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RESEARCH

Two Antarctic endophytic bacteria of *Colobanthus quitensis* show functional and genomic characteristics potentially responsible for plant growth promotion and cold tolerance

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Abstract
Bacterial endophytes reside in plant tissues and can promote plant growth under abiotic stresses. Complex microbial communities are associated with cold-adapted plants, but scarce information is available on the functional properties of Antarctic bacterial endophytes. This study aimed to investigate possible cold tolerance and plant growth promotion activities of two Antarctic bacterial endophytes by in vitro functional characterization and genome sequence analysis. *Ewingella* sp., *Pseudomonas* sp, and their bacterial consortium were cold tolerant and showed plant growth-promoting activity on tomato seedlings at low temperature. Phytohormones (indole-3-acetic acid) and proteases were produced by *Ewingella* sp. and *Pseudomonas* sp., respectively, while ammonia and siderophores were produced by both bacterial isolates and their consortium. *Ewingella* sp. and *Pseudomonas* sp. genomes encompassed genes possibly involved in plant growth promotion (e.g., auxin, cytokinin, ethylene, salicylic acid, and siderophore metabolism and transport) and genes related to bacterial metabolic processes that can contribute to plant growth-promoting activities, such as amino acid metabolism, iron transport, nitrogen metabolism, and lytic activities (amylases, cellulases, and proteases), phosphate metabolism, potassium transport, and zinc transport. Moreover, *Ewingella* sp. and *Pseudomonas* sp. encompassed genes possibly associated with bacterial cold tolerance that can contribute to cold stress mitigation in the plant host, such as cold shock- and heat shock-related proteins, lipid desaturases, polyamine metabolism, proline metabolism, proline and glycine betaine transport, reactive oxygen species detoxification, and trehalose metabolism. Antarctic bacterial endophytes include multiple characteristics to survive under cold conditions and some bacterial functions can contribute to plant growth promotion and stress mitigation at low temperature.

Keywords Cold stress · Bacterial endophytes · Antarctic bacteria · Plant growth-promoting characteristics · Bacterial genome · Bacterial genes

Introduction

Bacterial endophytes reside in plant tissues and some of them can promote plant growth and protection against abiotic and biotic stresses (Negi et al. 2024). In particular, bacterial endophytes can promote plant growth by solubilizing mineral nutrients (e.g., phosphorous, potassium, and zinc), fixing atmospheric nitrogen, and synthesizing phytohormones (auxins, cytokinins, and gibberellins), 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ethylene metabolism), siderophores, ammonia, and hydrogen cyanide under optimal growth conditions and abiotic stresses (Rana et al. 2020; Kandasamy and Kathirvel 2023; Negi et al. 2024). Moreover, bacterial endophytes can synthesize hydrolytic enzymes that can help in the penetration into plant tissues and the limitation of

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pathogen infections, such as amylase, cellulase, pectinase, protease, and xylanase (Rana et al. 2020; Negi et al. 2024). Endophytic microorganisms can also play key roles in plant protection against abiotic stresses (Kandasamy and Kathirvel 2023). In particular, complex microbial communities are associated with wild plants in cold environments and can contribute to plant growth under cold stress (Marian et al. 2022). Cold-tolerant bacterial endophytes can display positive effects on plant growth at low temperature in different crops, such as *Solanum lycopersicum*, *Triticum aestivum*, and *Vitis vinifera* (Nissinen et al. 2012; Acuña-Rodríguez et al. 2020). For example, *Duganella*, *Ewingella*, *Hafnia*, *Janthinobacterium*, *Pseudomonas*, and *Rahnella* genera were the most abundant cold-tolerant endophytic bacteria in Antarctic plants (*Colobanthus quitensis*), and their plant growth-promoting effects were demonstrated in tomato seedlings at low temperature (Perazzolli et al. 2022). In particular, *Ewingella* S1.OA.A_B6 (*Ewingella* sp.) and *Pseudomonas* S2.OTC.A_B10 (*Pseudomonas* sp.) decreased lipid peroxidation under cold stress in tomato plants by a complex reprogramming of phenolic compound metabolism (Licciardello et al. 2024). Cold-tolerant bacteria isolated from mountain areas are known to display functional activities related to plant growth promotion, such as ACC deaminase, nitrogen fixation, phosphorus solubilization, auxin (indole-3-acetic acid) production, and siderophore production (Subramanian et al. 2016; Li et al. 2021; Marian et al. 2022). Likewise, a cold-adapted bacterium isolated from cave soil (*Pseudochrobactrum kiredjianiae* A4) showed plant growth-promoting properties, such as ACC deaminase activity, indole-3-acetic acid production, phosphate solubilization, and siderophore production (Qin et al. 2017), suggesting that genomic knowledge is required to understand functional characteristics of cold-tolerant bacteria. For example, the genome analysis of cold-tolerant *Arthrobacter* sp. ERGS4:06, *Janthinobacterium* sp. ERGS5:01, *Chryseobacterium* sp. ERMR1:04, and *Rahnella* sp. ERMR1:05 revealed genes possibly related to plant growth promotion, such as nitrate and nitrite reductases, phosphate metabolism-related enzymes, siderophore receptors, and tryptophan synthases (Mukhia et al. 2022). Likewise, genomes of bacterial endophytes encompass genes related to nitrogen fixation, nitrogen metabolism, nitrate reduction, ammonia assimilation, phosphate transport, iron transport, siderophore transport, ethylene metabolism, tryptophan metabolism, and indole-3-acetic acid biosynthesis, as found in *Bacillus cereus* T4S (Adeleke et al. 2021) and *Variovorax paradoxus* S110 (Han et al. 2011). Moreover, cold-tolerant bacteria include traits for their survival at low temperature, such as genes related to cold shock and heat shock response, lipid desaturation, proline and glycine betaine metabolism, polyamine metabolism and transport, reactive oxygen species (ROS) detoxification, and trehalose metabolism (Raymond-Bouchard et al. 2018; Guo et al. 2020; Han et al. 2021; Teoh et al. 2021; Jiang et al. 2022). Thus, bacterial activities could have beneficial effects on plants against cold stress to detoxify ROS, accumulate protective solutes (e.g., proline, glycine betaine, and trehalose), and trigger hormone signaling (e.g., salicylic acid), (Ait Barka et al. 2006; Subramanian et al. 2015; Acuña-Rodríguez et al. 2020), but scarce information is available on functional and genomic characteristics of Antarctic bacterial endophytes. This study aimed (i) to investigate the plant growth promotion activity of two endophytic bacteria isolated from Antarctic plants on tomato seedlings at low temperature, (ii) to analyze cold tolerance and plant growth promotion characteristics in vitro, and (iii) to annotate bacterial genes potentially responsible for plant growth promotion and cold tolerance by genome sequencing analysis.

Materials and methods

Bacterial isolates and inoculum preparation

Ewingella sp. S1.OA.A_B6 (*Ewingella* sp.; NCBI accession number MZ089444) and *Pseudomonas* sp. S2.OTC.A_B10 (*Pseudomonas* sp.; NCBI accession number MZ089387) were previously isolated from surface-sterilized leaves of *C. quitensis* collected at King George Island of Maritime Antarctica (62° 14′ S, 58° 48′ W) during the summer season (February 2018) by incubation on solid peptone yeast extract medium (5 g L^{-1} peptone, 2 g L^{-1} yeast extract, and 15 g L^{-1} agar; Oxoid, Basingstoke, Hampshire, UK) at 25 ± 1 °C (Perazzolli et al. 2022). These two isolates were able to colonize tomato plants and mitigate cold stress at 4 ± 1 °C for 7 days by a complex reprogramming of phenolic compound metabolism (Licciardello et al. 2024). Bacterial isolates were stored in 40% glycerol at − 80 °C and grown at 25 ± 1 °C on solid peptone yeast extract medium, previously named Antarctic bacterial medium (Shivaji et al. 2013).

