Micromonospora*.

Thus, the isolated actinobacteria contain a wide range of biosynthetic genes involved in the synthesis of biologically active compounds. A wider range of biosynthetic genes was found in the genomes of streptomycetes than in other genera. These results coincide with the distribution of the antibiotic activity spectrum of the studied isolates.

Table 3. Enzymatic activities of actinobacteria associated with *Polytrichum strictum*

Genus	Isolate	Pectinolytic	Amylolytic	Protease	Lecithinase	Nitrate reduction	Lipase
Micromonospora sp.	Psp 67-01	+	+	+	–	–	+
	Psp 67-05	–	+	+	–	–	–
	Psp 67-08	–	+	+	–	–	+
	Psp 67-09	–	+	+	–	–	+
	Psp 67-10	–	–	–	–	–	–
	Psp 67-11.1	–	–	–	–	+	–
	Psp 67-11.2	–	–	–	–	+	–
	Psp 67-12	–	+	–	–	+	+
	Psp 67-16	+	+	+	–	+	–
	Psp 67-20	–	–	–	–	–	–
	Psp 67-22	–	+	–	–	–	–
	Psp 67-23	–	+	+	–	–	+
	Psp 67-24	+	+	+	–	+	–
	Psp 67-25	–	+	+	–	–	+
Streptomyces sp.	Psp 67-03	+	+	+	+	+	+
	Psp 67-13	–	+	+	+	–	+
	Psp 67-15	–	+	+	+	–	+
	Psp 67-19	–	+	+	+	–	+
	Psp 67-21	+	+	–	+	–	+
	Psp 67-27	–	+	–	–	+	+
	Psp 67-28	–	+	–	–	+	+
Kribbella sp.	Psp 67-02	–	+	+	–	+	+
Micrococcus sp.	Psp 67-29	–	+	–	–	+	–

Table 4. Antagonistic activity and screening of the biosynthetic genes of the actinobacteria associated with *Polytrichum strictum*

Genus	Isolate	Bs	Sa	MRSA	Ms	Ec	Pa	Prv	Ca	Ca12	Ps	Pc	At	Ea	Fo	An	PKS I	PKS II	NRPS	CYP 450	APH	HMG-CoA	oxyB
Micromonospora sp.	Psp 67-01	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
	Psp 67-05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–
	Psp 67-08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	+	–	–	–
	Psp 67-09	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Psp 67-10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–
	Psp 67-11.1	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
	Psp 67-11.2	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	–	–	–	–
	Psp 67-12	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	+	–	+	–
	Psp 67-16	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	+	+	–	+	+	–	–
	Psp 67-20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–
	Psp 67-22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
	Psp 67-23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–
	Psp 67-24	–	–	–	+	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	–	+	–
	Psp 67-25	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	–	–
	Psp 67-03	–	+	+	–	–	–	–	–	–	–	–	+	–	+	+	–	+	+	–	+	–	–
Streptomyces sp.	Psp 67-13	–	–	–	–	–	–	–	+	+	–	–	–	–	+	+	+	+	–	+	–	+	–
	Psp 67-15	+	–	–	–	–	–	–	+	+	–	–	–	–	+	+	+	+	–	+	+	+	–
	Psp 67-19	+	–	–	+	–	–	–	+	+	–	–	–	+	+	+	+	+	–	+	+	+	–
	Psp 67-21	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	+	+	–	–
	Psp 67-27	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	+	+	+	–
	Psp 67-28	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	+	–
Kribbella sp.	Psp 67-02	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	+	+
Micrococcus sp.	Psp 67-29	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–	+	+	+	–

Note: Bs — *B. subtilis* ATCC 31324, Sa — *S. aureus* ATCC 25923, MRSA — *S. aureus* 120, Ec — *E. coli* ATCC 25922, Pa — *P. aeruginosa* ATCC 9027, Prv — *P. vulgaris* ATCC 29905, Ms — *M. smegmatis* DSM 43286, Ca — *C. albicans* ATCC 885-653, Ca12 — *C. albicans* 12, Ps — *P. syringae* IMB 8511, Pc — *P. carotovorum* IMB 8982, At — *A. tumefaciens* IMB 8628, Ea — *E. amylovora* Mi2, Fo — *F. oxysporum* IMB 54201, An — *Aspergillus niger* IMB 16706.

