

The diversity and antibacterial activity of culturable actinobacteria isolated from the rhizosphere soil of *Deschampsia antarctica* (Galindez Island, Maritime Antarctic)

Item Type	Article
Authors	Tistechok, Stepan;Skvortsova, Maryna;Mytsyk, Yuliia;Fedorenko, Victor;Parnikoza, Ivan;Luzhetskyy, Andriy;Gromyko, Oleksandr
Citation	Polar Biol 44, 1859–1868 (2021). https://doi.org/10.1007/s00300-021-02924-2 .
DOI	10.1007/s00300-021-02924-2
Publisher	Springer
Journal	Polar Biology
Rights	Attribution 4.0 International
Download date	2026-10-09 09:18:27
Item License	http://creativecommons.org/licenses/by/4.0/
Link to Item	http://hdl.handle.net/10033/623073

Stepan Tistechok¹, Maryna Skvortsova¹, Yuliia Mytsyk¹, Victor Fedorenko¹, Ivan Parnikoza^{2,3}, Andriy Luzhetskyy^{4,5},
Oleksandr Gromyko¹

**The diversity and antibacterial activity of culturable actinobacteria isolated from the rhizosphere soil of
Deschampsia antarctica (Galindez Island, Maritime Antarctic)**

¹Department of Genetics and Biotechnology, Ivan Franko National University of Lviv, 75005 Lviv, Ukraine

²Institute of Molecular Biology and Genetics of NAS of Ukraine, 03143 Kyiv, Ukraine

³National Antarctic Scientific Center of Ukraine, 01601 Kyiv, Ukraine

⁴Department of Pharmaceutical Biotechnology, Saarland University, 66123 Saarbruecken, Germany

⁵Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), 66123 Saarbruecken, Germany

Corresponding author: Oleksandr Gromyko, e-mail: oleksandr.gromyko@lnu.edu.ua

ORCID ID:

S. Tistechok <https://orcid.org/0000-0003-2116-746X>

V. Fedorenko <https://orcid.org/0000-0002-7672-1897>

I. Parnikoza <https://orcid.org/0000-0002-0490-8134>

O. Gromyko <https://orcid.org/0000-0002-8107-0128>

Abstract

Antarctic actinobacteria, which can be isolated from both soils and marine sediments, demonstrate a wide range of antimicrobial activities as well as significant biosynthetic potential as the producers of biologically active compounds. However, the actinobacterial diversity of the Antarctic region has not yet been sufficiently studied. The present study sought to examine the diversity and antibacterial activity of culturable actinobacteria isolated from the rhizosphere soil of *Deschampsia antarctica* (É. Desv.), which was collected from Galindez Island, Maritime Antarctic. Among the actinobacteria isolated using a 16S rRNA gene sequence-based phylogenetic analysis process, five genera, namely *Streptomyces*, *Micromonospora*, *Umezawaea*, *Kribbella* and *Micrococcus*, were identified. To the best of our knowledge, this is the first report to describe the isolation and initial characterisation of members of the genus *Umezawaea* from the Antarctic. The isolated actinobacteria were assayed to determine their activity against gram-positive bacteria, gram-negative bacteria and yeast. Among the isolated strains, only 30.2% were able to inhibit the growth of at least one of the tested pathogens. The polymerase chain reaction-based screening of the biosynthetic genes revealed the presence of type I polyketide synthases (65.1%), type II polyketide synthases (25.6%) and nonribosomal peptide synthetases (9.3%) in the actinobacteria strains. The examination of the sensitivity/resistance to antibiotics profile of the actinobacteria strains revealed their high sensitivity in relation to the tested antibiotics. Taken together, the results showed that Antarctic actinobacteria demonstrate potential as the producers of natural bioactive compounds, which means that they represent a valuable prospect for further studies.

Keywords Antarctic actinobacteria, *Deschampsia antarctica*, Galindez Island, *Umezawaea* sp., biosynthetic potential

Declaration

Acknowledgements We thank the 21st Ukrainian Antarctic Expeditionary Group for collecting and transporting samples for the isolation of actinobacteria. In addition, we acknowledge Dr. Leticia Barrientos and the anonymous reviewer of the manuscript.

Funding This study was performed and partially funded under the State Target Scientific and Technical program of the research in Antarctica for 2011—2020 within project: Study of Actinomycetes from *Deschampsia antarctica* È. Desv. rhizosphere, Island Galindez (Antarctica) (contract dated October 1, 2018 № 19) and was supported by grant H/309-2003 from Ministry of Education and Science of Ukraine.

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

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and material Not applicable

Code availability Not applicable

Authors' contributions ST and OG conceived and planned the experiments. IP collected the samples. ST performed phylogenetic analysis; ST, MS and YM performed screening of the antagonistic activity, screening biosynthetic gene clusters and antibiotic sensitivity test. ST and OG processed the experimental data, performed the analysis, drafted the manuscript and designed the tables and figures. VF and AL aided in interpreting the results and worked on the manuscript. All authors read and approved the manuscript.

Introduction

One of nature's reactions to human activities involves the emergence of extremely dangerous forms of pathogenic microorganisms, including multidrug-resistant strains of bacteria or fungi (Tanwar et al. 2014). At the same time, nature represents an inexhaustible source of biologically active compounds, particularly those of microbial origin, which can be used as tools to combat dangerous pathogenic microorganisms (Tringali 2011). However, due to long-term screening studies involving a variety of natural habitats resulting in the detection of numerous already known compounds, the frequency of the detection of new bioactive compounds today is much reduced (Tulp and Bohlin 2005). This has prompted researchers to study the microbial diversity of previously poorly explored extremal biotopes, for example, Antarctica, and to assess the biotechnological potential of extremophiles with regard to the production of new biologically active compounds (Sivalingam et al. 2019).

Antarctica is characterised by extreme conditions, including periods of low temperature, high levels of ultraviolet radiation, nutrient deficiency and water deficit. Under such conditions, it is difficult for living beings to survive in most areas of the continent (Wynn-Williams 1996). Yet, the microbiota of Antarctic habitats thrives, apparently due to the evolutionary physiological adaptations of such organisms. A wide range of cold-shock proteins, cold-active enzymes, antifreeze enzymes and polysaccharides have been found in the cells of Antarctic microorganisms, which provide the microbial cells with resistance to significant temperature changes, etc. (Santiago et al. 2016; Muñoz et al. 2017). In addition, the rivalry in relation to nutrient sources in an environment characterised by high microbial diversity can give rise to unusual metabolic pathways, which can lead to the synthesis of new bioactive compounds, including antibiotics (Núñez-Montero and Barrientos 2018).

The phylum *Actinobacteria* represents one of the largest sources of biologically active compounds, including gram-positive bacteria with a high G+C content (Ventura et al. 2007; Katz and Baltz 2016). Representatives of this phylum exhibit a wide spectrum in terms of both their morphology (from coccoid of *Micrococcus* to the highly differentiated branched mycelia bacteria of the genus *Streptomyces*) and their physiological features or metabolic potential (Yadav et al. 2018). Actinobacteria, particularly the genus *Streptomyces*, are the producers of the largest number of antibiotics known today (Genilloud 2017).

The plant rhizosphere serves as a generous reservoir of microbial diversity. The root exudate, as an accessible food source, facilitates the development of many groups of microorganisms, thereby resulting in increased microbial diversity when compared with non-rhizosphere soil (Berendsen et al. 2012). *Deschampsia antarctica* (É. Desv.) is one of two endemic plants distributed across the coastal zone of the Antarctic islands (Alberdi et al. 2002). Several studies have sought to characterise the actinobacteria associated with *D. antarctica*, which have demonstrated good potential as a source of biologically active compounds (Lamilla et al. 2018; Silva et al. 2020). However, these prior studies have failed to reveal the potential of this microbiome as a site for the isolation of new producers of bioactive compounds.