To prepare the bacterial suspension for functional assays and seed inoculation, each isolate was grown overnight (18 h) in liquid peptone yeast extract medium (5 g L^{-1} peptone and 2 g L^{-1} yeast extract; Oxoid) at 25 ± 1 °C under orbital shaking at 200 rpm. Bacterial cells were collected by centrifugation (3500×*g* for 10 min) and washed three times with sterile 10 mM $MgSO_4$. The bacterial suspension was adjusted to 1.0×10^9 colony forming units (CFU) per unit of volume (CFU mL^{-1}) based on the conversion of optical density (OD) at 600 nm (OD_{600}) assessed with a spectrophotometer (Ultrospec 3100, GE Healthcare, Chicago, IL, USA) and previously optimized for each isolate (OD_{600} = 0.1 corresponded to 2.3×10^8 CFU mL^{-1} *Ewingella* sp., and 3.5×10^7 CFU mL^{-1} *Pseudomonas* sp.) (Perazzolli et al. 2022). The bacterial consortium was prepared by

mixing 1.0×10^9 CFU mL^{-1} of each bacterial isolate and bacterial viability was assessed at 25 ± 1 °C and 4 ± 1 °C. Briefly, an aliquot (100 µL) of each bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium) was inoculated in liquid peptone yeast extract medium (final volume of 5.0 mL) and incubated overnight (18 h) at 25 ± 1 °C or 4 ± 1 °C under orbital shaking at 200 rpm to determine cold tolerance and compatibility of the two isolates under non-stressed and cold-stressed conditions. Cultures were serially diluted, plated in the solid peptone yeast extract medium, and incubated at 25 ± 1 °C for one day. CFU of *Ewingella* sp. and *Pseudomonas* sp. were counted according to their different morphology, using a stereomicroscope (Leica M125 C, Leica, Wetzlar, Germany). Five replicates (bacterial cultures) were analyzed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

Assessment of plant growth promotion

Seeds of *Solanum lycopersicum* L. cultivar Moneymaker (Justseed, Wrexham, UK) were surface disinfected as previously reported (Perazzolli et al. 2022). Briefly, seeds were treated with 70% ethanol for 1 min, 2% sodium hypochlorite for 5 min, and 70% ethanol for 1 min, followed by three washes with sterile distilled water (3 min each) in a 15 mL tube with moderate shaking. Surface-disinfected seeds (50 seeds for each treatment) were treated with 5 mL of sterile 10 mM $MgSO_4$ (mock-inoculated) or inoculated with 5 mL of the respective bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium) by overnight (18 h) incubation at 25 ± 1 °C in a 15 mL tube under orbital shaking in the dark at 80 rpm. Seeds of each inoculation were transferred into a 100 cm^2 square dish (50 seeds for each dish; Sarstedt, Nümbrecht, Germany) containing 8 g L^{-1} water agar (Oxoid) and they were incubated for two days in a growth chamber (Kälte Klima Röhler, Bolzano, Italy) at 25 ± 1 °C with a photoperiod of 16 h light and 8 h dark to allow seed germination. Germinated seeds with the same root length (about 2 mm) were selected and five seeds were transferred along a line at 4 cm from the edge of a 100 cm^2 square dish (Sarstedt) containing 40 mL solid (8 g L^{-1} agar; Oxoid) half-strength Hoagland. Dishes were incubated in a vertical position for three days in the growth chamber at 25 ± 1 °C or 15 ± 1 °C with a photoperiod of 16-h light (photon flux density of 0.050 mmol sec^{-1} m^{-2}) and 8 h dark to assess plant growth promotion activity on tomato seedlings under non-stressed and cold-stressed conditions (Perazzolli et al. 2022), respectively. Root length was measured with a ruler 7 days after seed inoculation. Five replicates (dishes with five plants each) were analyzed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

Assessment of indole-3-acetic acid production

The production of the phytohormone indole-3-acetic acid by bacterial isolates was assessed as previously described (Gang et al. 2019) with slight modifications. Briefly, an aliquot (100 µL) of each bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium) was inoculated in liquid peptone yeast extract medium (final volume of 5.0 mL) supplemented with 0.5 g L^{-1} tryptophan (Acros organics, Geel, Belgium) and incubated at 25 ± 1 °C (non-stressed condition) or 4 ± 1 °C (cold-stressed condition) under orbital shaking at 200 rpm for 48 h. Cells were collected by centrifugation (3500×*g* for 10 min) and 1 mL of the supernatant was mixed with 1 mL of Salkowski reagent (10 mM $FeCl_3$ in 35% perchloric acid). The mixture was incubated at room temperature for 30 min in the dark. and the OD at 530 nm was determined for each sample using a spectrophotometer (Ultrospec 3100, GE Healthcare) to quantify the indole-3-acetic acid content (mg L^{-1}), using a calibration curve of 0, 5, 10, 20, 50, and 100 mg L^{-1} indole-3-acetic acid dissolved in liquid peptone yeast extract medium. Five replicates (bacterial cultures) were assessed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

Assessment of ammonia production

The production of ammonia by bacterial isolates was assessed as previously described (Campisano et al. 2015) with slight modifications. Briefly, an aliquot (100 µL) of each bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium) was inoculated in peptone water (final volume of 5.0 mL; 10 g L^{-1} peptone and 5 g L^{-1} NaCl in distilled water) and incubated at 25 ± 1 °C (non-stressed condition) or 4 ± 1 °C (cold-stressed condition) under orbital shaking at 200 rpm for 48 h. Cells were collected by centrifugation (3500×*g* for 10 min) and 1 mL of the supernatant was mixed with 1 mL of Nessler's reagent (Fluka, Sigma-Aldrich). The mixture was incubated at room temperature for 30 min and the OD at 420 nm was determined for each sample using a spectrophotometer (Ultrospec 3100, GE Healthcare). The ammonia content was quantified according to the following equation: $OD_{420} = 0.01077 + 0.0001708 \times$ ammonia (µg L^{-1}) (Zhao et al. 2019), and it was expressed as mg L^{-1}. Five replicates (bacterial cultures) were assessed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

Assessment of protease production

The production of proteases by bacterial isolates was assessed on skim milk agar (Oxoid) as previously described (Abubakr et al. 2012). For each bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium), four spots (5 µL each) were spotted on skim milk agar (Fluka, Sigma-Aldrich) in a Petri dish. Dishes were incubated at 25 ± 1 °C (non-stressed condition) or 4 ± 1 °C (cold-stressed condition) for three days and the formation of a clear halo around the colony indicated protease activity. The protease production was expressed as the halo area calculated by subtracting the colony area from the total halo area of each spot (Campisano et al. 2015). Five replicates (four spots for each bacterial culture) were assessed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

Assessment of siderophore production

The production of siderophores by bacterial isolates was assessed using Chrome azurol S (CAS) agar as previously described (Campisano et al. 2015). For each bacterial suspension (*Ewingella* sp., *Pseudomonas* sp, or bacterial consortium), four spots (5 µL each) were spotted on CAS agar in a Petri dish. Dishes were incubated at 25 ± 1 °C (non-stressed condition) or 4 ± 1 °C (cold-stressed condition) for three days and the formation of a clear halo around the colony indicated siderophore activity. The siderophore production was expressed as the halo area calculated by subtracting the colony area from the total halo area of each spot (Campisano et al. 2015). Five replicates (four spots for each bacterial culture) were assessed for each bacterial suspension and incubation temperature, and the experiment was carried out twice.

DNA extraction and sequencing

Each isolate was grown overnight (18 h) in liquid peptone yeast extract medium at 25 ± 1 °C under orbital shaking at 200 rpm and genomic DNA was extracted from 0.5 mL of the bacterial cultures using the FastDNA SPIN Kit for Soil (MP Biomedicals, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturers' instructions. DNA of *Ewingella* sp. and *Pseudomonas* sp. was quantified using a Qubit (Thermo Fisher Scientific) and its quality was checked using 1% w/v agarose gel electrophoresis. DNA samples were subjected to Illumina Standard genomic library construction protocol (Illumina, SanDiego, CA, USA) at Eurofins Genomics (Ebersberg, Germany) and paired-end reads of 150 nucleotides were obtained using an Illumina Novaseq 6000 instrument (Illumina).

Genome assembly and annotation

The genome assembly was carried out using the Bacflux workflow, designed specifically for the processing and analysis of bacterial genomic data sequenced with Illumina technology (Antonielli et al. https://doi.org/10.5281/zenodo.11143918). Briefly, raw reads were pre-processed to remove Illumina phiX contamination using bowtie2 v2.5.3 (Langmead et al. 2019), and adapters were then removed using fastp v23.4 (Chen 2023). Filtered reads were assembled into contigs with SPAdes v3.15.5 (Prjibelski et al. 2020) and quality control of de novo assembly was performed by filtering contigs based on a minimum length of 500 bp and minimum coverage of two, and a subsequent re-mapping filtered reads to contigs, using bowtie2 v2.5.3 (Langmead et al. 2019) and samtools (Danecek et al. 2021). The resulting BAM file was analyzed with QualiMap v2.3 (Okonechnikov et al. 2016). Contigs were subjected to local alignments against the NCBI nucleotide database (downloaded in November 2023) using BLAST + v2.15.0, contaminant contigs were identified and discarded with BlobTools v1.1.1 (Laetsch et al. 2020). Genome assembly quality was evaluated with Quast v5.2.0 (Gurevich et al. 2013), and completeness and contamination were assessed with CheckM v1.2.2 (Parks et al. 2015).

Taxonomic analysis was carried out using Genome Taxonomy Database-Tk v2.3.2 (Chaumeil et al. 2022) and the curated reference database v214.0. (https://gtdb.ecogenomic.org). Sequences were deposited at the NCBI (https://www.ncbi.nlm.nih.gov/sra; Bioproject number PRJNA1114625) under the accession number MZ089444 (*Ewingella* sp.) and MZ089387 (*Pseudomonas* sp.). The presence of plasmids was investigated with Platon v1.5.0 (Schwengers et al. 2020) according to BLAST + v2.15.0 search of sequencing data (https://zenodo.org/record/4066768/files/db.tar.gz).

