Highly specialized bacterial communities within three distinct rhizocompartments of Antarctic hairgrass (*Deschampsia antarctica* Desv.)

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Abstract

Antarctic hairgrass, *Deschampsia antarctica* Desv. (*Poaceae*) is one of the two flowering plants that have an established presence in Maritime Antarctica. It has adapted to varying edaphic and climatic conditions. *D. antarctica's* associations with soil-dwelling bacteria have long been suspected to add to its remarkable resilience. In this study three compartments within *D. antarctica* root system and soil have been investigated as microbial habitats: the rhizosphere (root-adjacent soil particles), the rhizoplane (root surface) and the endosphere (root interior). For this purpose, a modification for existing methods of bacterial extraction from cryophilic plant rhizocompartments was devised with the temperature sensitivity of the source material in mind. Next-generation targeted 16S rRNA gene amplicon sequencing along with a culture-based approach were employed to explore the bacterial community residing within those three rhizocompartments. Results showed that each of the

compartments housed a distinct bacterial community not only in terms of phylogenetic diversity but also concerning plant-beneficial and adaptive traits. Although the majority of cultivable bacteria displayed plant-growth promoting abilities like rock-phosphate solubilisation and phytohormone production (*Arthrobacter* spp.), some could be potential pathogens (*Clavibacter* sp.). This study highlights the need for amending the still scarce information on the microbiome of Antarctic flora but also gives tools and insight to explore it further.

Key words: Antarctica, bacterial community, *Clavibacter* sp., microbiome, plant growth promotion

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Abstract

Antarctic hairgrass, *Deschampsia antarctica* Desv. (*Poaceae*) is one of the two flowering plants that have an established presence in Maritime Antarctica. It has adapted to varying edaphic and climatic conditions. *D. antarctica's* associations with soil-dwelling bacteria have long been suspected to add to its remarkable resilience. In this study three compartments within *D. antarctica* root system and soil have been investigated as microbial habitats: the rhizosphere (root-adjacent soil particles), the rhizoplane (root surface) and the endosphere (root interior). For this purpose, a modification for existing methods of bacterial extraction from cryophilic plant rhizocompartments was devised with the temperature sensitivity of the source material in mind. Next-generation targeted 16S rRNA gene amplicon sequencing along with a culture-based approach were employed to explore the bacterial community residing within those three rhizocompartments. Results showed that each of the compartments housed a distinct bacterial community not only in terms of phylogenetic diversity but also concerning plant-beneficial and adaptive traits. Although the majority of cultivable bacteria displayed plant-growth promoting abilities like rock-phosphate solubilisation and phytohormone production (*Arthrobacter* spp.), some could be potential pathogens (*Clavibacter* sp.). This study highlights the need for amending the still scarce information on the microbiome of Antarctic flora but also gives tools and insight to explore it further.

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Introduction

Interactions between soil microbes and plants have been studied for over a century (Hartmann et al. 2008). They are considered among the most important factors determining plant health (Turner et al. 2013). The spectrum of those interactions is very wide, ranging from highly beneficial to severely deleterious, mainly for the plant (Ali et al. 2017). The interplay between those two partners takes place mostly in three compartments within the boundaries of root-adjacent soil and the plant root itself (Sasse et al. 2018). One of those compartments, the rhizosphere is the part of the soil that has been altered by root exudates so heavily that its microbial community does no longer resemble that of the bulk soil (Huang et al. 2014). It remains in direct contact with the second rhizocompartment, the rhizoplane which is the root surface. Its role is that of a selective gate to the root interior (Edwards et al. 2015). It consists of microbial cells, bound mostly within a biofilm structure and debris of plant cells, that are constantly sheading from the growing root (Lang et al. 2019). The interior of the root (root endosphere) is the third rhizocompartment and has been proven to house microbial cells, provided they do not trigger the plants' immune system or have ways to counteract its response (Turner et al. 2013). The majority of soil microbes display plant-beneficial rather than disease-causing traits. Among the most important features are growth promotion by nutrient availability increase (rock-bound nutrient solubilisation, nitrogen fixation) and phytohormone production (IAA, gibberellins) but also host defence from abiotic stresses (modulation of stress hormone levels) and negative biotic influences (synthesis of antimicrobials) (Doornbos et al. 2012; Yu et al. 2019).

Although highly important for the majority of plants on the globe, those beneficial interactions seem essential for plants in extreme environments (de Zelicourt et al. 2013). *D. antarctica* Desv. (*Poaceae*), the Antarctic hairgrass is one such plant. It has colonized coastal ice-free areas of the majority of the Antarctic islands and the Antarctic Peninsula (Maritime Antarctic) during the Holocene (Mosyakin et al. 2007). It has adapted to varying soil conditions,

96 growing in sites rich in nutrients (ornithogenic soils) and also in weathered post-glacial deposits
97 of volcanic origin (Znój et al. 2017), where nutrient availability is low and heavy metal
98 concentrations especially high (Park et al. 2012; Romaniuk et al. 2018). Climatic conditions
99 caused the Antarctic hairgrass to become resistant to freezing, drought, intense UV radiation
100 and high salt concentrations (Ruhland and Krna 2010). The secret to the resilience of *D.*
101 *antarctica* has been sought in its morphological, anatomical and biochemical adaptations
102 (Giełwanowska et al. 2005; Olave-Concha et al. 2005; Yudakowa et al. 2016). There were also
103 several attempts to probe its rhizomicrobiome for stress-alleviating traits (Tistechok et al. 2019;
104 Barrios et al. 2013). However, the three aforementioned major rhizocompartments in regards
105 to *D. antarctica* have never been investigated collectively, so the conclusions drawn in previous
106 works explain the phenomenon only in a limited extend.

107 In this paper, we provide a method for extracting microorganisms from three
108 rhizocompartments of cryophilic plants to be used in molecular and classical microbiology
109 analyses. The aim of investigation was to infer the phylogenetic biodiversity of bacteria residing
110 within the three rhizocompartments of *D. antarctica* and to examine the beneficial traits of
111 bacterial isolates derived from each of those compartments. For our hypothesis we assumed
112 that *D. antarctica* rhizosphere, rhizoplane and root endosphere house distinct bacterial
113 communities with each of the groups displaying varying intensities of plant-growth promoting
114 and adaptive features.

115 **Materials and Methods**

116 **Sampling and site description**

117 The study was carried out at the “H. Arctowski” Polish Antarctic Station, located in
118 Admiralty Bay, King George Island, Antarctica (South Shetlands archipelago in Maritime
119 Antarctica). Samples were collected during the austral summer of 2017–2018 at the Point
120 Thomas penguin colony: 62º09'45"S, 58º27'46"W;10 m a.s.l.; 100-120 m from the sea coast.

