



## Genomic and phenotypic characterization of *Rhodopseudomonas* sp. strain RCAM05734 isolated from Svalbard permafrost

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### Resume

We report the isolation and characterization of *Rhodopseudomonas* sp. strain RCAM05734 from the upper horizon of Holocene permafrost on the southern shore of Isfjorden, Svalbard. The strain was recovered on R2A at 5°C and sequenced using Oxford Nanopore long reads; assembly produced a single circular chromosome of 6.65 Mb (GC 63.9%) encoding 6,183 predicted proteins and 65 RNA genes (GenBank CP199742; SRA SRS26238712). Although the 16S rRNA gene shows high similarity (98%) to several *Rhodopseudomonas* type strains, average nucleotide identity values (78–80%) indicate that RCAM05734 is genomically distinct and may represent a novel species. The genome contains three *fix* loci and an almost complete *nif* gene complement, consistent with potential for nitrogen fixation under microaerobic conditions. Genes implicated in plant-associated functions (*acdS/acdR*, *iaaM/iaaH*), multiple cold-shock proteins, and accessory nod regulatory loci were also identified. RCAM05734 encodes extensive aromatic-compound catabolic pathways, including both catechol and protocatechuate branches of the  $\beta$ -ketoacid pathway and meta-cleavage enzymes. Phenotypic profiling (Biolog GEN III) revealed utilization of diverse mono- and disaccharides and selected carboxylic acids, limited halotolerance (growth at 1% NaCl) and activity at mildly acidic pH. Collectively, genomic and physiological data suggest that RCAM05734 harbors metabolic versatility relevant to carbon and nitrogen turnover, stress tolerance, and potential plant-growth promotion in cold Arctic conditions.

**Keywords:** *Rhodopseudomonas* sp., permafrost, phenotypic profiling, Oxford Nanopore long-read sequencing

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### Introduction

The Svalbard Archipelago represents one of the most climatically dynamic regions of the High Arctic, hosting some of the warmest permafrost at comparable latitudes [Ernakovich et al., 2022]. This thermal difference is caused by the influence of the West Spitsbergen Current and associated maritime air masses, which moderate ground temperatures and create

distinctive cryospheric conditions [Descamps et al., 2017]. As a result, permafrost in Svalbard is characterized not only by subzero stability but also by episodic thaw and freeze events within the active layer, strong seasonal shifts in light (polar day and night), and intense wind-driven redistribution of snow and soil [Osokin et al., 2012]. These interacting environmental drivers define an Arctic niche where microorganisms face

persistent stress yet maintain long-term viability. The rapid pace of climate warming in the Arctic has profound implications for permafrost degradation. As formerly frozen soils thaw, microbial communities are reactivated, releasing stored carbon and nitrogen and initiating transformations in sulfur and metal cycles [Spolaor et al., 2024].

The resulting greenhouse gas emissions and biogeochemical feedbacks have global relevance, underscoring the importance of characterizing permafrost microbiota in both ecological and functional terms [Ernakovich et al., 2022]. While permafrost has long been recognized as a repository of viable microbial life, our understanding of its community structure and metabolic capacity remains incomplete, particularly for lineages with complex and flexible lifestyles. Among bacterial taxa of interest, members of the genus *Rhodopseudomonas* (family Nitrobacteraceae, order Hyphomicrobiales) are especially notable. These purple non-sulfur bacteria are renowned for extraordinary metabolic versatility [Li et al., 2022]. They can perform anoxygenic photoheterotrophy in the presence of light and anoxia, shift to aerobic or anaerobic chemoheterotrophy in darkness, fix atmospheric dinitrogen, oxidize reduced sulfur and iron compounds, degrade aromatic substrates, and generate molecular hydrogen [Gallagher et al., 2025]. Their pigment systems, based on bacteriochlorophyll and diverse carotenoids, support adaptation to fluctuating light regimes, while robust regulatory networks allow them to tolerate oxidative, osmotic, and nutrient stresses.

