First record of the endophytic bacteria of *Deschampsia antarctica* Ė. Desv. from two distant localities of the maritime Antarctic

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Abstract

Endophytic bacteria, recognized for their beneficial effects on plant development and adaptation, can facilitate the survival of Antarctic plants in severe environments. Here we studied endophytes of the vascular plant *Deschampsia antarctica* Ė. Desv. from two distantly located regions in the maritime Antarctic: King George Island (South Shetland Islands) and Galindez Island (Argentine Islands). Bacterial group-specific PCR indicated presence of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes, Cytophaga-Flavobacteria* and *Actinobacteria* in root and leaf endosphere of *D. antarctica* sampled at four distinct sites of both locations. The diversity of endophytic bacteria was significantly higher in the leaves compared to the roots in plants from Galindez Island. Similarly, the diversity of endophytes was higher in the leaves rather than roots of plants from the King George Island. Twelve bacterial species were isolated from roots of *D. antarctica* of Galindez Island (the Karpaty Ridge and the Meteo Point) and identified by sequencing the 16S rRNA gene. Isolates were dominated by the *Pseudomonas* genus, followed by the genera *Bacillus* and *Micrococcus*. The vast majority of the isolates exhibited cellulase and pectinase activities, however, *Bacillus* spp. expressed neither of them, suggesting lack of genetic flow of these traits in endophytic bacilli in the maritime Antarctic. *Pseudomonas* sp. IMBG305 promoted an increase in the leaf number in most of the treated plant genotypes when compared with non-inoculated plants, and a rapid vegetation period of *D. antarctica* cultured *in vitro*, albeit the length of leaves in the treated plants was significantly lower, and flavonoid content leveled off in all treated plants. *D. antarctica* is known to develop diverse ecotypes with regard to ecological conditions, such as organic input, moisture or wind exposition. The *D. antarctica* phenotype could be extended further through the endophyte colonization, since phenotypic changes were observed in the inoculated *D. antarctica* plants grown *in vitro* in our study. Herewith, endophytes can contribute to plant phenotypic plasticity, potentially beneficial for adaptation of *D. antarctica*.

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Key words: endophytic bacteria, Antarctic hairgrass, Antarctica, plant growth promotion

Introduction

Vast majority of the Antarctic continent surface is continuously covered by ice, besides two percent of the area (Alberdi et al. 2002). Apart from the Antarctic oases, which rarely accumulate snow, core ice-free lands are distributed across the coastal regions of the Western Antarctic Peninsula and proximal rocky islands, being seasonally available for vascular plant colonization and growth. During the wintertime, these ice-free areas including those inhabited by plants are covered by snow. *Deschampsia antarctica* Ė. Desv., an Antarctic hairgrass, is one of two native vascular plants that inhabit this hostile environment (Alberdi et al. 2002, Parnikoza et al. 2011a) and face unfavorable conditions, such as low temperature, thaw-freezing cycles, permanent exposure to UV radiation during the summer season, as well as often deficiency of water in most of habitats (Alberdi et al. 2002, Parnikoza et al. 2011a). Successful colonization and stepwise distribution of the plant indicate presence of special adaptations. Biochemical, cytogenetic and molecular-genetic traits of Antarctic hairgrass have been studied to provide a deeper insight into such adaptations, however, they remain enigmatic (Parnikoza et al. 2011a, b; Ozheredova et al. 2015, Yudakova et al. 2016).

Microorganisms inhabiting the plant interior, endophytes, possess a number of beneficial functions for the host plants (for review *see* e.g. Brader et al. 2014). Endophytes have plant-growth promoting properties and enhance tolerance of plants against biotic and abiotic stresses (Brader et al. 2014, Hardoim et al. 2015). Endophytes are capable to synthesize extracellular polysaccharides, antifreeze and ice-binding proteins, which help plants to overcome the effects of low temperature stress and episodic freezing events. Endophytes are capable of modulating phytohormone levels and stimulating antioxidant activity, crucial in protection of plants from oxidative damage caused by UV and drought stress (Devi et al. 2017). Nutrient acquisition by plants due to the activity of bacterial nitrogenase, enzyme that provides fixation of atmospheric N_2, is another mechanism behind plant growth promotion (Hardoim et al. 2015). Plants can effect the endophytic colonization by specific root exudates and a selective plant defense response (Rosenblueth and Martínez-Romero 2006), which may play a crucial role in improving fitness of Antarctic plants in severe environments.

Endophytes of Antarctic vascular plants have been in the focus of several research groups with a specific emphasis on fungal symbionts (Rosa et al. 2009, Upson et al. 2009, Santiago et al. 2017). Fungal endophytes have positive effects on the Antarctic host plant *Colobanthus quitensis* (Kunth) Bartl. under water deficiency that resulted in lower oxidative stress, higher production of osmoprotective molecules

and increased net photosynthesis (Hereme et al. 2020), enhanced growth and flowering under UV-B (Ramos et al. 2018) and improved growth of *C. quitensis* under salt stress (Molina-Montenegro et al. 2020). Data on bacteria inhabiting tissues of Antarctic plants is scarce (Cid et al. 2017), although the contribution of bacteria to plant adaptation and growth promotion is equally important (Devi et al. 2017, Lally et al. 2017, Tamošiūnė et al. 2018). Generally, plant hosts considerably benefit from an association with bacterial endophytes (Ardanov et al. 2011, Kozyrovska 2013, Khan et al. 2020). Therefore, an advanced understanding on endophytic bacteria and their interaction with Antarctic vascular plants is necessary. Our research was initiated to investigate the correlation between plant phenotypic plasticity and the endophytic community structure. The purpose of the study was to assess the diversity of endophytic communities of *D. antarctica* grown at different localities in the maritime Antarctica, and the influence of selected endophytes on growth of *D. antarctica in vitro* germinated from seeds originating from the Antarctic region. To our best knowledge taxonomic composition, diversity, and plant growth-promoting properties of endophytic bacteria from *D. antarctica* inhabiting the central maritime Antarctic (Argentine Islands) are discussed for the first time in this study.