The sequenced genomes were annotated using Bakta v.1.9.3 (Schwengers et al. 2021). Gene annotations were manually inspected to identify genes possibly involved in plant growth-promoting, such as genes implicated in cytokinin metabolism, ethylene metabolism (e.g., ACC deaminase), iron uptake (e.g., siderophore metabolism and transport), tryptophan and indole-3-acetic acid metabolism that were previously associated with plant growth promotion activities in other bacteria (Han et al. 2011; Qin et al. 2017; Rana et al. 2020; Adeleke et al. 2021). Moreover, genes possibly related to nutrient acquisition (e.g., amino acid metabolism and transport, amylase production, cellulase production, iron transport, nitrate transport and reduction, nitrogen metabolism and transport, phosphate metabolism and transport, potassium transport, and zinc transport) and colonization of the plant host (e.g., protease production) were analyzed, since they were previously associated with possible plant growth promotion effects (Han et al. 2011;

Mushtaq et al. 2019; Rana et al. 2020; Adeleke et al. 2021; Mukhia et al. 2022; Negi et al. 2024).

Putative genes involved in bacterial cold tolerance were also searched, such as genes encoding cold shock-related proteins, heat shock-related proteins, and lipid desaturases (Kube et al. 2013; Guo et al. 2020), as well as genes implicated in polyamine metabolism and transport, proline and glycine betaine transport, proline metabolism, ROS detoxification, salicylic acid metabolism and transport, and trehalose metabolism (Mercado-Blanco et al. 2001; Raymond-Bouchard et al. 2018; Han et al. 2021; Teoh et al. 2021; Jiang et al. 2022; Mukhia et al. 2022). These genes can contribute to the cold tolerance of bacterial cells and they can support the host plant in the mitigation of cold stress, controlling ROS homeostasis and synthesizing protective solutes (e.g., proline, glycine betaine, and trehalose) or stress-related hormones (e.g., salicylic acid) (Ait Barka et al. 2006; Subramanian et al. 2015; Acuña-Rodríguez et al. 2020). Genes possibly involved in antimicrobial resistance and virulence were searched on gene annotations, and the results were confirmed by mapping to the CARD database (https://card.mcmaster.ca/) with BBMap v. 39.06 (Bushnell 2014) and screening with ABRicate v.1.0.1 (https://github.com/tseemann/abricate/blob/master/LICENSE). Genes displaying more than 80% of covered reads were selected as potentially relevant for contributing to antimicrobial resistance or virulence in *Ewingella* sp., and *Pseudomonas* sp.

Phylogenomic analysis

For *Ewingella* sp. and *Pseudomonas* sp. core-phylogenomics analysis, the whole-genome sequences of 83 type-strains (belonging to *Yersiniaceae* and *Hafniaceae* families) and 46 type-strains (belonging to *Pseudomonas* spp. reported by Girard et al. 2021 for *P. fluorescens* group) were downloaded from the RefSeq genome database of NCBI (https://www.ncbi.nlm.nih.gov/refseq/; available on November 2024), respectively. Genomes selected were reannotated with Prokka (version 1.13) (Seemann 2014). The average nucleotide identity (ANI) (Goris et al. 2007) was calculated using pyani tool (https://github.com/widdowquinn/pyani).

Phylogenomic analysis was performed using Roary pipeline (Page et al. 2015) setting 70% as blast similarity and 99% as number of strains enclosed. For *Ewingella* sp. and *Pseudomonas* sp., 87 and 107 core-genes shared with the outgroup *Pseudomonas taeanensis* MS-3 and *Ewingella americana* CCUG14506 were used to build the trees with RAxML (version 8.2.12) (Stamatakis 2014) using GTR-GAMMA as statistical method, respectively. The phylogenomic trees were visualized with ggtree R package (Xu et al. 2022).

Statistical analysis

Statistical analyses of functional assays were carried out using Past 4.03 software (Hammer et al. 2001), and the Mann–Whitney test was used to detect significant differences ($P \leq 0.05$) in the pairwise comparisons among inoculation conditions (mock, *Ewingella* sp., *Pseudomonas* sp., and bacterial consortium) and incubation temperatures (25 ± 1 °C and 4 ± 1 °C).

Results

Ewingella sp. and *Pseudomonas* sp. display plant growth-promoting activities under cold conditions

Antarctic bacterial endophytes promoted plant growth of tomato seedlings, and root length was longer in *Ewingella* sp.-, *Pseudomonas* sp-, or bacterial consortium-inoculated seedlings compared to mock-inoculated seedlings at 15 ± 1 °C (cold-stressed condition), but not at 25 ± 1 °C (non-stressed condition; Fig. 1). To test plant growth-promoting activities of Antarctic bacterial endophytes, the production of indole-3-acetic acid, ammonia, protease, and siderophores was evaluated in vitro at 25 ± 1 °C and 4 ± 1 °C (Fig. 2). *Ewingella* sp. and *Pseudomonas* sp. growth were comparable in the pure culture and the bacterial consortium under non-stressed (25 ± 1 °C) and cold-stressed (4 ± 1 °C) conditions (Fig. S1), indicating cold tolerance of both Antarctic bacteria and compatibility in the consortium at two temperature regimes.

Indole-3-acetic acid was produced by *Ewingella* sp. and the bacterial consortium at 25 ± 1 °C and 4 ± 1 °C in vitro, but not by *Pseudomonas* sp. (Fig. 2A). The production of indole-3-acetic acid was higher at 25 ± 1 °C compared to 4 ± 1 °C for each bacterial suspension, and it was higher in the pure culture of *Ewingella* sp. compared to the bacterial consortium at each incubation temperature. Ammonia was produced by *Ewingella* sp. and *Pseudomonas* sp. grown as pure culture or bacterial consortium in vitro, and ammonia production was lower in *Pseudomonas* sp. compared to *Ewingella* sp. and bacterial consortium at each incubation temperature (Fig. 2B). Moreover, ammonia production was higher at 25 ± 1 °C compared to 4 ± 1 °C for each bacterial suspension. Proteases were produced by *Pseudomonas* sp. and the bacterial consortium, but not by *Ewingella* sp. in vitro (Fig. 2C). Protease production was higher at 25 ± 1 °C compared to 4 ± 1 °C for each bacterial suspension, and it was higher in *Pseudomonas* sp. compared to the bacterial consortium at 4 ± 1 °C. Siderophores were produced by *Ewingella* sp. and *Pseudomonas* sp. grown as pure culture or bacterial consortium at 25 ± 1 °C in vitro

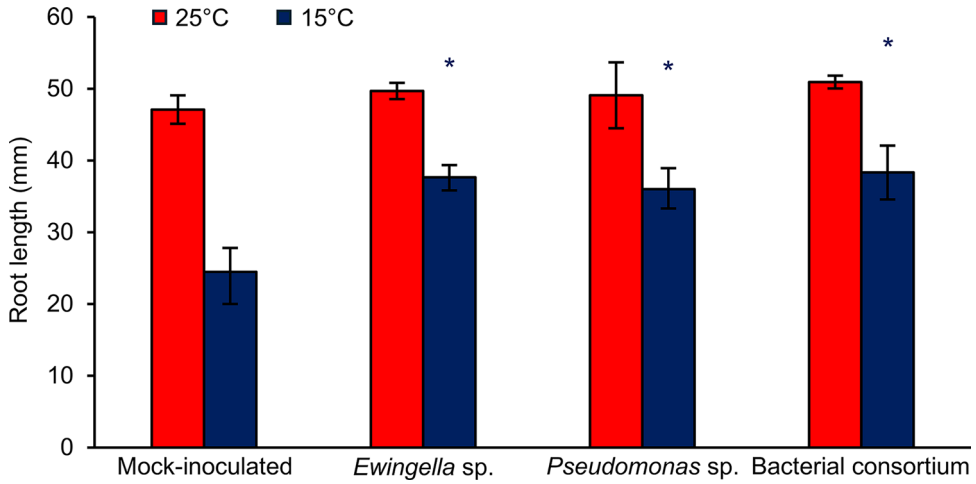

Fig. 1 Plant growth-promoting activity of Antarctic endophytic bacteria on tomato seedlings. Tomato seeds were treated with 10 mM $MgSO_4$ (mock-inoculated) or inoculated with *Ewingella* sp. S1.OA.A_B6 (*Ewingella* sp.), *Pseudomonas* sp. S2.OTC.A_B10 (*Pseudomonas* sp.), and the consortium of the two isolates (bacterial consortium). Plants were grown at 15 ± 1 °C and root length was measured 7 days after seed inoculation. Mean and standard error values of ten replicates (dishes with five plants each) from the two experiments are reported for each treatment. Asterisks indicate significant differences between mock-inoculated and bacterium-inoculated plants at 15 ± 1 °C, according to the Mann–Whitney test ($P \leq 0.05$). No differences between plants inoculated with each pure culture and the bacterial consortium were found, according to the Mann–Whitney test ($P > 0.05$)

(Fig. 2D). Siderophore production was higher at 25 ± 1 °C compared to 4 ± 1 °C for each bacterial suspension, and it was lower in *Ewingella* sp. compared to the bacterial consortium at both incubation temperatures.