In soil ecosystems, *Rhodopseudomonas* are additionally recognized for their roles in nitrogen cycling, bioremediation of xenobiotics, and potential plant-microbe interactions [Do Thi et al., 2024; Mutharasaiah et al., 2012]. Despite this metabolic breadth, the ecological distribution of *Rhodopseudomonas* remains incompletely resolved. Reports of isolates typically originate from soils, sediments, aquatic habitats, and plant-associated environments, whereas cryogenic ecosystems have received far less attention [Pfennig, 1969; Ramana et al., 2012; Hiraishi, Ueda, 1994; Guro et al., 2023]. The detection of a *Rhodopseudomonas* strain in Arctic permafrost therefore expands the known ecological range of the genus into extreme polar habitats, where survival requires cold adaptation, tolerance of freeze-thaw stress, and metabolic strategies compatible with long-term dormancy and intermittent resource availability. Microorganisms in permafrost may persist for centuries, becoming active again when liquid water becomes available during the summer thaw. Facultative phototrophs, such as *Rhodopseudomonas*, may be particularly well positioned to exploit these transient niches, coupling light utilization, versatile carbon

metabolism, and nitrogen fixation under variable conditions.

In this study we combined long-read genome sequencing with biochemical profiling to describe *Rhodopseudomonas* sp. strain RCAM05734 from Svalbard permafrost. Our goals were to determine its relationship to described taxa and to document key functional loci relevant to nitrogen fixation, aromatic metabolism, and environmental stress responses.

### Materials and Methods

Strain RCAM05734 was isolated from the top layer of permafrost, which during the Holocene Climate Optimum represented an active topsoil horizon. Core of permafrost sediments obtained by drilling a borehole (78.09856° N, 14.23299° E, 43.0 MASL) on the southern shore of Isfjorden bay was used. A suspension was prepared by mixing 0.4 g of permafrost core sample with 5 mL of sterile 0.9% NaCl. Aliquots of 100 µL were plated in duplicate onto Reasoner's 2A (R2A) agar (Difco™, USA) and incubated at 5°C. To obtain a pure isolate, a single colony was picked and restreaked twice onto fresh R2A agar plates. Strain was deposited in the Russian Collection of Agricultural Microorganisms (RCAM, <https://rcam.arriam.ru/>) and stored at -80°C in the automated Tube Store (Liconic Instruments, Liechtenstein).

For DNA extraction, a single colony was inoculated into liquid R2A medium and incubated for 48 h at 28°C. To obtain the whole-genome sequence of RCAM05734, we applied an Oxford Nanopore long-read sequencing workflow with standard downstream processing. Sequencing libraries were prepared with the SQK-LSK109 ligation kit (Oxford Nanopore Technologies, (ONT), UK) and the EXP-NBD104 barcoding kit, with the DNA-shearing step omitted. Sequencing was carried out on a MinION device with ONT R9.4.1 flow cell. Basecalling, demultiplexing, and adapter trimming were performed using Guppy (v 5.0.11), and quality control of the raw reads was provided by NanoStat (v 1.6.0) [De Coster et al., 2018]. The Oxford Nanopore Technologies reads were filtered using Filtlong (v 0.2.1; <https://github.com/rrwick/Filtlong>), where reads shorter than 1,000 bp were discarded and the lowest-quality 5% of bases were removed.

Assembly was performed with Flye v2.9, and consensus polishing was conducted using Medaka (v1.8.0, ONT) and the raw reads [Kolmogorov et al., 2019]. Genomic statistics for the final assembly were measured using QUAST (v 5.0.2) [Gurevich et al., 2013]. Annotation was carried out with Prokka (v1.3) [Seemann, 2014]. The whole-genome assembly of strain RCAM05734 has been deposited in GenBank under accession number CP199742. Raw sequencing reads have been deposited in the NCBI Sequence Read Archive

(SRA) under accession number SRS26238712 (BioSample: SAMN50694219).

Comparative taxonomic analyses were performed using 16S rRNA BLASTn and average nucleotide identity (ANI) calculations with OrthoANI in the OAT software package [Altschul et al., 1990; Lee, 2016]. Evolutionary distances were calculated using the maximum composite likelihood method in the MEGA X software package [Tamura et al., 2021]. Bootstrap analysis with 500 replicates was used to estimate cluster support. Large-scale study of phenotypic properties of the isolates were performed using microassay system GEN III MicroPlate (BioLog, USA) which analyses the ability of bacteria to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity (71 carbon source and 23 chemical sensitivity assays). The analyses were performed according to the manufacturer's recommendations.

