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Pseudomonas rossensis sp. nov., a novel psychrotolerant species produces antimicrobial agents targeting resistant clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

The extreme conditions of the Antarctic environment have driven the evolution of highly specialized microbial communities with unique adaptations. In this study, we characterized five *Pseudomonas* isolates from James Ross Island, which displayed notable taxonomic and metabolite features. Phylogenomic analysis revealed that strain P2663^T occupies a distinct phylogenetic position within the *Pseudomonas* genus, related to species *Pseudomonas svalbardensis, Pseudomonas silesiensis, Pseudomonas mucoides, Pseudomonas prosekii*, and *Pseudomonas gregormendelii*. The novelty of five Antarctic isolates was further confirmed through analyses of housekeeping genes, ribotyping, and REP-PCR profiling. MALDI-TOF MS analysis identified 11 unique mass spectrometry signals shared by the Antarctic isolates, which were not detected in other related species. Additionally, chemotaxonomic characterization, including fatty acid composition, demonstrated similarities with related *Pseudomonas* species. Phenotypic assessments revealed distinctive biochemical and physiological traits.

In-depth genomic analysis of strain P2663^T uncovered numerous genes which could be involved in survival in extreme Antarctic conditions, including those encoding cold-shock and heat-shock proteins, oxidative and osmotic stress response proteins, and carotenoid-like pigments. Genome mining further revealed several biosynthetic gene clusters, some of which are associated with antimicrobial activity. Functional assays supported the antimicrobial capabilities of this novel species, showing antagonistic effects against clinical isolates of *Pseudomonas aeruginosa*, possibly mediated by tailocins (phage tail-like particles).

This comprehensive polyphasic study characterized a new cold-adapted species, for which we propose the name *Pseudomonas rossensis* sp. nov.

Introduction

Antarctica is one of the most isolated regions on Earth, characterized by extreme environmental conditions, including severe cold, dryness, and nutrient scarcity. These factors not only constrain the resident microbiota but also limit scientific exploration and research in these areas (Cowan and Tow, 2004; Niederberger et al., 2008). The local soil biodiversity is additionally shaped by freeze-thaw cycles, extreme aridity, and high salinity (Priddle and Heywood, 1980). Despite the inhospitable conditions, Antarctic microbial diversity is unexpectedly

Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; CDS, protein-coding sequence; COG, clusters of orthologous group; dDDH, digital DNA-DNA hybridization; FAME, fatty acid methyl ester; GGDC, genome to genome distance calculator; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MIC, minimal inhibition concentration; MiGA, microbial genomes atlas; PCA, plate count agar; REP-PCR, repetitive extragenic palindromic PCR; TSA, tryptone-soya agar; WGS, whole-genome sequencing.

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high, though still underexplored (Chown et al., 2015). Advances in accessibility and molecular techniques over recent decades have dramatically expanded our understanding of Antarctic microbiota, leading to the identification of numerous psychrophilic and psychrotolerant species from this region (Holochová et al., 2020; Králová et al., 2021).

The genus *Pseudomonas* encompasses a large and diverse group of gram-negative bacteria. It is one of the most heterogeneous genera within the *Pseudomonadota* phylum, and its classification has been revised multiple times with the advent of new molecular methods (García-Valdés and Lalucat, 2016; Garrido-Sanz et al., 2017; Mulet et al., 2010; Palleroni, 2015). Currently, more than 350 *Pseudomonas* species have been described with validly published names, with many new taxa emerging in recent years (LPSN; http://www.bacterio.net/pseudomonas.html, accessed August 2024). Members of this genus are frequently isolated from a wide range of environmental niches and exhibit diverse lifestyles. While the majority inhabit natural environments, some species are recognized as pathogens of plants, animals, and humans (Palleroni, 2015; Smith et al., 2009). In Antarctica, a considerable number of *Pseudomonas* strains could be isolated from soil, rocks, or water samples (García-Valdés and Lalucat, 2016; Gilichinsky et al., 2007; Laybourn-Parry and Pearce, 2016; Scherer and Neuhaus, 2006). Some of them could be associated with the previously described species, while others have been defined as novel taxa. We could mention *Pseudomonas proteolytica, Pseudomonas antarctica*, or *Pseudomonas meridiana* which were sampled from Antarctic cyanobacterial and algae populations (Reddy et al., 2004) whereas *Pseudomonas leptonychotis* was firstly isolated from Wedell seals (Nováková et al., 2020). From abiotic habitats including water, rocks, soil or sediments has been described *Pseudomonas extremaustralis, Pseudomonas gregormendelii, Pseudomonas prosekii, Pseudomonas deceptionensis*, or *Pseudomonas versuta* (Carrión et al., 2011; Kosina et al., 2013, 2016; López et al., 2009; See-Too et al., 2017). The broad distribution of *Pseudomonas* species across Antarctica exemplifies the genus' remarkable adaptive and survival capabilities.

This resilience is primarily attributed to their extensive metabolic flexibility, which enables them to utilize diverse organic compounds as energy sources and produce a wide array of secondary metabolites (Singh et al., 2016). Mesophilic strains of *Pseudomonas* have been widely recognized for their roles as plant growth promoters (Rehm, 2008; Zamioudis et al., 2013) and as biocontrol agents that protect crops from pathogenic bacteria and fungi (Diallo et al., 2011; Mannaa et al., 2017). These ecological functions are often facilitated by the production of various secondary metabolites, including antimicrobial agents (Chatterjee et al., 2017; Fernandez et al., 2017; Jayaseelan et al., 2014; Lavermicocca et al., 2002; Raaijmakers and Mazzola, 2012) or siderophores (Harrison and Buckling, 2009; Sah et al., 2017). Despite the extensive research on mesophilic *Pseudomonas* species, cold-adapted variants from Antarctica remain underexplored in terms of their biotechnological potential and ability to produce antimicrobial compounds. Nevertheless, several studies have highlighted the capacity of psychrophilic isolates, including pseudomonads and enterobacteria, to produce antimicrobials, some of which exhibit activity against clinically relevant species (Barros et al., 2013; Sánchez et al., 2010; Snopková et al., 2022; Wong et al., 2011). Given their metabolic versatility, Antarctic *Pseudomonas* strains represent a promising group for further exploration identifying novel antimicrobial compounds.

In the current study, we focused on characterizing a unique collection of Antarctic *Pseudomonas* isolates. Utilizing a polyphasic approach that incorporated various genotypic and phenotypic techniques, we propose the designation of a novel species, *Pseudomonas rossensis* sp. nov., with the type strain P2663^T (= CCM 8880^T, LMG 32503^T). Comprehensive genomic analysis of strain P2663^T revealed the presence of numerous genes which could be associated with stress responses and survival under extreme Antarctic conditions, as well as previously uncharacterized biosynthetic gene clusters. Importantly, all members of this newly identified species show significant potential as sources of novel clinically relevant antimicrobials targetig *Pseudomonas aeruginosa* strains isolated from hospitalized patients, including resistant variants.

Material and methods

Bacterial strains, isolation, and cultivation

Five *Pseudomonas* sp. strains characterized through the study were isolated in the deglaciated northern part of James Ross Island, Antarctica during the Czech Antarctic Research Program. The strains were chosen for detailed analyses based on distinct phenotypical differences from the previously described *Pseudomonas* species. Sampling was carried out as already mentioned (Sedláček et al., 2017) and the pure cultures were maintained at −70 °C until analysis. Details, including the characteristics of the sampling sites, can be found in Table S1. Reference strains of the phylogenetic relative species, namely *P. prosekii* CCM 7990^T and CCM 8389, *P. gregormendelii* CCM 8506^T and CCM 8507, *Pseudomonas silesiensis* CCM 9194^T, and *Pseudomonas mucoides* CCM 9396^T were retrieved from the Czech Collection of Microorganisms, Brno, Czech Republic (https://ccm.sci.muni.cz/en). *Pseudomonas svalbardensis* was not included in the collection because its species description coincided with the finalization and submission of our manuscript. Clinical isolates of *P. aeruginosa* used for antagonistic assays were requested from the clinical isolates collection stored and maintained by the Institute for Microbiology of Faculty of Medicine, Masaryk University and the St. Anne's University Hospital Brno, Czech Republic. The Antarctic strains and type strains of reference species were cultivated on Plate Count Agar (PCA; Oxoid) at 20 °C for 48 h. Indicator *P. aeruginosa* strains were propagated on tryptone-soya agar (TSA; Oxoid) at 37 °C overnight. Cultivation conditions specific to various tests are mentioned in the corresponding method section.