The present study sought to identify the actinobacteria strains isolated from the rhizosphere soil of *D. antarctica* collected from Galindez Island, Maritime Antarctic. Moreover, it also sought to investigate their potential in relation to the synthesis of antimicrobial agents against gram-positive bacteria, gram-negative bacteria and yeast.

Material and methods

Actinobacteria strains and growth conditions

A total of 43 actinobacteria-like strains were isolated from the rhizosphere soil of *D. antarctica* collected from Galindez Island, Maritime Antarctic (65.245875°S, 64.257505°W) during the 21st Ukrainian Antarctic Expedition in 2017 (Tistechok et al. 2019). These strains were obtained from the Microbial Culture Collection of Antibiotic Producers (MCCAP) of Ivan Franko National University of Lviv (LNU).

Oatmeal medium (OM) (oat flour: 40 g L⁻¹, agar: 16 g L⁻¹, pH 7.2) was used for the cultivation of the actinobacteria strains. For the isolation of the total DNA, liquid tryptic soy broth was used (TSB; Sigma-Aldrich, St. Louis, MO, USA).

16S rRNA gene sequencing and analysis

The strains were cultured in TSB medium for 3–5 days at a temperature of 28 °C and a shaking rate of 180 rpm. The total DNA was isolated by means of the salting-out procedure, as described by Kieser et al. (2000). The amplification of the 16S rRNA gene was carried using the following primers: 8F (5'-AGAGTTTGATYMTGGCTCAG-3') and 1510R (5'-TACGGYTACCTTGTTACGACTT-3'). The polymerase chain reaction (PCR) was performed in a total volume of 50 µl, including 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 10x PCR buffer, 0.5 µl of DNA polymerase (5 U µl⁻¹) and 36.0 µl of Milli-Q laboratory-grade water. The PCR parameters involved 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 then extension at 72 °C for 90 s. A final extension was performed at 72 °C for 10 min. The amplified PCR products of the 16S rRNA were visualised in 1% agarose gel, purified using the QIAquick Gel Extraction Kit (Qiagen, Venlo, Netherlands) and then sequenced with the primers used for amplification by Sanger sequencing method in Eurofins Genomics (GATC Services). The forward and reverse sequences were assembled using Geneious software version 9.1.3 (Kearse et al. 2012).

The analysis of the 16S rRNA gene sequence of the actinobacteria isolates was performed using RDP Release 11 (Wang et al. 2007). The closest related species to the 16S rRNA were identified on the basis of the Basic Local Alignment Search Tool search data available in the National Center for Biotechnology Information's database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and then obtained from the GenBank using Multiple Sequence Comparison by Log-Expectation (Edgar 2004). The phylogenetic trees were constructed using two making algorithms, namely the neighbour-joining (NJ) (Saitou and Nei 1987) and maximum-likelihood (ML) (Felsenstein 1981) algorithms, in MEGA X (Kumar et al. 2018). The evolutionary distances were computed according to the Kimura two-parameter method

(Kimura 1980), while the robustness of the trees topology was evaluated by means of the bootstrap test (1000 replicates) (Felsenstein 1985).

The sequences of the 16S rRNA were deposited in the GenBank, and their identification numbers are presented in the table (Online Resource 1).

Antibiotic sensitivity test

Twelve different standard antibiotic discs (HiMedia, India), namely penicillin G (10 µg), streptomycin (10 µg), gentamicin (10 µg), moxifloxacin (5 µg), tetracycline (30 µg), vancomycin (30 µg), teicoplanin (30 µg), lincomycin (15 µg), erythromycin (15 µg), nystatin (100 iu), rifampicin (5 µg) and itraconazole (30 µg), were used against the isolated actinobacteria in order to check the antibiotic sensitivity pattern on the OM agar medium. The spores of the actinobacteria isolates were washed from the surface of OM agar plates using saline solution (0.9% sodium chloride) and then collected. Some 100 µl of the ten-fold dilution of the spore suspension of the strains were plated on OM agar plates. The antibiotic discs were placed on the plates and then incubated at 28 ± 1 °C for 7 days. All the experiments were performed in triplicate. The antibiotic sensitivity was observed by measuring the inhibition zone diameters (Williams et al. 1989). The actinobacteria isolates were considered to be either sensitive (S; an inhibition zone > 10 mm), intermediate (I; an inhibition zone of 5–9.9 mm) or resistant (R; an inhibition zone of 0.0–4.9 mm) to an antibiotic (Passari et al. 2015).

Screening for antimicrobial activity

The antimicrobial activity screening was performed against *Bacillus subtilis* ATCC3132, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 13883, *Proteus vulgaris* ATCC 29905 and *Candida albicans* ATCC 885-653, which were all obtained from the MCCAP of LNU. The isolated strains were inoculated onto OM agar at seven strains per plate around the perimeter and then 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⁻¹. Then, 100 µl of the cultures were added to 5 ml of soft agar and poured onto OM plates containing actinobacteria isolates. LB soft agar (10 g L⁻¹ tryptone, 10 g L⁻¹ NaCl, 5 g L⁻¹ yeast extract and 7 g L⁻¹ agar) and Sabouraud soft agar (10 g L⁻¹ peptone, 40 g L⁻¹ dextrose and 7 g L⁻¹ agar) were used for bacteria and *C. albicans*, respectively. The plates were incubated overnight at 28 °C for *C. albicans* and 37 °C for bacteria. The antimicrobial activity was observed with the appearance of zones revealing the growth inhibition of the test cultures. All the experiments were performed in triplicate.

Screening of biosynthetic genes

The total DNA of the isolated strains was screened for the presence of the biosynthetic genes responsible for the synthesis of type I polyketide synthases (PKS I), type II polyketide synthases (PKS II) and nonribosomal peptide synthetases (NRPS). The degenerate primers K1F and M6R specifically targeted toward the PKS I ketosynthase (KS) domain and the methylmalonyl transferase domains, the primers A3 and A7R targeted toward the NRPS adenylation domains (Ayuso-Sacido and Genilloud 2005) and the primers KS α and KS β targeted toward the PKS II-conserved sequences in the KS α and KS β domains (Ayuso et al. 2005) were used. They are all listed in Table 1. The PCR reaction was performed in a total volume of 50 µl, as mentioned above. The PCR program included template denaturation at 95 °C (5 min), followed by 40 cycles of denaturing at 95 °C for 30 s, annealing for PKS I, PKS II and NRPS at 55°C, 58 °C and 59 °C, respectively, for 60 s, extension at 72 °C for 2 min, and the completion of the DNA synthesis at 72 °C for 10 min. The presence of biosynthetic genes was determined based on the appearance of amplicons of appropriate sizes following the visualisation of the PCR products in agarose gel. The PCR products were visualised as described above.

Results

Phylogenetic characteristics of the actinobacteria strains

In a previous study, we isolated 43 actinobacteria-like strains (Tistechok et al. 2019). A phylogenetic analysis based on the 16S rRNA gene sequences confirmed that all the strains are representatives of the phylum *Actinobacteria*. Among them, we identified members of five genera, including *Streptomyces* (17 strains), *Umezawaea* (11 strains), *Micromonospora* (9 strains), *Kribbella* (5 strains) and *Micrococcus* (1 strain) (Online Resource 1). The topology of the phylogenetic trees constructed using the NJ (Fig. 1) and ML algorithms demonstrated that the 16S rRNA gene sequences of the isolated strains formed tight clades with the 16S rRNA of several representatives of the respective genera.