## Results and Discussion

During the initial isolation and cultivation of strain RCAM05734, distinct growth characteristics were observed. Colonies appeared after 5 days at 5°C, characterized as slow-growing, transparent, and approximately 0.5 mm in diameter. Sequencing yielded 150,391 reads with an average length of 4,593.6 bp, an N50 of 13,478 bp, and a mean read quality of 12.2. After filtering, 66,827 reads remained, with an average length of 9,304 bp, an N50 of 15,648 bp, and a mean read quality of 13.4. Assembly produced a single circular contig representing the chromosome. The total size of the assembly is 6,649,164 bp with an average coverage of 92× and a GC content of 63.92%. Annotation identified 6,248 genes, including 6,183 protein-coding sequences and 65 RNA genes (58 tRNAs, 6 rRNAs, and 1 tmRNA). The 16S rRNA gene of RCAM05734 was most similar to the type strains *Rhodopseudomonas pseudopalustris* DSM 123 (98.06% identity; GenBank NR\_122099.1), *Tardiphaga robiniae* R-45977 (97.99%; NR\_117178.1), and *R. faecalis* (97.99%; NR\_024971.1) (Fig 1).

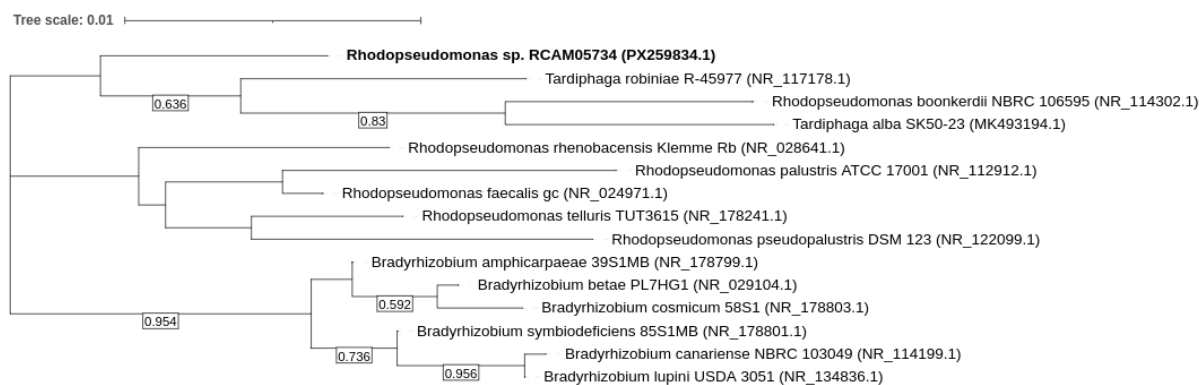


Fig 1. Phylogenetic tree generated by the maximum composite likelihood method, using partial 16S ribosomal RNA gene sequences of strain RCAM05734 and type strains of closely related species

Despite this relatively high 16S rRNA similarity, ANI values to the closest type strains were substantially lower than the accepted species-level cutoff (95-96%): 78.59% with *R. pseudopalustris* DSM 123 (GCF\_900110435.1), 80.30% with *T. robiniae* R-45977 (GCA\_013359755.1), and 77.90% with *R. faecalis* JCM 11668 (GCF\_003217325.1). The combination of high 16S rRNA gene similarity and low ANI values indicates that RCAM05734 is genomically distinct from previously described species and may represent a novel species within the genus *Rhodopseudomonas* (Fig 2.).