The site was supplied with water from melting snow patches with washings of penguin guano deposits, thus characterized by large nutrients content, especially nitrogen and potassium compounds (Łachacz et al. 2018).The site was also strongly influenced by salty marine aerosols, moist, with negligible human influence. Fifteen specimens of *D. antarctica* were collected with the root adjacent soil with the use of sterile tools into sterile plastic containers and transported frozen (-20°C) to the laboratory in Institute of Biochemistry and Biophysics, Polish Academy of Sciences (IBB PAS).

Bacterial extraction

Bacterial cells were extracted from the three rhizocompartments of *D. antarctica* with a procedure developed to accommodate psychrophilic microorganisms (Online Resource 1). The procedure was done on 3 *D. antarctica* specimens' roots.

Extraction of rhizosphere soil bacteria

To analyse the microbiome of the root- adjacent soil a sample of the soil was carefully removed from between the roots with a sterile spatula onto a pre-sterilized and pre-weighed aluminium foil piece. Approx. 1 g of the soil was weighted and placed in a 50-mL conical tube containing 20 mL of sterile and cool (4°C) dilution liquid composed of 1% (wv^{-1}) glycerol, Tween 80 (10 ppm) and 0.1% tetrasodium pyrophosphate (further termed GTP). The suspension was then shaken for 30 min in a Tornado^{TM} Vortexer at 2000 oscillation min^{-1} at 4°C. The tubes were then placed in a VWR Ultrasonic Cleaner USC-TH filled with chilled water and sonicated for 60 s. The tubes were vortexed afterwards for 30 s to suspend detached cells. After brief centrifugation (1 min; 126 *g* ; 4°C) the suspension was submitted to further analysis.

Extraction of the root-attached bacteria (rhizoplane bacteria)

To detach the loosely adhering soil from the roots (and leave only the tightly bound bacteria) the root system was washed in 60 mL of sterile PBS solution (8 g NaCl L^{-1}, 2 g KCl

L^{-1}, 2.77 g $Na_2HPO_4 \cdot 12H_2O$ L^{-1}, 1 g KH_2PO_4 L^{-1}) by shaking for 30 min in the aforementioned shaker (1000 oscillation min^{-1}; 4°C) and then rinsed 3 times in 5 mL of sterile and cooled PBS by vortexing. The washed root system was then placed in a 15-mL tube containing 10 mL of GTP liquid and submitted to the above - mentioned procedure (shaking, ultrasonication and vortexing). The root system was then removed from the tube and the remaining liquid containing root-associated bacteria was submitted to further testing.

Extraction of root-dwelling bacteria (endosphere bacteria)

Washed roots were sterilized by incubation in cooled 10% H_2O_2 solution for 5 min, then rinsed 3 times in the GTP diluent. The so surface-sterilized roots were placed in a pre-cooled sterile mortar. 2.5 mL of GTP was added with 0.6 g of sterile, sharp, garnet sand (Lysing Matrix A) and gently ground with a pestle, allowing the sharp angular garnet pieces to comminute the roots to an amorphous pulp. The liquid inside the mortar was used for further analysis.

DNA extraction

Environmental DNA extraction

Rhizosphere soil DNA was extracted using the PowerSoil® DNA isolation kit according to manufacturer protocol. An approx. 0.2 g of soil was used in triplicate. DNA solutions were kept at 4°C for further analysis. The GTP liquid containing rhizoplane bacteria was passed through a sterile 47-mm Whatman polycarbonate filter (0.22-µm pore size). The DNA from the filter-trapped bacteria was extracted using the PowerWater® DNA isolation kit according to manufacturer protocol and kept at 4°C. Root endosphere bacterial DNA was extracted from the comminuted root pulp using the PowerSoil® DNA isolation kit according to manufacturer protocol. DNA solutions were kept at 4°C for further analysis.

Bacterial strain DNA extraction

DNA from pure bacterial strains was extracted from a single colony. The procedure involved suspending the colony with sterile toothpick in 100 µL sterile MiliQ Water containing 5% of Chelex® 100 resin (Sigma) and 5% of garnet sand Lysing Matrix A (MP Biomedicals). The 1.5-mL tube with the mentioned suspension was shaken in a Qiagen Retsch TissueLyserIIfor 5 min at 39Hz – 1800 oscillation min^{-1}. The tubes were then placed in a heating block and heated for 5 min at 99°C. Then the tubes were centrifuged for 3 min at 13000 *g*. 100 µL of the clear supernatant was taken and used for PCR amplifications.

16S rRNA gene fragment amplification and sequencing

The phylogenetic study was performed by sequencing and analysis of prokaryotic 16S ribosomal RNA gene. A fragment of the16S rRNA gene containing the V3 and V4 variable regions was amplified using gene-specific primers: 16S_V3-F and 16S_V4-R positions 341-357F and 785-805R, respectively, according to *Escherichia coli* 16S rRNA gene reference sequence (Klindworth et al. 2013). Illumina Nextera XT overhang adapter nucleotide sequences were included in addition to the 16S rRNA gene-specific sequences, which allowed sample indexing and pooling. Each PCR amplification was done in triplicate using KAPA HiFi PCR kit (Roche) in a final volume of 20 µL per reaction according to the manufacturer's instructions. Obtained PCR products were pooled in equimolar ratio and indexed using Nextera XT barcodes (Illumina, San Diego, USA). Amplicon libraries were pooled and sequenced on Illumina MiSeq instrument (Illumina, San Diego, USA) in the DNA Sequencing and Oligonucleotide Synthesis Laboratory (IBB, PAS). Sequencing was done in paired-end mode (2 × 300 bp) with the use of a v3 (600 cycles) chemistry cartridge which allowed the generation of long paired reads fully covering 16S V3–V4 amplicons.

Amplification of 16S rRNA gene fragment from pure strains was performed using universal primers 27F and 1492R (Lane 1991). PCR amplification reaction conditions were as follows: 1 min of 95°C initial denaturation followed by 30 cycles of 95°C for 15 s, 55°C annealing for 15

s and elongation 72°C for 1 min, using DreamTaq polymerase (Thermo Scientific-Fermentas). Obtained PCR products (~1500 bp for 16S rRNA gene fragment were purified using Exonuclease I/Alkaline phosphatase mix (Thermo Scientific-Fermentas). 16S rRNA gene amplicons were sequenced using internal 16S rRNA gene primers: 341F, 518R and 928F (Weidner et al. 1996) with the use of BigDye Terminator v.3.1 chemistry and ABI3730xl genetic analyser at the DNA Sequencing Laboratory (IBB PAS). Sequencing reads were manually corrected and assembled into contigs using Seqman software (DNAStar).