Material and Methods

Sampling

Sampling of the above and below-ground parts of *D. antarctica* was performed during the austral summer 2014 (19th Ukrainian Antarctic expedition) and 2017/18 (22nd Ukrainian Antarctic expedition) in two regions of the maritime Antarctica: the Point Thomas oasis, King George Island (South Shetland Islands) and in Galindez Island (Argentine Islands) located 400 km to the South from King George Island (Fig. 1a). Three specimens of *D. antarctica* with a near root substrata (leptosols) were collected from each sampling point (Fig. 1b, c), packed in sterile plastic boxes and transported to the laboratory of the Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine (NASU). The sampling points are marked on Fig.1 and described in Table 1.

A PCR-based assessment of endophyte diversity

Roots and leaves of *D. antarctica* were surface-sterilized in 70% ethanol for 1 min. and in 6% calcium hypochlorite for 20 min. followed by washing three times for 5 min. in sterile distilled water. DNA was isolated from the surface-sterilized plant material using Power Plant DNA isolation kit (MoBio Labs, USA). Nucleic acids were quantified and qualified by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wimington, DE). Group-specific bacterial primers were used for the corresponding bacterial DNA amplification (Table 2). All PCR amplifications were carried out using the same PCR mix (final volume 20 μL), which contained 10 pmol of each primer, 20 ng of template DNA, and the BioMix buffer (Neogen, Ukraine). Amplifications were carried out with a Thermal Cycler T-CY (CreaCon Technologies, The Netherlands) equipped with a heated lid. The DNA samples were amplified using the following parameters: initial denaturation at 94°C for 5 min., 35 cycles of 94°C for 45 s, 54-69°C (Table 2) for 45 s and 72°C for 1 min., followed by a final extension at 72°C for 5 min. The PCR products were separated in 2.0% agarose gel.

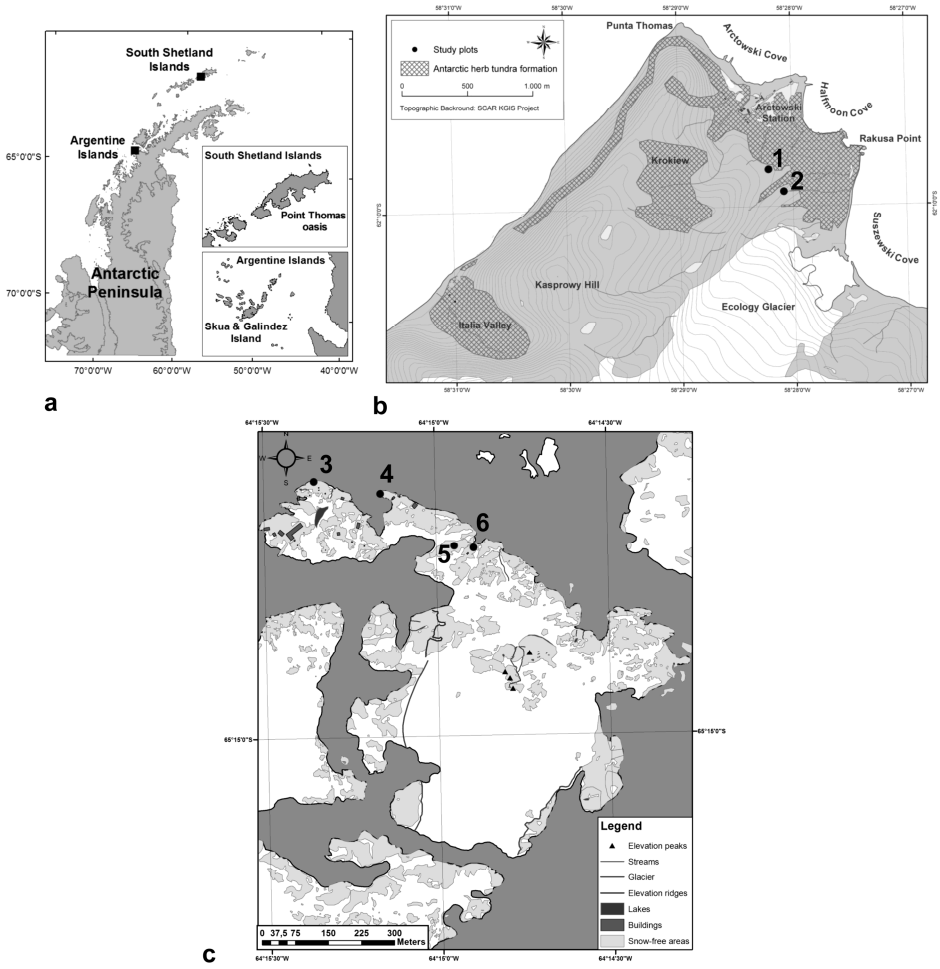

Fig. 1. Sampling regions of *Deschampsia antarctica* Ė. Desv. (a) sampling locations on the Point Thomas, King George Island: 1 – Puchalski grave, 2 – Near Ecology Glacier; (b) and on Galindez Island: 3 – Meteo Point; 4 – Magnit Cape; 5 – Karpaty Ridge; 6 – Cemetry Ridge (c).

Isolation of endophytic bacteria

Endophytic bacteria were isolated from roots of the plants sampled at the Karpaty Ridge and the Meteo Point on Galindez Island (points 5 and 3 on Fig. 1b). For isolation of endophytic bacteria, *D. antarctica* plants were surface-sterilized as described above. The plant material was crushed in a sterile mortar with a pestle, serially diluted and inoculated (0.1 mL) on KB (King et al. 1954), LB (Bertani 1951) and M9 (Miller 1972) agar media and incubated at 10°C for 21 days. Pure cultures of each isolate were initiated on the respective media for DNA isolation, and long-term stock cultures were stored at -80°C.