The genome of RCAM05734 encodes three distinct *fix* loci rather than a single operon: *fixABCX*, *fixJL*, and a separate *fixNOQPGHIS* cluster. In addition, a near-complete *nif* complement is present, including the

core structural and assembly genes (*nifHDK*, *nifUSTB*, *nifHQVW*, *nifENX*, *nifZ*, *nifO*) and the regulatory genes *nifA* and *nifL*. No canonical Nod factor backbone genes (*nodA*, *nodB*, *nodC*) were detected. However, several regulatory and accessory nod loci were identified (*nodD*, *nodG*, *nodM*, *nodN*, *nodP*, *nodQ*, *nodT*, *nodV*, *nodW*, *nodI*, *nodE*). Together, this gene set indicates substantial genetic potential for nitrogen fixation and for supporting nitrogenase activity under microaerobic conditions (through multiple *fix* clusters and the complete *nif* machinery). Beyond symbiotic loci, additional genes with potential roles in plant-microbe interactions and stress tolerance were detected. These include multiple cold-shock proteins (*cspA*, *cspC*, *cspD*, *cspG*), the ACC deaminase gene *acdS* with its regulator *acdR*, and genes

associated with indole-3-acetic acid (IAA) biosynthesis (*iaaM*, *iaaH*), with tryptophan biosynthesis genes (*trpA*, *trpB*) and an aldehyde dehydrogenase (*aldh*). Taken together, these features point to both abiotic stress adaptation and the capacity for plant-growth-promoting activities such as ACC deamination and IAA production. In addition, the genome encodes a near-complete set of genes for aromatic compound catabolism. Both catechol and protocatechuate branches of the  $\beta$ -ketoacid pathway are present, including *cataA*, *catB*, *catC*-like, *pcaD*, *pcaI/pcaJ*, and *atoA/atoD*-like, as well as *pcaG/pcaH*, *pcaB*, and *pcaC*. Peripheral entry points are represented by *pobA/PHBH* and shikimate/quinic enzymes, while a dedicated 4-hydroxyphenylacetate

(HPA) to homoprotocatechuate (HPC) cluster (*hpaC*, HPCD, downstream isomerases and dehydrogenases) is also present. Additional loci encode enzymes for meta-cleavage (catechol 2,3-dioxygenase, HMSH, HOPDA hydrolase) and for homogentisate/gentisate turnover (*hppD*, *hmgA*, *maiA*, fumarylacetoacetate hydrolase family). Collectively, these genes indicate broad potential for degradation of lignin-derived and phenolic compounds. Together, these loci suggest broad versatility for aromatic ring cleavage and assimilation, supporting growth on diverse phenolic substrates and enhancing ecological fitness in plant-associated or soil environments.

