Genomic characterization of two Arctic, psychrophilic bacterial strains – representatives of a new taxonomic genus (*Gelidimonas*) within the family *Oxalobacteraceae*

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INTRODUCTION

Despite the harsh environmental conditions, polar regions are inhabited by metabolically diverse microorganisms. This microbial diversity supports a wide array of metabolic traits and biochemical processes that play a crucial role in shaping biogeochemical cycles [1]. There is growing concern about the impact of climate change on polar biota and the potential acceleration of microbial activity [2]. Of particular concern is nitrous oxide (N_2O), a greenhouse gas primarily of microbial origin, with denitrifying bacteria representing its main natural source - accounting for approximately 70% of global emissions [3].

In this study, we present detailed characteristics of two denitrifying psychrophilic bacterial strains isolated from ornithogenic soil collected in the Spitsbergen Island (Norway), at a breeding colony of the marine planktivorous bird *Alle alle* (little auk), identified as a hotspot of enhanced N_2O emissions (Fig. 1) [4]. In-depth physiological, genomic and phylogenetic analyses revealed specific properties and unexpected taxonomic position of the strains.

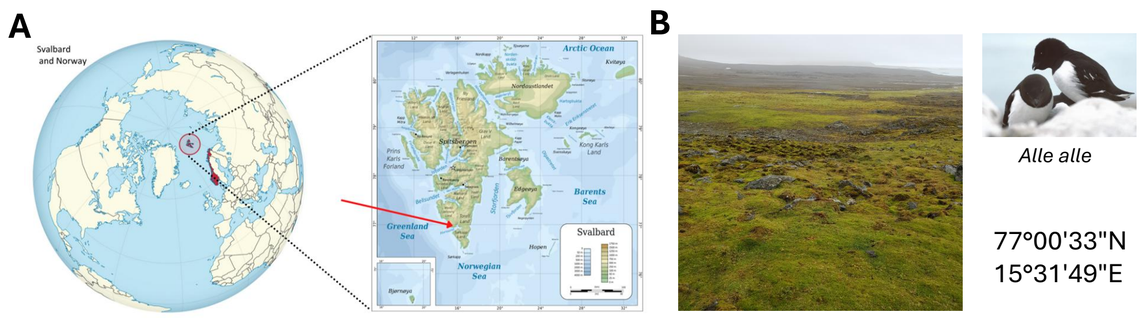

Figure 1. (A) Map showing Svalbard (Norway), with ithe sampling site indicated by a red arrow. **(B)** Photograph of the sampling site – ornithogenic soil influenced by a little auk (*Alle alle*) colony.

RESULTS

CELL MORPHOLOGY AND PHYSIOLOGY

Cell morphology

- **Gram-negative, rod-shaped** (Fig. 2);
- D2 - 1.8-2 µm long, 0.5 µm wide;
- D11 - 2–32 µm long, 0.8 µm wide;
- motile – **polar flagella**;
- nonsporulating;
- circular white colonies, convex with entire edges, smooth (R2A medium).

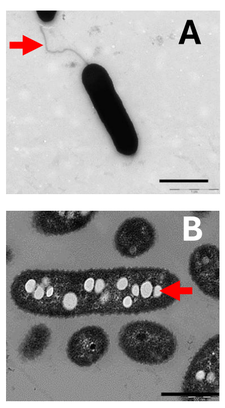

Figure 2. D2 cell morphology **(A)** and ultractructure **(B)**. Arrows indicate: polar flagella (A) and intracellular polyhydroxyalkanoates (PHA) granules (B).

Physiological properties

- growth at 5-20 °C (**opt. 10 °C**) – typical for **psychrophiles**;
- pH 5.5–8.0 (**opt. pH 6.0 for D2, pH 6.0-6.5 for D11**);
- both strains tolerate aerobic and anaerobic conditions;
- **D2 grows on Nutrient Agar and R2A,** but not on LB and TSB. **D11 grows on all tested media**;
- antibiotic resistance: both strains: Lincomycin-resistant; D2 also resistant to rifamycin;
- oxidase activity: **negative**;
- catalyze activity: **positive**.

Nitrogen metabolism

- both strains capable of **N_2O emission** *via* denitrification processes;
- D2 is capable of **nitrogen (N_2) fixation** (Fig. 3).

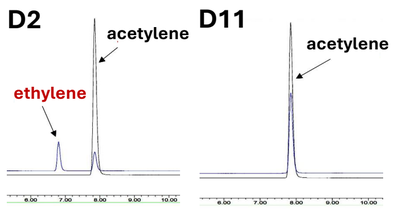

Figure 3. Detection of N_2 fixation ability. Reduction of acetylene to ethylene by the nitrogenase complex.