#	Sample location	Coordinates	*Short description*
1.	King George Island, Point Thomas, Puchalski grave	62° 9.811' S, 58° 28.146' W	On the top of the Puchalski grave, Total Vegetation Cover (TVC) 90%, *D. antarctica* – 50%, *Colobantus quitensis* – 1%, Bryophytes – 10%, *Usnea antarctica* – 5-10%, *Ochrolechia* sp. – 5%, 28 m.a.s.l.
2.	King George Island, Point Thomas near Ecology Glacier	62° 9.989' S, 58° 28.097' W	Area of Glacier periphery with gravel, initial stage of vegetation colonization, TVC 1%, small cover of *D. antarctica* 0.5%, *C. quitensis* – 0.4%, Bryophytes – 0.1%, *D. antarctica*, 50 m a.s.l.
3.	Galindez Island, Meteo Point	65° 14.687' S, 64° 15.348' W	On the rocky coast of the Marina Point near Meteorological station, TVC 1%, *D. antarctica* 0.5%, *Sanionia* sp. 0.5%, gravel, 13 m.a.s.l.
4.	Galindez Island, Magnit Cape	65° 14.704' S, 64° 15.155' W	Top of the coastal rock, TVC 5-25%, *D. antarctica* 4-24%, Bryophytes 1%, on limpet shells, 6 m.a.s.l.
5.	Galindez Island, Karpaty Ridge	65° 14.766' S, 64° 14.951'W	The Karpaty Ridge, N slope of central part of the ridge, *Polytrychium strictum* Bridel moss bank, TVC 80% with incorporation of *Sanionia georgicouncinata* (Müll. Hal.) Ochyra, 65°14.768' S, 64°14.959' W, 17 m.a.s.l.
6.	Galindez Island, Cemetry Ridge	65° 14.770' S, 64° 14.874' W	Top of the Cemetery Ridge near VLF, TVC 5-40%, *D. antarctica* 4-30%, bryophytes 1-10%, limpets, gravel, 17 m.a.s.l.

Table 1. Characteristics of sampled points of *Deschampsia antarctica* E. Desv. on the Point Thomas, King George and Galindez Islands.

Isolation of bacterial DNA, PCR of the 16S rRNA fragment and subsequent phylogenetic analysis of the isolates

Bacterial DNA isolation was performed with innuSPEED bacteria/fungi DNA isolation kit (Analytik Jena AG, Germany) according to manufacturer's instructions. The 16S rRNA gene was amplified using standard primers 27F and 1492R (Fredriksson et al. 2013), which span nearly the full-length 16S rRNA gene (about 1400 bp). The PCR mix (final volume 20 µL) contained 10 pmol of each primer, 20 ng of template DNA, and the BioMix buffer (Neogen, Ukraine). The PCR conditions were as follows: denaturing at 95°C for 5 min., denaturing at 94°C for 45 s, annealing at 56°C for 30 s, and elongation at 72°C for 60 s. The last three steps were

repeated 30 times, and the final elongation was performed at 72°C for 10 min. Amplification was carried out with a Thermal Cycler T-CY (CreaCon Technologies, The Netherlands). The PCR products were sequenced by the Sanger method (Sanger et al. 1977) using Big Dye Terminator Sequencing Standard Kit v3.1 (Applied Biosystems, USA) and 3130 Genetic Analyser (Applied Biosystems). The 16S rDNA sequences were annotated using BLAST (The Basic Local Alignment Search Tool) searches of NCBI (The National Center for Biotechnology Information, USA) Gen Bank's (US National Library of Medicine, Bethesda, Maryland, USA) non-redundant nucleotide database. The sequences were subsequently submitted to GenBank®(NIH) under accession numbers MG916945-MG916956. Sequence alignment and phylogenetic assessment of the isolates based on the 16S rRNA gene was performed in the MEGA X software (Kumar et al. 2018). Phylogenetic assessment of the isolates was done using the Neighbor-Joining method (Saitou and Nei 1987). Statistical significance of the taxa clustering was evaluated by the bootstrap test (500 replicates) (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004).

Target group	Primer: Sequence (5`-3`)	AT, °C	Amplicon size, bp	Reference
Alphaproteobacteria	Alf28f: ARCGAACGCTGGCGGCA Alf684r: TACGAATTTYACCTCTACA	69	674	Mühling et al. 2008
Betaproteobacteria	Beta359f: GGGGAATTTTGGACAATGGG Beta682r: ACGCATTTCACTGCTACACG	63	342	
Gammaproteobacteria	Gamma395f: CMATGCCGCGTGTGTGAA Gamma871r: ACTCCCCAGGCGGTCDACTTA	54	496	
Firmicutes	Firm350f: GGCAGCAGTRGGGAATCTTC Firm814r: ACACYTAGYACTCATCGTTT	57	483	
Actinobacteria	ACT235f: CGCGGCCTATCAGCTTGTTG ACT878r: CCGTACTCCCCAGGCGGGG	54	643	Stach et al. 2003
Cytophaga-Flavobacteria	CF315-F: ACKGGYACTGAGAYACGG CF967-R: GGTAAGGTTCCTCGCGTA	55	382	Chen et al. 2008

Table 2. Summary of group-specific 16S rRNA gene PCR primers, the annealing temperatures used in the PCR reactions and the length of the amplicons.