Culture dependent techniques

Spread plate method

100 µL aliquots of the decimal dilutions of the aforementioned rhizospheric, rhizoplane and endosphere bacteria suspensions were plated on pre-cooled R2A agar plates prepared with 10% Antarctic soil extract (Zdanowski et al. 2013). The plates were incubated at 4°C for 6 weeks to assure complete colony development.

Strain picking and characteristics

40 colonies from plates seeded with a particular material type were picked and purified by re-streaking onto fresh R2A agar plates (120 strains in total). They were subcultured in liquid R3A medium (Reasoner and Geldreich 1985) and frozen in 15% glycerol solution at -80°C. Basic strain characteristics were investigated to place the strains into groups within one material type. Gram reactions were analysed by the non-staining KOH method. Catalase activity was determined by exposing the colonies to a 3% H_2O_2 solution and observation of bubble formation. Oxidase presence was tested with Biomerieux Oxidase solution. Cell morphology was investigated in a light microscope after crystal violet staining.

Phosphate solubilisation

Pure bacterial strains were tested in terms of phosphate solubilizing abilities on the NBRIP medium (Nautiyal 1999) containing per L: 10 g glucose, 5 g Ca_3PO_4, 5 g $MgCl_2 \cdot 6H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$, 0.1 g Yeast extract. 5 µL of bacterial suspensions were placed on the agar surface and incubated at 10°C for 2 weeks. Clearing around the bacterial growth was scored as positive for phosphate solubilisation. To investigate if the solubilizing substance production was constitutive or induced by phosphate deficiency the strains were inoculated in the same manner but on a NBRIP medium supplemented with 0.5 g of KH_2PO_4 L^{-1}.

Acid production

Strains were inoculated into 3 mL of liquid NBRIP medium (pH 6.8) without any phosphates and incubated for 2 weeks. After that period the pH of the spent medium was measured with the help of a pH meter (Hanna).

Biofilm formation

Biofilm formation was assessed by a colorimetric assay with crystal violet after (Burton et al. 2007) with slight modification. The strains were inoculated in 4 wells each on a microtiter plate containing the aforementioned R3A medium supplemented with 3 g of sucrose L^{-1} and 3 g glycerol L^{-1}. After a week-long incubation at 10°C the medium was removed and the wells were washed 3 times with 200 µL of PBS buffer. Plate wells were then stained for 15 min with a 0.4% aqueous solution of crystal violet. The staining solution was removed and the wells washed 3 times with PBS buffer. After drying the plate for 15 min the biofilm bound crystal violet was solubilized in each well by the addition of 200 µL of 33% acetic acid. The absorbance of the solution was read at 630 nm in a Varioscan plate reader (Thermofisher Scientific).

Indole-3-acetic acid production

Obtained bacterial strains were inoculated into 3 mL of R3A medium containing 0.1 g of L-tryptophan L^{-1} (Rodrigues et al. 2016). After 2 weeks of incubation, the culture was centrifuged at 12 000 rpm for 3 min and the clear supernatant was mixed with Salkowski reagent (1 mL of 0.5M $FeCl_3$ and 50 ml of 35% H_2SO_4) in a 3:2 ratio. After 30 min of incubation in the dark, the solution was distributed into microtiter plates and absorbance was read at 520 nm in a Varioscan plate reader.

Nitrogen-free medium growth

The growth in a liquid medium with or without a nitrogen source was assessed in a minimal salts medium containing L^{-1}: 1.1 g Na_2HPO_4, 1 g KH_2PO_4, 1 g NH_4Cl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2$, 0.05 g Yeast extract, 0.01 g Fe-citrate, 0.005 g $NaMoO_4 \cdot 2H_2O$. 1.5 g sodium lactate, sucrose and mannitol L^{-1} each were supplemented as carbon sources. NH_4Cl was omitted for the nitrogen-deficient version. The medium was distributed into wells of a microtiter plate (150 µL each) and inoculated with suspension of the strains in 0.9% saline. The absorbance was measured in a plate reader (600 nm) at the beginning and at the end of a 2 week incubation period at 10°C.

Cellulolytic activity

Cellulolytic activity of pure bacterial strains was assessed by inoculating an agar plate containing R2A medium amended with 5 g of carboxymethylcellulose sodium salt $L^{-1}$with 5 µL of bacterial suspension and incubating it for 2 weeks at 10°C. After that time the plate was flooded with an aqueous solution of Congo Red (0.1%) and stained for 20 min, rinsed afterwards with 1M NaCl. Clear halos around bacterial growth were scored as positive reactions.

Chitinolytic assay

Chitin agar was prepared according to Atlas (2010). 5 g of chitin powder from crab shells (Sigma) was dissolved in 200 mL of cooled 37% HCl. The resulting liquid was poured into a 1.5 L of cooled deionized water, mixed thoroughly and let sit overnight to precipitate colloidal chitin. The chitin was washed 5 times with deionized water by repeated centrifugation and resuspension in fresh dH_2O and finally suspended in 1L of deionized water. pH was adjusted by 3% NaOH. Then mineral salts were added to make a mineral salts medium (see above) and 15 g agar L^{-1}, autoclaved and poured into Petri dishes. Bacteria were inoculated as mentioned earlier. Those creating clear zones were scored as positive for chitinolysis.

Data analysis

Calculations, charts and graphs were made in Excel (MS Office for Windows) and R software. Obtained Ilumina reads were quality checked using FastQC software (www.bioinformatics.babraham.ac.uk/projects/fastqc, (Wingett and Andrews 2018)). Paired-end data (FASTQ files) were uploaded to the EzBioCloud 16S-based Microbiome Taxonomic Profile (MTP) virtual platform (ChunLab Inc., Seoul, Korea). The data quality was again checked. Sequences of low quality with regard to read length (<100 bp or >2000 bp) and averaged Q values less than 25 were filtered out. Remaining reads were aligned and classified using PKSSU4.0 version database and Open reference UCLUST_MC2 for OTUs picking at 97% cut-off. Chimeric and non-target amplicons (chloroplast, mitochondrial, and archaeal) were automatically discarded. Raw classification results are contained in Online Resource 2. Good's coverage of library was applied to assess if the richness in each sample was sufficiently covered. Bacterial community richness was measured by present OTU abundance and alpha diversity indices: ACE (abundance coverage estimator) and Chao1 (Yoon et al. 2017). Illumina reads were deposited in the NCBI Sequence Read Archive (SRA) as BioProject PRJNA639928.