Genomic features of Antarctic bacterial isolates

A total of 13.35 and 13.83 megabase (Mbp) were sequenced for *Ewingella* sp. and *Pseudomonas* sp. genome, and the guanine-cytosine (GC) content was 51.57% and 60.63%, respectively (Supplementary Table S1). Mapping results revealed that 99.90% and 99.95% of sequenced reads were aligned on *Ewingella* sp. (13,340,286 mapped reads) and *Pseudomonas* sp. (13,829,530 mapped reads) reference genomes, corresponding to a coverage of 436.7 ± 111.3 and 309.7 ± 87.9, respectively (mean ± standard deviation of the coverage of each nucleotide site). The genome of *Ewingella* sp. (NCBI accession number MZ089444) was assembled in 38 contigs (18 longer than 50,000 bp, 4 longer than 25,000 bp, 2 longer than 10,000 bp, 8 longer than 1000 bp, and 6 shorter than 1000 bp) and the genome of *Pseudomonas* sp. (NCBI accession number MZ089387) was assembled in 71 contigs (35 longer than 50,000 bp, 11 longer than 25,000 bp, 9 longer than 10,000 bp, 6 longer than 5000 bp, 5 longer than 1000 bp, and 5 shorter than 1000 bp). The genome assembly of *Ewingella* sp. and *Pseudomonas* sp. showed N50 values of 300,118 and 242,701, and N90 values of 100,900 and 50,103 base pairs, respectively, with L50 values of three and ten, and L90 values of 14 and 34, indicating a high level of assembly quality. Gene prediction results indicated that the genomes of *Ewingella* sp. and *Pseudomonas* sp. included 4148 and 5983 predicted genes, five and three rRNA genes, 62 and 58 tRNA genes, respectively. Moreover, no plasmids were found in *Ewingella* sp. and *Pseudomonas* sp. sequencing data. Phylogenomic analysis showed that *Ewingella* sp. and *Pseudomonas* sp. are phylogenetically close to *Hafnia psychrotolerans* type-strain and *Pseudomonas yamanorum* type-strain, respectively (Fig. 3). Moreover, ANI values calculated for *Ewingella* sp. and *Pseudomonas* sp. with each genome used in the phylogenomic were lower than 95%, as possible indication of novel species for both Antarctic endophytic bacteria (Supplementary Tables S2 and S3).

Ewingella sp. and *Pseudomonas* sp. genes are possibly implicated in plant growth promotion and cold tolerance

Ewingella sp., and *Pseudomonas* sp. gene prediction revealed genes possibly involved in bacterial metabolism and in plant growth promotion (Fig. 4A; Supplementary Tables S4 and S5), such as those involved in amino acid metabolism and transport (20 and 38 genes, respectively), cytokinin metabolism (no genes and one gene, respectively), ethylene metabolism (no genes and one gene, respectively), iron transport (23 and 23 genes, respectively), nitrate transport and reduction (19 and 13 genes, respectively), nitrogen metabolism and transport (*Ew_04885*, *Ew_07575*, *Ew_09205*, *Ew_09510*, *Ew_13070*, *Ew_17685*, *Ew_17690*, *Ew_18510*, *Ew_19895*, *Ew_19900*, *Pseudomonas*

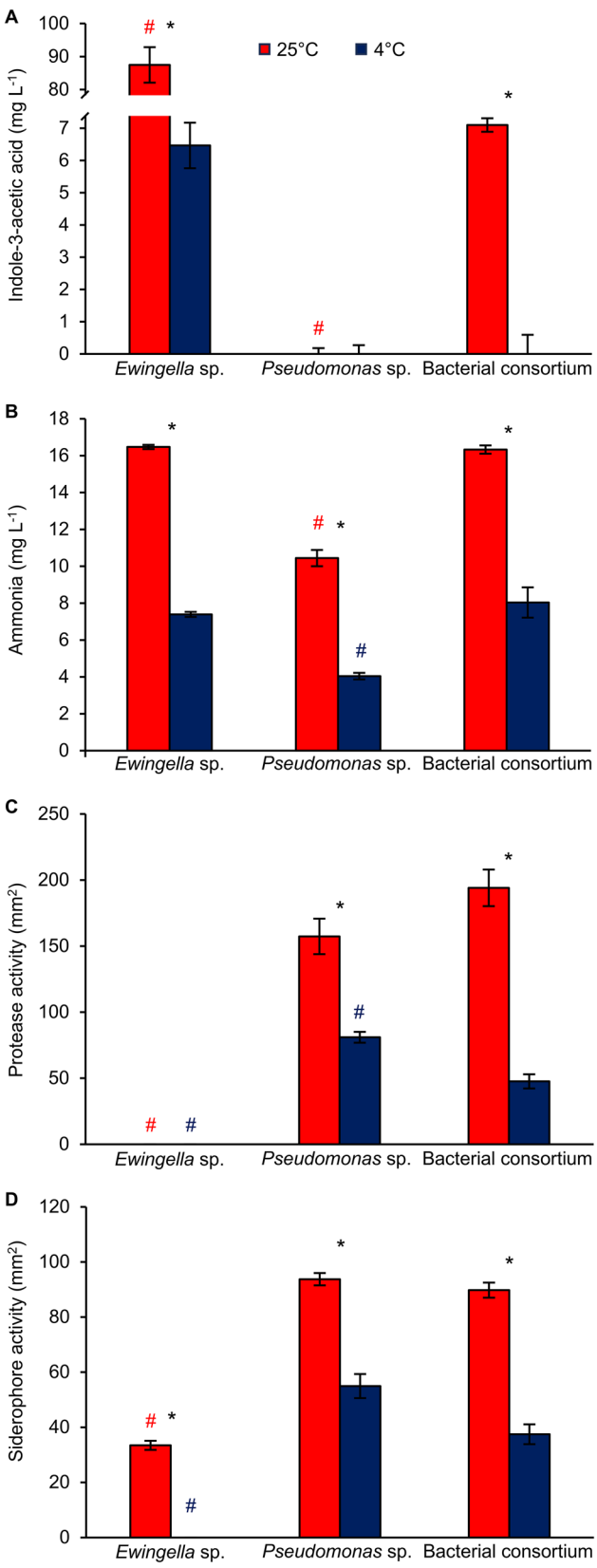

Fig. 2 Plant growth-promoting activities of Antarctic endophytic bacteria in vitro. Production of indole-3-acetic acid (**A**), ammonia (**B**), proteases (**C**), and siderophores (**D**) by *Ewingella* sp. S1.OA.A_B6 (*Ewingella* sp.), *Pseudomonas* sp. S2.OTC.A_B10 (*Pseudomonas* sp.), and the consortium of the two isolates (bacterial consortium) was assessed at 25 ± 1 °C (red bars) or 4 ± 1 °C (blue bars). Mean and standard error values of ten replicates (bacterial cultures) from the two experiments are reported for each treatment. For each incubation temperature, colored hashtags indicate significant differences between each pure culture and the bacterial consortium, according to the Mann–Whitney test ($P \leq 0.05$). For each bacterial suspension, asterisks indicate significant differences between 25 ± 1 and 4 ± 1 °C, according to the Mann–Whitney test ($P \leq 0.05$)

sp._02095, Pseudomonas sp._02100, Pseudomonas sp._03400, Pseudomonas sp._05620, Pseudomonas sp._14920, Pseudomonas sp._14925, Pseudomonas sp._14930, Pseudomonas sp._19110, Pseudomonas sp._20560, Pseudomonas sp._20665, Pseudomonas sp._29300, Pseudomonas sp._29305, and *Pseudomonas sp._293200*), phosphate metabolism and transport (23 and 26 genes, respectively), potassium transport (16 and 14 genes, respectively), siderophore metabolism and transport (five and eleven genes, respectively), tryptophan and indole-3-acetic acid production (ten and ten genes, respectively), and zinc transport (ten and nine genes, respectively). Moreover, *Ewingella* sp., and *Pseudomonas* sp. encoded lytic enzymes, such as amylases (e.g., *Ew_09095*, *Ew_09095*, and *Ps_26740*), cellulases (e.g., *Ps_26825*), and proteases (*Ew_03420, Ew_04210, Ew_05410, Ew_06950, Ew_07790, Ew_08185, Ew_08190, Ew_08480, Ew_08960, Ew_08975, Ew_09280, Ew_09285, Ew_09855, Ew_09880, Ew_10820, Ew_11030, Ew_11515, Ew_12955, Ew_12995, Ew_13155, Ew_13185, Ew_13330, Ew_13610, Ew_16155, Ew_18060, Ew_18635, Ew_19735, Ps_00500, Ps_01675, Ps_02750, Ps_03245, Ps_03350, Ps_03360, Ps_03565, Ps_05785, Ps_07095, Ps_07900, Ps_08340, Ps_08725, Ps_09440, Ps_09455, Ps_09465, Ps_09470, Ps_10165, Ps_10255, Ps_11560, Ps_12295, Ps_12300, Ps_13250, Ps_14270, Ps_14970, Ps_15390, Ps_16140, Ps_16670, Ps_17030, Ps_17160, Ps_17165, Ps_17270, Ps_17770, Ps_17775, Ps_20480, Ps_21710, Ps_22445, Ps_22830, Ps_22835, Ps_24875, Ps_25405, Ps_26225, Ps_26830, Ps_29755, Ps_29760,* and *Ps_30610*).