Antibiotic sensitivity assay

The actinobacteria isolates were tested to determine their sensitivity to antibiotics with different mechanisms of action (Table 2). All the isolates were sensitive to the aminoglycoside antibiotics (i.e. streptomycin, gentamicin) and quinolone antibiotic moxifloxacin. Moreover, most of the isolates were also sensitive to the macrolide antibiotic erythromycin (95.4%) and the glycopeptide antibiotic teicoplanin (93.0%). One common feature of the studied actinobacteria strains was their nystatin resistance. The sensitivity of the isolates to the rest of the tested antibiotics was generally related to the genus. For example, the strains of the genus *Streptomyces* were resistant to the β -lactam antibiotic penicillin G, except for the strain Da 62-13. Only half of the *Streptomyces* sp. strains exhibited intermediate sensitivity to tetracycline and itraconazole. In terms of the rest of the antibiotics, the bacteria of this genus proved sensitive. All the isolates of the genus *Umezawaea* showed resistance or intermediate sensitivity to itraconazole, while three isolates showed intermediate sensitivity to penicillin G and one isolate showed intermediate sensitivity to rifampicin. With regard to the rest of the antibiotics, the isolates of this genus proved sensitive. All the isolates of the genera *Micromonospora* and *Kribbella* were sensitive to penicillin G, rifampicin and itraconazole. Further, most were resistant to lincomycin. The strains of the genus *Kribbella* were resistant to vancomycin. The strain *Micrococcus* sp. Da 62-3, like all the other isolates, was resistant to penicillin G, in addition to being resistant to vancomycin.

Analysis of antimicrobial activity of the actinobacteria strains

All 43 strains were tested with regard to their antimicrobial activities against gram-positive bacteria, gram-negative bacteria and yeast. Among the 43 strains, only 13 (30.2%) were able to inhibit the growth of at least one of the tested pathogens (Table 2). Most of the strains inhibited the growth of gram-positive bacteria. Seven (16.2%) and eight (18.6%) strains showed antagonistic activity against the gram-positive bacteria *B. subtilis* and *S. aureus* respectively. Among them, six isolates belong to the genus *Streptomyces*, while six isolates belong to the genus *Micromonospora*. Only one strain, namely *Umezawaea* sp. Da 62-2, showed antagonistic activity against the gram-negative *E. coli* bacteria. No antagonists against the other gram-negative bacteria (*P. aeruginosa*, *K. pneumonia* and *P. vulgaris*) and the *C. albicans* were found.

Screening of biosynthetic genes

The screening of the isolated actinobacteria for the presence of the biosynthetic genes of the PKS I, PKS II and NRPS was performed using specific primers. The PKS I were present in 28 (65.1%) out of 43 isolated strains, whereas the PKS II and NRPS were detected in only 11 (25.6%) and 4 (9.3%) strains, respectively (Table 2). Among the isolated strains of the genus *Streptomyces*, 58.8%, 23.5% and 5.8% were positive for the screening of the PKS I, PKS II and NRPS, respectively. In one strain, namely *Streptomyces* sp. Da 62-26, all the studied biosynthetic genes were present. All the members of the genus *Micromonospora* were positive for the PKS I screening, although the PKS II and NRPS were identified in only one strain. Seven of the 11 strains of the genus *Umezawaea* were positive for the PKS type I or

II screening. The strain *Umezawaea* sp. Da 62-1.1 showed positive amplification with the PKS I, PKS II and NRPS primers. Among the isolates of the genus *Kribbella*, three strains were positive for the screening of the PKS I, while one strain (Da 62-41) was positive for the screening of the PKS II and NRPS. No biosynthetic genes were detected in the member of the genus *Micrococcus*.

Discussion

The microbial diversity of the Antarctic has been the subject of research interest for about 120 years (Wynn-Williams et al. 1990); however, the region still has significant potential in relation to the detection of new microorganisms with the potential to produce natural products, including antibiotics (Silva et al. 2020). We focused on studying the diversity and biosynthetic potential of the culturable actinobacteria associated with *D. antarctica* (Galindez Island, Maritime Antarctic). Molina-Montenegro et al. (2019) used a metagenomic analysis to demonstrate that *Actinobacteria* are the dominant bacterial phyla in the *D. antarctica* rhizosphere. It is clear that these bacteria play an important role in plants' adaptation to the conditions that characterise the Antarctica, since they produce a wide range of phytostimulants (Tistechok et al. 2019). The actinobacteria isolated from the rhizosphere soil of this plant were found to be represented by five genera: *Streptomyces*, *Umezawaea*, *Micromonospora*, *Kribbella* and *Micrococcus*. All the selected strains were grouped into two major clades. All the *Streptomyces* sp. strains fell under one clade, while the *Umezawaea* sp., *Micromonospora* sp., *Kribbella* sp. and *Micrococcus* sp. strains were grouped together and so formed another large clade. Among the isolated strains, the rare actinobacteria of the genus *Umezawaea* (family *Pseudonocardiaceae*) were most closely related to the genus *Micromonospora* (family *Micromonosporaceae*) (Fig. 1), which is consistent with the findings of prior studies (Sen et al. 2014). The largest group of isolated strains was affiliated with the genus *Streptomyces* (40%), although most species of this genus are widespread in terrestrial ecosystems, especially in soils, where they play an important ecological role as soil-forming microorganisms (Goodfellow and Williams 1983).

The second largest group contained the strains of the genus *Umezawaea* (26%). To date, more than 20 genera of actinobacteria have been isolated from various environmental niches in the Antarctic (O'Brien et al. 2004; Lo Giudice et al. 2007; Lee et al. 2012; Cheah et al. 2015; Tomova et al. 2015; Lavin et al. 2016). However, this is the first report concerning the isolation of actinobacteria of the genus *Umezawaea* from Antarctic environments. Previously, only two type strains of this genus have been described. As a result of the reclassification of the strain *Saccharothrix tangerinus* MK27-91F2(T) using a polyphase taxonomic approach, the first species of the genus *Umezawaea*, the *U. tangerina* strain MK27-91F2(T) (Labeda and Kroppenstedt 2007), and the endophytic strain *U. endofitica* (Chu et al. 2015) were described. Additionally, an analysis of the 16S rRNA sequences revealed that several isolates were affiliated with this genus (Liu et al. 2017). Interestingly, all the isolates of this genus were morphologically different. Consequently, there is a high probability that among these strains of the genus *Umezawaea* sp., new species of this genus can be found (which is the subject of our on-going research). Moreover, among the isolates, five strains of the genus *Kribbella* sp. were identified. More than 30 species of this genus have been isolated from different environments (<https://www.bacterio.net/genus/kribbella>). However, in Antarctic habitats, this genus of actinobacteria has only recently been isolated (Silva et al. 2020). A significant number of rare actinobacteria genera (Fig. 2) associated with *D. antarctica* are not typical and so do not correlate with prior studies (Yadav et al. 2018). This fact increases the likelihood of identifying rare genera of actinobacteria, which is important because they are considered to be a valuable source of new secondary metabolites (Tiwari and Gupta 2013).

Information concerning the spectrum of antibiotic resistance is valuable when seeking to comprehensively study the properties of natural isolates, especially rare genera, as potential sources of new antibiotic resistance genes. Equally

important is the fact that antibiotic resistance genes can be associated with antibiotic synthesis clusters (Mak et al. 2014). However, there are many antibiotic resistance mechanisms, and antibiotic resistance genes can be found both inside antibiotic synthesis gene clusters and outside of them. Thus, information concerning different mechanisms of action of antibiotic resistance can prove useful in terms of predicting the classes of antibiotics capable of producing these strains. Yet, it is important to note that strains resistant to glycopeptide antibiotics (e.g. Da 62-69, Da 62-21, Da 62-22, Da 62-61 and Da 62-3) may not produce them, as such resistance to glycopeptide antibiotics can often be inducible (Peterson and Kaur 2018). In turn, the lack of detected antimicrobial activity among these strains indicates that these clusters are most likely not expressed under standard conditions and so require a certain stress factor (Tomm et al. 2019).