Strain sequence chromatogram files were analysed using FinchTV ver. 1.4.0 (Geospiza, Akron, USA). Consensus sequences were obtained with Seqman Pro ver. 9.1 software (DNAStar,

Madison, USA). 16S rRNA gene fragments sequences were aligned against 16S reference sequence database GenBank using BLAST (Altschul et al. 1990). Multiple sequence alignments were performed using ClustalW program. Phylogenetic trees were constructed using Mega-X software (Kumar et al. 2018). Sequences were deposited in GenBank under accession numbers: MT622197-MT622202.

Results

Good's library coverage presented in Fig 1A was ≥99% for all samples. Valid reads and OTU (operational taxonomy unit) numbers obtained for the rhizosphere, rhizoplane and endosphere were as follows (respectively): 67918 and 2959; 68042 and 3013; 17924 and 2116 (Fig. 1B), whereas ACE (abundance coverage estimator) and Chao1 diversity index displayed the following values: 3203.4 and 3090.3; 3275.9 and 3142.7; 2344.8 and 2254.1. Fifteen major (sequence abundance contribution >1%) bacterial phyla were identified in the three rhizocompartments of *D. antarctica* (Fig. 1E). Present in all three compartments in considerable amounts were members of the following phyla: *Proteobacteria* (rhizosphere – 26%, rhizoplane – 27.6 %, root endosphere – 19.9%), *Bacteroidetes* (36.6%; 21.9%, 20.6%), *Verrucomicrobia* (6.8%, 13.2%, 11.7%), *Actinobacteria* (8.1%, 10%, 5.5%), *Sacharibacteria* (7.6%, 4.2%, 6.2%), *Parcubacteria* (3%, 3.5%, 2.9%). The abundance of several phyla sequences was gradually reduced towards the root endosphere: *Acidobacteria* (7.2% > 2.1% > 1.3%), *Armatimonadetes* (2.8% > 0.9% > 0.5%), *Chlorobi* (0.9% > 0.36% > 0.1%), *Gemmatimonadetes* (2.9% > 1% > 0.5%), *Planctomycetes* (5.3% > 2.5% > 1.3%). *Cyanobacteria* (5.2%) were enriched in the rhizoplane and displayed their highest abundance in this compartment. *Fibrobacteres* showed their highest numbers in the root endosphere.

Rhizosphere community family rank sequence percentage (Fig. 2) was the highest for the following groups: *Chthoniobacteraceae* (6.9%), *Tepidisphaeraceae* (3.1%), *Acidobacteraceae* (2.51%), *Bryobacteraceae* (2.26%), *Micropepsacaeae* (1.55%),

312 *Solibacteraceae* (0.9%), *Gemmatimonadetes* (2.02%). *Chthoniobacteraceae* were also present
313 in the rhizoplane (6.44%), together with the *Comamonadaceae* (5.0%), *Microcolaceae*
314 (4.64%), *Sphingomonadaceae* (2.70%), *Verrucomicrobiaceae* (2.19%) and *Rhodobacteraceae*
315 (0.96%). Root endosphere community consisted mostly of *Flavobacteriaceae* (15.55%),
316 *Polyangiaceae* (6.18%), *Cytophagaceae* (4.87%), *Microbacteriaceae* (4.75%) and
317 *Hyphomicrobiaceae* (4.15%) family members.

318 The highest percentage of phosphate solubilizing strains was observed for the
319 endosphere and the rhizosphere (90% and 87.5% respectively). 60% of rhizoplane strains
320 displayed this ability. The gradual decrease of phosphate solubilizing strain contribution was
321 observed when there were phosphate anions present: 85%, 22.5% and 5% for the rhizosphere,
322 rhizoplane and endosphere isolates respectively. Mean pH of the spent phosphate - free medium
323 gradually deceased for strains from each compartment: rhizosphere – 5.79, rhizoplane 5.13,
324 endosphere 4.59 (Fig. 3a). The relatively high percentage of chitin utilizing strains was
325 observed in the rhizoplane bacteria (17.5%), whereas the highest cellulase activity was noted
326 in the endosphere (55% carboxymethylcellulose degrading strains) (Fig. 3b). Rhizosphere
327 bacterial strains displayed on average good growth on the minimal medium amended with a
328 nitrogen source (A_{600} =0.86) albeit on the nitrogen - free medium cell densities were reduced
329 by half (N-/N+ = 0.54). Rhizoplane and endosphere strains displayed lower cell densities on
330 each of the media, however, the ratio was substantially higher for both cases (0.96 and 1.00
331 respectively) (Fig. 3c). Rhizoplane bacterial isolates displayed on average the highest
332 biofilming ability (A_{630} = 0.15) in the crystal violet assay, followed by root endosphere bacteria
333 (A_{630} = 0.12) and rhizosphere bacteria (A_{630} = 0.11). Highest IAA phytohormone production
334 was on average observed in the rhizosphere derived strains (A_{540} = 0.3), lowest in the
335 rhizoplane-derived strains (A_{540} = 0.19) (Fig. 3d).

Six strains were chosen as representatives of groups that emerged in clustering by morpho-physiological features of the isolates (data not shown) and subjected to identification based on 16S rRNA sequence similarity. Rhizosphere isolate R16 was closely related (98.04% sequence similarity) to *Arthrobacter cryoconiti* strain Cr6-08, whereas isolate R9 was related (98.11% sequence similarity) to *Flavobacterium resistens* strain BD-b365. Rhizoplane isolate P27 16S rRNA gene sequence showed closest resemblance (99.13%) to *Flavobacterium aquidurense* strain WB 1.1-56, isolate P18 was closely related to *Acidovorax defluvii* strain BSB411 (98.86% sequence similarity) and isolate P12 had phylogenetic similarity to *Pseudomonas frederiksbergensis* strain DSM 13022 (99.53% sequence similarity). Isolate E21 representing the most numerous group of endospheric isolates was closely related to *Clavibacter michiganensis* subsp. *phaseoli* strain LPPA 982 (99.59% sequence similarity) (Fig. 4).

Discussion

Investigations of three rhizocompartments of land plants have been conducted before, some with the use of ultrasonic rhizoplane bacterial detachment. We improved on the procedure by adding two surfactants in bacteria-safe concentrations (Tween 80 and tetrasodium pyrophosphate) (Yoon and Rosson 1990; Velji and Albright 1986) to further facilitate the detachment of root-surface bound bacteria but also to better detach the bacteria from soil and root-pulp particles.

