Genomic adaptations of novel halotolerant bacteria from extreme North Greenland

Miguel Ángel Salinas García[1,2,3], *Anders Priemé[2,1,3]

[1] Centre for Exolife Sciences (CELS), Niels Bohr Institute, University of Copenhagen, Jagtvej 155A, DK-2200, Copenhagen, Denmark

[2] Center for Volatile Interactions (VOLT), Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

[3] Section of Microbiology, Department of Biology University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

***Corresponding author**: Anders Priemé (aprieme@bio.ku.dk)

Keywords: extremophile, Greenland, halophile, halotolerant, genome, adaptations

ABSTRACT

Peary Land, northern-most Greenland, is a cold desert region whose soil microbiome remains underexplored. This is the first study to explore the halotolerant soil microbiome of this region with culture-dependent methods. Forty-nine taxonomically diverse bacterial isolates were obtained from dry biological soil crust, surface soil and permafrost soil. Nine isolates were selected for whole genome sequencing; these genomes showed adaptations to their cold, saline native environment, such as cold shock proteins and a large number of osmoprotectant transporters. The genomes also contained several biosynthetic gene clusters, including a large variety of clusters involved in the production of antimicrobial compounds. Furthermore, most of the sequenced genomes showed low digital DNA-DNA hybridisation to their closest relatives, suggesting that they represent novel species. Overall, this study demonstrates the unexplored potential of High Arctic deserts as a source of novel halotolerant bacteria and their secondary metabolites.

INTRODUCTION

Peary Land in extreme North Greenland is the northern-most landmass of our planet. The interior of this remote and uninhabited peninsula is characterized by very sparse vegetation, very low precipitation and very low temperatures (Mølgaard 1982). Due to the low precipitation, most of Peary Land is ice-free and vegetation is mainly found along the moist northern coast bordering the icy Arctic Ocean and in small depressions in the landscape fed by water from melting snowpack. In addition, Peary Land is home to one of the oldest permafrost formations in the world, located in Kap København and around 2 million years old (Fernandez-Guerra et al. 2023). Other notable locations include Cape Morris Jessup, the most northern point of mainland Greenland, and Citronen Fjord, which contains rich deposits of zinc and lead and is being considered for exploitation (Ironbark Zinc ltd.).

Due to the low precipitation and sparse vegetation, the soils in Peary Land are generally thin, alkaline and salt-rich, and poor in carbon, nitrogen and phosphorus (Stimmler et al. 2022). Surface soils of the High Arctic desert of Peary Land tend to harden and crack due to lack of water and evaporation, creating the typical patterns found in deserts (Charlier 1969). In contrast to warm or hot deserts, UV radiation is relatively low, with noon UV index averages of 2-3 in July (Acker and Leptoukh 2007; Hovila et al. 2009).

Biological soil crusts are common in hot and cold deserts and have been defined as communities of organisms dwelling on the soil surface. These communities create an almost living 'skin' of intimate associations between soil particles and photoautotrophic (cyanobacteria, algae, lichens, bryophytes) and heterotrophic (bacteria, fungi, archaea) organisms (Weber et al. 2022). Biological soil crusts regularly experience low water activity and are hence expected to harbour halophilic, salt-loving, and halotolerant taxa. These biological crusts prevent soil erosion and retain moisture, properties relevant in desert regions where bioremediation of biological soil crusts could be particularly effective (Guida et al. 2023).

Permafrost soil remains frozen for at least two consecutive years and is covered by the active layer, i.e., soil that thaws during summer. Despite the frozen conditions, permafrost soils are home to a large diversity of microorganisms (Jansson and Taş 2014). In permafrost and other frozen environments, liquid brine inclusions (cryopegs) form due to salt fractionation (Iwahana et al. 2021). In contrast to the bulk permafrost, nutrients and microorganisms can move in the brine, enabling microbial activity. These brines can reach salt concentrations near saturation, but nevertheless provide a liquid environment for microorganisms to inhabit (Steven et al. 2006). Permafrost soil, and especially the cryopegs within, has been a source of many

halophilic microorganisms described in the literature (Niederberger et al. 2009; Shcherbakova et al. 2009; Spirina et al. 2017). Microorganisms isolated from these environments can serve as models in astrobiology (Nicholson et al. 2013), and their enzymes and metabolites have biotechnological and industrial applications (Flores-Gallegos et al. 2019; Parvizpour et al. 2021).