Enzymatic activity of isolates

The endophytic isolates were examined for cellulolytic and pectinolytic activities by plate assays. The Congo red test was used for the extracellular cellulase activity. Inoculation was carried out by using a platinum needle to transfer the bacterial cells to the center of the plates containing the minimal medium A (Miller 1972) supplemented with 10 $g \cdot L^{-1}$ carboxymethylcellulose and 20 $g \cdot L^{-1}$ agar. The inoculated plates were incubated for 96 h at 20°C, and a 10 mL aliquot of Congo red dye (2.5 $g \cdot L^{-1}$) was then added to each plate. After 15 min., the solution was discarded, and the cultures were washed with 10 mL of 1 M NaCl. Cellulase production was indicated by the appearance of a pale halo with orange edges, indicative of areas of hydrolysis (Wood 1981). To test pectinase activity, bacteria were inoculated on minimal agar with sodium polygalacturonate as described in (Starr et al. 1977) and cultivated 3 days. Production of polygalacturonate degradation enzymes was determined by the ability of colonies to form grooves on the surface of potassium-stabilized polypectate gel as a consequence of polygalacturonic acid degradation.

Effect of Pseudomonas sp. IMBG305 inoculation on performance of D. antarctica

Various genotypes of *D. antarctica* (G/D12-1, G/D12-2a, S22, R35, Y66) (Navrotska et al. 2017) were cultivated in the presence of the bacterial endophyte *Pseudomonas* sp. IMBG305. Plant seeds were collected during the 9^{th}, 11^{th}, 12^{th}, 14^{th}, 17^{th}, and 18^{th} Ukrainian Antarctic expeditions on the Argentine Islands Archipelago (years 2004-2014), germinated, and cultivated *in vitro* at the temperature of 17–19°C and under a 16-h photoperiod. Plants were cultivated *in vitro* for 1–4 years before they were used for the study. *D. antarctica* genotypes G/D12-2a, G/D12-1, R35 and S22 have diploid chromosome number (2n = 26). Plants of the Y66 genotype have a hypotriploid chromosome number 2n = 36–38 and contain a significant number of aneuploid cells and a small percentage of diploid and haploid cells.

Pseudomonas sp. IMBG305 isolated from *D. antarctica* (Meteo Point, Galindez Island), presenting cellulase and pectinase activity, was selected for inoculation. It was grown in the KB medium to the density of 10^8 CFU mL^{-1}. Bacterial cells were washed and suspended in a sterile 0.9% sodium chloride. Tillering nodes (1.5-2.0 cm) were aseptically cut from *in vitro* plants grown for a month, immersed in the bacterial suspension, and incubated at room temperature for 30 min. After the inoculation, tillering nodes were wiped with sterile paper and placed on Gamborg and Eveleigh (B5) medium with naphthylacetic acid (0.01 $mg \cdot L^{-1}$). Plants were incubated at 150 µmol m^{-2} s^{-1} provided by luminescent lamps in a photoperiod of 16/8 hours (illumination/darkness), at a humidity of 70% and a temperature of 18ºC for 8 weeks (Zahrychuk et al. 2012). Tillering nodes, immersed in sterile 0.9% sodium chloride instead of bacterial suspension, were incubated in the same conditions as a control. Number and length of leaves, as well as concentration of flavonoids were measured in experimental and control plants.

Quantification of flavonoids

Leaves were dried at 60ºC in an air circulating oven. Then, 0.25-0.5 g of dry mass was ground with powdered glass, and 10 ml of methanol was added to 0.25 g of plant material and left for 24 h for extraction. Fluorescent probes and controls were prepared for each sample. A fluorescent probe included 3 mL of water, 50 µL of the methanol extract and 0.5 mL of zirconium (IV) oxynitrate (0.2% water solution); control probes included 3.5 mL of water and 50 µL of the extract. Optical density of the probe was measured against the control at the wavelength of 397.6 nm. Concentration of flavonoids was evaluated by the formula:

$$A = (D \times 3.052) \div m,$$

where A – concentration of flavonoids in mg g^{-1} of dry mass analyzed by rutin; D – optical density of the probe; *3.052* – thickness of the cuvette wall (mm); m – mass of dried plant material (mg).

Data analysis

Parameters of inoculated and non-inoculated plants were compared with the Mood median test (Pollard 1982) and Student's t-test (p-value = 0.05).

Results

PCR-based assessment of the endophyte diversity

Results of group-specific PCR that describes taxonomic composition of the endophyte bacterial communities of Antarctic hairgrass are shown in Fig. 2. Bacteria belonging to *Alpha-*, *Beta-*, *Gammaproteobacteria*, *Firmicutes*, *Cytophaga-Flavobacteria* and *Actinobacteria* were found in leaf and root tissue of *D. antarctica*. Composition of bacterial communities in leaves and roots differed in several cases (Fig. 2).

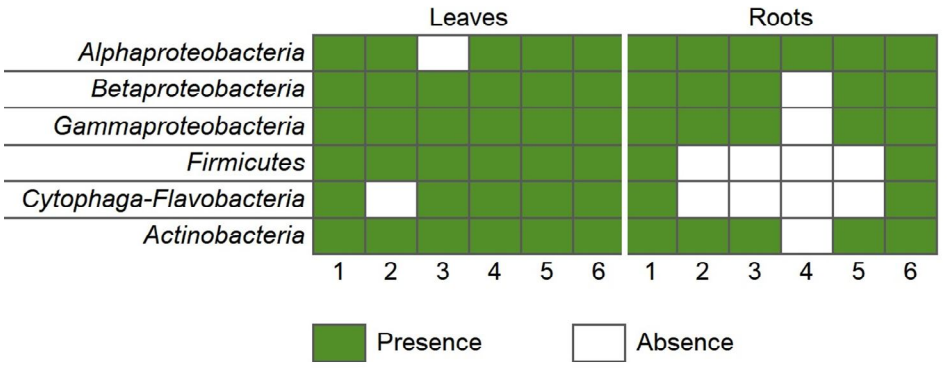

Fig. 2. Taxonomic composition (phylum level) of unculturable endophytic bacteria colonizing leaves and roots of *Deschampsia antarctica* established with group-specific qualitative PCR. 1 – King George Island, Point Thomas, Puchalski grave; 2 – King George Island, Point Thomas, Near Ecology Glacier; 3 – Galindez Island, Meteo Point; 4 – Galindez Island, Magnit Cape; 5 – Galindez Island, Karpaty Ridge; 6 – Galindez Island, Cemetry Ridge.