The rhizosphere

The rhizosphere of *D. antarctica* has not been analysed previously in such detail by Illumina targeted 16S rRNA gene amplicon sequencing, so the available literature provides only limited background for the discussion of our results. This is especially true for the OTU numbers, which were exceptionally high (>3000) in this study, caused probably by the higher

resolution of the method. Reports to date provide OTU numbers in the *D. antarctica* rhizosphere in the range of 552-732 (Teixeira et al. 2010). However, the rhizosphere of *D. antarctica* contained several phyla previously reported from Antarctic soils (Newsham et al. 2019; Tytgat et al. 2016; de Scally et al. 2016). The most striking however is the absence of some phyla representatives that have been abundantly present in other studies. The Firmicutes phylum has featured prominently in the rhizosphere of *D. antarctica* from different sites at Admiralty Bay shore (Teixeira et al. 2010). Most studies attribute their presence to a heavy impact of animal excreta like penguin guano (Kim et al. 2012; Grzesiak et al. 2020). In this study it was apparent that the *D. antarctica* rhizosphere was mostly *Firmicutes* free. This could indicate that the guano deposition did not occur in an immediate proximity to the plant. Examined rhizosphere showed hallmarks of a more mature and established pedosphere, namely highly diverse taxa, most notably rarely cultivated ones like *Acidobacteria*, *Parcubacteria*, *Armatimonadetes*, *Verrucomicrobia* and the elusive WS6 candidate phylum (Jangid et al. 2013). Therefore, *D. antarctica* roots could, due to stabilization of the ground, facilitate an establishment of a more niche-diverse habitat for various bacteria. The most abundant phyla, namely *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* have been reported as a staple in Antarctic soils on many occasions (Teixeira et al. 2010; Newsham et al. 2019; Tytgat et al. 2016; de Scally et al. 2016). Especially, the frequently isolated genus *Arthrobacter* has been in focus as a plant beneficial microbe (Dsouza et al. 2015). In our study, it also constituted the bulk of the strains isolated from *D. antarctica* rhizosphere. It had pronounced phosphate solubilizing abilities presumably secreting siderophores or weak acids like HCN, hence the marginal pH drop on phosphate - free medium, which facilitated the dissolution of the $Ca_3(PO_4)_2$ precipitate (de Serrano et al. 2016). Some strains produced a water - soluble pink pigment, likely indicative of a cation scavenging compound (Saha et al. 2013). Furthermore, rhizosphere isolates were very efficient IAA producers, even more so than the endospheric ones. Presumably, enhanced root growth

385 due to phytohormone stimulation is beneficial for those bacteria, perhaps providing more
386 nutritious exudates to the surrounding soil (Jiang et al. 2012).

387 *The rhizoplane*

388 Several phyla have diminished in percentile contribution in the rhizoplane compartment
389 compared to the rhizosphere. Most notably *Acidobacteria*, *Planctomyces* and the candidate
390 phylum WS6. Although not much is yet known about the former, depletion of *Acidobacteria*
391 and *Planctomyces* in the rhizoplane have been observed for rice plants (Edwards et al. 2015).
392 Cells belonging to those phyla were presumably not bound by the multispecies biofilm
393 engulfing the *D. antarctica* roots, as this is a requirement for bacteria to be included in the
394 rhizoplane community (van der Heijden and Schlaeppi 2015). The second requirement is to be
395 able to compete in the presence of elevated nutrient levels provided by plant root exudates (van
396 der Heijden and Schlaeppi 2015). This could explain the reduction of the aforementioned taxa
397 as they tend to be oligotrophic in nature (Kielak et al. 2016; Jenkins and Staley 2013).
398 Considerably enriched in the rhizoplane compartment were the *Cyanobacteria*, mostly those
399 belonging to the *Microcoleaceae* family and *Actinobacteria*. *Cyanobacteria* have been reported
400 to produce biofilms in plant rhizocompartments as well as phytohormones (Ahmed et al. 2014).
401 They have also been confirmed, together with *Actinobacteria*, to produce antifungal
402 exometabolites in the rhizoplane of agricultural plants (Domracheva et al. 2010). Among the
403 *Proteobacteria*, members of the family *Comamondaceae* reached their highest percentage
404 contribution in the rhizoplane. This phenomenon has also been observed in the rhizoplane of
405 *Rehmania glutinosa*, a medicinal legume from China (Wu et al. 2018). The members of the
406 rhizoplanic *Comamonadaceae* cultivated in this study belonged to the *Acidovorax* genus and
407 some isolates expressed considerable chitin degradation abilities, making them potential fungal
408 antagonists (Hoster et al. 2005). The biofilming abilities of the rhizoplane strains were the
409 highest on average compared to strains from other compartments, albeit the intensity of biofilm

formation differed extensively between isolates, suggesting there is a fraction of biofilm non-formers benefiting from the actions of the biofilm formers (Yang et al. 2011). Surprisingly, a high percentage of *Verrucomicrobia* have been detected in the rhizoplane of *D. antarctica*. Those bacteria were rarely considered as plant-beneficial, however, a recent study (Bünger et al. 2020) indicated their major involvement in growth promotion of rice.

The endosphere

Root-endosphere bacterial community displays the lowest OTU numbers among the three compartments in *D. antarctica* and a simple UPGMA clustering showed its community has more in common with the rhizoplane than the rhizosphere, suggesting that a considerable variety of rhizoplane bacteria has access to the root interior. Several endospheric taxa revealed in this study are known to hold cellulase producing members like the phylum *Fibrobacteres* (Rosenberg 2014) and the family *Cytophagaceae* (McBride et al. 2014), but also the family *Microbacteriaceae* (Koeck et al. 2014). Representatives of the latter have been cultivated in this study. Based on the 16S rRNA gene sequence similarity they belonged to the species *C. michiganensis*, which is a widely recognized plant pathogen (Eichenlaub et al. 2007). This is the first report of *C. michiganensis* infecting *D. antarctica* roots to our knowledge. Isolates of *C. michiganensis* displayed cellulolytic abilities, presumably for root entry and as a virulence factor. They also exhibited growth without a nitrogen source suggesting an efficient use of the available trace amounts of nitrogen compounds or even atmospheric nitrogen fixation capabilities, although there are no reports that this genus has this ability. Furthermore, the *Clavibacter* spp. isolates, which constituted a majority of endosphere isolates, displayed phosphate solubilizing abilities, presumably due to organic acid excretion marked by a considerable pH drop. This ability was halted by the addition of free phosphate ions. This observation could shed a light on the interaction between this bacterium and the Antarctic hairgrass. The endospheric bacterial cell, when provided with nutrients (*inter alia* phosphate

ions) does not lower the pH of the environment thus do not harm the plant tissue. This suggests that the Antarctic *Clavibacter* spp. strains have lost their pathogenic traits due to selection and have adapted the role of a benign commensal (Gartemann et al. 2003). Residing within the *D. antarctica* roots were also members of the family *Polyangiaceae*, known cellulose degraders but also producers of several biologically active compounds, mostly antifungal, antibacterial and antibiofilming substances (Garcia and Müller 2014). Likewise enriched in the endosphere were members of the family *Hyphomicrobiaceae*, which include a number of nitrogen - fixing species like *Devosia neptuniae*, a symbiont of aquatic legumes (Rivas et al. 2002). The largest group residing within the *D. antarctica* roots were members of the family *Flavobacteriaceae*. Those bacteria have often been observed in plant roots, however, their function as endophytes remains elusive, even more so for Antarctic flora, which merits further research (Liu et al. 2011; McBride 2014).