Genome assembly and annotation

Whole genome sequence (WGS) of the type strain P2663^T was determined using MiSeg (2 × 300 bp) technology. Low-quality reads were trimmed with Trimmomatic software v0.32 and reads were assembled via de novo assembler IDBA-UD v1.1.1 under default parameters. WGS was deposited in the GenBank and the GOLD database under acc. no. JBBWCB000000000 and Ga0136137, respectively. Annotation of the P2663^T genome was performed by the Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016), DOE-JGI Annotation pipeline v5.0 (Chen et al., 2017), and RAST pipeline v2.0 (Aziz et al., 2008). Biosynthetic clusters encoding secondary metabolites were predicted by antiSMASH v6.1.1 (Blin et al., 2021). Ortholog clusters were found by the OrthoVenn3 webtool using a modified OrthoMCL heuristic approach (Sun et al., 2023). CRISPRs were identified during the DOE-JGI Annotation process (Chen et al., 2017). Prophage and tailocin regions were predicted by PHASTER (Arndt et al., 2016). In silico prediction of resistance genes was performed during the RAST annotation process.

Phylogenomic and phylogenetic analyses

To verify the taxonomic status of P2663^T, the sequence of the gene encoding 16S rRNA was analyzed using the EzTaxon server (http://ezbiocloud.net/eztaxon; Yoon et al., 2017a) and WGS was submitted to the Microbial Genomes Atlas (MiGA; accessed 12th June 2024), analysed using the Type (Strain) Genome server (TYGS) (Meier-Kolthoff et al., 2022), and online version of DFAST_QC (Elmanzalawi et al., 2025). Additionally, digital DNA-DNA hybridization (dDDH) values between P2663^T and related type *Pseudomonas* species (genomes acc. no. are listed in Table 1) were re-calculated via Genome to Genome Distance Calculator (GGDC) v3.0 (Meier-Kolthoff et al., 2022). Average nucleotide identity (ANI) values were determined via the ANI calculator webtool using reciprocal best hits (two-way ANI) (Rodriguez-R and

Table 1
Genomic compassion between *P. rossensis* sp. nov. and related *Pseudomonas* species.

Species	Strain	GenBank	Similarity of				Ref.
		Acc. No.	16S rDNA	ANI	AAI	dDDH	
Pseudomonas svalbardensis	PMCC 200367^T	GCA_030053115.1	99.42 %	94.57 %	96.43 %	60.10 %	(Ge et al. 2024)
Pseudomonas mucoides	P154a^T	GCA_015461845.1	99.18 %	87.64 %	90.38 %	47.30 %	(Duman et al., 2019)
Pseudomonas silesiensis	A3^T	GCA_001661075.1	99.18 %	88.25 %	90.04 %	44.00 %	(Kaminski et al., 2018)
Pseudomonas gregormendelii	LMG 28632^T	GCA_017114825.1	99.03 %	85.93 %	88.81 %	41.50 %	(Kosina et al., 2016)
Pseudomonas prosekii	LMG 26867^T	GCA_900105155.1	98.29 %	86.45 %	89.81 %	43.40 %	(Kosina et al., 2013)

Whole genome sequences of the related *Pseudomonas* spp. were downloaded from GenBank database under dedicated accession numbers.

Konstantinidis, 2016) and EZBioCloud ANI Calculator (Yoon et al., 2017b).

Whole genome phylogenetic tree was calculated from a total of 357 reference genomes from genus *Pseudomonas* which was obtained from the NCBI RefSeq database (accessed 18th October 2024; O'Leary et al., 2016). List of analyzed genomes could be shown in Table S2. The pan-genome analysis was performed with BPGA v1.3.0 (Chaudhari et al., 2016), with amino acid sequences clustered using USEARCH (Edgar, 2010), with an identity cut-off of 50 %. This value offers ideal trade-off between identification of distant orthologues and detection of false homologies and was recently successfully used in *Pseudomonas* phylogeny (Nováková et al., 2023). Core genes were concatenated and aligned with MUSCLE. Distance matrix was calculated using Kimura substitution model and result phylogenomic tree was reconstructed using Neighbor-joining algorithm.

Genes encoding 16S rRNA, *rpo*D, and *rpo*B in all five analyzed strains were amplified via PCR using primers 27F plus 1541R, PsEG30F plus PsEG90R, and LAPS plus LAPS27, respectively (Ait Tayeb et al., 2005; Devereux and Wilkinson, 2004; Mulet et al., 2010). The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Sequencing was performed at the Eurofins MWG Operon sequencing facility. The partial sequences were manually trimmed using Lasergene Sequence Analysis Software (DNASTAR) to remove low-quality bases. To build the phylogenetic tree, relevant sequences of the related species were downloaded from the GenBank database (acc. no. are listed in Table S3), and further phylogenetic analyses were performed in MEGA v11.0 (Tamura et al., 2021). The multiple sequential alignments for the concatenated sequences were performed using the MUSCLE algorithm under default parameters (Edgar, 2004). Phylogenetic tree was inferred using the maximum-likelihood method with the Tamura-Nei gamma distance model (Tamura and Nei, 1993).

The phylogenetic affinity among the analyzed strains and related *Pseudomonas* spp. was further determined by automated *Eco*RI ribotyping using a RiboPrinter Microbial Characterization System (DuPont Qualicon) according to the manufacturer's instructions. Numerical analysis and dendrogram construction were performed via BioNumerics v7.6. Repetitive extragenic palindromic PCR with the REP primers (REP-PCR) was also performed, as previously described by Gevers et al. (2001) with modifications by Švec et al. (2008). REP-PCR fingerprints were analyzed and clustered using BioNumerics v7.6.

Morphology, physiology, and biotyping

The colony morphology was assessed after cultivation on TSA for 48 h at 25 °C. The cellular morphology of the type strain P2663^T was determined in detail via transmission electron microscopy according to Holochová et al. (2020) using a Morgagni 268D Philips (FEI Company) electron microscope (20 randomly selected cells were analyzed).

Phenotypic characterization was performed using conventional biochemical tests, protocol A of the GEN III MicroPlate™ kit (Biolog), and the API ZYM and API 20 NE kits (bioMérieux) according to the manufacturer's instructions. The incubation step was performed at 25 °C for 48 h (GEN III MicroPlate™) or 18 h (API kits). Within the kits, the susceptibility/resistance to selected antibiotics was assessed qualitatively. However, precise antimicrobial susceptibility testing was performed by microdilution method (see below).

MALDI-TOF MS analysis

Protein fingerprints by MALDI-TOF MS were acquired with an Ultraflextreme instrument (Bruker Daltonics) by following the ethanol/formic acid extraction protocol (Freiwald and Sauer, 2009). As many as 15 independent mass spectra were acquired for each sample, and only signals present in the minimum of 11 of these mass spectra were used for the identification and cluster analysis. The mass spectral data were compared with entries in the latest version of the Biotyper database v10.0.0.0 (containing 9607 references). A MALDI-TOF MS-based dendrogram was constructed with the software Biotyper v3.1 (Bruker Daltonics) using Pearson's product-moment similarity coefficient and the unweighted pair group method with arithmetic average (UPGMA) as a grouping method.

Antimicrobial susceptibility

In vitro antimicrobial susceptibility was detected via microdilution method using plates with dehydrated antibiotics (Erba Lachema). Suspension preparation and plate inoculation was performed according to the manufacturer's instructions. The minimal inhibition concentration (MIC) was tested for the following antibiotics, ampicillin/sulbactam (1/0.5–128/64 mg/mL), piperacillin (1–128 mg/mL), piperacillin/tazobactam (1/4–128/4 mg/mL), ceftazidime (0.12–16 mg/mL), aztreonam (0.12–16 mg/mL), meropenem (0.12–16 mg/mL), gentamicin (0.25–32 mg/mL), amikacin (0.5–64 mg/mL), colistin (0.12–16 mg/mL), ciprofloxacin (0.06–8 mg/mL), tigecycline (0.06–8 mg/mL), and trimethoprim/sulfamethoxazole (0.06/1.19–4/76 mg/mL); in antibiotics with two active compounds the concentration of both compounds is presented. The plates were incubated at 25 °C for 18 h and another 20 h at room temperature (manufacturer's recommendation for slowly growing strains). The MIC was the lowest concentration of antibiotic where no visible growth was observed (except trimethoprim/sulfamethoxazole, where MIC was assessed as ≥80 % growth inhibition compared to the control). Interpretation of results was performed according to EUCAST guidelines with regard to genus *Pseudomonas* (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 10.0, 2020). The breakpoints represent the highest concentration of given antibiotic with therapeutic effect, achievable in human plasma and therefore applicable for treatment.

Polar lipids, quinones

Polar lipids and quinones were analyzed as service in the DSMZ on the cooperation base. Polar lipids were extracted according to the integrated procedure of Tindall (1990a, 1990b). Respiratory quinones were extracted and analyzed as described previously by Vieira et al. (2021) and their identity was confirmed by mass spectrometry.