As mentioned above, actinobacteria are considered interesting as potential producers of biologically active natural compounds, including antibiotics. These bacteria are able to produce from 30 to 50 secondary metabolites with a wide range of biological activities, including antimicrobial activities (Baltz 2019). The Antarctic actinobacteria have shown great potential as producers of bioactive natural products, despite the adverse environmental conditions they face (lack of moisture, lack of nutrients and extreme temperature changes) (Liu et al. 2013). The ability to inhibit the growth of gram-negative and gram-positive bacteria, as well as the growth of fungi, has been described in relation to different genera of actinobacteria (Núñez-Montero and Barrientos 2018). Members of the genera *Streptomyces* and *Micromonospora* were found to be antagonists of only gram-positive bacteria. Only one isolate of the genus *Umezawaea*, namely Da 62-2, inhibited *E. coli*. The other isolates did not demonstrate antagonistic activity. The production of secondary metabolites by actinobacteria depends on the available nutrient sources (carbon, nitrogen, metal ions, etc.) and the growing conditions (Ruiz et al. 2010; Souagui et al. 2019). For the screening of antibacterial activity, an OM medium was used. It is clear that the use of different media to culture strains will expand the range of antagonists among the isolated strains with regard to pathogenic microorganisms. The identification of genes that may indicate the presence of biosynthetic systems (i.e. PKS and NRPS) within the studied strains may confirm this assumption, as it is known that these biosynthetic systems may be responsible for the synthesis of a wide range of bioactive compounds (Hertweck et al. 2007; Winn et al. 2016). However, to confirm the role played by the identified biosynthetic systems in the production of antimicrobial compounds, the structures of those secondary metabolites capable of producing isolated actinobacteria strains will need to be identified.

The PCR screening of biosynthetic gene clusters for the major classes of biologically active compounds (e.g. antibiotics) represents one of the methods available for evaluating the biotechnological potential of microorganisms (Minowa et al. 2007). Most of the screened strains showed the presence of PKS and NRPS biosynthetic genes, many of which also exhibited antimicrobial activity against microbial pathogens. Similar findings were reported by Encheva-Malinova et al. (2014), although in this work these genes were only screened among the isolates of the genus *Streptomyces*. Nonetheless, the strains *Streptomyces* sp. Da 62-11 and Da 62-15 that were negative for the PKS I, PKS II and NRPS biosynthetic genes also demonstrated antimicrobial activity. However, the absence of the PKS and NRPS biosynthetic genes does not reduce the antagonistic activity of the isolates, which means that the compounds produced by these strains are probably synthesised by means of other biosynthetic mechanisms (Liu et al. 2016). Similarly, the strains were positive for PKS I, PKS II, or NRPS amplification (e.g. Da 62-1.1, Da 62-41, etc.), although no antimicrobial activity was detected. This is unsurprisingly, as either a significant number of biosynthetic gene clusters are not expressed in laboratory conditions (Rutledge and Challis 2015) or our strains produced compounds without activity against the tested pathogenic strains. Most recently, Rego et al. (2020) assessed the diversity of biosynthetic genes (PKS and NRPS) in Maxwell Bay, Antarctica. The results of their study, coupled with our results, demonstrate the potential of some Antarctic areas as sources of possible producers of biologically active compounds.

In summary, the results obtained in the present study complement the existing data concerning the diversity and antibacterial activity of culturable Antarctic actinobacteria. A high number of the strains isolated from the *D. antarctica* rhizosphere belong to rare actinobacteria of the genus *Umezawaea*, which has not been isolated from other environmental niches in Antarctica, thereby indicating that Antarctic vascular plants form their own unique microflora (Molina-Montenegro et al. 2019). The combined screening of antibacterial activity and genes encoding the biosynthetic genes (PKS and NRPS) in the isolated strains reveals their biosynthetic potential as producers of natural bioactive compounds. However, the results presented in this work are not conclusive, which means that further studies are required. In particular, it is necessary to confirm our assumption that new species have emerged among the isolates of the genus *Umezawaea*. It is also important to determine which antibiotic resistance genes are also present in the genomes of the isolated actinobacteria, especially in the rare genera. Additionally, it is necessary to evaluate the spectrum and biological activity of the metabolites that produce these rare actinobacteria.

REFERENCE

1. Alberdi M, Bravo L, Gutie'rrrez A et al (2002) Ecophysiology of Antarctic vascular plants. *Physiol Plant* 115:479–486. <https://doi.org/10.1034/j.1399-3054.2002.1150401.x>
2. Ayuso A, Clark D, González I et al (2005) A novel actinomycete strain de-replication approach based on the diversity of polyketide synthase and nonribosomal peptide synthetase biosynthetic pathways. *Appl Microbiol Biotechnol* 67:795–806. <https://doi.org/10.1007/s00253-004-1828-7>
3. Ayuso-Sacido A, Genilloud O (2005) New PCR Primers for the Screening of NRPS and PKS-I Systems in Actinomycetes: Detection and Distribution of These Biosynthetic Gene Sequences in Major Taxonomic Groups. *Microb Ecol* 49:10–24. <https://doi.org/10.1007/s00248-004-0249-6>
4. Baltz RH (2019) Natural product drug discovery in the genomic era: realities, conjectures, misconceptions, and opportunities. *J Ind Microbiol Biotechnol* 46:281–299. <https://doi.org/10.1007/s10295-018-2115-4>
5. Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* 17(8):478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>
6. Cheah Y, Lee LH, Chieng CYC, Wong VLCM (2015) Isolation, identification and screening of actinobacteria in volcanic soil of deception island (the Antarctic) for antimicrobial metabolites. *Polish Polar Res* 36:67–78.
7. Chu X, Liu B-B, Gao R et al (2015) *Umezawaea endophytica* sp. nov., isolated from tobacco root samples. *Antonie Van Leeuwenhoek* 108:667–672. <https://doi.org/10.1007/s10482-015-0522-3>
8. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>
9. Encheva-Malinova M, Stoyanova M, Avramova H et al (2014) Antibacterial potential of streptomycete strains from Antarctic soils. *Biotechnol Biotechnol Equip* 28:721—727. <https://doi.org/10.1080/13102818.2014.947066>
10. Felsenstein J (1981) Evolutionary trees from DNA sequences: a Maximum Likelihood Approach. *J Mol Evol* 17:368–376. <https://doi.org/10.1007/BF01734359>
11. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
12. Genilloud O (2017) Actinomycetes: still a source of novel antibiotics. *Nat Prod Rep* 34:1203–1232. <https://doi.org/10.1039/c7np00026j>
13. Goodfellow M, Williams S (1983) Ecology of actinomycetes. *Annu Rev Microbiol* 37:189–216. <https://doi.org/10.1146/annurev.mi.37.100183.001201>
14. Hertweck C, Luzhetskyy A, Rebets Y, Bechthold A (2007) Type II polyketide synthases: Gaining a deeper insight into enzymatic teamwork. *Nat Prod Rep* 24:162–190. <https://doi.org/10.1039/b507395m>
15. Katz L, Baltz RH (2016) Natural product discovery: Past, present, and future. *J Ind Microbiol Biotechnol* 43:155–176. <https://doi.org/10.1007/s10295-015-1723-5>
16. Kearse M, Moir R, Wilson A et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649. <https://doi.org/10.1093/bioinformatics/bts199>
17. Kieser B, Buttner M, Charter K, Hopwood B (2000) *Practical Streptomyces genetics*. John Innes Foundation, Norwich

18. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120. <https://doi.org/10.1007/BF01731581>
19. Kumar S, Stecher G, Li M et al (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Bio Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
20. Labeda DP, Kroppenstedt RM (2007) Proposal of *Umezawaea* gen. nov., a new genus of the *Actinosynnemataceae* related to *Saccharothrix*, and transfer of *Saccharothrix tangerinus* Kinoshita et al. 2000 as *Umezawaea tangerina* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 57:2758–2761. <https://doi.org/10.1099/ijs.0.64985-0>
21. Lamilla C, Braga D, Castro R et al (2018) *Streptomyces luridus* So3.2 from Antarctic soil as a novel producer of compounds with bioemulsification potential. *PLoS ONE* 13(4):e0196054. <https://doi.org/10.1371/journal.pone.0196054>
22. Lavin PL, Yong ST, Wong CMVL, De Stefano M (2016) Isolation and characterization of Antarctic psychrotroph *Streptomyces* sp. strain INACH3013. *Antarct Sci* 28:433–442. <https://doi.org/10.1017/S0954102016000250>
23. Lee LH, Cheah YK, Sidik SM et al (2012) Molecular characterization of Antarctic actinobacteria and screening for antimicrobial metabolite production. *World J Microbiol Biotechnol* 28:2125–2137. <https://doi.org/10.1007/s11274-012-1018-1>
24. Liu C, Jiang Y, Wang X et al (2017) Diversity, Antimicrobial Activity, and Biosynthetic Potential of Cultivable Actinomycetes Associated with Lichen Symbiosis. *Microb Ecol* 74:570–584. <https://doi.org/10.1007/s00248-017-0972-4>
25. Liu J-T, Lu X, Liu X et al (2013) Bioactive natural products from the antarctic and arctic organisms. *Mini Rev Med Chem* 13:617–626. <https://doi.org/10.2174/1389557511313040013>
26. Liu L, Salam N, Jiao JY et al (2016) Diversity of Culturable Thermophilic Actinobacteria in Hot Springs in Tengchong, China and Studies of their Biosynthetic Gene Profiles. *Microb Ecol* 72:150–162. <https://doi.org/10.1007/s00248-016-0756-2>
27. Lo Giudice A, Bruni V, Michaud L (2007) Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. *J Basic Microbiol* 47:496–505. <https://doi.org/10.1002/jobm.200700227>
28. Mak S, Xu Y, Nodwell JR (2014) The expression of antibiotic resistance genes in antibiotic-producing bacteria. *Mol Microbiol* 93:391–402. <https://doi.org/10.1111/mmi.12689>
29. Minowa Y, Araki M, Kanehisa M (2007) Comprehensive analysis of distinctive polyketide and nonribosomal peptide structural motifs encoded in microbial genomes. *J Mol Biol* 368:1500–1517. <https://doi.org/10.1016/j.jmb.2007.02.099>
30. Molina-Montenegro MA, Ballesteros GI, Castro-Nallar E et al (2019) A first insight into the structure and function of rhizosphere microbiota in Antarctic plants using shotgun metagenomic. *Polar Biol* 42:1825–1835. <https://doi.org/10.1007/s00300-019-02556-7>
31. Muñoz PA, Márquez SL, González-Nilo FD et al (2017) Structure and application of antifreeze proteins from Antarctic bacteria. *Microb Cell Fact* 16:138. <https://doi.org/10.1186/s12934-017-0737-2>
32. Núñez-Montero K, Barrientos L (2018) Advances in Antarctic Research for Antimicrobial Discovery: A Comprehensive Narrative Review of Bacteria from Antarctic Environments as Potential Sources of Novel

- Antibiotic Compounds Against Human Pathogens and Microorganisms of Industrial Importance. *Antibiotics* (Basel) 7:90. <https://doi.org/10.3390/antibiotics7040090>
33. O'Brien A, Sharp R, Russell NJ, Roller S (2004) Antarctic bacteria inhibit growth of food-borne microorganisms at low temperatures. *FEMS Microbiol Ecol* 48:157–167. <https://doi.org/10.1016/j.femsec.2004.01.001>
 34. Passari AK, Mishra VK, Saikia R et al (2015) Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. *Front Microbiol* 6:273. <https://doi.org/10.3389/fmicb.2015.00273>
 35. Peterson E, Kaur P (2018) Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front Microbiol* 9:2928. <https://doi.org/10.3389/fmicb.2018.02928>
 36. Rego A, Sousa AGG, Santos JP, et al (2020) Diversity of Bacterial Biosynthetic Genes in Maritime Antarctica. *Microorganisms* 8(2):279. <https://doi.org/10.3390/microorganisms8020279>
 37. Ruiz B, Chavez A, Forero A et al (2010) Production of microbial secondary metabolites: Regulation by the carbon source. *Crit Rev Microbiol* 36:146–167. <https://doi.org/10.3109/10408410903489576>
 38. Rutledge P, Challis G (2015) Discovery of microbial natural products by activation of silent biosynthetic gene clusters. *Nat Rev Microbiol* 13:509–523. <https://doi.org/10.1038/nrmicro3496>
 39. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
 40. Santiago M, Ramírez-Sarmiento CA, Zamora RA, Parra LP (2016). Discovery, Molecular Mechanisms, and Industrial Applications of Cold-Active Enzymes. *Front Microbiol* 7:1408. <https://doi.org/10.3389/fmicb.2016.01408>
 41. Sen A, Daubin V, Abrouk D et al (2014) Phylogeny of the class *Actinobacteria* revisited in the light of complete genomes. The orders '*Frankiales*' and *Micrococcales* should be split into coherent entities: proposal of *Frankiales* ord. nov., *Geodermatophilales* ord. nov., *Acidothermates* ord. nov. and *Nakamurellales* ord. nov. *Int J Syst Evol Microbiol* 64(Pt 11):3821–3832. <https://doi.org/10.1099/ij.s.0.063966-0>
 42. Silva LJ, Crevelin EJ, Souza DT et al (2020) Actinobacteria from Antarctica as a source for anticancer discovery. *Sci Rep* 10:13870. <https://doi.org/10.1038/s41598-020-69786-2>
 43. Sivalingam P, Hong K, Pote J, Prabakar K (2019) Extreme Environment Streptomyces: Potential Sources for New Antibacterial and Anticancer Drug Leads?. *Int J Microbiol* 2019:5283948. <https://doi.org/10.1155/2019/5283948>
 44. Souagui S, Djoudi W, Boudries H et al (2019) Modeling and Statistical Optimization of Culture Conditions for Improvement of Antifungal Compounds Production by *Streptomyces albidoflavus* S19 Strain of Wastewater Origin. *Antiinfect Agents* 17:39–49. <https://doi.org/10.2174/2211352516666180813102424>
 45. Tanwar J, Das S, Fatima Z, Hameed S (2014) Multidrug resistance: an emerging crisis. *Interdiscip Perspect Infect Dis*. <https://doi.org/10.1155/2014/541340>
 46. Tistechok S, Skvortsova M, Luzhetskyy A et al (2019) Antagonistic and Plant Growth Promoting Properties of Actinomycetes from Rhizosphere *Deschampsia antarctica* É. Desv. (Galindez Island, Antarctica). *Ukrainian Antarctic Journal* 18:169–177. [https://doi.org/10.33275/1727-7485.1\(18\).2019.140](https://doi.org/10.33275/1727-7485.1(18).2019.140)