Ewingella sp., and *Pseudomonas* sp. genomes included potential functions related to cold stress tolerance (Fig. 4B; Supplementary Tables S4 and S5), such as cold shock-related proteins (*Ew_08035*, *Ew_08195*, *Ew_10140*, *Ew_14315*, *Ew_18455*, *Ps_04795*, *Ps_05725*, *Ps_12290*, *Ps_23070*, *Ps_26100*, and *Ps_26340*), heat shock-related proteins (*Ew_07810, Ew_11950, Ew_15980, Ew_15985, Ew_17365, Ew_17370, Ps_00150, Ps_00170, Ps_08665, Ps_15375, Ps_15380, Ps_20800, Ps_21665, Ps_27765,* and *Ps_27850*), lipid desaturases (*Ew_06560*, *Ew_06565*, *Ew_06570, Ps_11975*, *Ps_12160*, *Ps_15605*, *Ps_15610*,

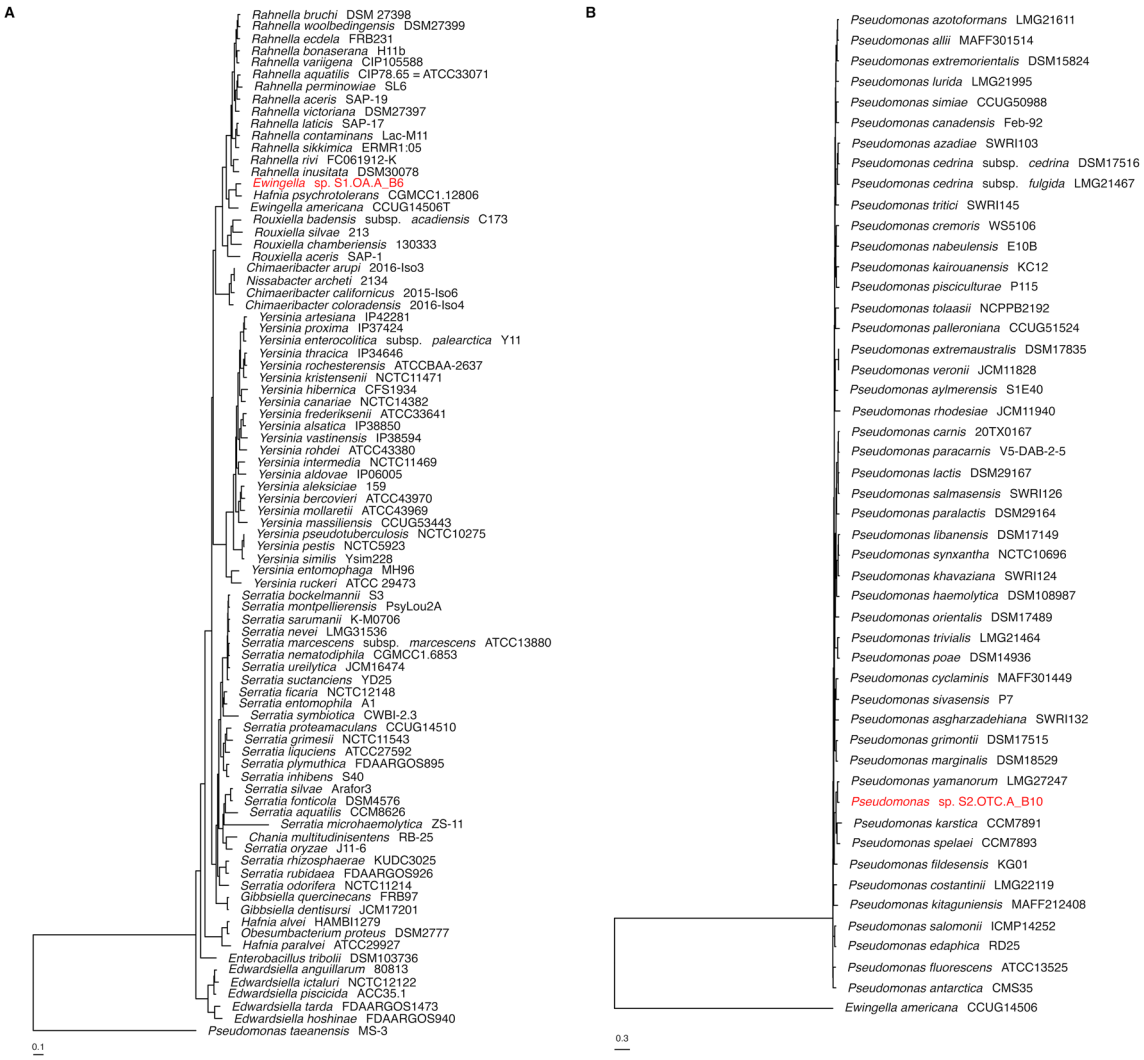

Fig. 3 Phylogenomic analysis of Antarctic endophytic bacteria. Phylogenomic trees of *Ewingella* sp. S1.OA.A_B6 (**A**) and *Pseudomonas* sp. S2.OTC.A_B10 (**B**) were obtained with 87 core-genes (shared with the outgroup *Pseudomonas taeanensis* MS-3 and 83 type-strains belonging to *Yersiniaceae* and *Hafniaceae* families) and 107 core-genes (shared with the outgroup *Ewingella americana* CCUG14506 and 46 type-strains belonging to *Pseudomonas* genus) using the GTRGAMMA statistical method of RAxML, respectively

Ps_15665, and *Ps_30300*), and genes involved in polyamine metabolism and transport (seven and 26 genes, respectively), proline and glycine betaine transport (*Ew_02180*, *Ew_05940*, *Ew_09460*, *Ew_20095*, *Ew_20100*, *Ew_20105*, *Ew_20110*, *Ps_00665*, *Ps_00720*, *Ps_03080*, *Ps_03085*, *Ps_03090*, *Ps_03095*, *Ps_06495*, *Ps_06515*, *Ps_12145*, *Ps_17500*, *Ps_17920*, *Ps_17950*, *Ps_17955*, and *Ps_19865*), proline metabolism (*Ew_00500*, *Ew_00510*, *Ew_05945*, *Ew_18250*, *Ps_05590*, *Ps_05605*, *Ps_05915*, *Ps_07550*, *Ps_12005*, *Ps_12010*, *Ps_17505*, *Ps_20090*, *Ps_20095*, *Ps_22585*, and *Ps_24800*), ROS detoxification (e.g., *Ew_00085*, *Ew_01940*, *Ew_03575*, *Ew_09450*, *Ew_16485*, *Ew_17245*, *Ps_01635*, *Ps_03380*, *Ps_07080*, *Ps_08430*, *Ps_09330*, *Ps_16885*, *Ps_22480*, *Ps_24275*, *Ps_28485*, *Ps_29405*, *Ps_30040*, *Ps_30080*), salicylic acid metabolism and transport (*Ew_04400*, *Ps_11375,* and *Ps_18745*), and trehalose metabolism (*Ew_10530*, *Ew_10535*, *Ew_20625*, *Ew_20630*, *Ew_20635*, *Ps_18235*, *Ps_18245*, *Ps_23285*, and *Ps_23290*). However, genes related to nitrogen fixation, pectin and xylan lysis, gibberellin metabolism, and hydrogen cyanide production were not found in *Ewingella* sp., and *Pseudomonas* sp. genomes. Moreover, nine and twelve antimicrobial resistance genes were found in *Ewingella* sp. and *Pseudomonas* sp., respectively, while no virulence genes were found (Supplementary Tables S4, S5 and S6).