4 Discussion

The plant rhizosphere is the "home" to various microorganisms, including actinobacteria (Berendsen et al., 2012). Mosses are one of the dominant components of the vegetation in the Antarctic (Parnikoza et al., 2018). The microbial diversity in soils associated with mosses may be greater than in soils free of plants (Kaštovská et al., 2005). However, some similar studies do not confirm this view (Learn-Han et al., 2012), and the problem requires further research. Moreover, so far, there are almost no data on the biotechnological potential of actinobacteria associated with mosses in Antarctica (Learn-Han et al., 2012; Raymond, 2016). This work focuses on the study of the diversity and characterization of actinobacteria associated with *P. strictum*. In our previous work, the biosynthetic potential of Antarctic actinobacteria isolated from the rhizosphere of *Deschampsia antarctica* É. Desv. 1837 (Poaceae) was demonstrated (Tistechok et al., 2019).

Moreover, the spectra of genera of actinobacteria associated with *P. strictum* and *D. antarctica* (unpublished data) are almost indistinguishable. The similarity of the spectra of the genera of actinobacteria between two different samples could be influenced by the same soil composition, as the samples were collected at a short distance from each other, and also the same isolation methods and media were used. This is confirmed by previously published data (Molina-Montenegro et al., 2019).

Most of the actinobacterial isolates belonged to the genus *Micromonospora* (14 strains). This is not typical because the usual dominant genus in populations of soil actinobacteria is *Streptomyces*. Most isolates of the genus *Micromonospora* were isolated after pretreatment with phenol, commonly used to isolate members of this genus (Hayakawa et al., 1991). These actinobacteria are widespread in various ecosystems, including desert soils and Antarctica (Hirsch et al., 2004; Carro et al., 2019). In addition, *Micromonospora* strains are a potential source of bioactive compounds, including important drugs such as aminoglycoside antibiotics (gentamicin, sagamicin, megalomicin, galomycin etc.) (Boumehira et al., 2016).

Seven isolates were affiliated to the genus *Streptomyces*. The genus is widespread in the terrestrial ecosystems, especially in the soils, where it plays a significant role in soil formation. Also they are known as one of the most important sources of biologically active compounds, mainly antibiotics (Chater, 2013).

The isolates of the genera *Micrococcus* and *Kribbella* are also typical members of the soil microbiota. It should be noted that *Micrococcus* spp. are common on animals and human skins. The members of this genus are among the few bacteria that can synthesize long-chain (C21–C34) aliphatic hydrocarbons, which have a great potential in the biochemical industry (Kocur et al., 2006). *Kribbella* is a rare genus of actinobacteria that includes only 33 described species (List of Prokaryotic names with Standing in Nomenclature). This genus of actinobacteria was recently isolated from the rhizosphere of *D. antarctica* on King George Island (Silva et al., 2020).

The analysis of the cultural properties of natural isolates of microorganisms is a part of understanding their physiological characteristics and selecting the optimal conditions for cultivation in the laboratory. The periods of extremely low temperatures could have induced adaptive mechanisms in Antarctic microorganisms, the main of which are the cold-shock proteins, cold-active enzymes, antifreeze enzymes, and polysaccharides, etc. (Raymond, 2016; Muñoz et al., 2017). We found eight psychrotrophic isolates that grew well in the range of 4–37 °C and may be of interest for further study of the mechanisms of their cold resistance.

However, low temperatures are not the only stress factor on Galindez Island. Antarctic soils are characterized by increased salinity which has a negative effect on plant growth and the ecosystem in general (Vasileva-Tonkova et al., 2014). It is known that microorganisms play an important role in reducing the adverse effects of salinity (Yasmin et al., 2020). Among the studied isolates, there were four halophilic strains, the members of the genera *Streptomyces* and *Micromonospora*.These isolates grew in the presence of 7.5% NaCl, and they can be used to further study the mechanisms of the salinity tolerance. Acidity is another crucial characteristic of the soil. The soils of Galindez Island are weakly acidic (pH

4–6) (Parnikoza et al., 2017). This can be caused by the activity of living organisms, e.g., mosses, which actively metabolize Nitrogen (Neina, 2019). Although most isolates grew well in the range of pH 6–10, two isolates (*Streptomyces* sp. Psp 67-13 and Psp 67-15) could grow in a much wider range of pH values (pH 4–12).