Fatty acid methyl esters analysis

Cellular fatty acid methyl esters analysis was performed from biomass of all analyzed Antarctic isolates and related reference type strains. All cultures were grown on the TSA (BD Difco) at 28 ± 2 °C for 48 h (except the strain CCM 7990^T, CCM 9194^T, and CCM 9396^T which were cultivated for 24 h) to reach the late exponential stage of growth according to the four quadrants streak method (Sasser, 1990). The extraction of fatty acid methyl esters and subsequent analysis using the Agilent 7890B gas chromatograph was performed according to the standard protocol of the Sherlock MIDI Identification System (MIDI Sherlock v6.2, MIDI Database RTSBA 6.21).

Inhibition assay

An overlay plate assay, according to Snopková et al. (2022) was used for the detection of antagonistic interactions of the investigated strains towards clinical isolates of *P. aeruginosa*. Briefly, the producer strain (i.e., one of the Antarctic isolates) was grown on TSA (Oxoid) supplemented with mitomycin C (final concentration 5 μg ml^{-1}) at 4 °C for 7 days. Macrocolonies were killed by chloroform vapours and overlayed with soft TSA (0.7 %, w/v agar) containing approximately 10^8 CFU/ml of the indicator strain (i.e., clinical *P. aeruginosa* isolate). Plates were cultivated at 37 °C overnight, and subsequently, inhibition zones were scored based on the presence and character of the inhibition zone. All positive results (i.e., those with inhibition zone presented) were performed in two independent replications.

Results and discussion

Phylogenomic and phylogenetic analysis

All five investigated Antarctic isolates were initially assigned to the genus *Pseudomonas* based on their basic phenotypic characteristics and 16S rRNA gene similarity. However, these strains exhibited distinct differences that set them apart from the previously described *Pseudomonas* species. Upon further examination, the strain P2663^T demonstrating the most distinct characteristics, particularly in terms of genetic uniqueness and antimicrobial activity, was chosen for sequencing. A novelty status of the Antarctic isolate P2663^T was supported by genome-based analysis using MiGA platform with a *P*-value 0.0054. TYGS analysis revealed that all dDDH values to curated type-strain genomes were below 70 %, the cut-off value for species delineation. These results were further confirmed by DFAST_QC, an alternative to MiGa and TYGS that provides quick taxonomic identification based on NCBI Taxonomy. The recommended ANI threshold value of 95 % (Gomila et al., 2015; Jain et al., 2018) was not reached with any closest strains proposed by the tool.

To further investigate the taxonomic placement of P2663^T, a pan-genome analysis of the genus *Pseudomonas* was performed. Core-genome-based taxonomy placed a proposed type strain P2663^T close to the environmental *Pseudomonas* species such as *P. silesiensis, P. gregormendelii, P. prosekii, P. mucoides*, and *P. svalbardensis* (Fig. 1). The number of core genes among all analyzed species within the genus *Pseudomonas* was 198, the number of unique genes in *P. rossensis* was 242. Finally, genomic distances between P2663^T and related type strains re-calculated by GGDC confirmed that the dDDH values were below 70 %, see Table 1 (Meier-Kolthoff et al., 2022). The ANI values re-calculated by the ANI calculator webtool (Rodriguez-R and

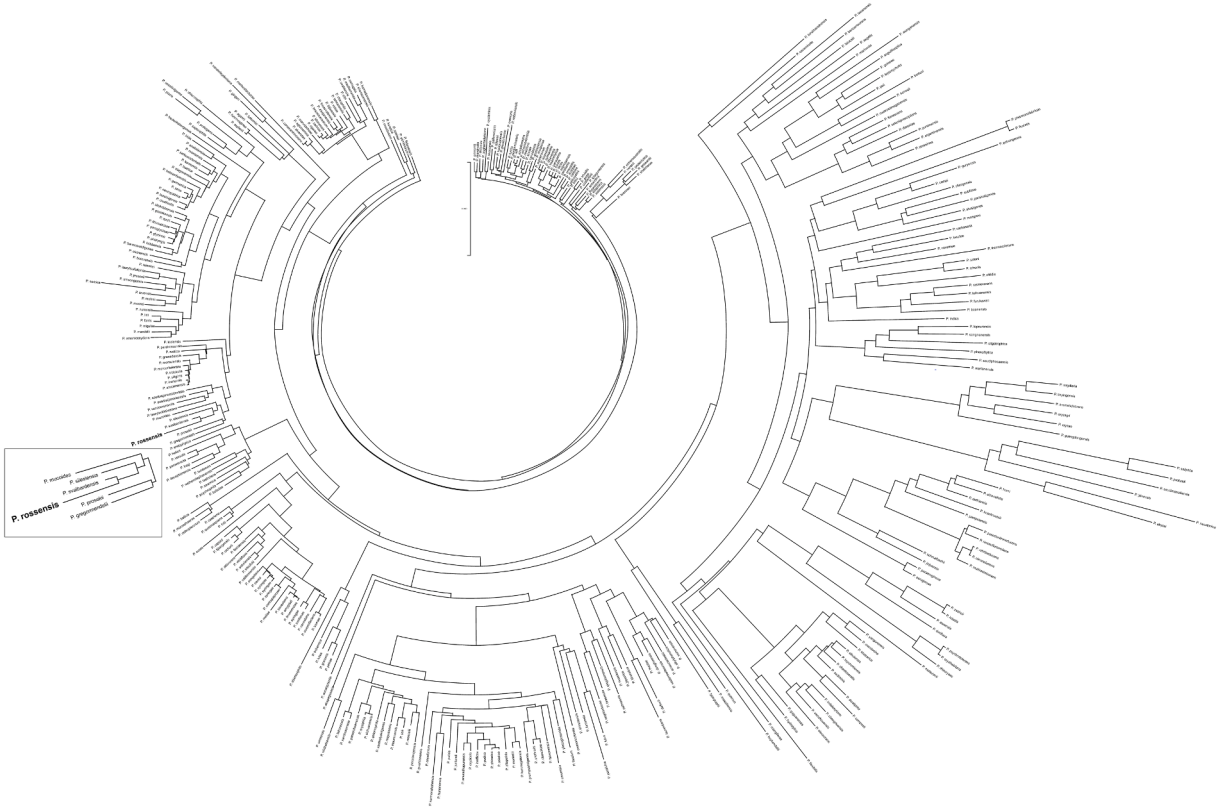

Fig. 1. Phylogenetic tree based on whole genome sequences.
The tree was constructed using the core genome of 357 validly described *Pseudomonas* species. The inset shows an enlarged area highlighting *Pseudomonas rossensis* P2663^T and its closest relatives. The core genome shared among all species within the genus *Pseudomonas* consists of 198 genes, while the P2663^T genome contains 242 unique genes. Genomes sequences were adopted from NCBI RefSeq database, their acc. no. are listed Table S2.

Konstantinidis, 2016) reached 94.57–85.93 % for related type strains of *Pseudomonas* spp. Analogical results were delivered through AAI analysis, where amino acid identity ranged between 96.43–88.81 %. Summary results are listed in Table 1. The rrs analysis and comparative genomics methods support our hypothesis that strain P2663^T represents a novel species.

Nevertheless, a polyphasic approach is preferred for the delineation of *Pseudomonas* species (Mulet et al., 2010; Peix et al., 2018). Hence, taxonomic characterization of the investigated isolates employed comprehensive analyses of housekeeping genes and fingerprint methods. The following analyses were conducted with all five analyzed Antarctic isolates to confirm their novel taxonomic status. The isolates were compared with related species, including *P. silesiensis, P. mucoides, P. gregormendelii*, and *P. prosekii*. The description of *P. svalbardensis* was published concurrently with the finalization and submission of our manuscript; consequently, we included only phylogenetic analysis for this species, excluding ribotyping and REP-PCR methods. Maximum-likelihood phylogenetic tree calculated based on concatenated *rpo*B,16S rRNA, and *rpo*D genes separated the cluster of Antarctic isolates from another validly described *Pseudomonas* species (Fig. S1). Both employed fingerprint methods, ribotyping and REP-PCR approach, yielded consistent results that supported the newly taxonomic status of the investigated strains. Ribotype patterns of all Antarctic isolates could be unambiguously distinguished from reference strains of related *Pseudomonas* spp., and at the same time, showed high similarity within the cluster of the proposed novel species (Fig. 2). Similarly, the REP-PCR fingerprints of the Antarctic strains formed the coherent cluster standing parallel to related *Pseudomonas* spp. (Fig. 3). Although the fingerprints of P2708, P2781, and P2663^T exhibited very similar profiles, the presence of unique bands together with different sample locations and time intervals among samplings confirmed their independent character.

The results of all phylogenomic and phylogenetic analyses were highly consistent and confirmed the novelty of the Antarctic isolates.