47. Tiwari K, Gupta RK (2013) Diversity and isolation of rare actinomycetes: an overview. *Crit Rev Microbiol* 39:256–294. <https://doi.org/10.3109/1040841X.2012.709819>
48. Tomm HA, Ucciferri L, Ross AC (2019) Advances in microbial culturing conditions to activate silent biosynthetic gene clusters for novel metabolite production. *J Ind Microbiol Biotechnol* 46:1381–1400. <https://doi.org/10.1007/s10295-019-02198-y>
49. Tomova I, Stoilova-Disheva M, Lazarkevich I, Vasileva-Tonkova E (2015) Antimicrobial activity and resistance to heavy metals and antibiotics of heterotrophic bacteria isolated from sediment and soil samples collected from two Antarctic islands. *Frontiers in Life Science* 8:348–357. <https://doi.org/10.1080/21553769.2015.1044130>
50. Tringali C (2011) *Bioactive Compounds from Natural Sources: Natural Products as Lead Compounds in Drug Discovery* (2nd ed.). CRC Press, Boca Raton
51. Tulp M, Bohlin L (2005) Rediscovery of known natural compounds: nuisance or goldmine?. *Bioorg Med Chem* 13:5274–5282. <https://doi.org/10.1016/j.bmc.2005.05.067>
52. Ventura M, Canchaya C, Tauch A et al (2007) Genomics of *Actinobacteria*: Tracing the Evolutionary History of an Ancient Phylum. *Microbiol Mol Biol Rev* 71(3):495-548. <https://doi.org/10.1128/MMBR.00005-07>
53. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>
54. Williams ST, Sharpe ME, Holt JG (1989) *Bergey's Manual of Systematic Bacteriology*, Vol. 4. MD: Williams and Wilkins, Baltimore
55. Winn M, Fyans JK, Zhuo Y, Micklefield J (2016) Recent advances in engineering nonribosomal peptide assembly lines. *Nat Prod Rep* 33:317–347. <https://doi.org/10.1039/c5np00099h>
56. Wynn-Williams DD (1990) Ecological aspects of Antarctic microbiology. *Advances in Microbial Ecology* 11:71–146. https://doi.org/10.1007/978-1-4684-7612-5_3
57. Wynn-Williams DD (1996) Response of pioneer soil microalgal colonists to environmental change in Antarctica. *Microb Ecol* 31:177–188. <https://doi.org/10.1007/BF00167863>
58. Yadav AN, Verma P, Kumar S, et al (2018) Actinobacteria from Rhizosphere: Molecular Diversity, Distributions, and Potential Biotechnological Applications. In: Singh BP, Gupta VK, Passari AK (eds) *New and Future Developments in Microbial Biotechnology and Bioengineering: Actinobacteria: Diversity and Biotechnological Applications*. Elsevier, Amsterdam, pp 13–41

Table 1 Primers used for screening the biosynthetic genes

Type	Primer name	Primer (5' - 3')	Expected product, bp	Reference
PKS I	K1F	TSAAGTCSAACATCGGBCA	1200 – 1400	Ayuso-Sacido and Genilloud 2005
	M6R	CGCAGGTTSCSGTACCAGTA		
PKS II	KS α	TSGRCTACRTCAACGGSCACGG	700-800	Ayuso et al. 2005
	KS β	TACSAGTCSWTCGCCTGGTTC		
NRPS	A3F	GCSTACSYSATSTACACSTCSGG	700	Ayuso-Sacido and Genilloud 2005
	A7R	SASGTCVCCSGTSGCGTAS		

Abbreviations: PKS I – type I polyketide synthases, PKS II – type II polyketide synthases and NRPS – nonribosomal peptide synthetases

Table 2 Antimicrobial activity, antibiotic sensitivity pattern and presence of the biosynthetic genes of the actinobacteria isolated from the rhizosphere soil of *Deschampsia antarctica*

Genus	Strain No	Antimicrobial activity (AI)								Antibiotic sensitivity										Biosynthetic genes			
		Bs	Sa	Ec	Pa	Kp	Pv	Ca	P	S	G	M	Te	V	Ti	L	E	N	R	I	PKS I	PKS II	NRPS
Streptomyces sp.	Da62-7	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-11	-	+	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-12	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-13	-	-	-	-	-	-	-	S	S	S	S	I	S	S	S	S	R	S	S	+	+	-
	Da62-15	-	+	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-16	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	S	-	-	-
	Da62-23	-	+	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	S	+	+	-
	Da62-25	-	+	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	S	+	-	-
	Da62-26	-	+	-	-	-	-	-	I	S	S	S	I	S	S	S	S	R	S	I	+	+	+
	Da62-27	-	-	-	-	-	-	-	R	S	S	S	I	S	S	S	S	R	S	S	+	+	-
	Da62-28	+	+	-	-	-	-	-	R	S	S	S	I	S	S	S	S	R	S	R	+	-	-
	Da62-32	-	-	-	-	-	-	-	R	S	S	S	R	S	S	S	S	R	S	S	-	-	-
	Da62-33	-	-	-	-	-	-	-	R	S	S	S	I	S	S	S	S	R	S	S	+	-	-
	Da62-43	-	-	-	-	-	-	-	R	S	S	S	I	S	S	I	S	R	S	R	-	-	-
	Da62-44	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	I	+	-	-
	Da62-45	-	-	-	-	-	-	-	R	S	S	S	I	S	S	S	S	R	S	S	-	-	-
	Da62-47	-	-	-	-	-	-	-	I	S	S	S	I	S	S	S	S	R	S	S	+	-	-
Umezawaea sp.	Da62-1.1	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	I	+	+	+
	Da62-1.2	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-2	-	-	+	-	-	-	-	R	S	S	S	S	S	S	S	S	R	I	R	+	+	-
	Da62-5	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	R	+	-	-
	Da62-6	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	S	R	+	+	-
	Da62-8	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	R	+	+	-
	Da62-37	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-38	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	I	-	-	-
	Da62-39.1	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	R	-	-	-
	Da62-39.2	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	R	+	-	-
	Da62-40	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	R	S	R	+	+	-
	Da62-17	+	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	I	R	+	-	-
	Da62-24	-	-	-	-	-	-	-	R	S	S	S	S	S	S	I	S	R	R	R	+	+	+
	Da62-30	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	I	R	+	-	-
	Da62-31	+	-	-	-	-	-	-	R	S	S	S	S	S	S	R	S	R	I	R	+	-	-
	Da62-34	+	+	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	R	R	+	-	-
	Da62-35	+	-	-	-	-	-	-	R	S	S	S	I	S	S	S	S	R	R	R	+	-	-
Micromonospora sp.	Da62-42	+	+	-	-	-	-	-	R	S	S	S	S	S	S	R	S	R	I	R	+	-	-
	Da62-49	-	-	-	-	-	-	-	R	S	S	S	S	R	I	R	S	R	R	R	+	-	-
	Da62-50	+	-	-	-	-	-	-	R	S	S	S	R	S	S	R	S	R	R	R	+	-	-
	Da62-4	-	-	-	-	-	-	-	R	S	S	S	S	I	S	R	S	R	I	I	-	-	-
	Da62-21	-	-	-	-	-	-	-	R	S	S	S	S	R	I	R	S	R	R	I	+	-	-
	Da62-22	-	-	-	-	-	-	-	R	S	S	S	I	R	I	R	I	R	R	R	+	-	-
	Da62-41	-	-	-	-	-	-	-	R	S	S	S	I	R	S	R	I	I	R	R	-	+	+
Micrococcus sp.	Da62-48	-	-	-	-	-	-	-	R	S	S	S	S	S	S	S	S	R	R	R	+	-	-
	Da62-3	-	-	-	-	-	-	-	R	S	S	S	S	R	S	I	S	R	I	S	-	-	-

1 Bs *B. subtilis*, Sa *S. aureus*, Ec *E. coli*, Pa *P. aeruginosa*, Kp *K. pneumoniae*, Pv *P. vulgaris*, Ca *C. albicans*.