Conclusions

The rhizobiome of *D. antarctica* houses distinct bacterial communities in each rhizocompartment. Bacteria in these rhizocompartments have unique adaptive features, many of which bear plant-beneficial traits. Used methods proved successful for discovering this phenomenon in *D. antarctica* and can be applicable for cryophilic plants or to plants in general after appropriate modification (temperature, salinity etc.). This study is also the first to report *C. michiganensis* infection of Antarctic hairgrass roots. Although there is ongoing research on Antarctic plants' rhizosphere microbes, the information is still scarce. We hope that the methodology and insight provided in this paper will help promote further investigations of the topic.

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677 Figure 5. Phylogenetic tree constructed using partial 16S rRNA gene sequences of the
678 *Deschampsia antarctica* root-associated bacterial strains. The tree was built using the neighbor
679 joining method. Letters in the strains name indicate the rhizocompartment of origin: R –
680 rhizosphere, P – rhizoplane, E – root endosphere.

681

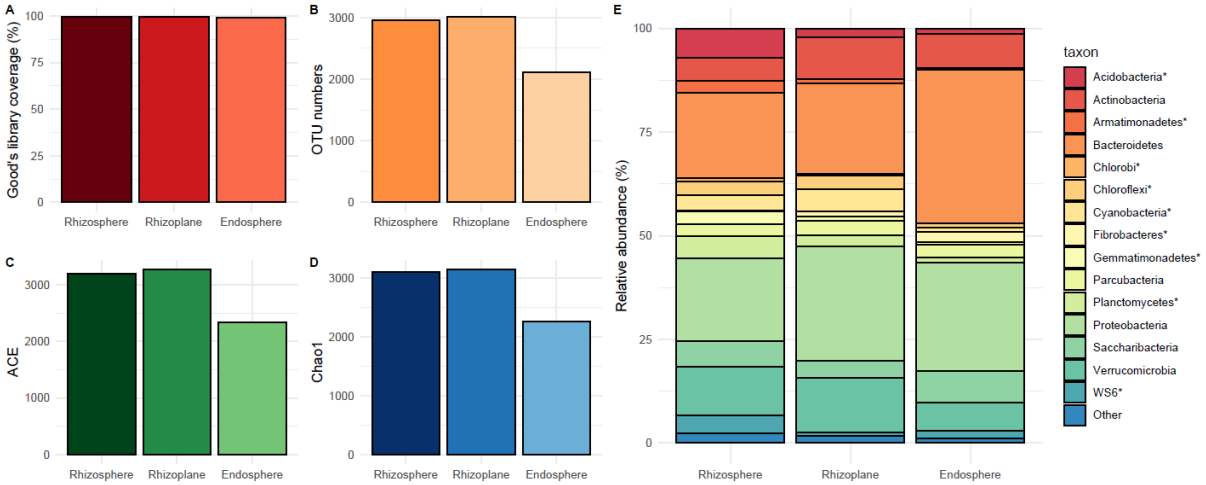


Figure 1. Bacterial community indices based on targeted 16S rRNA gene sequencingin three *Deschampsia antarctica* root-associated zones: rhizosphere, rhizoplane and root endosphere. 1A – Good's library coverage. 1B – number of unique OTUs. 1C - abundance coverage estimator (ACE). 1D – Chao1 richness index. 1E – phylogenetic diversity on phylum level; asterisks indicate those phyla whose abundance by sequence numbers changed within the three rhizocompartments more than 3-fold.

	Rhizosphere	Rhizoplane	Endosphere	
Acidobacteria/*Acidobacteriaceae*	2.51	0.13	0.14	***
Acidobacteria/*Bryobacteraceae*	2.26	0.14	0.06	***
Planctomycetes/*Tepidisphaeraceae*	3.14	0.80	0.68	*
Proteobacteria/*Micropepsaceae*	1.55	0.42	0.40	*
Proteobacteria/*Haliangiaceae*	1.06	0.42	0.34	*
Acidobacteria/*Solibacteraceae*	0.91	0.28	0.18	**
Gemmatimonadetes/*Gemmatimonadaceae*	2.02	0.64	0.33	**
Actinobacteria/*Acidimicrobiaceae*	0.06	0.04	0.00	**
Verrucomicrobia/*Chthoniobacteraceae*	6.96	6.44	2.42	*
Cyanobacteria/*Microcoleaceae*	2.21	4.64	0.92	**
Verrucomicrobia/*Verrucomicrobiaceae*	0.83	2.19	0.61	*
Actinobacteria/*Parviterribacteraceae*	0.04	1.21	0.11	***
Proteobacteria/*Comamonadaceae*	1.36	5.00	2.20	*
Proteobacteria/*Sphingomonadaceae*	0.92	2.70	1.66	*
Acidobacteria/*Blastocatellaceae*	0.19	0.88	0.73	*
Proteobacteria/*Rhodobacteraceae*	0.13	0.96	0.88	**
Proteobacteria/*Hyphomicrobiaceae*	0.92	3.32	4.15	*
Actinobacteria/*Microbacteriaceae*	1.39	3.61	4.75	*
Proteobacteria/*Polyangiaceae*	1.77	3.21	6.18	*
Actinobacteria/*Micromonosporaceae*	0.07	0.35	1.17	***
Bacteroidetes/*Cytophagaceae*	1.03	1.96	4.87	*
Bacteroidetes/*Flavobacteriaceae*	2.04	5.72	15.55	**

690

691 Figure 2. Distribution of chosen bacterial family-rank groups in each rhizocompartment of
692 *Deschampsia antarctica*. *** family rank groups whose percentage abundance varied more
693 than 15-fold between the compartments; ** family rank groups whose percentage abundance
694 varied 5 to 10-fold between the compartments; * family rank groups whose percentage
695 abundance varied 3 to 5-fold between the compartments.