The diversity of endophytic bacteria at the phylum level was higher in leaves than in roots of the plants from the two sites of Galindez Island (the Karpaty Ridge and the Magnit Cape) and one site of King George Island (the Point Thomas, the Puchalski grave). The difference was especially noticeable in plants from the site of the Magnit Cape (Galindez Island). Homogeneity of endophytic community structure was observed in developed *D. antarctica* coenoses within distant locations, *e.g.*, the plants from King George Island, Point Thomas, Puchalski grave, Galindez Island, and Cemetry Ridge each had similar endophytic communities in both roots and leaves.

Diversity of cultivated endophyte bacteria

Twelve bacterial cultures were isolated from surface-sterilized roots of *D. antarctica* collected from the Karpaty Ridge (isolates IMBG299 - 303) and the Meteo Point (isolates IMBG304 - 310) on Galindez Island (points 3 and 5 in Fig. 1). Nine of the isolates were gram-negative rods belonging to the *Pseudomonas* genus (*Gammaproteobacteria*) based on the 16S rRNA sequence analysis with the closest homologs in the NCBI database. Phylogenetic analyses resulted in classification of the isolate IMBG299 as *P. gramilis*, IMBG301 as *P. asturiensis*, and IMBG307 as *P. rhodesiae* (Fig. 3).

The other isolates were highly similar with more than one known species and can be classified as *Pseudomonas* sp. Isolates IMBG305, IMBG302, IMBG310 had identity of >99.2% with known *Pseudomonas* species. Nevertheless, they formed a separate cluster in the phylogenetic tree, which indicates the higher relatedness between the three strains of bacteria.

Comparison of the nucleotide sequences revealed that the closest homologs of the isolate IMBG304 belong to several species, *Micrococcus luteus, M. yunnanensis* and *M. aloeverae* (*Actinobacteria*). Therefore, the isolate IMBG304 was classified as *Micrococcus* sp. Bacteria belonging to *Bacillus* spp. (*Firmicutes*), are the closest homologs of the isolates IMBG306 and IMBG309. The phylogenetic tree reveals that the IMBG309 forms a cluster with *Bacillus subtilis*, and IMBG306 is similar with *B. stratosphericus*, *B. aerius* and *B. altitudinis*, therefore classified as *Bacillus* sp.

Enzymatic activity of the isolates

The isolates belonging to *Pseudomonas* spp., except for *P. graminis* IMBG299, exhibited the tested enzymatic activities (Table 3). *Pseudomonas* sp. IMBG300, *Pseudomonas* sp. IMBG305, *P. rhodesiae* IMBG307 and *Pseudomonas* sp. IMBG310 both had pectinase and cellulase activities. Gram-positive bacilli isolates had no pectinase or cellulase activities.

Effect of Pseudomonas sp. IMBG305 inoculation on performance of the in vitro D. antarctica

Cultivation of *D. antarctica,* belonging to G/D12-2a, G/D12-1, R35, S22, and Y66 genotypes in the presence of *Pseudomonas* sp. the IMBG305 resulted in significant decrease of leaf length in all genotypes tested (Fig. 4A).