Fatty acids (FA) profiles

- FA profiling plays a significant role in **chemotaxonomy**. The presence and ratios of different fatty acids can serve as valuable markers for distinguishing between different species, genera, and even higher taxonomic groups;
- D2 and D11 have **similar but not identical FA profiles** (Table 1);
- both contain e.g. C12:0, C13:0, C14:0, C17:0 (saturated); C16:1 ω7c, C18:2 ω6,9 (unsaturated) and both lack C16:0 and C18;
- D2 and D11 differ in the relative abundance of individual FA fractions, e.g. in D11 – dominance of C16:1 ω7c, iC16:0; in D2 – dominance of iC17:0, C18:2 ω6,9, C12:0, C13:0, C14:0, C17:0, and hydroxy FAs.

Table 1. Fatty acids composition profiles of D2 and D11 strains.

FA (%)	D2	D11
Saturated		
C12:0	3.54	1.02
C13:0	3.46	2.57
C14:0	3.44	1.55
C16:0	-	-
C17:0	5.23	2.11
C18:0	-	-
Unsaturated		
C16:1 ω7c	20.41	55.32
C18:2 ω6.9	30.69	13.37
Hydroxy		
C10:0 2-OH	3.32	1.36
C12:0 2-OH	TR	TR
C12:0 3-OH	8.89	5.06
C14:0 3-OH	5.92	2.29
Branched-chain		
iC16:0	8.33	11.68
iC17:0	2.61	1.66

GENOMICS

Genome size and content

The chromosomes of D2 and D11 are similar in size (3.85 Mbp and 3.67 Mbp, respectively) and G+C content (55.3% and 55.4%, respectively) (Table 2). They also exhibit a high degree of sequence identity, with an Average Nucleotide Identity (ANI) of 94.50%, and conserved synteny (Fig. 4). Both strains harbor an almost identical plasmid (plasmid 1; 26.6 kb), while strain D11 additionally carries a second plasmid (plasmid 2) of 26.3 kb.

Table 2. Genome descriprion of strains D2 and D11.

Property	D2 (chromosome/plasmid 1)	D11 (chromosome/plasmid 1/plasmid 2)
Genome size (bp)	3 855 409 / 26 349	3 673 767 / 26 352 / 16 319
G+C content (%)	55.3 / 52.4	55.4 / 52.2 / 54.4
No. plasmids	1	2

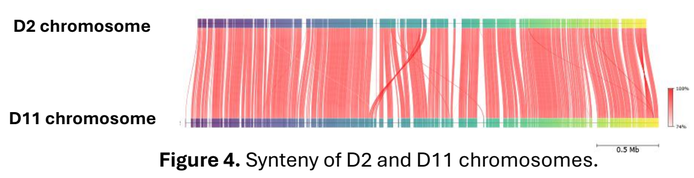

Figure 4. Synteny of D2 and D11 chromosomes.

Nitrogen and carbon dioxide fixation genes

D2 encodes (i) **a molybdenum nitrogenase complex** (**for N_2 fixation**) (Fig. 5) (related enzyme complex is encoded by bacteria of the genus *Herbaspirillum; Betaproteobacteria*) and (ii) **RuBisCO** (**for CO_2 assimilation**).

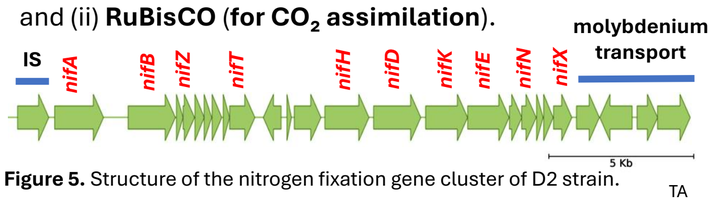

Figure 5. Structure of the nitrogen fixation gene cluster of D2 strain.

Extrachromosomal replicons

- plasmid 1 is an the **RK2-type** (IncP), posibly broad host range (BHR) replicon (Fig. 6);
- plasmid 1 encodes TrfA replication initiation protein; plasmid 2 - RepA protein;
- both contain toxin-antitoxin (TA) systems;
- both have relaxase genes = **mobilizable** but not self-transmissible replicons;
- no genes for antibiotic resistance, detoxification, or secondary metabolism.

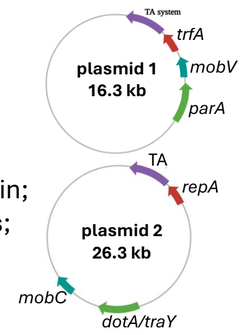

Figure 6. Schematic representation of plasmids.