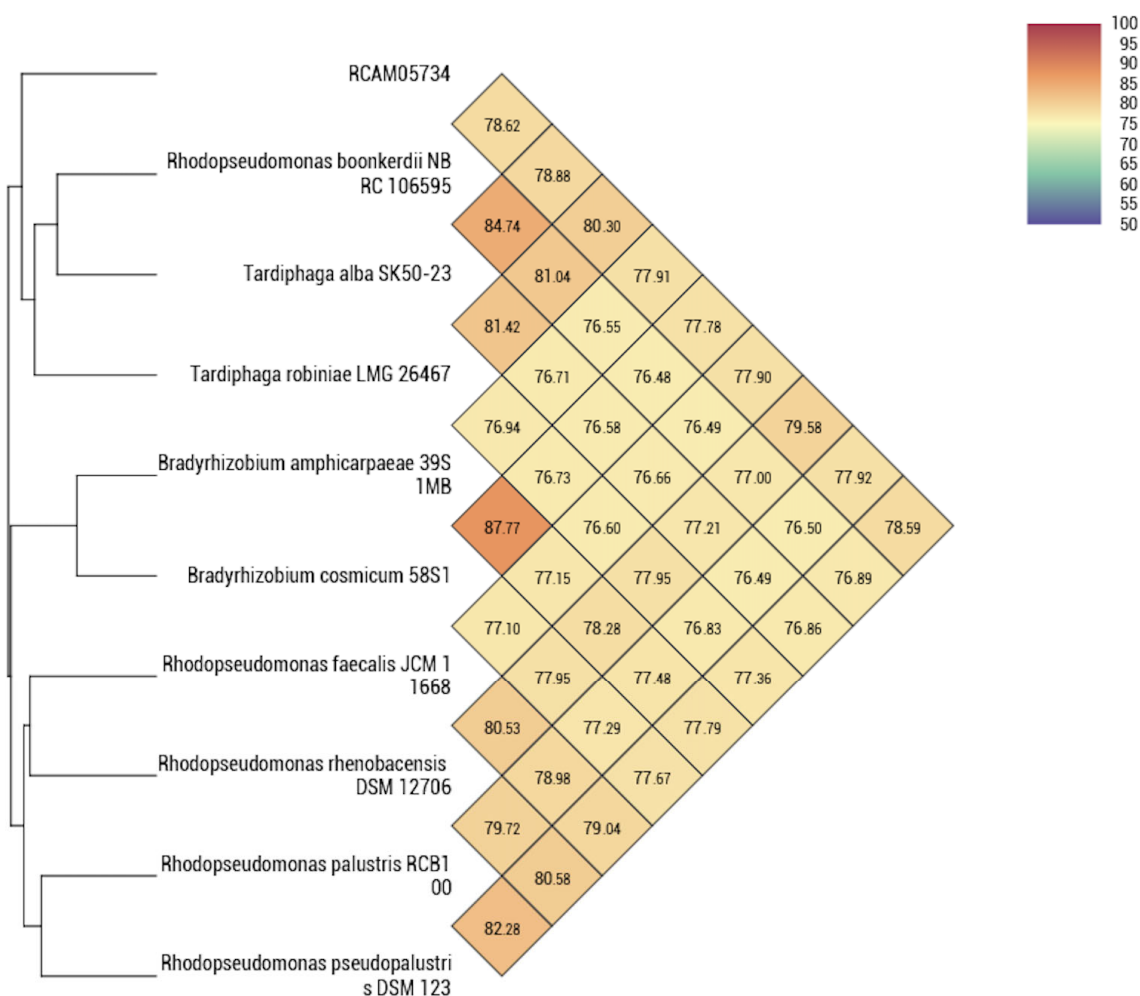


Fig 2. Heatmap of Orthologous Average Nucleotide Identity between strain RCAM05734 and relatives type strains from genus *Rhodopseudomonas*, *Tardiphaga* and *Bradyrhizobium*, calculated using OAT software

Biolog GEN III testing indicated utilization of a broad range of carbohydrates and related compounds, including dextrin, gentiobiose, D-turanose, D-fructose,

D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, and D-fructose-6-phosphate. The strain also metabolized sugar derivatives and carboxylic acids such

as D-glucuronic acid, glucuronamide, L-malic acid, acetoacetic acid, mucic acid, alpha-ketoglutaric acid, L-histidine, and sodium butyrate. Positive utilization was observed for additional substrates including gentiobiose and D-turanose, while sodium bromate elicited only a weak reaction. Positive reactions were recorded in the presence of several antimicrobial or chemical agents, indicating tolerance under assay conditions; these included minocycline, troleandomycin, rifamycin SV, lincomycin, nalidixic acid, Niaproof 4, aztreonam, and potassium tellurite, and the strain also tolerated guanidine HCl. Growth was detected at pH 5 and pH 6 and in medium containing 1% NaCl, but no growth occurred in media containing 4–8% NaCl. Overall, the profile indicates versatile metabolism across mono- and disaccharides, sugar derivatives, selected carboxylic acids and amino acids, together with tolerance to several inhibitory chemicals, limited halotolerance, and activity at mildly acidic pH; sodium bromate is poorly utilized.

In summary, the genome of strain RCAM05734 is relatively compact and contains gene suites indicative of broad metabolic capabilities. Although 16S rRNA similarity to described taxa is quite high, low ANI values suggest it may represent a novel *Rhodopseudomonas* species. The presence of multiple *fix* clusters and an almost complete *nif* gene set suggests potential for nitrogen fixation under microaerobic conditions. Accessory *nod* loci and plant-associated genes (*acdS/acdR*, *iaaM/iaaH*) are consistent with possible plant-microbe interactions. Genes for aromatic compound degradation (both  $\beta$ -keto adipate branches, HPA/HPC pathways, and a meta-cleavage pathway), together with a wide substrate range in Biolog assays, point to metabolic versatility relevant to soil and plant-associated niches, although the strain exhibited limited halotolerance and sensitivity to low pH. Together, these features suggest that *Rhodopseudomonas* sp. RCAM05734 may contribute to nitrogen cycling, plant growth promotion, and the turnover of lignin-derived aromatics in its native environment.

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