Basic genome characterization of P2663^T

The draft genomic data of the P2663^T strain contained 543 contigs with N50 42298 bp. The genome size reached 6511781 bp, number of protein-coding sequences (CDSs) was 6048, and GC content was 58.68 mol.%. The genome size and number of CDSs are consistent with close relative species *P. silesiensis, P. mucoides, P. gregormendelii, P. prosekii*, and *P. svalbardensis* (Ge et al., 2024; Duman et al., 2019; Kaminski et al., 2018; Kosina et al., 2013; Kosina et al. 2016), which ranged between 5.8 and 6.8 Mb with 5621–6088 CDSs. The genome size of the environmental pseudomonads could reach up to 8 Mb, which provides their huge metabolic plasticity and enables colonization of various habitats (García-Valdés and Lalucat, 2016). Putative functions were assigned to 4674 (77.28 %) of CDSs in the P2663^T genome, the remaining 1374 (22.71 %) of CDSs were annotated as hypothetical proteins. The most frequent Clusters of Orthologous Groups (COGs) categories were E (Amino acid transport and metabolism; 10.42 % of total CDSs), K (Transcription; 8.42 %), T (Signal transduction mechanisms; 6.40 %), C (Energy production and conversion; 6.25 %), and P (Inorganic ion transport and metabolism; 5.51 %), see Table S4. The genome contained 57 tRNAs, three rRNAs (two 5S rRNA and one 16S rRNA), 181 other RNA genes, and one CRISPR sequence. Ten prophage regions were identified, of which four were assigned as intact, an additional five as incomplete, and one sequence was assessed as questionable (Table S5). From those, one region (GOLD acc. no. Ga0136137_11636–11658) has been previously characterized as a tailocin gene cluster encoding phage tail-like particle with finely tuned antimicrobial activity (Snopková et al., 2022). The cluster spacing of 20.6 kb encoded 24 genes exhibiting homology to the tail proteins of myophages.

Biosynthetic potential of the proposed species

Secondary metabolite-related clusters were identified via antiSMASH webtool (Blin et al., 2021). The most abundant clusters were connected to the siderophores production (namely pyoverdine with a total of four clusters, histicorrugatin with four clusters, and fabrubactin) and antifungal compounds production (fengycin, fragin, and lankacidin C). One cluster was associated with carotenoid-like pigment APE Vf and an additional five clusters exhibited no significant homology to the previously described secondary metabolites (summary results could be shown Table S6). Siderophore-related genes were further annotated via RAST webtool; a total of 61 genes were likely associated with iron acquisition by siderophores, from them 36 were connected pyoverdine and an additional 14 achromobactin synthesis pathways. Yellow-green fluorescent pigment pyoverdine is the main high-affinity iron scavenging molecule among fluorescent pseudomonads, but numerous additional siderophores, including pyochelin, quinolobactin, achromobactin, and histicorrugatin have been described (Berti and Thomas, 2009; Cornelis and Matthijs, 2002; Meyer et al., 2008; Zhang and Rainey, 2013). The last mentioned could be classified within the category of suspected photoactive siderophores (Butler et al., 2021). On the other hand, no reference for fabrubactin production by pseudomonads

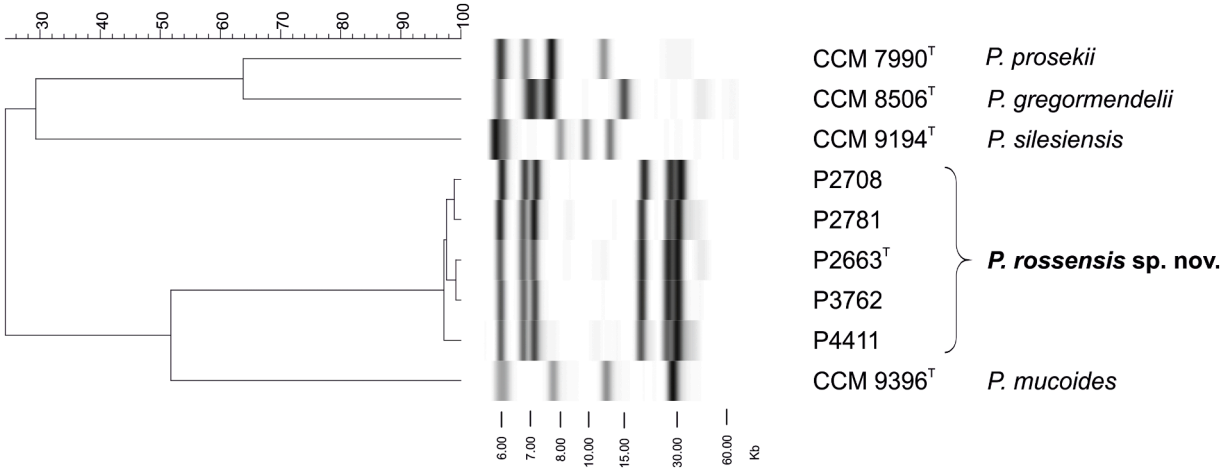

Fig. 2. Ribotype patterns of *Pseudomonas rossensis* sp. nov. and reference strains of related *Pseudomonas* spp.
Dendrogram based on cluster analysis of *Eco*RI ribotype patterns obtained using the RiboPrinter system from strains of *Pseudomonas rossensis* sp. nov. and types of phylogenetically closest *Pseudomonas* spp. (*P. svalbardensis* was non-included in the analysis due to concurrently species description and manuscript submission). The dendrogram was calculated using Pearsońs correlation coefficients with the UPGMA clustering method.

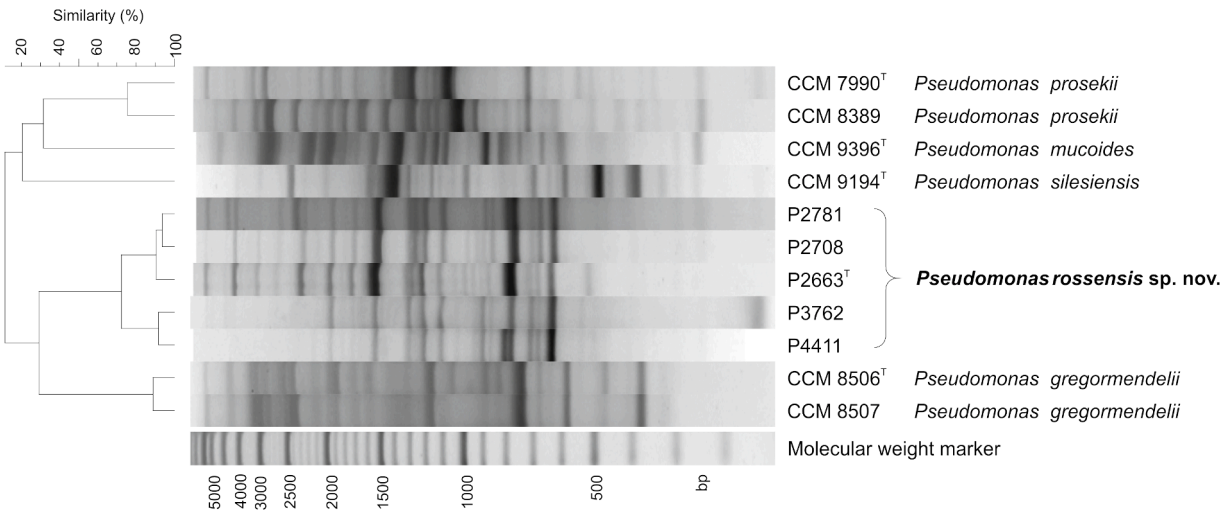

Fig. 3. Cluster analysis of REP-PCR fingerprints of the studied Antarctic strains and the phylogenetically related *Pseudomonas* spp. type and reference strains. The dendrogram was calculated using Pearson's correlation coefficients with the UPGMA clustering method in BioNumerics v7.6. *P. svalbardensis* was non-included in the analysis due to concurrently species description and manuscript submission.

could be found; the siderophore was detected in several *Agrobacterium* spp. (Vinnik et al., 2021). Similarly, the detected genes connected to antimicrobial agents have been previously reported for gram-positive bacteria, such as *Bacillus* spp. or *Streptomyces* spp. (Patel et al., 2011). However, the low similarity compared to the original antimicrobial agent clusters (7–37 %) raises doubts about the functionality of the clusters. Experimental verification of gene function, however, was beyond the scope of this study.

