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

Stepan Tistechok1 · Maryna Skvortsova1 · Yuliia Mytsyk1 · Victor Fedorenko1 · Ivan Parnikoza2,3 · Andriy Luzhetskyy4,5 · Oleksandr Gromyko1

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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 non-ribosomal 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

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 extremet 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′-AGAGTTTGATYMTGGCTC AG-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 10×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 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 was 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 25,922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 13,883, *Proteus vulgaris* ATCC 29,905 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 was 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 non-ribosomal 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 KSα and KSβ targeted toward the PKS II-conserved sequences in the KSα and KSβ 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.

| Type   | Primer name | Primer (5′–3′)                          | Expected product, bp | Reference                        |
|--------|-------------|-----------------------------------------|----------------------|----------------------------------|
| PKS I  | K1F         | TSAAGTCSAACATCGGBCA                     | 1200–1400            | Ayuso-Sacido and Genilloud (2005) |
|        | M6R         | CGCAGGTSCSGTGACAGTA                     |                      |                                  |
| PKS II | KSα         | TSGRCTCACTCAACGGGSCACGG                 | 700–800              | Ayuso et al. (2005)              |
|        | KSβ         | TACSACTCWSCTGCCCTTGTC                  |                      |                                  |
| NRPS   | A3F         | GCSTACYSYSATSTACACSTCGG                 | 700                  | Ayuso-Sacido and Genilloud (2005) |
|        | A7R         | SASGTCCCGTGCCGTSAS                     |                      |                                  |

*PKS I* type I polyketide synthases, *PKS II* type II polyketide synthases and *NRPS* non-ribosomal peptide synthetases.
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 aminoglycosides and tetracycline, and intermediate sensitivity to tetracycline and itraconazole. 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.

All the isolates of the genus *Streptomyces* 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 genus *Umezawaea* were sensitive to the β-lactam antibiotic penicillin G, except for the strain Da 62–13. Only half of the *Umezawaea* strains exhibited intermediate sensitivity to tetracyclines 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 genus *Streptomyces* were sensitive to the β-lactam antibiotic 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*) 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
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 was used as outgroup. Node numbers indicate percent bootstrap support with 1000 resampling. Bar 0.05 substitutions per nucleotide position

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 Antarctic, 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.
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 | Antibiotic sensitivity | Biosynthetic gene |
|------------------|------------|------------------------|------------------------|-------------------|
|                  |            | Bs1 Sa Ec Pa Kp Pv Ca  | P2 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 R S I | + + +          |
|                  | Da62-1.2   | − − − − − − − − − − − | S S S S S S S S R S S | − − −          |
|                  | 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     | − − − − − − − − − − − | S S S S S S S S R S R | + + −          |
|                  | Da62-8     | − − − − − − − − − − − | S S S S S S S S S S R S I | − − −          |
|                  | Da62-37    | − − − − − − − − − − − | S S S S S S S S S S R S I | − − −          |
|                  | Da62-38    | − − − − − − − − − − − | S S S S S S S S S S R S I | − − −          |
|                  | Da62-39.1  | − − − − − − − − − − − | S S S S S S S S S S R S R | − − −          |
|                  | Da62-39.2  | − − − − − − − − − − − | S S S S S S S S S S R S R | + − −          |
|                  | Da62-40    | − − − − − − − − − − − | S S S S S S S S S S R S R | + − −          |
| **Micromonospora sp.** | Da62-17   | + − − − − − − − − − − − | R S S S S S S S S R S 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 S S R I R | + − −          |
|                  | Da62-31    | + − − − − − − − − − − − | R S S S S S S S S S 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 | + − −          |
|                  | Da62-42    | + + − − − − − − − − − − | R S S S S S S S S S S R I R | + − −          |
|                  | Da62-49    | − − − − − − − − − − − | R S S S S R S R S R R R | + − −          |
|                  | Da62-50    | + − − − − − − − − − − − | R S S S R S S S R R R R | + − −          |
| **Kribbella sp**  | Da62-4     | − − − − − − − − − − − | R S S S S S S S 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 R R R | − − −          |
|                  | Da62-48    | − − − − − − − − − − − | R S S S S S S S S R R R R | + − −          |
| **Micrococcus sp.** | Da62-3     | − − − − − − − − − − − | R S S S S S S S S R I S | − − −          |

1*Bs B. subtilis, Sa S. aureus, Ec E. coli, Pa P. aeruginosa, Kp K. pneumoniae, Po P. vulgaris, Ca C. albicans.* Antimicrobial activity screening: “+” inhibitory zone is present, “−” no inhibitory zone. 2*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). 3*PKS 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.
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 *Saccarothrix 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. endophita* (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 ongoing 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 30–50 secondary metabolites with a wide range of biological activities, including antimicrobial activities (Balz 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.

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

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Authors’ contribution 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.

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Declarations

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

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