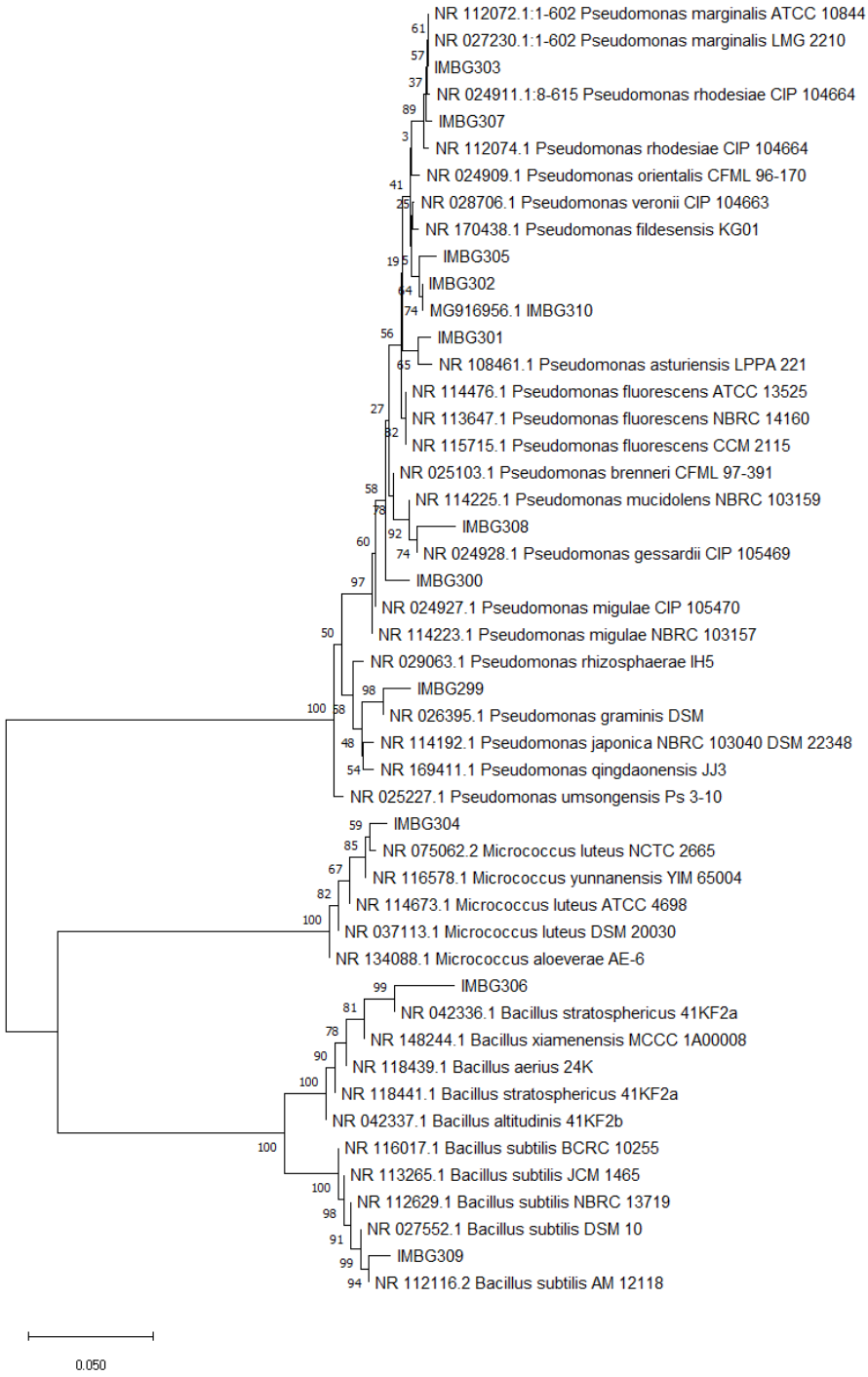

Fig. 3. Phylogenetic tree generated from *endophyte isolates* and their closest homologs according to the NCBI database at sequence similarity threshold of 97%. Bootstrap values are based on 500 replications. The scale bar represents the nucleotide substitution per site.

Endophytic isolate	Enzymatic activity	
	Pectinase	Cellulase
**Pseudomonas graminis* IMBG 299	-	-
**Pseudomonas sp.* IMBG 300	+	+
**Pseudomonas asturiensis* IMBG 301	-	+
**Pseudomonas sp.* IMBG 302	-	+
**Pseudomonas sp.* IMBG 303	+	-
***Micrococcus sp.* IMBG 304	-	-
***Pseudomonas sp.* IMBG 305	+	+
***Bacillus sp.*IMBG 306	-	-
***Pseudomonas rhodesiae* IMBG 307	+	+
***Pseudomonas sp.* IMBG 308	-	-
***Bacillus subtilis* IMBG 309	-	-
***Pseudomonas sp.* IMBG 310	+	+

Table 3. Enzymatic activity of endophytic bacteria isolated from root tissue of *Deschampsia antarctica*: *Galindez Island, Karpaty Ridge; **Galindez Island, Meteo Point.

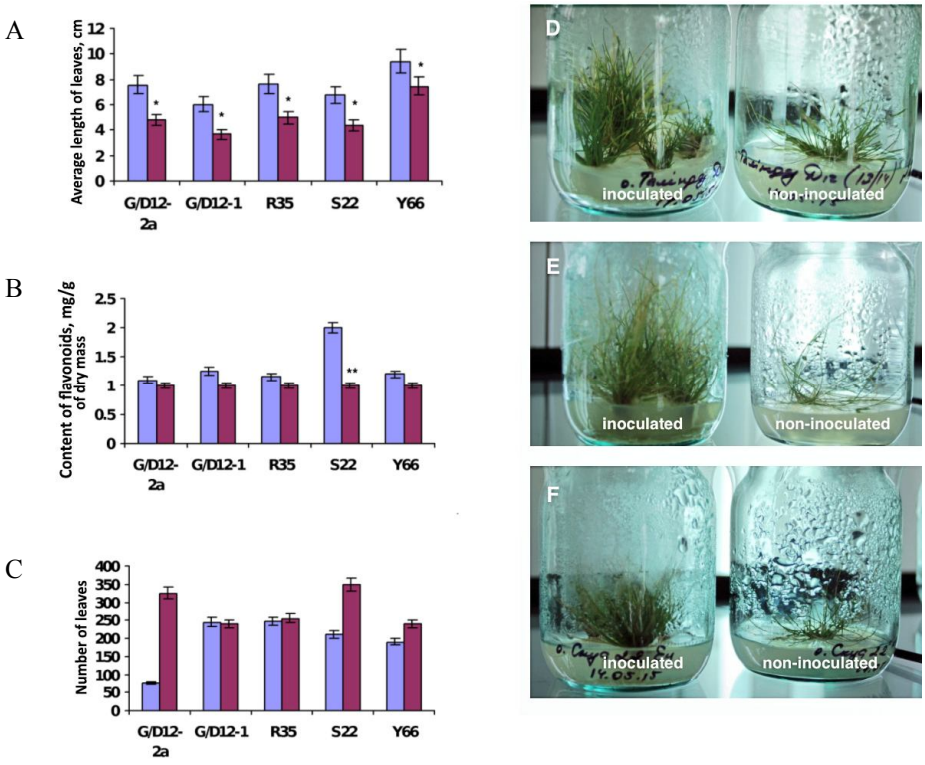

Fig. 4. Effect of *Pseudomonas* sp. IMBG 305 inoculation on performance of genotypes of *D. antarctica*: A - average length of leaves (cm); B - content of flavonoids of dry mass (mg g^{-1}); C - number of leaves; D - growth of inoculated and non-inoculated G/D12-a; E - growth of inoculated and non-inoculated Y66; F - growth of inoculated and non-inoculated S22. * indicates $\Delta dl >$ limit value α=0.05% of χ^2 (chi-square) distribution. ** indicates that p-value derived by t-test is < 0.05.

Paired comparison of leaf length (Δdl) of inoculated and non-inoculated plants by mood median test indicated higher values than the limit value α=0.05% (3.84 cm) of χ^2 (chi-square) distribution in all options of the experiment (Δdl > α=0.05%). The concentration of flavonoids in leaves of all treated plants was on average 1 mg g^{-1} (Fig. 4B) of dry mass with significant decrease in inoculated S22 genotype compared to non-inoculated plants (p-value = 0.0002). The number of leaves per individual in three genotypes (G/D12-2a, S22, Y66) inoculated with the IMBG305 was increased (Fig. 4C-F), which indicates a cushion increase, and a more rapid vegetation period.