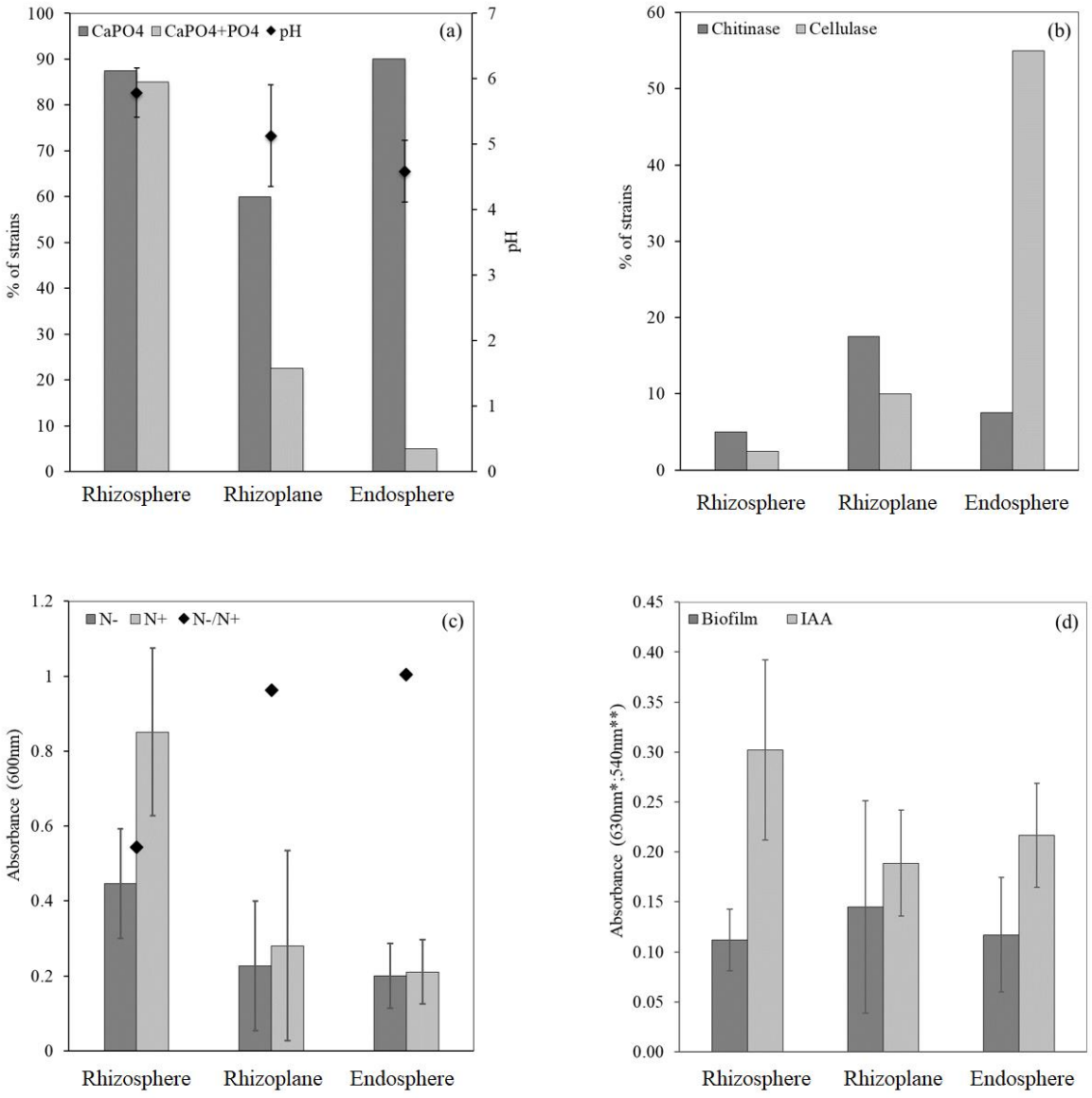
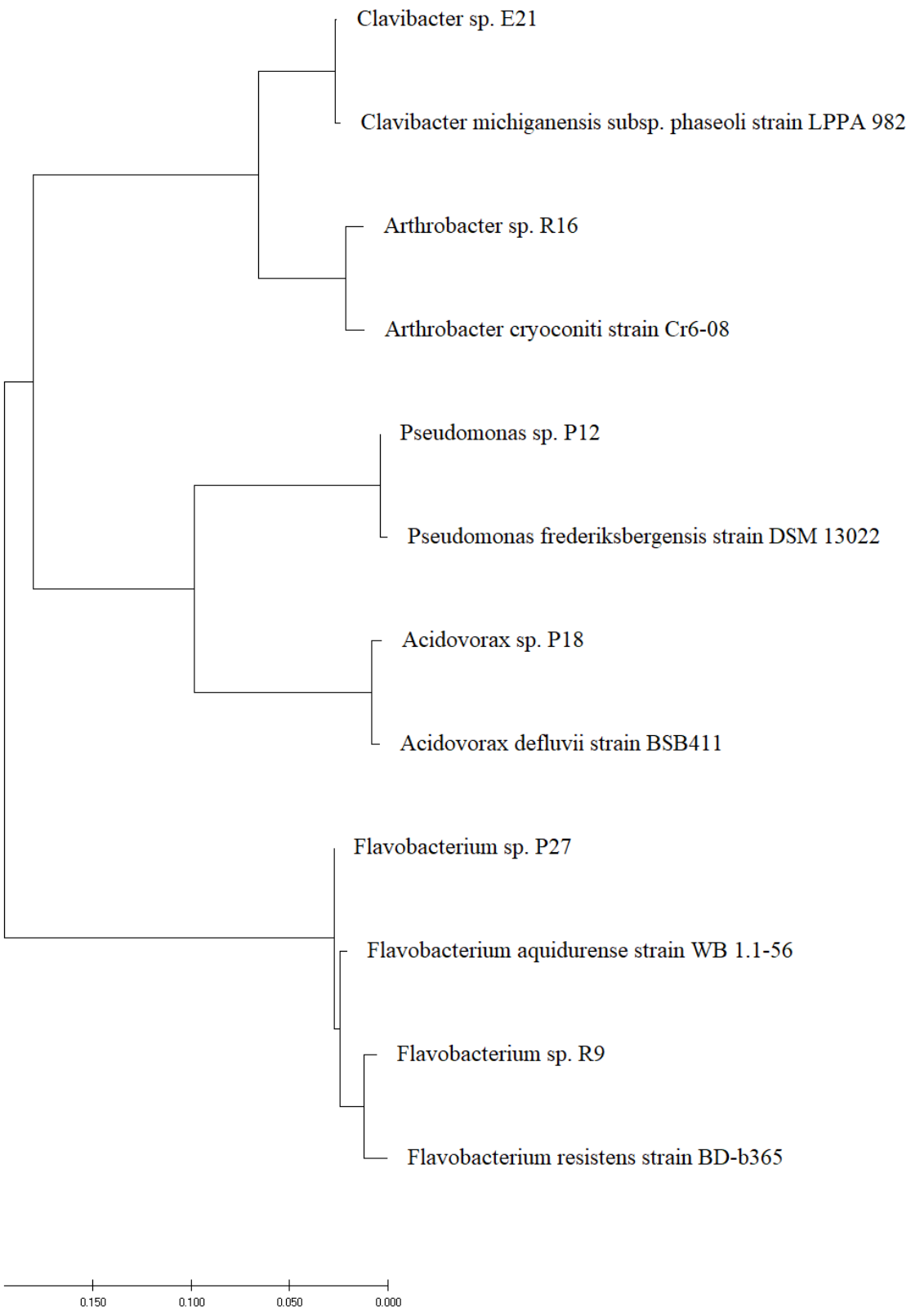