The rhizosphere microorganisms play a significant role in the growth and development of plants. A broad range of bioactive compounds (antibiotics, enzymes, phytohormones, etc.) produced by plant-associated actinobacteria can promote plant growth and protection against phytopathogens (Berendsen et al., 2012). These properties cause the formation of close mutualistic interactions in the system "plant-microorganism". Furthermore, actinobacteria can hydrolyze difficult to access substrates and improve the nutrition of the symbiont plants (Sathya et al., 2017). The isolates studied in this work produced cellulase, amylase, caseinase, lipase, nitrate reductase, and pectinase activities. Such enzymatic activity can provide the symbiont plants with available nutrient sources, contributing to their tolerance to extreme environmental conditions. In addition, the enzymes that can be active at low temperatures may be promising for use in food biotechnology (Muñoz et al., 2017). However, this aspect still requires further research.

Antarctic actinobacteria can produce a wide range of antibiotics despite extreme polar conditions, including new compounds (Núñez-Montero & Barrientos, 2018). The composition of the nutrient medium has a significant effect on the production of bioactive secondary metabolites. Because of this, the study of the antimicrobial activities of actinobacteria associated with *P. strictum* was performed on three different media: NL19, DNPM, and ISP2. The approach has been demonstrated to be efficient (Axenov-Gribanov et al., 2016). Streptomycetes produced antimicrobial compounds on all used media. *Micromonospora* spp. and *Kribella* sp. Psp 67-02 in most cases inhibited the growth of some test cultures after cultivation on NL19 and DNPM media. The studied isolates had a significantly smaller spectrum of antimicrobial activity against used pathogens than isolates from more favorable biotopes (Gromyko, 2012). At the same time, most of them inhibited the growth of at least one experimental culture of gram-positive bacterium or yeast. Among the streptomycetes isolates, we found antagonists of MDR clinical strains of *C. albicans* and *S. aureus*, which may be promising for screening of the antistaphylococcal or anticandidal compounds. A limited number of isolates inhibited the growth of phytopathogenic bacteria, while most isolates showed antifungal activity. As mentioned above, mosses predominate in more acidic environments, promoting the growth of filamentous fungi (Rosa et al., 2019). Therefore, it can lead to selection pressure and natural selection of symbiotic/associated actinobacteria that can compete with fungi by producing antifungal compounds.

The screening of the biosynthetic genes involved in synthesizing a wide range of bioactive compounds (e. g. antibiotics) can reveal the biotechnological potential of isolated microorganisms. (Winn et al., 2016). The biosynthetic genes of types I and II PKS, CYP 450, and APH, which are responsible for the production of a significant number of antibiotics with different mechanisms of action, were identified in most of the isolated actinobacteria. Similar findings were reported by Encheva-Malinova et al. (2014). In the genomes of isolates belonging to the genus *Streptomyces* sp. more biosynthetic gene clusters were found than in members of the genus *Micromonospora*, in accordance with the fact that streptomycetes are leaders in the production of antibiotic compounds (Liu et al., 2018). A large number of biosynthetic genes in *Kribbella* sp. Psp 67-2 and *Micrococcus* sp. Psp 67-29 were also identified. However, the presence in the genomes of the studied isolates of many biosynthetic genes does not always correlate with the spectrum of their antimicrobial activities. For example, the isolate *Micromonospora* sp. Psp 67-05 was positive for PKS type I, NRSP, and CYP 450 genes screening but did not inhibit any used test culture. Similar results were obtained with other isolates of the genus *Micromonospora* (Psp 67-20, Psp 67-22, Psp 67-23). In the genome of the isolate *Kribbella* sp Psp. 67-2 all the biosynthetic genes except NRPS were detected, but this strain showed only antifungal activity against phytopathogenic fungi. The absence of correlation between antimicrobial

activity and the presence of biosynthetic genes might have several reasons. The studied biosynthetic genes may be in a cryptic state, or their expression was insufficient to detect antimicrobial activity. Moreover, these biosynthetic genes may be involved in the production of compounds that have no activity against the used test cultures (Tran et al., 2019).

Thus, the presented results complement the existing data on the diversity of actinobacteria in Antarctic biotopes. Furthermore, analysis of enzymatic and antimicrobial activities, as well as a screening of the biosynthetic genes, demonstrated the potential of isolated actinobacteria as producers of a wide range of biologically active compounds.