Antimicrobial activity screening:

“+” inhibitory zone is present, “-” no inhibitory zone. 2P penicillin G (10 µg), S streptomycin (10 µg), G gentamicin (10 µg), M moxifloxacin (5 µg), Te tetracycline (30 µg), V vancomycin (30 µg), Ti teicoplanin (30 µg), L lincomycin (15 µg), E erythromycin (15 µg), N nystatin (100 IU), R rifampicin (5 µg), I itraconazole (30 µg). Degree of susceptibility: > 10 mm – sensitive (S); 5.0–9.9 mm – intermediate (I); 0.0–4.9 mm – resistant (R). 3PKS I type I polyketide synthases, PKS II type II polyketide synthases, NRPS non-ribosomal peptide synthetases. Biosynthetic genes screening: “+” PCR product is present, “-” no PCR product

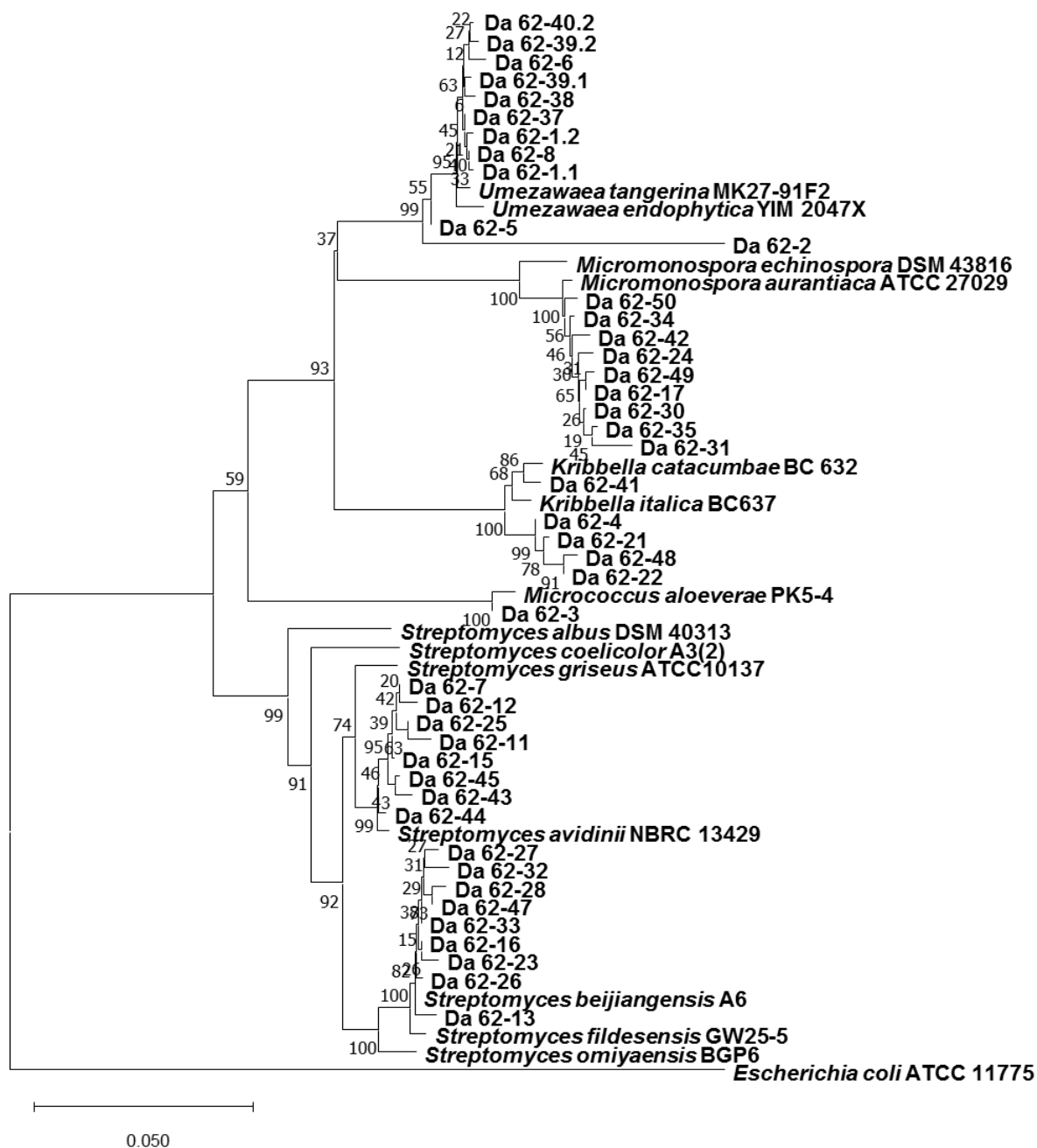


Fig. 1 Neighbour-joining tree derived from 16S rRNA gene sequences (~ 1300 bp) showing the relationship of actinobacteria isolated from the rhizosphere soil of *D. antarctica* collected from the Galindez Island, Maritime Antarctica. *Escherichia coli* ATCC11775

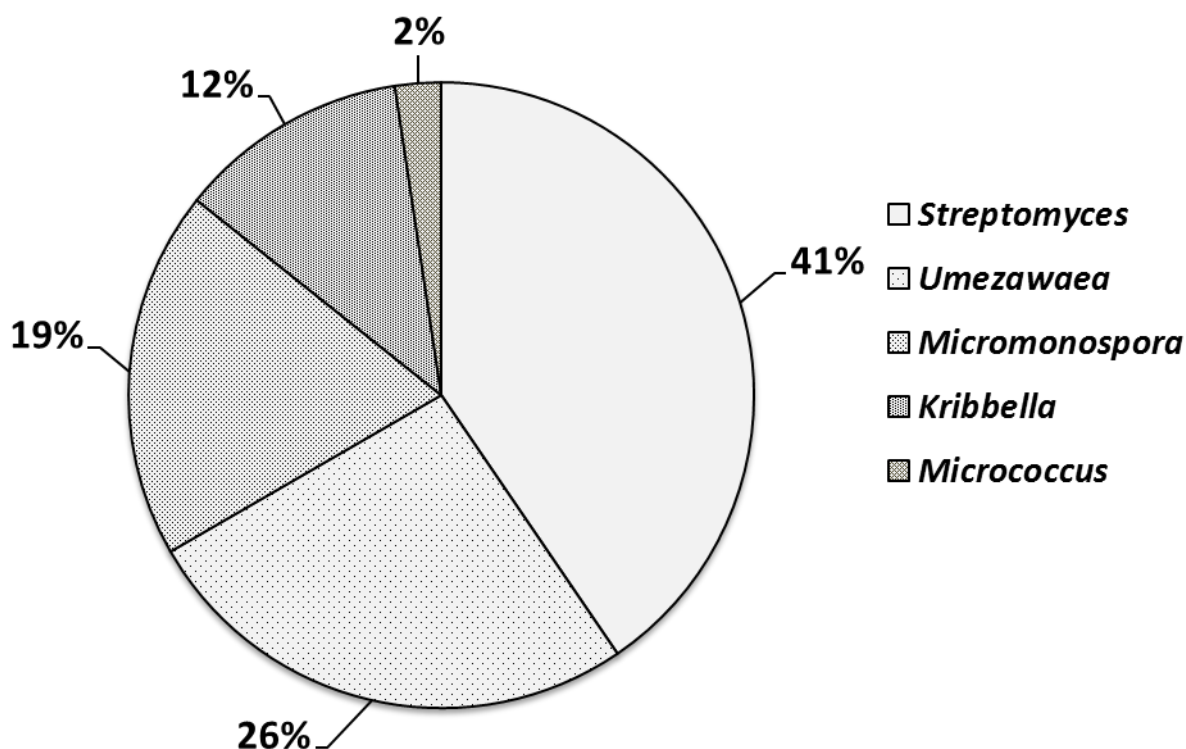


Fig. 2 Distribution of actinobacteria isolated from the rhizosphere soil of *D. antarctica* based on 16S rRNA gene sequences

Table S1 Actinobacteria strains isolated from the rhizosphere soil of *D. antarctica*.