OrthoMCL analysis showed that a proposal type P2663^T shared around 65 % of gene clusters with the relative species (see Fig. 4). Total of 16 gene clusters were unique for the strain. Most of them encoded non-annotated clusters but genes related to metabolic process were also identified. On the other hand, 32 clusters related to various metabolic processes were shared by all analyzed *Pseudomonas* spp. except for P2663^T (i.e., shared by *P. mucoides* P154a^T, *P. silesiensis* A3^T, *P. prosekii* LMG 26867^T, *P. gregormendelii* LMG 28632^T, and *P. svalbardensis* PMCC 200367^T). The similarity matrix for pairwise genome comparison showed *P. svalbardensis* PMCC 200367^T as the strain sharing the highest

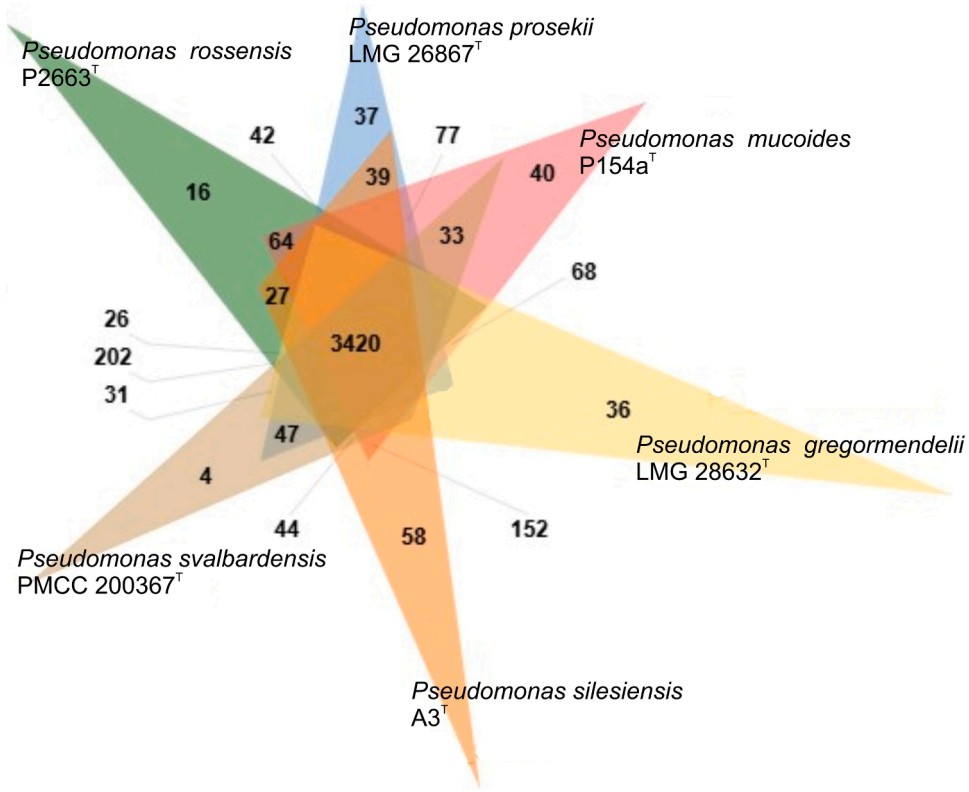

Fig. 4. Venn diagram showing the core genome of P2663^T and type strains of related *Pseudomonas* spp.
The numbers represent the gene clusters shared by a dedicated group of species. A total of 22 gene clusters were unique for the P2663^T genome. All genomes were downloaded from GenBank database, acc. no. are listed in Table 1.

proportion of the ortholog clusters (total of 5340 proteins; Fig. 5).

Genomic insight into cold adaptation strategies

To identify the possible coping strategy of Antarctic isolates to survive harsh local conditions, we deeply analyzed the gene composition in the P2663^T genome. The major emphasis has been taken on the stressor-related genes, genes encoding exosaccharides, and biofilm-related products. Precise gene mining revealed the presence of a total of 214 genes could be involved in the stress response (Table S7). From them, six genes were related to cold shock proteins (namely Csp A, C, D, and G), and another 16 genes corresponded to the heat shock response proteins (all from dnaK gene family). Both groups of proteins serve as chaperons responding to adaptation to a rapid temperature shift which is typical for the Antarctic environment (Fuchs et al., 2013). Freezing periods characterized by a lack of free water could be overcome by the production of freeze-protective solutes, such as sugars, polyols, or glycine betaine (Wargo, 2013). Forty genes related to osmotic stress were predicted in the P2663^T genome. Production of osmoregulated periplasmic glucans and betaine has been proposed. Genes involved in oxidative stress were the most abundant category of stress-related genes; a total of 101 genes encoded, besides others, catalase, superoxide dismutase, peroxidase, or proteins reacting with NO. Another survival strategy employs the production of extracellular polymeric substances and subsequent biofilm formation, which successfully protect bacteria against unfavorable environmental conditions (Goodchild et al., 2004; Phadtare, 2004). Genes which could be involved in the alginate pathway were identified in the P2663^T genome. Protection against high UV doses and reactive oxygen species could be mediated by carotenoid-like pigments production (Rastogi et al., 2010; Schöner et al., 2016). Part of the cluster encoding yellow aryl polyene APE Vf pigment was also detected.

Compared to related *Pseudomonas* species, *P. rossensis* P2663^T exhibits a distinctive gene profile concerning the presence of stress-related genes (see Fig. 6; Table S7). Multiple copies of genes involved in osmoprotection and handling freeze-thaw cycles were detected, such as *betA* (choline dehydrogenase) and *proU* (proline glycine betaine ABC transporter). The production of glycine betaine from choline is common among *Pseudomonas* species; however, in *P. rossensis*, as well as in other Antarctic isolates like *P. extremaustralis* 14-3b, an increased number of such genes was documented (Raiger Iustman et al., 2015). Another significant difference lies in the oxidative stress response, where multiple catalase genes (*HPII* and *HPIIb*) and genes encoding flavohemoproteins (e.g., AUX 17) have been identified. These genes are crucial for metabolic versatility and adaptation to Antarctic conditions. Even *P. svalbardensis*, which was isolated from a similar environment, does not possess such copies of these genes.

Phenotypic characterization

Antarctic isolates proposed as novel species were motile gram-negative rods. The strains grew on all basal agar media and produced yellow-greenish pigment pyoverdine on King B medium. Colonies on TSA medium were light beige, flat, smooth, approx. 2 mm in diameter. Cultivation of the strains was possible even in temperatures below zero (−2 °C), and all strains preferred lower temperatures (<30 °C) for their growth. The psychrotolerant isolates were able to grow in the presence of 3 % (w/v) NaCl in the medium, utilized various carbohydrates as a carbon source, and were able to degrade some biomacromolecules. Further detailed morphological, physiological, and biochemical characterization of the investigated isolates is given in the species description section. Physiological and biochemical tests useful for species distinguish from the related *Pseudomonas* spp. are listed in Table 2. Although we did not have physical access to the type strain of *P. svalbardensis*, we identified the following differences between the species based on the literature review. *P. rossensis* exhibits fluorescence, does not grow in 4 % NaCl, and shows negative assimilation of arabinose. In contrast, *P. svalbardensis* does not produce fluorescent pigment on King B medium, can grow in up to 7 % NaCl, and assimilates arabinose, as demonstrated in the API 20NE tests (Ge et al., 2024).

MALDI-TOF MS profiles of the investigated strains revealed 31 signals within the *m/z* range 2–14 kDa that were common to all five Antarctic isolates from which 11 were absent from the protein profiles of the four related *Pseudomonas* spp. tested in this study (non-included *P. svalbardensis*). The Biotyper database assigned with low confidence P2708 and P4411 to *P. mandelii* and *P. frederiksbergensis*, respectively (Biotyper log/scores/ between 1.700 and 1.999) whereas strains P2663^T, P2781, and P3762 remained unclassified. After the Biotyper database was extended with MALDI-TOF MS profiles of P2663^T, all four

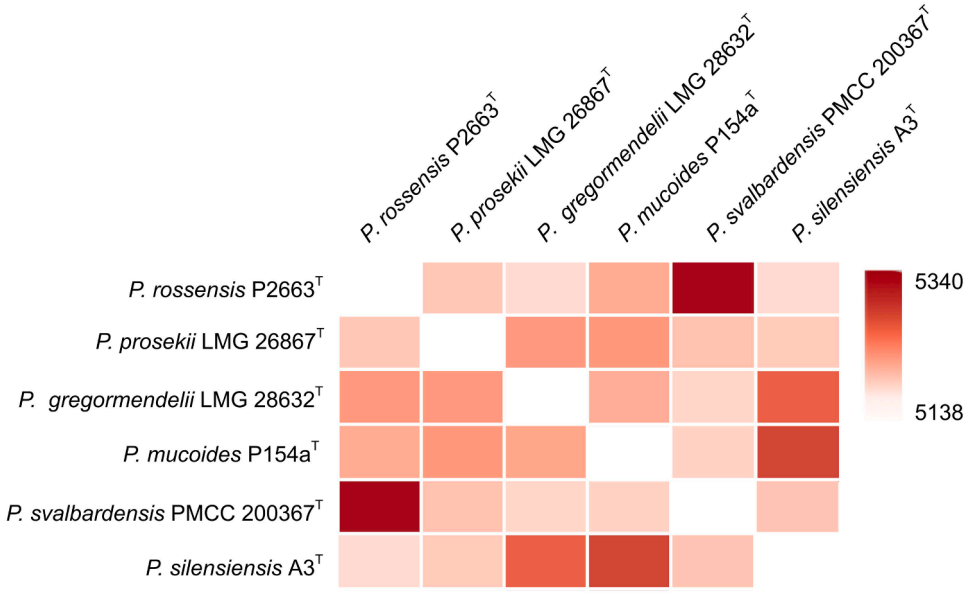

Fig. 5. Pairwise heatmap highlighting the similarity of the genomes of P2663^T and type strains of related *Pseudomonas* spp. based on orthologous gene clusters. The colour scale indicates the number of orthologous gene clusters between genomes. All genomes were downloaded from GenBank database, acc. no. are listed in Table 1.