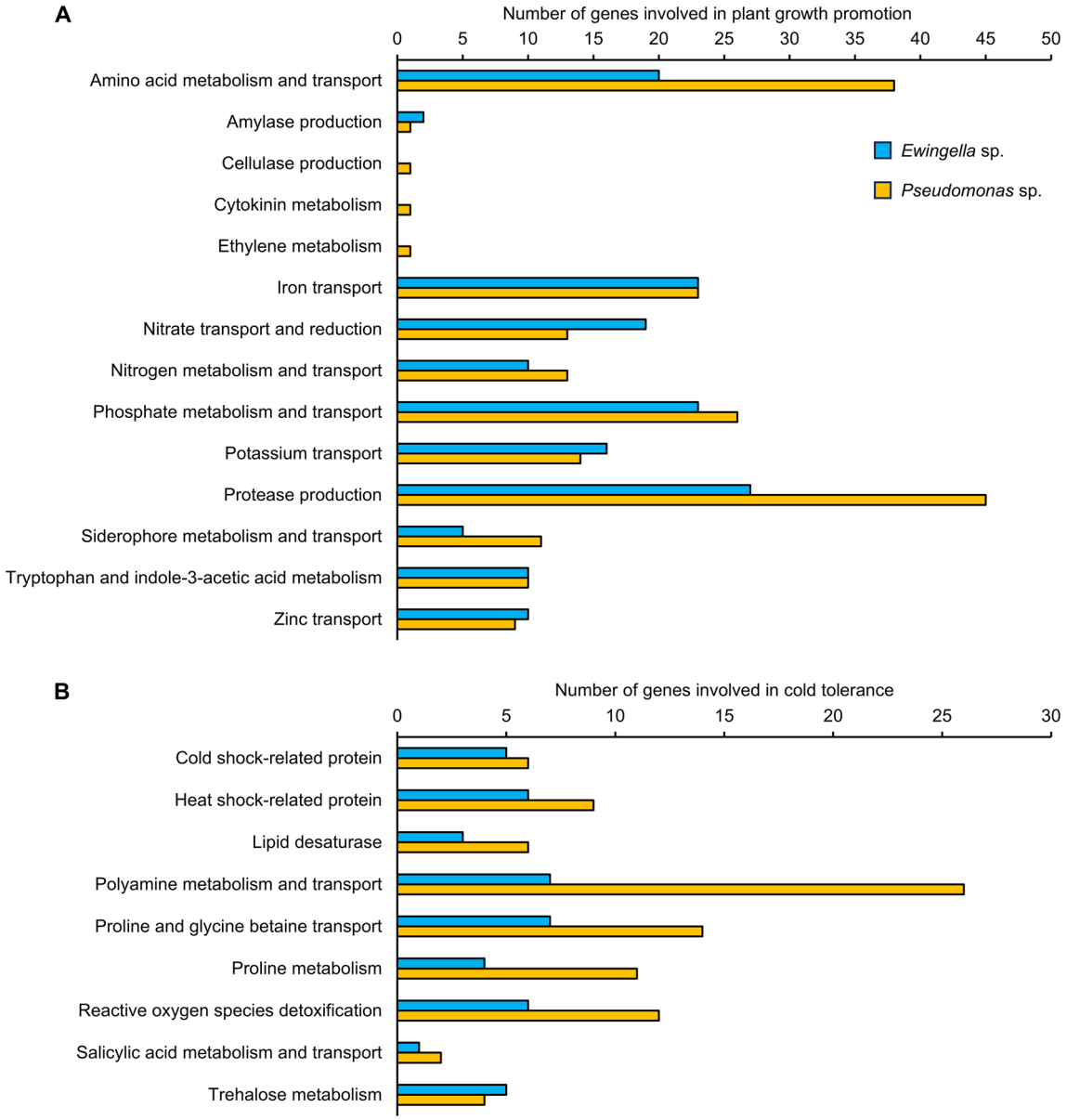

Fig. 4 Genes of Antarctic endophytic bacteria involved in plant growth promotion and cold tolerance. The number of genes involved in functional categories of bacterial metabolism and possibly related to plant growth promotion (**A**) and cold tolerance (**B**) are reported for *Ewingella* sp. S1.OA.A_B6 (yellow bars) and *Pseudomonas* sp. S2.OTC.A_B10 (blue bars) according to genome annotation results

Discussion

Antarctic bacterial endophytes showed plant growth-promoting activities on tomato plants at low temperature, and root length was longer in *Ewingella* sp.-, *Pseudomonas* sp-, or bacterial consortium-inoculated seedlings compared to mock-inoculated seedlings at 15 ± 1 °C. The plant growth promotion activity on tomato seedlings under cold-stressed condition (15 ± 1 °C), but not under non-stressed condition (25 ± 1 °C), indicated that Antarctic bacterial endophytes could better display beneficial properties when host plants are under non-optimal conditions. This result was coherent with the principle that non-optimal conditions frequently reveal higher beneficial effects exerted by associated microbes compared to optimal conditions (Rahman et al. 2018; Riva et al. 2021). Moreover, *Ewingella* sp. and *Pseudomonas* sp. can grow at 25 ± 1 °C and 4 ± 1 °C as pure culture and bacterial consortium in vitro, indicating cold tolerance of the two Antarctic bacterial endophytes and compatibility in the bacterial consortium at both temperature regimes. Moreover, ammonia and siderophore production were found in both bacterial isolates and their consortium

in vitro, while the production of indole-3-acetic acid and proteases in vitro was lower in the bacterial consortium compared to the pure culture, indicating possible metabolic competition of the two isolates. To better understand the possible functional properties and metabolic interactions of Antarctic bacterial endophytes, *Ewingella* sp. and *Pseudomonas* sp. genomes were sequenced and they revealed a GC content of 51.57% and 60.63%, in agreement with previous *Ewingella* sp. and *Pseudomonas* sp. genomic analyses, respectively (Liu et al. 2020b; Hu et al. 2023). Gene prediction revealed 4148 genes in *Ewingella* sp. and 5983 genes in *Pseudomonas* sp., in agreement with the previous reports on *Ewingella* sp. and *Pseudomonas* sp. genomes (Liu et al. 2020b; Hu et al. 2023). In particular, the *Ewingella* sp. genome encompassed genes possibly involved in indole-3-acetic production, such as a bifunctional indole-3-glycerol-phosphate synthase (*Ew_04160*) (Adeleke et al. 2021). Indole-3-acetic acid is a phytohormone belonging to the auxin class that can be synthesized by bacterial endophytes and plays crucial roles in promoting plant growth, cell elongation, root development, and stress tolerance (Rana et al. 2020). Indole-3-acetic acid was produced by *Ewingella* sp. and the bacterial consortium at 25 ± 1 °C and 4 ± 1 °C, but not by *Pseudomonas* sp. Moreover, the bacterial consortium showed a lower content of indole-3-acetic acid compared to the pure culture of *Ewingella* sp., indicating that *Pseudomonas* sp. might metabolize indole-3-acetic acid or its precursor. *Pseudomonas* sp. genome encompassed no genes for indole-3-acetic production, but a gene encoding tryptophan 2,3-dioxygenase (*Ps_21400*) involved in the degradation of tryptophan (Kurnasov et al. 2003), as a possible explanation of the lower content of indole-3-acetic acid in the bacterial consortium compared to the pure culture of *Ewingella* sp. Genome mining of Antarctic bacterial endophytes revealed that *Ewingella* sp. and *Pseudomonas* sp. genomes included genes potentially involved in the biosynthesis of other plant growth-related hormones, such as a cytokinin riboside 5′-monophosphate phosphoribohydrolase of *Pseudomonas* sp. (*Ps_17050*), which converts inactive cytokinin nucleotides into active free compounds (Chen et al. 2022). Cytokinins are involved in plant development, growth, and stress tolerance, and bacterial endophytes are known to produce cytokinin-like compounds (Negi et al. 2024). Moreover, *Ewingella* sp. and *Pseudomonas* sp. genomes encompassed genes involved in salicylic acid metabolism and transport (*Ew_04400*, *Ps_11375*, and *Ps_18745*), as a possible mechanism of cold stress mitigation in the plant host (Mishra and Baek 2021), and *Pseudomonas* sp. encompassed a gene encoding ACC deaminase (*Ps_18525*), which can decrease ethylene content (Negi et al. 2024), as found in plant growth-promoting bacteria that improve plant tolerance to abiotic stresses (Li et al. 2021).

Ammonia was produced by *Ewingella* sp. and *Pseudomonas* sp. grown as pure culture or bacterial consortium in vitro, but no genes associated with nitrogen fixation were found in *Ewingella* sp. and *Pseudomonas* sp. genomes. Ammonia can be produced as a byproduct of amino acid catabolism in a peptide-rich medium (Reitzer 2005) and genes potentially involved in amino acid degradation (*Ew_02040*, *Ew_03000*, *Ew_11185*, *Ew_16980* *Ps_00700*, *Ps_01440*, *Ps_01585*, *Ps_02625*, *Ps*_15680, and Ps_*17615*) and nitrogen metabolism were found in *Ewingella* sp. and *Pseudomonas* sp. genomes, suggesting a role of cold-tolerant endophytes in nitrogen cycling in Antarctic environments. Moreover, hydrolytic enzymes can help in the penetration of bacterial endophytes into plant tissues (Rana et al. 2020; Negi et al. 2024), and genes encoding amylases (two and one genes, respectively), cellulases (zero and one gene, respectively), and proteases (27 and 45 genes, respectively) were found in *Ewingella* sp., and *Pseudomonas* sp. Proteases were produced by *Pseudomonas* sp. and the bacterial consortium, but not by *Ewingella* sp., indicating different transcriptional or post-transcriptional activation of lytic enzymes. Moreover, genes encoding enzymes involved in macronutrient and micronutrient metabolism and transport were found in *Ewingella* sp. and *Pseudomonas* sp. genomes, and they were previously associated with possible plant growth-promoting activities (Han et al. 2011; Mushtaq et al. 2019; Rana et al. 2020; Adeleke et al. 2021; Mukhia et al. 2022; Negi et al. 2024), such as those implicated in amino acid metabolism and transport, iron transport, nitrate transport and reduction, nitrogen metabolism and transport, phosphate metabolism and transport, potassium transport, siderophore metabolism and transport (five and eleven genes in *Ewingella* sp., and *Pseudomonas* sp., respectively), phosphate transport, potassium transport, and zinc transport. In particular, siderophore production by *Ewingella* sp. and *Pseudomonas* sp. was shown in pure culture and bacterial consortium in vitro, corroborating the possible contribution of endophytic bacteria to iron solubilization and uptake (Han et al. 2011; Rana et al. 2020; Adeleke et al. 2021).