5 Conclusions

A total of 23 actinobacterial isolates associated with dominant Antarctic species *P. strictum* were isolated. Among them, members of the four genera *Streptomyces* (7 isolates), *Micromonospora* (14 isolates), *Kribbella* (1 isolate), and *Micrococcus* (1 isolate) were identified. Eight psychrotrophic strains from different genera capable of growing in the range of 4—37 °C were isolated. The optimum growth conditions for all isolates were in the range of pH 6-10 and a temperature of 28—37 °C. Some isolates, such as *Streptomyces* sp. Psp 67-13 and Psp 67-15, grew at pH 4-12 and a temperature of 4—45 °C. Four isolates grew in the presence of 7.5% NaCl. A significant number of the isolates showed cellulase, protease, lipase, nitrate reductase, lecithinase, and pectinase activities. Among the studied actinobacteria isolates, six antagonists of gram-positive bacteria, three antagonists of typical and MDR strains of *C. albicans*, and one isolate active against MRSA were found. Some strains were active against phytopathogenic microorganisms, namely three strains against *E. amylovora*, one strain against *A. tumefaciens*, and one strain against *P. carotovorum*. More than half of the isolates showed antifungal activity against *F. oxysporum* and *A. niger*. The biosynthetic genes (PKS I and II, NRPS, CYP450, APH, HMG-CoA, and oxyB) involved in synthesizing a wide range of bioactive compounds were found in more than 80% of isolates.

Antarctic actinobacteria isolated in this study demonstrate potential as producers of a wide range of biologically active compounds. Further research of these actinobacteria may lead to the identification of previously unknown biologically active compounds.

Author contributions. OG conceived and planned the experiment. IP collected the sample and determined the moss species. ST performed the isolation, phylogenetic analysis, and contributed to the final version of the manuscript. IR performed the determination of cultural properties and screening of antimicrobial activity, OA performed the screening of biosynthetic genes. OG analysed the data, designed the tables and figures, and wrote the manuscript with input from IR and OA. VF and AL aided in interpreting the results. All authors read and approved the manuscript.

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

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Виділення і характеристика культурабельних актинобактерій, асоційованих з *Polytrichum strictum* (о. Галіндез, морська Антарктика)

Реферат. Метою роботи є оцінка різноманіття актинобактерій, асоційованих з *Polytrichum strictum* — видом домінантом поширеного антарктичного угруповання торф'янистих мохів, та їхня характеристика як продуцентів біологічно активних речовин. Ізоляти актинобактерій виділяли шляхом прямого висівання та попередньої обробки зразків розчином фенолу та прожарюванням. Культуральні властивості досліджували з використанням діагностичних середовищ. Антимікробну активність вивчали методом подвійних культур. Філогенетичний аналіз базувався на аналізі послідовності гена 16S рРНК. Скринінг біосинтетичних генів здійснювали за допомогою ПЛР з використанням специ-

фічних праймерів. Зі зразків *P. strictum* ізольовано 23 штами актинобактерій 4 родів: *Streptomyces* (7 ізолятів), *Micromonospora* (14 ізолятів), *Kribbella* (1 ізолят) та *Micrococcus* (1 ізолят). Виявлено вісім психротрофних штамів усіх ідентифікованих родів. Оптимум pH середовища для росту становив від 6 до 10. Чотири ізоляти росли в присутності 7,5% NaCl. Значна кількість ізолятів виявляла широкий спектр ферментативних активностей. Серед досліджених ізолятів актинобактерій виявлено антагоністів широкого спектру патогенних мікроорганізмів, у тому числі мультирезистентного штаму *Candida albicans* та метицилін-резистентного штаму *Staphylococcus aureus*. Деякі штами затримували ріст фітопатогенних бактерій: три штами — *Erwinia amylovora*, по одному штаму — *Agrobacterium tumifaciens* і *Pectobacterium carotovorum*. Більше половини ізолятів виявляли антифунгальну активність проти *Fusarium oxysporum* і *Aspergillus niger*. Гени біосинтезу, що беруть участь у синтезі широкого спектру біоактивних сполук, були виявлені у більш ніж 80% ізолятів. Антарктичні актинобактерії, виділені в цьому дослідженні, демонструють потенціал як продуценти широкого спектру біологічно активних сполук. Подальші дослідження цих ізолятів можуть призвести до ідентифікації раніше невідомих біологічно активних сполук.

Ключові слова: антарктичні актинобактерії, *Polytrichum strictum*, антимікробна активність, біосинтетичні гени