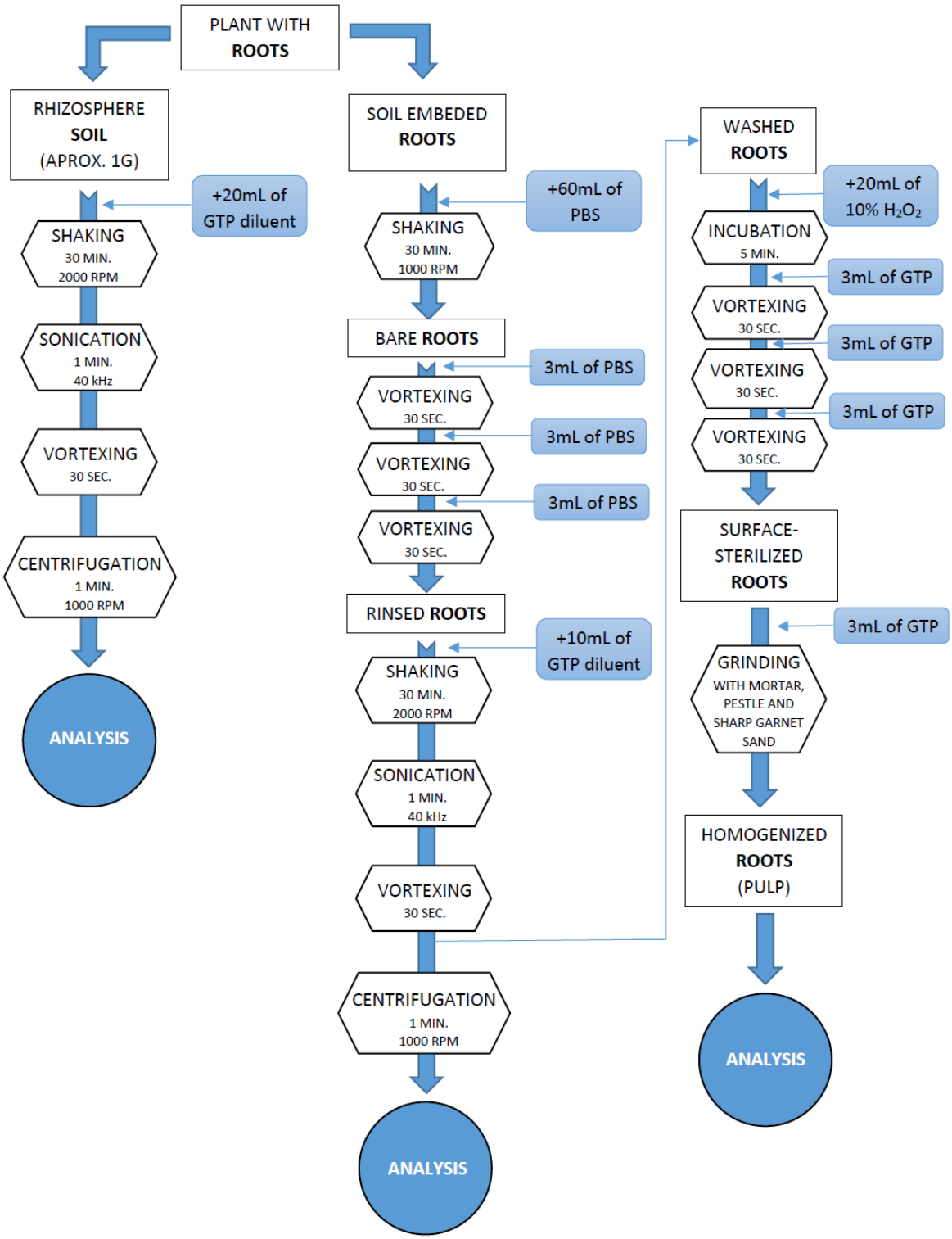
Figure 3. Plant-beneficial and adaptive features of bacterial strains isolated from three rhizocompartments of *Deschampsia antarctica*; a) percentage of strains within each compartment displaying calcium phosphate solubilizing abilities with added free phosphates (light grey bars) or without (dark grey bars) added free phosphates; black diamond indicates pH of the spent liquid medium without any phosphates, mean values (n=40) with error bars. b) percentage of strains displaying chitinase (light grey bars) and cellulase activities (dark grey bars). c) bacterial growth given as absorbance values (A_{600}) on minimal salts medium with (N+) or without (N-) added nitrogen (NH_4^+); black diamonds represent the ratio between A_{600} values obtained on N- and N+ media. d) biofilm formation (dark grey bars) and IAA production (light grey bars) by isolated strains given as absorbance values: * absorbance wavelength used for the biofilm assay, ** absorbance wavelength used for the IAA production assay. Mean values were given (n=40) with error bars.


Clavibacter sp. E21
Clavibacter michiganensis subsp. phaseoli strain LPPA 982
Arthrobacter sp. R16
Arthrobacter cryoconiti strain Cr6-08
Pseudomonas sp. P12
Pseudomonas frederiksbergensis strain DSM 13022
Acidovorax sp. P18
Acidovorax defluvii strain BSB411
Flavobacterium sp. P27
Flavobacterium aquidurense strain WB 1.1-56
Flavobacterium sp. R9
Flavobacterium resistens strain BD-b365
0.150
0.100
0.050
0.000

709

710

Figure 5. Phylogenetic tree constructed using partial 16S rRNA gene sequences of the *Deschampsia antarctica* root-associated bacterial strains. The tree was built using the neighbor joining method. Letters in the strains name indicate the rhizocompartment of origin: R – rhizosphere, P – rhizoplane, E – root endosphere.



ESM1. Online resource 1.