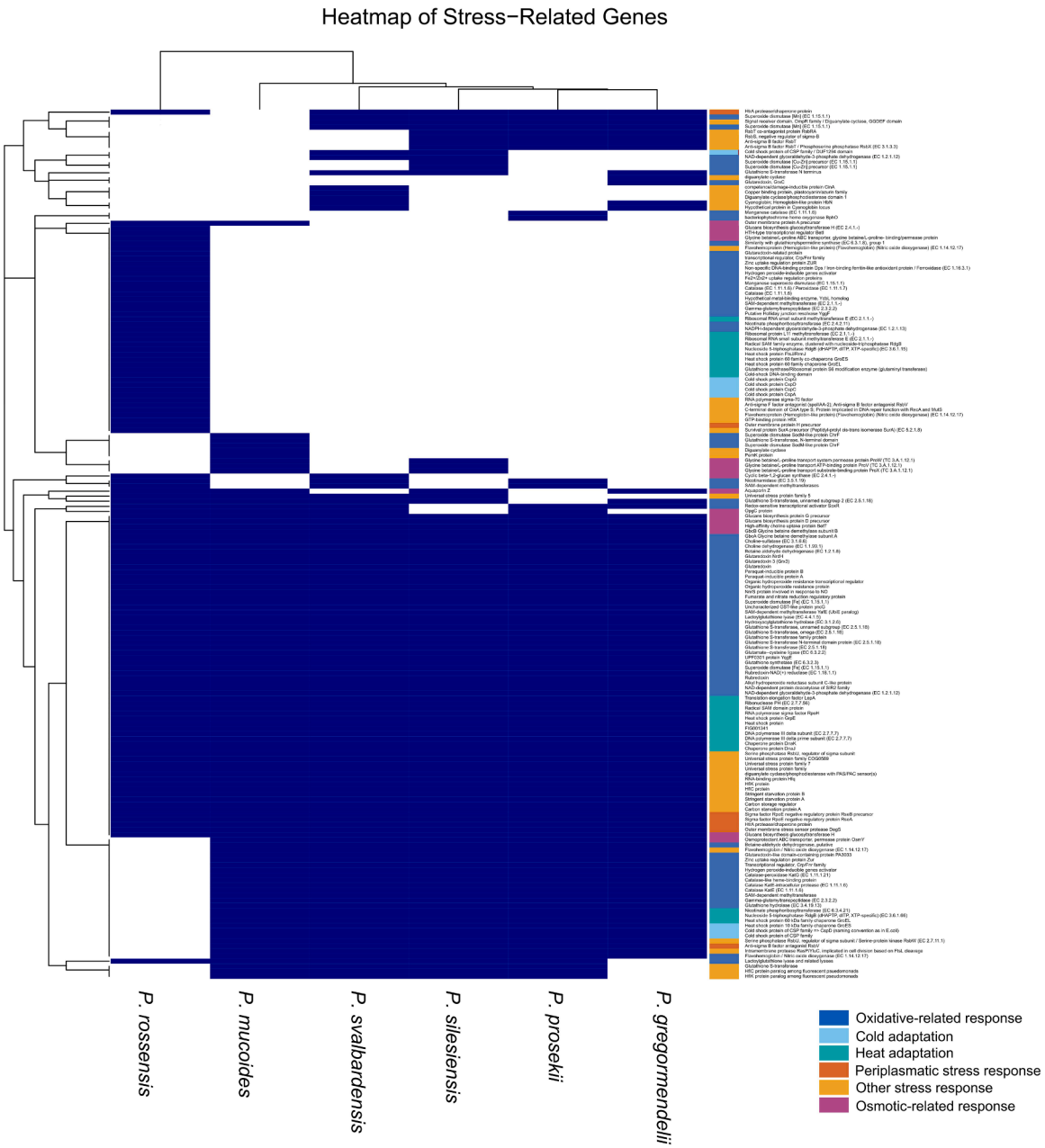

Fig. 6. Comparison of the stress-related genes in the genomes of P2663^T and type strains of related *Pseudomonas* spp.
The colour scale, displayed on the right side of the figure, corresponds to distinct categories of stress-related genes. All genomes were downloaded from GenBank database, acc. no. are listed in Table 1.

remaining Antarctic strains were assigned to the corresponding species with high confidence (Biotyper log/scores/ greater than 2.000). The mutual similarity between the MALDI-TOF MS profiles of the proposed novel species was reflected by a dendrogram (see Fig. 7), where the strains formed a separate cluster that was distinct from the remaining *Pseudomonas* spp.

The polar lipids of P2663^T strain were typical for pseudomonads, including aminophospholipid, phosphatidylglycerol, diphosphatidylglycerol, aminolipid, phospholipid, and lipid (Fig. 8). The major quinones of P2663^T were Q9 (93.1 %) with Q8 (6 %) and the minor part represented Q10 (0.5 %) and Q7 (0.4 %).

Analysis of the fatty acid methyl esters of isolates and the closest relatives are presented in Table 3. Results are consistent with other phylogenetically relative *Pseudomonas* species (Kaminski et al., 2018; Palleroni, 2015; Vancanneyt et al., 1996). The most abundant fatty acids were Summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\ \omega6c$; 36.0 %, on average), $C_{16:0}$ (33.8 %) and Summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$, 6.4 %). Hydroxylated fatty acids as $C_{10:0}$ 3OH, $C_{12:0}$ 2OH and $C_{12:0}$ 3OH were also presented. All isolates showed quite similar profiles of fatty acids, however they differed in the amount of cyclopropane-hexadecanoic acid (C17:0 cyclo). In comparison to the closest relatives (non-included *P. svalbardensis*), the isolates showed lower amounts of fatty acids in Summed features 8.

Antibiotic susceptibility

In vitro microdilution method revealed the susceptibility of the proposed species to the aminoglycosides (amikacin, and gentamicin), some β-lactam antibiotics (piperacillin/tazobactam and meropenem), colistin, and ciprofloxacin, however, ciprofloxacin showed only

Table 2
Phenotypic differentiation of suspected novel species and reference strains of related *Pseudomonas* spp.

Test	1*	2	3	4	5
Nitrate reduction	+	+	+	−	−
Gas from nitrate	+	+	−	−	−
Lysine decarboxylase	+	−	−	−	+
Fluorescein (King B medium)	+	−	+	+	−
Acid from mannitol (OF test)	+	+	−	−	−
Growth in 4.0 % NaCl (4 days)	−	+	+	+	w
Malonate utilisation	−	+	+	+	+
Hydrolysis of: Gelatine	+#	+#	−	+#	−
Caseine	+	−	−	+	−
Lecithine	−	−	−	+	−
API 20NE: Arabinose assimilation	−	+	w	+	−
N-acetyl-glucosamine assimilation	w	+	−	+	−

1. *Pseudomonas rossensis* sp. nov., 2. *Pseudomonas mucoides* CCM 9396^T, 3. *Pseudomonas silesiensis* CCM 9194^T, 4. *Pseudomonas prosekii* CCM 7990^T, 5. *Pseudomonas gregormendelii* CCM 8506^T. *P. svalbardensis* was non-included in the analysis due to concurrently species description and manuscript submission.

* data are uniform for all isolates of *Pseudomonas rossensis* sp. nov.; +, positive; w, weak; −, negative.

Negative gelatine in API 20 NE kit.

intermediate results, i.e., susceptible when treated with an adjusted dosage of antibiotic only. The results for piperacillin were strain dependent, however, most of the isolates were resultant (3 susceptible strains, 1 intermediate, and 1 resistant strain). The ampicillin/sulbactam (β-lactam antibiotic) and trimethoprim/sulfamethoxazole seem to be overall resistant, similarly to *P. aeruginosa*, but contrastingly to some other *Pseudomonas* species (*Pseudomonas stutzeri* in case of trimethoprim/sulfamethoxazole). All strains were resistant to aztreonam and ceftazidime (β-lactam antibiotics). Summary results for all Antarctic strains are shown in Table S8. In the P2663^T genome, genes corresponding to the detected antimicrobial profile were found. Three genes encoding β-lactamases class C (GOLD database genes acc. no. Ga0136137_112974, Ga0136137_112388, and Ga0136137_115267) were detected in the P2663^T genome. An additional 11 genes were involved in the production of multidrug efflux pumps, mostly from RND superfamily which could mediate resistance to various types of antibiotics (Lorusso et al., 2022).