Ewingella sp. and *Pseudomonas* sp. genomes included genes potentially associated with cold tolerance through synthesis and transport of compatible solutes, such as genes related to proline and glycine betaine transport (seven and 14 genes, respectively), proline metabolism (four and eleven genes, respectively), and trehalose metabolism (five and four genes, respectively). Moreover, *Ewingella* sp. and *Pseudomonas* sp. genomes encompassed genes encoding cold shock-related proteins (five and six genes, respectively), heat shock-related proteins (six and nine genes, respectively), and ROS detoxification processes (six and twelve genes, respectively) to allow bacterial survival under cold stress (Raymond-Bouchard et al. 2018; Guo et al. 2020; Han et al. 2021; Teoh et al. 2021; Jiang et al. 2022). Moreover, the presence

of genes related to the synthesis and transport of compatible solutes, ROS detoxification, and synthesis of stress-related hormones (e.g., salicylic acid) can contribute to the possible role of endophytic bacteria in the mitigation of cold stress in plant hosts (Ait Barka et al. 2006; Subramanian et al. 2015; Acuña-Rodríguez et al. 2020). For example, soluble sugar content and proline content in tomato plants are known to increase after inoculation of endophytic bacteria (*Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21) (Wang et al. 2016). Likewise, trehalose (Liu et al. 2020a) and glycine betaine (Karabudak et al. 2014) were previously associated with cold tolerance in tomato plants. Furthermore, *Ewingella* sp. and *Pseudomonas* sp. genomes included genes encoding lipid desaturases and genes involved in polyamine metabolism and transport, which can contribute to the adjustment of membrane fluidity (Upchurch 2008) and cold stress tolerance (Gill and Tuteja 2010). Phylogenomic analysis and ANI calculation showed that *Ewingella* sp. is phylogenetically close to *Hafnia psychrotolerans* type-strain, which is susceptible to being reclassified as *Ewingella psychrotolerans* (Ludwig et al. 2021), while *Pseudomonas* sp. belongs to the *P. fluorescens* group previously characterized by Girard et al. (2021). However, further genetic and metabolic studies are required to verify that these Antarctic endophytic bacteria could belong to novel species. Moreover, further functional studies on cold-stressed plants inoculated with Antarctic bacteria are required to better characterize the mechanisms of plant growth promotion and the possible contribution of bacterial colonization in cold stress mitigation of the plant host. Moreover, antimicrobial resistance genes were found in *Ewingella* sp. and *Pseudomonas* sp., including those encoding multidrug transporters and multidrug resistance proteins, indicating possible resistance against antimicrobial molecules. *Ewingella* sp., and *Pseudomonas* sp. sequencing data include no plasmids, and further studies are required to exclude potential effects of the DNA extraction protocol and to verify the presence of extrachromosomal antibiotic resistance and virulence genes in these bacterial isolates.

Conclusion

Functional assays of two Antarctic bacterial endophytes revealed indole-3-acetic acid production and protease production by *Ewingella* sp. and *Pseudomonas* sp., respectively, while ammonia and siderophore production were found in both bacterial isolates and their consortium. Genomic analysis of *Ewingella* sp. and *Pseudomonas* sp. highlighted genes possibly involved in plant growth promotion (e.g., auxin, cytokinin, ethylene, salicylic acid, and siderophore metabolism) and genes related to bacterial metabolic processes that can contribute to plant growth-promoting activities (e.g., amino acid metabolism, iron transport, lytic activities, nitrogen metabolism, phosphate metabolism, potassium transport, and zinc transport). Moreover, *Ewingella* sp. and *Pseudomonas* sp. genomes encompassed genes possibly associated with bacterial cold tolerance that can contribute to cold stress mitigation in the plant host, such as cold shock- and heat shock-related proteins, lipid desaturases, proline and glycine betaine metabolism, ROS detoxification, and trehalose metabolism. Although further experiments are required to validate gene functions using *Ewingella* sp. and *Pseudomonas* sp. mutants, these results indicate possible strategies for the survival of cold-tolerant endophytic bacteria under harsh environmental conditions and suggest that some bacterial functions can contribute to plant growth promotion and cold stress mitigation in the plant host.

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Author contributions GL and CS carried out the experiments in vitro. GL and LA carried out the genome analyses. IL carried out the phylogenomic analyses. GL, LA, IL and MP analyzed the data and contributed to data interpretation. MP and GL conceived the study and designed the experiments. GL, LA, and MP wrote the manuscript. All the authors revised and approved the final manuscript.

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Data availability Genome sequencing data were deposited at the NCBI database (https://www.ncbi.nlm.nih.gov/sra) under the BioProject number PRJNA1114625.

Declarations

Competing interests The authors declare no competing interests.

References

Abubakr MAS, Hassan Z, Muftah MAI, Sherifah NRSA (2012) Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). Afr J Microbiol Res 6:6358–6364. https://doi.org/10.5897/ajmr12.702

Acuña-Rodríguez IS, Newsham KK, Gundel PE et al (2020) Functional roles of microbial symbionts in plant cold tolerance. Ecol Lett 23:1034–1048. https://doi.org/10.1111/ele.13502

Adeleke BS, Ayangbenro AS, Babalola OO (2021) Genomic analysis of endophytic *Bacillus cereus* t4s and its plant growth-promoting traits. Plants 10:1776. https://doi.org/10.3390/plants10091776

Ait Barka E, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252. https://doi.org/10.1128/AEM.01047-06

Bushnell B (2014) BBMap: a fast, accurate, splice-aware aligner. LBL Publication, San Francisco

Campisano A, Pancher M, Puopolo G et al (2015) Diversity in endophyte populations reveals functional and taxonomic diversity between wild and domesticated grapevines. Am J Enol Vitic 1:12–21. https://doi.org/10.5344/ajev.2014.14046

Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH (2022) GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics 38:5315–5316. https://doi.org/10.1093/bioinformatics/btac672

Chen S (2023) Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. iMeta 2:1–5. https://doi.org/10.1002/imt2.107

Chen L, Jameson GB, Guo Y et al (2022) The LONELY GUY gene family: from mosses to wheat, the key to the formation of active cytokinins in plants. Plant Biotechnol J 20:625–645. https://doi.org/10.1111/pbi.13783

Danecek P, Bonfield JK, Liddle J et al (2021) Twelve years of SAMtools and BCFtools. GigaScience 10:1–4. https://doi.org/10.1093/gigascience/giab008

Gang S, Sharma S, Saraf M et al (2019) Analysis of indole-3-acetic acid (IAA) production in *Klebsiella* by LC-MS/MS and the Salkowski method. Bio-Protoc 9:e3230. https://doi.org/10.21769/bioprotoc.3230

Gill SS, Tuteja N (2010) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5:26–33. https://doi.org/10.4161/psb.5.1.10291

Girard L, Lood C, Höfte M et al (2021) The ever-expanding pseudomonas genus: description of 43 new species and partition of the *Pseudomonas putida* group. Microorganisms 9:1–24. https://doi.org/10.3390/microorganisms9081766

Goris J, Konstantinidis KT, Klappenbach JA et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0

Guo DJ, Singh RK, Singh P et al (2020) Complete genome sequence of Enterobacter roggenkampii ED5, a nitrogen fixing plant growth promoting endophytic bacterium with biocontrol and stress tolerance properties, isolated from sugarcane root. Front Microbiol 11:580081. https://doi.org/10.3389/fmicb.2020.580081

Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistic software package for education and data analysis. Palaeontol Electron 4:1–9

Han JI, Choi HK, Lee SW et al (2011) Complete genome sequence of the metabolically versatile plant growth-promoting endophyte *Variovorax paradoxus* S110. J Bacteriol 193:1183–1190. https://doi.org/10.1128/JB.00925-10

Han SR, Kim B, Jang JH et al (2021) Complete genome sequence of *Arthrobacter* sp. PAMC25564 and its comparative genome analysis for elucidating the role of CAZymes in cold adaptation. BMC Genom 22:1–14. https://doi.org/10.1186/s12864-021-07734-8

Hu YQ, Zeng YX, Du Y et al (2023) Comparative genomic analysis of two Arctic *Pseudomonas* strains reveals insights into the aerobic denitrification in cold environments. BMC Genom 24:1–16. https://doi.org/10.1186/s12864-023-09638-1

Jiang L, Peng Y, Seo J et al (2022) *Subtercola endophyticus* sp. nov., a cold-adapted bacterium isolated from *Abies koreana*. Sci Rep 12:1–13. https://doi.org/10.1038/s41598-022-16116-3

Kandasamy GD, Kathirvel P (2023) Insights into bacterial endophytic diversity and isolation with a focus on their potential applications—a review. Microbiol Res 266:127256. https://doi.org/10.1016/j.micres.2022.127256