Discussion

Severe growing conditions of *D. antarctica* necessitate special adaptations enabling the survival and distribution of this plant species in the Antarctica. Previous studies have not revealed any specific biochemical, cytogenetic or molecular-genetic traits in these plants explaining the endurance (Parnikoza et al. 2011a, b; Ozheredova et al. 2015). Plant adaptation can be achieved by not only their own specific traits, but also by assistance of their plant-associated organisms. In particular, endophytic microbiota that provide plant growth promotion can be crucial for fitness of Antarctic hairgrass in the unfavorable environment.

This study focused on the diversity of endophytic communities of *D. antarctica* growing in the North and Central maritime Antarctica, and their influence on the physiological and growth parameters of *D. antarctica*.

A PCR-based assay revealed that endophytic bacterial communities of *D. antarctica* are diverse and composed of bacteria belonging to *Proteobacteria*, *Firmicutes*, *Cytophaga-Flavobacteria* and *Actinobacteria,* being consistent with findings for other plant species. Dominance of these phyla has been found earlier, for example, in the roots and leaves of *Olea europaea* L. (Müller et al. 2015), *Paeonia sect. Moutan* (Yang et al. 2017), *Senecio vulgaris* L. (Cheng et al. 2019), *Distichlis spicata* L. Greene*, Pluchea absinthioides* (Hook. & Arn.) H. Rob.*, Gaultheria mucronata* (L.f.) Hook. & Arn., and *Hieracium pilosella* L. (Zhang et al. 2019). Similarly, *Proteobacteria* and *Firmicutes* were earlier reported as the dominant component of the plant endosphere (Hardoim et al. 2015, Santoyo et al. 2016). A recent study based on 16S rRNA gene sequence analysis showed the dominance of the phylum *Proteobacteria* and presence of the *Firmicutes* and *Actinobacteria* phyla members (Zhang et al. 2020).

Bacterial communities inhabiting the root tissue of plants collected on the Point Thomas Oasis near Ecology Glacier (King George Island), the Magnit Cape and the Karpaty Ridge (Galindez Island), had lower diversity than those in the leaves. Presence of diverse communities was earlier reported in various plant tissues for fungal (Bayman et al. 1997, Li et al. 2020) and bacterial (Mano et al. 2007) endophytes. Endophytic root communities isolated from *Oryza sativa* L. differed from those derived from leaves and seeds, except for one isolate, which was identified as *Micrococcus luteus* (Mano et al. 2007). Bacterial populations inhabiting roots and leaves of *D. antarctica* can be shaped by diverse environmental factors, affecting each plant organ, and being enriched with new bacteria from diverse sources. The aboveground parts of the Antarctic hairgrass, along with associated microbiota, face low temperatures or temperature fluctuations, intense UV radiation and oxidative stress, while the roots of the plant are less sub-

jected to these factors. Composition of endophytic communities populating each plant organ is influenced by the arrival of new epiphytic bacteria (Mano et al. 2006, Mano and Morisaki 2008). The endophytes colonize root tissues mainly from the rhizosphere, whereas leaves become colonized predominantly by bacteria arriving from the leaf surfaces, resulting in development of distinct bacterial populations in each organ. Moreover, the difference in diversity could be explained by specific conditions in substrates (Zaets and Kozyrovska 2012), *e.g.*, the high content of heavy metals in soils in some localities of the maritime Antarctic was shown to affect diversity and composition of microbial communities (Chong et al. 2009, Gran-Scheuch et al. 2020). Bacterial communities of leaves and roots of *D. antarctica* sampled from the Puchalski grave point (King George Island) and the Cemetry Ridge (Galindez Island) were composed of similar bacteria taxa. However, our qualitative PCR did not allow to identify or quantify individual members of these communities, and the ratio of specific taxa in roots and leafs in these samples can vary. Analysis of endophytic communities of *Arabidopsis thaliana* by 454 pyrosequencing revealed relative abundance of bacterial classes discussed in our study, which varied between root and leaf tissue (Bodenhausen et al. 2013). The relative proportion of *Actinobacteria* was higher in roots (about 30% on average), while *Alpha-* and *Gammaproteobacteria* had higher percentages in leaves (about 28% and 14%). The ratio of bacterial taxa at lower taxonomic levels (families, genera) can vary in different plant organs, as shown for plants growing in Patagonia and Atacama deserts (Zhang et al. 2019). Similarly, distinct community composition was revealed at the order level in root and leaf tissues of grassland plants (Toju et al. 2019).

Diversity of the cultivated endophytes of *D. antarctica* is much lower than the diversity estimated with culture-independent techniques. Among the isolates, only *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria* were found. The genus *Pseudomonas* was found to be dominant among the identified isolates, followed by *Bacillus* and *Micrococcus*. The majority of bacteria (95-99%) inhabiting natural environments, such as soil, water, or plants, is hard or impossible to cultivate due to fastidious or unknown growth requirements (Alain and Querellou 2009). On the other hand, *Pseudomonas* is a widely-distributed bacterial genus that is usually easy to cultivate on common nutrient media. *Pseudomonas* has been frequently found in different Antarctic environments (seawater, freshwater, marine sediments and soils) (Higuera-Llantén et al. 2018, Vásquez-Ponce et al. 2018), which indicates the adaptation of these bacteria to extremely cold environments. *Pseudomonas* was predicted to be present in the endosphere of Antarctic hairgrass plants (Zhang et al. 2020). *Pseudomonas* spp. are often isolated from the endosphere of plants, such as domestic apples (Miliute et al. 2016), *Achyranthes aspera* L. (Devi et al. 2017), *Piper nigrum* L. (Jasim et al. 2013) and a grass species related to Antarctic hairgrass, *Deschampsia flexuosa* (L.) Trin., growing in the subarctic aeolian sand dune area (Poosakkannu et al. 2015). Denaturing gradient gel electrophoresis analysis revealed that members of *Pseudomonadales* (*Pseudomonas* and *Psychrobacter*) and *Rhizobiales* were dominant in the tissues of *D. antarctica* (Cid et al. 2017). Abundance of pseudomonads in endophyte communities has been estimated by metagenomics, as well. High proportion of operational taxonomic units (OTUs) from roots and leaves of *Gaultheria mucronata* belonged to *Pseudomonadaceae* (Zhang et al. 2019). The abundance of reports indicates that members of *Pseudomonas* spp. are frequently involved in plant-microbe interactions.