Antimicrobial potential of novel species

Antimicrobial resistance is a major global health problem (White and Hughes, 2019). Our previous research demonstrated that Antarctic pseudomonads produce inhibition agents such as low-molecular-weight pyocins and phage tail-like particles (tailocins), which inhibit other Antarctic isolates or phytopathogenic pseudomonads (Snopkova et al., 2020; Snopková et al., 2021, 2022). Tailocins have been described as promising antimicrobial agents selectively targeting pathogenic species (Baltrus et al., 2021; Bhattacharjee et al., 2022; Redero et al., 2018). Modification on the molecular level has been applied for retargeting tailocins to generate novel bactericidal protein complexes (Williams et al., 2008). To clarify the clinical potential of the proposed species, we tested the antagonistic activity of P2663^T against the set of clinical isolates of *P. aeruginosa*. In hospitals, *P. aeruginosa* is a feared nosocomial pathogen causing (not only) ventilator-associated pneumonia, catheter-related infections, burn and wound infections, or septicemia with high mortality (Bassetti et al., 2021). This pathogen is a member of the so-called ESKAPE group, which highlights major pathogens responsible for life-threatening nosocomial infections with a high proportion of multidrug-resistant strains (Mulani et al., 2019). Of 96 well-characterized clinical isolates tested (see Table S9), seven (7.3 %) were antagonized by P2663^T. All inhibition zones detected during overlay plate assay were very narrow (appr. 1–2 mm) with sharp edges suggesting inhibition mediated by a high-molecular-weight antimicrobial agent such as tailocin. However, tailocin production by P2663^T has

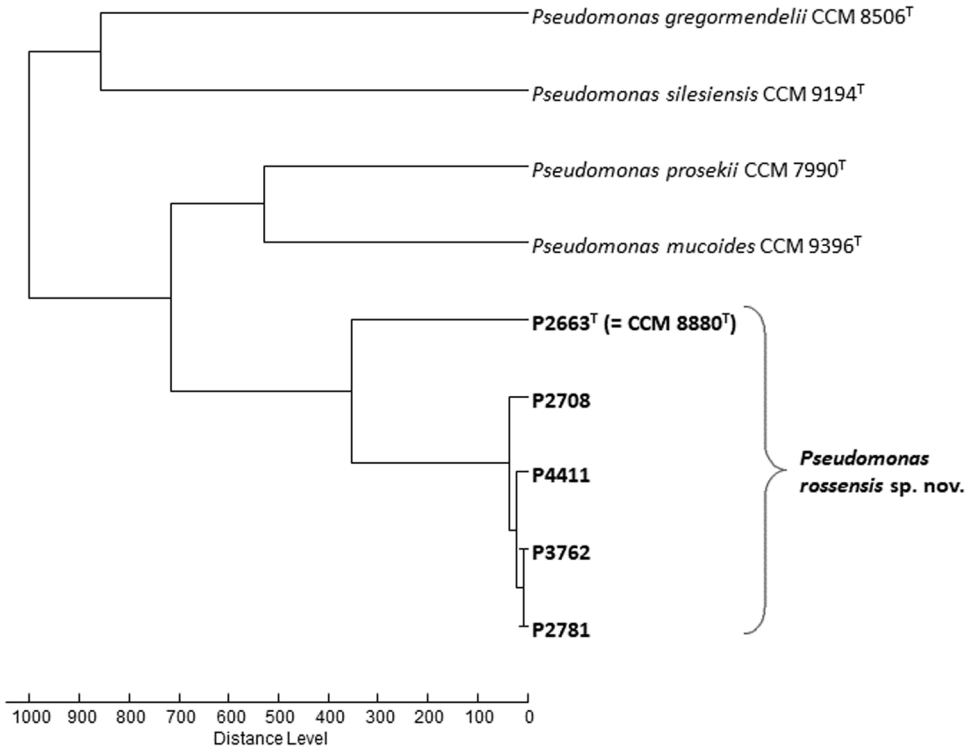

Fig. 7. Dendrogram obtained by cluster analysis of MALDI-TOF mass spectra of *P. rossensis* sp. nov. strains and related *Pseudomonas* spp. type strains.
The dendrogram was performed using Pearson's product-moment coefficient as a measure of similarity and the unweighted pair group average linked method (UPGMA) as a grouping method. Distance is displayed in relative units. *P. svalbardensis* was non-included in the analysis due to concurrently species description and manuscript submission.

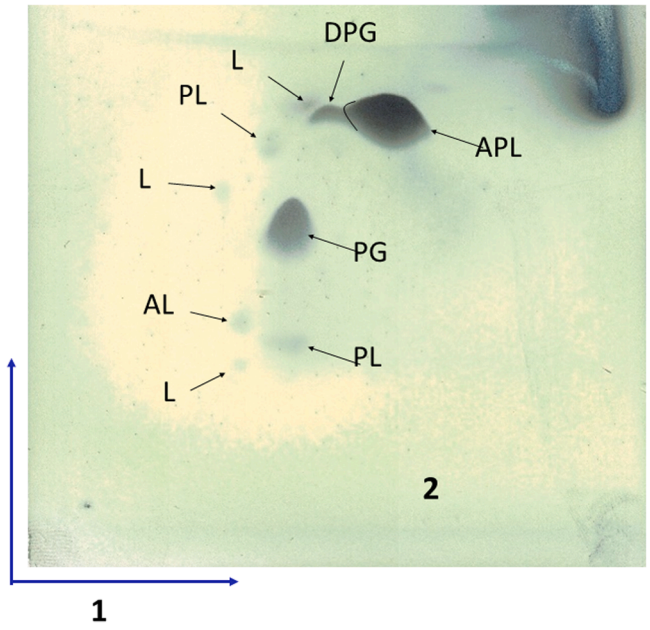

Fig. 8. Polar lipid profile of *P. rossensis* sp. nov. P2663^T.
Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; AL, aminolipid; APL, aminophospholipid; L, lipid; PL, phospholipid. The APL next to DPG may correspond to phosphatidylethanolamine (PE, as this is expected for the genus). However, spot shape and migration behaviour are not typical for PE.

been observed during the previous study (Snopková et al., 2022) a direct crosslink between tailocins and the detected killing activity has exceeded the scope of the study. Three *P. aeruginosa* isolates inhibited by P2663^T were resistant to fluoroquinolones (strains 11BC and 16BF were resistant to ofloxacin and ciprofloxacin, strain 49 CC was resistant to ofloxacin), one strain (16BF) was resistant to gentamicin (see Table 4). All clinical strains inhibited by P2663^T produce various virulence factors, such as siderophores or haemolysins and further were able to form biofilm. For detection of the intraspecies variability in the inhibition spectrum, we tested seven above-mentioned *P. aeruginosa* for sensitivity to other strains of the proposed novel species. All Antarctic strains were capable to antagonize clinical isolates, but the overall killing spectrum was unique for each isolate (the number of inhibited clinical isolates varied from two to seven).

Conclusion

A detailed investigation of five *Pseudomonas* strains isolated from James Ross Island, Antarctica, has elucidated their unique taxonomic status. Through a rigorous polyphasic approach, combining phylogenomic, phylogenetic, and phenotypic methodologies, we have proposed a new species, *P. rossensis* sp. nov., with the type strain P2663^T (= CCM 8880^T, LMG 32503^T). In-depth genomics analyses of P2663^T has revealed several genes associated with adaptation to the extreme Antarctic environment, including those linked to cold-shock and heat-shock protein production, oxidative and osmotic stress responses, and the synthesis of carotenoid-like pigments, which may contribute to the survival under harsh conditions. Furthermore, the pristine environment of James Ross Island represents a promising source of bioactive compounds, including novel antimicrobials. This is especially relevant in

Table 3
Cellular fatty acid composition (%) of *Pseudomonas rossensis* sp. *nov.* P2663^T, P2708, P2781, P3762, P4411 and its closest phylogenetic relatives.