Transposable elements

- D2 encodes 5 transposase genes (dominantly IS*200*/IS*600*), D11 encodes 11 (more diverse); both strains share IS*200*/IS*605*-, IS*3*-, and IS*1182*-family elements;
- D11 contains a novel putative **Tn*7*-family transposon** (25.5 kb; site specific integration within a YifB/ComM superfamily ATPase gene), encoding a **Sir2-HerA antiphage defense system** and numerous hypothetiocal proteins of unknown function (Fig. 7).

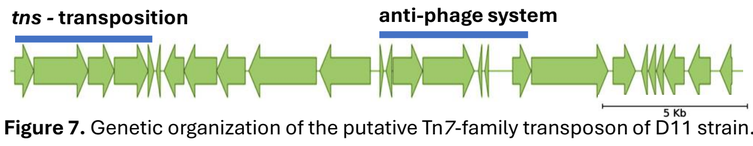

Figure 7. Genetic organization of the putative Tn*7*-family transposon of D11 strain.

PHYLOGENY

Detection and distribution of related strains

Analysis of **360 polar metagenomes** revealed that:

- D2- and D11-related strains have broad distributions (marine environments, saline lakes, soils, freshwater, and permafrost);
- D2-related strains are primarily associated with soil isolates but are also detected in permafrost;
- D11-related strains show strong dominance and wide occurrence in permafrost environments, especially in Europe and North America (Fig. 8).

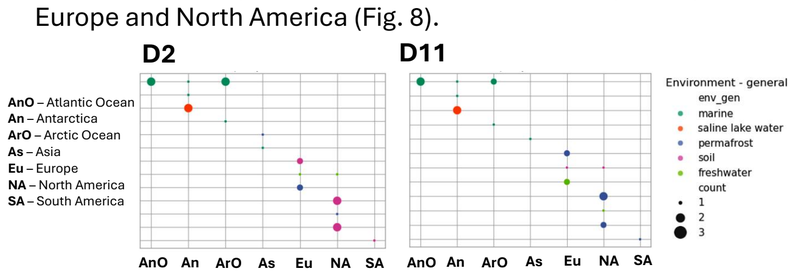

Figure 8. Distribution of strains related to D2 and D11 across cold environments.

Taxonomic position

Sequence comparisons indicated that D2 and D11 are members of the family *Oxalobacteraceae* (*Betaproteobacteria*). According to the ANI (below 95%) and Pairwise Percentage of Conserved Proteins (POCP (60–80%) values, D2 and D11 represent two separate species. Phylogeny based on 375 conserved protein sequences from all defined members of *Oxalobacteraceae* revealed that D2 and D11, together with several other strains, should be included into a separate genus. We propose establishing a novel genus, ***Gelidimonas*** (*gelidus* - cold, icy, and *monas* - a single-cell organism) (Fig. 9).

Proposed species names:

Gelidimonas diazotrophica – D2
Gelidimonas denitrificans – D11

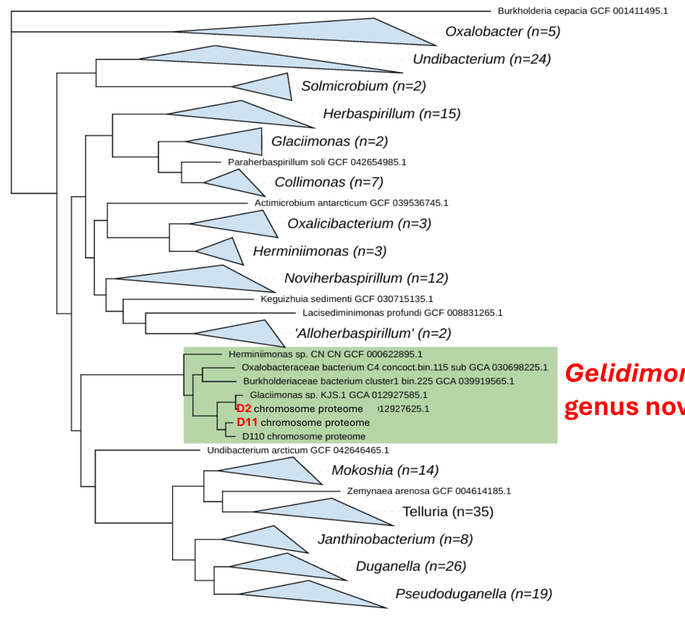

Figure 9. Phylogenetic tree of the family *Oxalobacteraceae* with the indicated proposed novel genus *Gelidimonas*.

CONCLUSIONS

- Comparative analyses of 16S rRNA, whole genomes, core gene proteins, and fatty acid profiles support reclassification of several bacterial strains and the establishment of a novel genus (***Gelidimonas***).
- Phylogeny based solely on 16S rRNA sequence analysis may lead to incorrect conclusions and misclassification of strains.
- D2 and D11 contain numerous mobile genetic elements, indicating HGT events in polar environments.

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