Plant cell wall-depolymerizing enzyme activities are important for endophytic life-

style, enabling plant colonization by a number of endophytic species (Sessitsch et al. 2012). Among the isolated bacteria in our study, 50% had cellulase activity, 40% had pectinase activity, and 30% of isolates exhibited activity of both enzymes. Cellulase and pectinase activities are likely to facilitate the bacterial penetration into plant tissues or migration from one plant organ to another (James et al. 2002, Reinhold-Hurek et al. 2006). The isolates *B. subtilis* IMBG309 and *Bacillus* sp. IMBG306 exhibited no activities of pectinase or cellulase, although cellulose activity is common for *Bacillus* spp. isolated from various environments (Soares et al. 1999, Gupta et al. 2015), including endophytic *Bacillus* and *Paenibacillus* strains from the medicinal plant *Lonicera japonica* Thunb. (Zhao et al. 2015). Bacteria lacking plant cell wall-depolymerizing enzyme activities could enter the *D. antarctica* endosphere through stomata, lenticels, wounds, areas of emergence of lateral roots, (Huang 1986) or transmit vertically in plant seeds (Hardoim et al. 2015).

Endophytes are known to affect plant performance *via* phytohormone synthesis, increased stress tolerance, and enhanced nutrient acquisition (Devi et al. 2017, Lally et al. 2017). *Pseudomonas* sp. IMBG305 that produces cellulase and pectinase enzymes was chosen for the *in vitro* experiment assessing the effect of growth stimulation on *D. antarctica.* Inoculation of the hairgrass with *Pseudomonas* sp. IMBG305 resulted in higher number of leaves (in 1.3 – 4.4 times higher compared to control), cushion increase, and more rapid vegetation period, albeit the length of leaves in the treated plants was significantly lower (in 1.3 – 1.6 times). *D. antarctica* is known to develop diverse ecotypes in regard of ecological conditions: organic input, moisture or wind exposition (Giełwanowska and Szczuka 2005, Nuzhyna et al. 2019). Phenotypic changes of the inoculated *D. antarctica* grown in the *in vitro* conditions evidence the influence of endophytic bacteria on the plants` phenotype. Phenotypic changes of the inoculated *D. antarctica* plants could have positive influence on plant survival in the Antarctic environment, where vegetation is affected by intense winds and nutrient deprived soils (Convey 1996, Robinson et al. 2003).

As a result of *Pseudomonas* sp. IMBG305 inoculation, the concentration of flavonoids in leaves decreased to the same concentration in all Antarctic hairgrass genotypes. Endophytes are capable of modulating the expression of host genes and consequently affect the production of metabolites, which may improve the fitness in the harsh environment (Jha 2019). Modulation of flavonoid biosynthesis in all studied hairgrass genotypes in our study likely occurred due to inoculation with the endophyte, which is in line with other studies. For example, inoculation of rice (*Oryza sativa*) plants with *Azospirillum* strains resulted in a decrease of flavonoids such as dihydroflavone/dihydroflavonol, a flavone, and an apigenin derivative, whereas quantities of dihydroflavones/dihydroflavonols increased in the root tissue (Chamam et al. 2013). In another study a root hair-associated Antarctic strain of *Pseudomonas* sp. was found, to solubilize phosphates *in vitro* and to promote root development of *D. antarctica* (Berríos et al. 2013).

A change in the secondary metabolite yield as a result of endophyte inoculation has been previously shown in *Hyptis suaveolens* (Jha 2019). Inoculation with endophytes may promote a shift from secondary metabolite production to protein synthesis (Jha 2019). Such shift fits the Protein Competition Model (PCM), which postulates a negative correlation between protein and polyphenol production (Jones and Hartley 1999). The PCM takes into account the competition between protein and phenolic synthesis that may limit common resource: phenylalanine. The tight coordination of protein production and polyphenol metabolism was confirmed by transcriptome analysis on *Arabidopsis thaliana*

under different nitrogen regimes (Scheible et al. 2004). Competition between chlorophyll and epidermal polyphenol in woody plants goes in line with PCM as well (Meyer et al. 2006). Prioritizing production of secondary metabolites over proteins can occur as a result of low nitrogen input that limits the protein synthesis in plants (Affendy et al. 2010), and an improved nutrition can cause the reverse pattern. The decreased flavonoid content in the leaves of *D. antarctica* after inoculation with *Pseudomonas* sp. IMBG305 can be caused by reduced demand for flavonoids resulting from plant-defensive and plant growth-promoting properties of the endophyte.

Conclusion

Bacterial communities derived from surface-sterilized leaf tissues of *D. antarctica* from the Northern and Central maritime Antarctic consisted of five to six bacterial phyla common among endophytic communities. Root communities exhibited a lower diversity with *Proteobacteria* as an obligate member. Cultured endophytes had a lower diversity and were represented mainly by the *Pseudomonas* genus, followed by *Bacillus* and *Micrococcus*. The majority of isolated bacteria produced either cellulose or pectinase, which are important enzymes in endophytic lifestyle. Remarkably, the representatives of *Firmicutes* did not possess cellulase and pectinase activities, in contrast to homologous species in mainland plants. The endophytic *Pseudomonas* sp. IMBG305 changed the phenotype of *D. antarctica* cultured *in vitro*, which can affect plant adaptation to the stressful conditions of the Antarctic.

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