Strain no. Fatty acid	P2663^T	P2708	P2781	P3762	P4411	CCM 9396^T	CCM 9194^T	CCM 7990^T	CCM 8506^T
$C_{10:0}$ 3OH	3.7	3.8	5.1	3.9	4	4.3	3.6	3	3
$C_{12:0}$	4.8	4.6	5	4.3	4.5	4.1	6.4	4.4	4.2
$C_{12:0}$ 2OH	3.9	3.8	3.7	4.1	4.2	4.1	2.1	3.7	4.4
$C_{12:0}$ 3OH	4.8	4.9	5.7	4.9	5.2	5.2	4.4	4.7	5.2
$C_{16:0}$	34.8	34.4	32.3	33.1	34.4	34.5	31.6	31.4	32.8
$C_{17:0}$ cyclo	4.3	3.7	1.5	3.7	3.2	1.2	1.6	3.3	4.8
$C_{18:0}$	TR	TR	TR	TR	TR	ND	TR	1.4	TR
Sum. feature 3*	34.7	36.2	36	37.3	36.1	38.1	35.3	32.8	33.8
Sum. feature 8*	6.4	6.2	8	5.8	5.7	7	12.5	13.4	8.8

Abbreviations: TR, trace amount (<1 %); ND, not detected. All data were taken from this study using cell grown to the late exponential phase (48 h, except 24 h for CCM 9396^T, CCM 9194^T, and CCM 7990^T) on TSBA medium at 30 °C. *P. svalbardensis* was non-included in the analysis due to concurrently species description and manuscript submission.
* Summed features are groups of two fatty acids that cannot be separated by gas chromatography using the MIDI system. Summed features 3 contains $C_{16:1}$ *ω*7c/ $C_{16:1}$ *ω*6c, Summed features 8 contains $C_{18:1}$ *ω*7c /$C_{18:1}$ *w*6c.

Table 4
Capacity of novel Antarctic species, *Pseudomonas rossensis*, inhibit clinical isolates of *P. aeruginosa*.

Clinical isolate		Isolate characterization					Isolate sensitivity to Antarctic strain				
		Resistance to	Biofilm formation	Production of							
				Haemolysins	Elastase	Siderophores	P2663^T	P2708	P2781	P3762	P4441
P. aeruginosa	11BC	OFXN, CIP		+	+	+	I				
P. aeruginosa	11CC		+	+	+	+	I	I			I
P. aeruginosa	12CC		+	+	+	+	I				
P. aeruginosa	49CC	OFXN	+	+	+	+	I	I			
P. aeruginosa	16FB	OFXN, CIP, CN	+++	+	+		I	I			
P. aeruginosa	95GA		+++	+	+		I		I	I	I
P. aeruginosa	32MB		+	+	+	+	I	I	I	I	I

Phenotypic characteristics of clinical isolates are listed, including resistance to antibiotics, biofilm formation capacity, and production of additional virulence factors (siderophores tested: pyoverdine and pyochelin; for further details, see study (Olejnickova et al., 2014). *Abbreviations*: OFXN, ofloxacin; CIP, ciprofloxacin, CN, gentamicin; plus indicate production of exoenzymes or ability of biofilm formation (biofilm production was scaled: + weak producers; ++, medium producers; +++, strong producers of biofilm).

light of the global challenge posed by antimicrobial resistance. The genomic analysis of P2663^T also identified several biosynthetic gene clusters, some of which encode for products with unknown functions or putative antimicrobial proteins. Finally, all strains of the novel species exhibited inhibitory activity against clinical isolates of *P. aeruginosa*, a notoriously difficult-to-treat nosocomial pathogen. The results summarized in the study underscore the taxonomic significance of cold-adapted Antarctic pseudomonads and highlight their potential for yielding novel antimicrobial agents.

Description of Pseudomonas rossensis sp. nov

The description of the species is based on five strains. Cells are Gram-stain-negative, non-spore-forming shorter rods, pleomorphic, occurring separately or in irregular clusters, and motile with a bundle of polar flagella (Fig. S1). Cell width ranged from 0.81 μm to 1.21 μm and length from 2.09 μm to 3.21 μm in size. Colonies on TSA medium are circular, with whole margin, flat, smooth, glistening, and light beige, approx. 2 mm in diameter after two days of cultivation at 25 °C. Aerobic growth is observed on R2A agar, Brain Heart Infusion agar, Mac Conkey agar (Merck), Endo agar (HiMedia), LB agar (Oxoid), Mueller-Hinton agar (Oxoid), Nutrient agar (Oxoid), Blood agar (Oxoid), TSA agar (Oxoid) and PCA agar (Oxoid) at 25 °C. Growth is observed between −2 °C and 30 °C, but not at 35 °C. Cells grow well in the pH range 6.0–8.0, pH 5.0 and pH 9.0 inhibit growth. Good growth in TSA broth in the presence of 2 % NaCl (w/v) and weak in the presence of 3 % NaCl; the presence of 4 % NaCl inhibits growth. Acid production (aerobic) from glucose, fructose, mannitol, and xylose, while fermentation of glucose in OF test medium is negative. Catalase, oxidase, esterase (C4), esterase lipase (C8), alkaline phosphatase, acid phosphatase, leucine arylamidase (weak), valine arylamidase and trypsin positive by API ZYM. Simmons citrate utilization, nitrate reduction with gas production, fluorescein (King B medium), and lysine decarboxylase positive. Gelatine, casein, and tyrosine hydrolysis positive (with brown exopigment on tyrosine agar). Acid production (aerobic) from maltose negative. Pyocyanin (King A medium), haemolysis, urease, indole, H_2S production, malonate, and acetamide utilization negative. Hydrolysis of Tween 80, ONPG, starch, esculin, lecithin, and DNA negative. Lipase (C14), cystine arylamidase, alpha-chymotrypsin, naphthol-AS-BI-phosphohydrolase, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, *N*-acetyl-betaglucosaminidase, alpha-mannosidase and alpha-fucosidase negative by API ZYM. On API 20 NE is positive assimilation of glucose, mannose (weak), mannitol, *N*-acetyl-glucosamine (weak), gluconate, caprate, malate and citrate, but negative arabinose, maltose, adipate and phenyl-acetate assimilation. Variable phenotypic reactions of *P. rossensis* strains are listed in Table S10. Determination of MIC through standard protocol revealed the sensitivity of the Antarctic strains to piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, meropenem, gentamicin, amikacin, colistin, ciprofloxacin, tigecycline and strain-dependent sensitivity to ampicillin/sulbactam, ceftazidime, aztreonam, and trimethoprim/sulfamethoxazole.

All strains were positive for the utilization (Biolog, GEN III MicroPlate) of D-trehalose, sucrose, *N*-acetyl-D-glucosamine, alpha-D-glucose, D-mannose, D-fructose, D-galactose, D-fucose (weak), L-fucose (weak), inosine, D-mannitol, D-arabitol, glycerol, D-fructose-6-PO4 (weak), L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine (weak), L-pyroglutamic acid, L-serine, pectin, D-galactonic acid lactone (weak), D-gluconic acid, glucuronamide, methyl pyruvate, L-lactic acid, citric acid, alpha-keto-glutaric acid, L-malic acid, bromo-succinic acid, tween 40, gamma-amino-butyric acid, alpha-hydroxy-D, L-butyric acid, propionic acid and acetic acid as carbon sources. Positive growth in the presence of troleandomycin, rifamycin SV, lincomycin, niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue, and potassium tellurite. Negative for the utilization of dextrin, D-maltose, D-cellobiose, gentiobiose, D-turanose, stachyose, D-raffinose, alpha-D-lactose, D-melibiose, beta-methyl-D-glucoside, D-salicin, *N*-acetyl-beta-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, D-sorbitol, myo-inositol, D-glucose-6-PO4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, quinic acid, p-hydroxy phenylacetic acid, D-lactic acid methyl ester, alpha-hydroxy-butyric acid, and alpha-keto-butyric acid. Negative growth in the presence of 4 % NaCl, 1 % sodium lactate, fusidic acid, D-serine, minocycline, quanidine HCl, nalidixic acid, lithium chloride, aztreonam, sodium butyrate, and sodium bromate. The Biolog system identified all above-mentioned isolates only to the genera level as *Pseudomonas* sp. The major fatty acids are Summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c), $C_{16:0}$ and Summed feature 8 ($C_{18:1}$ ω7c/$C_{18:1}$ ω6c).

The type strain is P2663^T (= CCM 8880^T = LMG 32503^T). The DNA G + C content of strain P2663^T is 58.68 mol%. The majority of characteristics of the type strain P2663^T are in agreement with the species description. The strain-dependent test results of P2663^T are presented in Table S10.

Pseudomonas rossensis (ross.en'sis. N.L. fem. adj. *rossensis,* pertaining to James Ross Island, isolation site).

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (verze GPT-4) in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Kateřina Snopková: Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft, Supervision. **Karel Sedlář:** Methodology, Data curation, Visualization, Formal analysis, Writing – review & editing. **Dana Nováková:** Investigation, Methodology, Writing – review & editing. **Eva Staňková:** Investigation, Visualization, Writing – review & editing. **Ivo Sedláček:** Formal analysis, Validation, Writing – review & editing. **Ondřej Šedo:** Investigation, Methodology, Writing – review & editing. **Veronika Holá:** Formal analysis, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2025.100353.

Data availability

Data will be made available on request.

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