Actinobacterial isolates	Sequence ID	Query score, %	Closest homologue strains, Name and Sequence ID
Umezawaea sp.Da 62-1.1	MN810924	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Umezawaea sp. Da 62-1.2	MN810925	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Umezawaea sp. Da 62-2	MN810926	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Micrococcus sp. Da 62-3	MT662128	100,0	<i>Micrococcus luteus</i> KUK 2-23/ MN826453; <i>Micrococcus yunnanensis</i> SJU9/ MN511766
Kribbella sp.Da 62-4	MN810927	99,0	<i>Kribbella</i> sp. NBRC 104209/ AB447491; <i>Kribbella ginsengisoli</i> Gsoil 001/ NR112567
Umezawaea sp. Da 62-5	MN810928	100,0	<i>Umezawaea tangerina</i> 1w5/ KF554225; <i>Umezawaea tangerina</i> 1g10/ KF554189
Umezawaea sp. Da 62-6	MN810929	100,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Streptomyces sp. Da 62-7	MN810930	99,0	<i>Streptomyces</i> sp. Ats18/ JQ977419; <i>Streptomyces</i> sp. RP-1-4/ MN180864
Umezawaea sp. Da 62-8	MT662129	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Streptomyces sp. Da 62-11	MN810931	99,0	<i>Streptomyces avidinii</i> IHBA 9319/ KR085837; <i>Streptomyces avidinii</i> 173271/ EU570553
Streptomyces sp. Da 62-12	MN810932	98,0	<i>Streptomyces</i> sp. Ats18/ JQ977419; <i>Streptomyces</i> sp. RP-1-4/ MN180864
Streptomyces sp. Da 62-13	MN810933	99,0	<i>Streptomyces</i> sp. A48/ AB856292; <i>Streptomyces</i> sp. Soll14/ AJ308572

Streptomyces sp. Da 62-15	MN810934	100,0	<i>Streptomyces</i> sp. RP-1-1/ MN180861; <i>Streptomyces</i> sp. RP-1-4/ MN180864
Streptomyces sp. Da 62-16	MT662130	100,0	<i>Streptomyces fildesensis</i> CMAA 1654/ MH241009; <i>Streptomyces</i> sp. A48/ AB856292
Micromonospora sp. Da 62-17	MN810935	100,0	<i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263; <i>Micromonospora aurantiaca</i> S 10-1/ MH588238
Kribbella sp. Da 62-21	MN810936	99,0	<i>Kribbella ginsengisoli</i> Gsoil 001/ NR112567; <i>Kribbella</i> sp. NBRC 104209/ AB447491
Kribbella sp. Da 62-22	MN810937	99,0	<i>Kribbella</i> sp. strain VKM Ac-2539/ MT525348; <i>Kribbella</i> sp. NBRC 104209/ AB447491
Streptomyces sp. Da 62-23	MN810938	99,0	<i>Streptomyces</i> sp. A48/ AB856292; <i>Streptomyces</i> sp. Soll14/ AJ308572
Micromonospora sp. Da 62-24	MN810939	99,0	<i>Micromonospora</i> sp. 7K504/ MG770777; <i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263
Streptomyces sp. Da 62-25	MN810940	99,0	<i>Streptomyces</i> sp. RP-1-4/ MN180864; <i>Streptomyces</i> sp. RP-1-1/ MN180861
Streptomyces sp. Da 62-26	MN810941	99,0	<i>Streptomyces</i> sp. A48/ AB856292; <i>Streptomyces</i> <i>fildesensis</i> CMAA 1654
Streptomyces sp. Da 62-27	MN810942	98,0	<i>Streptomyces</i> sp. A48/ AB856292; <i>Streptomyces</i> <i>fildesensis</i> CMAA 1654
Streptomyces sp. Da 62-28	MN810943	99,0	<i>Streptomyces</i> sp. MI-3.3 Y1/ FN550118; <i>Streptomyces</i> sp. A48/ AB856292
Micromonospora sp. Da 62-30	MN810944	100,0	<i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263; <i>Micromonospora aurantiaca</i> S 10-1/ MH588238
Micromonospora sp. Da 62-31	MN810945	99,0	<i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263; <i>Micromonospora aurantiaca</i> S 10-1/ MH588238
Streptomyces sp. Da 62-32	MN810946	99,0	<i>Streptomyces</i> sp. Es23/ JQ977438; <i>Streptomyces</i> sp. Ms36/ JQ977453
Streptomyces sp. Da 62-33	MT662131	100,0	<i>Streptomyces beijiangensis</i> / JX122145; <i>Streptomyces</i> sp. A48/ AB856292
Micromonospora sp. Da 62-34	MN810947	99,0	<i>Micromonospora</i> sp. KK4-8/ LC158512; <i>Micromonospora aurantiaca</i> B017/ KM678241
Micromonospora sp. Da 62-35	MN810948	99,0	<i>Micromonospora aurantiaca</i> R9-550/ JQ660004; <i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263
Umezawaea sp. Da 62-37	MT662132	100,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea tangerina</i> RAMS14/ MK176314
Umezawaea sp. Da 62-38	MN810949	100,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Umezawaea sp. Da 62-39.1	MN810950	100,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea tangerina</i> RAMS14/ MK176314
Umezawaea sp. Da 62-39.2	MN810951	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Umezawaea sp. Da 62-40.2	MN810952	99,0	<i>Umezawaea tangerina</i> MK27-91F2/NR024687; <i>Umezawaea endophytica</i> YIM 2047D/ KP984505
Kribbella sp. Da 62-41	MT662133	100,0	<i>Kribbella sandramycini</i> QT237/ MT072122; <i>Kribbella catacumbae</i> BC 632/ KJ875928
Micromonospora sp. Da 62-42	MN810953	98,0	<i>Micromonospora aurantiaca</i> 110B(2018)/ CP031263; <i>Micromonospora</i> sp. ALFb5/ KF876226
Streptomyces sp. Da 62-43	MN810954	99,0	<i>Streptomyces</i> sp. VTT E-052901/ EF093112; <i>Streptomyces avidinii</i> IHB A 9319/ KR085837
Streptomyces sp. Da 62-44	MN810955	100,0	<i>Streptomyces cirratus</i> IHB B 10402/ KR233768; <i>Streptomyces avidinii</i> IHB B 10281/ KR233763
Streptomyces sp. Da 62-45	MN810956	99,0	<i>Streptomyces</i> sp. MVTs171-2/ EU931084; <i>Streptomyces</i> sp. OSM13/ EU931074
Streptomyces sp. Da 62-47	MN810957	100,0	<i>Streptomyces fildesensis</i> CMAA 1654;

			<i>Streptomyces</i> sp. A48/ AB856292
Kribbella sp. Da 62-48	MN810958	99,0	<i>Kribbella</i> sp. NBRC 104209/ AB447491; <i>Kribbella ginsengisoli</i> Gsoil 001/ NR112567
Micromonospora sp. Da 62-49	MN810959	99,0	<i>Micromonospora aurantiaca</i> R9-550/ JQ660004; <i>Micromonospora aurantiaca</i> S 10-1/ MH588238
Micromonospora sp. Da 62-50	MN810960	99,0	<i>Micromonospora</i> sp. e36/ AY360162; <i>Micromonospora</i> sp. 206112/ EU437818