Karabudak T, Bor M, Özdemir F, Türkan I (2014) Glycine betaine protects tomato (*Solanum lycopersicum*) plants at low temperature by inducing fatty acid desaturase7 and lipoxygenase gene expression. Mol Biol Rep 41:1401–1410. https://doi.org/10.1007/s11033-013-2984-6

Kube M, Chernikova TN, Al-Ramahi Y et al (2013) Genome sequence and functional genomic analysis of the oil-degrading bacterium *Oleispira antarctica*. Nat Commun 4:2156. https://doi.org/10.1038/ncomms3156

Kurnasov O, Jablonski L, Polanuyer B et al (2003) Aerobic tryptophan degradation pathway in bacteria: novel kynurenine formamidase. FEMS Microbiol Lett 227:219–227. https://doi.org/10.1016/S0378-1097(03)00684-0

Laetsch DR, Blaxter ML, Leggett RM (2020) BlobTools: interrogation of genome assemblies. F1000Res 1287:1–16. https://doi.org/10.12688/f1000research.12232.1

Langmead B, Wilks C, Antonescu V, Charles R (2019) Scaling read aligners to hundreds of threads on general-purpose processors. Bioinformatics 35:421–432. https://doi.org/10.1093/bioinformatics/bty648

Li M, Wang J, Yao T et al (2021) Isolation and characterization of cold- adapted PGPB and their effect on plant growth promotion. J Microbiol Biotechnol 31:1218–1230. https://doi.org/10.4014/jmb.2105.05012

Licciardello G, Doppler M, Sicher C et al (2024) Metabolic changes in tomato plants caused by psychrotolerant Antarctic endophytic bacteria might be implicated in cold stress mitigation. Physiol Plant 176:e14352. https://doi.org/10.1111/ppl.14352

Liu T, Ye X, Li M et al (2020a) H2O2 and NO are involved in trehalose-regulated oxidative stress tolerance in cold-stressed tomato plants. Environ Exp Bot 171:103961. https://doi.org/10.1016/j.envexpbot.2019.103961

Liu Z, Sheng H, Okorley BA et al (2020b) Comparative genomic analysis provides insights into the phylogeny, resistome, virulome, and host adaptation in the genus *Ewingella*. Pathogens 9:1–23. https://doi.org/10.3390/pathogens9050330

Ludwig W, Viver T, Westram R et al (2021) Release LTP_12_2020, featuring a new ARB alignment and improved 16S rRNA tree for prokaryotic type strains. Syst Appl Microbiol 44:126218. https://doi.org/10.1016/j.syapm.2021.126218

Marian M, Licciardello G, Vicelli B et al (2022) Ecology and potential functions of plant-associated microbial communities in cold environments. FEMS Microbiol Ecol 98:fiab161. https://doi.org/10.1093/femsec/fiab161

Mercado-Blanco J, Van Der Drift KMGM, Olsson PE et al (2001) Analysis of the pmsCEAB gene cluster involved in biosynthesis

of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. J Bacteriol 183:1909–1920. https://doi.org/10.1128/JB.183.6.1909-1920.2001

Mishra AK, Baek KH (2021) Salicylic acid biosynthesis and metabolism: a divergent pathway for plants and bacteria. Biomolecules 11:705. https://doi.org/10.3390/biom11050705

Mukhia S, Kumar A, Kumari P, Kumar R (2022) Psychrotrophic plant beneficial bacteria from the glacial ecosystem of Sikkim Himalaya: genomic evidence for the cold adaptation and plant growth promotion. Microbiol Res 260:127049. https://doi.org/10.1016/j.micres.2022.127049

Mushtaq S, Shafiq M, Ashraf T et al (2019) Characterization of plant growth promoting activities of bacterial endophytes and their antibacterial potential isolated from citrus. J Anim Plant Sci 29:978–991

Negi R, Sharma B, Kumar S et al (2024) Plant endophytes: unveiling hidden applications toward agro-environment sustainability. Folia Microbiol 69:181–206. https://doi.org/10.1007/s12223-023-01092-6

Nissinen RM, Männistö MK, van Elsas JD (2012) Endophytic bacterial communities in three arctic plants from low arctic fell tundra are cold-adapted and host-plant specific. FEMS Microbiol Ecol 82:510–522. https://doi.org/10.1111/j.1574-6941.2012.01464.x

Okonechnikov K, Conesa A, García-Alcalde F (2016) Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics 32:292–294. https://doi.org/10.1093/bioinformatics/btv566

Page AJ, Cummins CA, Hunt M et al (2015) Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31:3691–3693. https://doi.org/10.1093/bioinformatics/btv421

Parks DH, Imelfort M, Skennerton CT et al (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. https://doi.org/10.1101/gr.186072.114.Freely

Perazzolli M, Vicelli B, Antonielli L et al (2022) Simulated global warming affects endophytic bacterial and fungal communities of Antarctic pearlwort leaves and some bacterial isolates support plant growth at low temperatures. Sci Rep 12:1–13. https://doi.org/10.1038/s41598-022-23582-2

Prjibelski A, Antipov D, Meleshko D et al (2020) Using SPAdes De Novo assembler. Curr Protoc Bioinf 70:1–29. https://doi.org/10.1002/cpbi.102

Qin Y, Fu Y, Kang W et al (2017) Isolation and identification of a cold-adapted bacterium and its characterization for biocontrol and plant growth-promoting activity. Ecol Eng 105:362–369. https://doi.org/10.1016/j.ecoleng.2017.04.045

Rahman MM, Flory E, Koyro HW et al (2018) Consistent associations with beneficial bacteria in the seed endosphere of barley (*Hordeum vulgare* L.). Syst Appl Microbiol 41:386–398. https://doi.org/10.1016/j.syapm.2018.02.003

Rana KL, Kour D, Kaur T et al (2020) Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie Van Leeuwenhoek/int J Gener Mol Microbiol 113:1075–1107. https://doi.org/10.1007/s10482-020-01429-y

Raymond-Bouchard I, Goordial J, Zolotarov Y et al (2018) Conserved genomic and amino acid traits of cold adaptation in subzero-growing Arctic permafrost bacteria. FEMS Microbiol Ecol 94:1–13. https://doi.org/10.1093/femsec/fiy023

Reitzer L (2005) Catabolism of amino acids and related compounds. EcoSal plus. https://doi.org/10.1128/ecosalplus.3.4.7

Riva V, Mapelli F, Dragonetti G et al (2021) Bacterial inoculants mitigating water scarcity in tomato: the importance of long-term in vivo experiments. Front Microbiol. https://doi.org/10.3389/fmicb.2021.675552

Schwengers O, Barth P, Falgenhauer L et al (2020) Platon: identification and characterization of bacterial plasmid contigs in short-read draft assemblies exploiting protein sequence-based replicon distribution scores. Microbial Genomics 6:1–12. https://doi.org/10.1099/mgen.0.000398

Schwengers O, Jelonek L, Dieckmann MA et al (2021) Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microbial Genomics 7:000685. https://doi.org/10.1099/MGEN.0.000685

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Shivaji S, Begum Z, Shiva Nageswara Rao SS et al (2013) Antarctic ice core samples: culturable bacterial diversity. Res Microbiol 164:70–82. https://doi.org/10.1016/j.resmic.2012.09.001

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Subramanian P, Mageswari A, Kim K et al (2015) Psychrotolerant endophytic *Pseudomonas* sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* Mill.) by activation of their antioxidant capacity. Mol Plant Microbe Interact 28:1073–1081. https://doi.org/10.1094/MPMI-01-15-0021-R

Subramanian P, Kim K, Krishnamoorthy R et al (2016) Cold stress tolerance in psychrotolerant soil bacteria and their conferred chilling resistance in tomato (*Solanum lycopersicum* Mill.) under low temperatures. PLoS ONE 11:1–17. https://doi.org/10.1371/journal.pone.0161592

Teoh CP, Lavin P, Lee DJH et al (2021) Genomics and transcriptomics analyses provide insights into the cold adaptation strategies of an Antarctic bacterium, *Cryobacterium* sp. SO1. Polar Biol 44:1305–1319. https://doi.org/10.1007/s00300-021-02883-8

Upchurch RG (2008) Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. Biotech Lett 30:967–977. https://doi.org/10.1007/s10529-008-9639-z

Wang C, Wang C, Gao YL et al (2016) A consortium of three plant growth-promoting rhizobacterium strains acclimates *Lycopersicon esculentum* and confers a better tolerance to chilling stress. J Plant Growth Regul 35:54–64. https://doi.org/10.1007/s00344-015-9506-9

Xu S, Li L, Luo X et al (2022) Ggtree: a serialized data object for visualization of a phylogenetic tree and annotation data. iMeta 1:5–8. https://doi.org/10.1002/imt2.56

Zhao Y, Shi R, Bian X et al (2019) Ammonia detection methods in photocatalytic and electrocatalytic experiments: how to improve the reliability of NH3 production rates? Adv Sci 6:1802109. https://doi.org/10.1002/advs.201802109