Microbial life in Peary Land soils require special adaptations to the low temperatures, low water activity and low nutrient conditions; including modifications to cellular membranes, proteins, genetic material and metabolism (Sarma et al. 2023). The extremophiles that thrive under these conditions are known as psychrophiles or psychrotolerant (organisms that grow below 10ºC) and xerotolerant (organisms that can grow at water activity below 0.8, where 1 is pure water) (Lebre et al. 2017). Halophiles are a group of xerotolerants that have evolved to grow under high salt conditions with low water activity (Stevenson et al. 2015). Halophilic microorganisms have previously been isolated from non-Polar deserts such as the Atacama Desert (Ruginescu et al. 2019), but very little is known about halophilic and halotolerant microorganisms in Polar deserts.

Previous studies have shown that the Greenland Ice Sheet is a promising source of biosynthetic gene clusters (Jaarsma et al. 2023). With this study, we aim to extend this potential to different Peary Land soils, whose microbiome remains underexplored and may harbour novel bacterial isolates with potential applications in biotechnology. We hypothesize that i) permafrost soil, biological soil crust and surface soil in Peary Land are inhabited by novel culturable halophilic and halotolerant bacteria, ii) the genomes of these bacteria reveal adaptations to life at low water activity and iii) Northern Greenland represents a potential source of secondary bacterial metabolites.

MATERIALS AND METHODS

Sampling sites

The Kap København formation (sampled at N82.341460, W21.256961, 160 meters above sea level, m.a.s.l.) (**Fig. 1a**), consists of a 100-m thick succession of shallow marine sediments which were deposited during an interglacial period 2 million years ago. The deposits were later uplifted and exposed subaerially about 1 million years ago, at which time the deposits became permafrost (Fernandez-Guerra et al. 2023). Samples were extracted using a sterilized shovel from the permafrost ca. 4 meter below the surface accessed from the side of the formation, which was exposed due to recent erosion. Within minutes of extraction, the extracted permafrost material was opened using a sterile chisel and subsamples from the freshly exposed

material were immediately transferred to sterile plastic tubes where they were kept until initiation of the isolation work at our laboratories.

At Citronen Fjord (**Fig. 1b**), conditions are desert-like with very sparse vegetation consisting of scattered specimens of Arctic Willow (*Salix arctica*) and Entire-leaved Mountain Avens (*Dryas integrifolia*). Here, samples of biological soil crust were obtained from a site devoid of vegetation (N83.085782, W28.2496755, 28 m.a.s.l.). Biological soil crust was scooped from the surface using a sterile spoon and transferred to sterile plastic tubes.

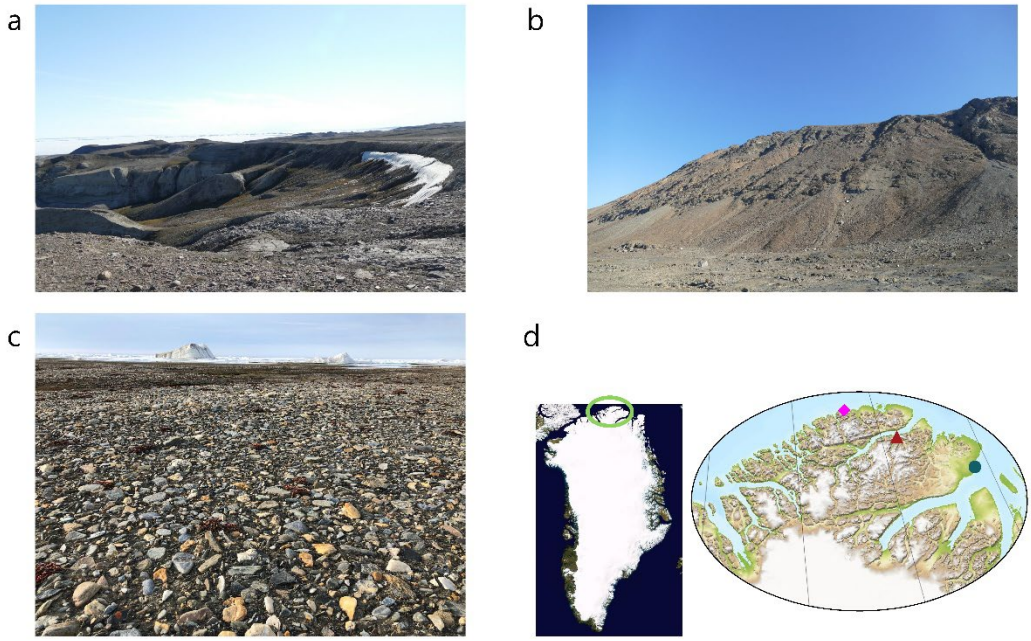


Fig. 1 *Sampling sites at Kap København (a) where permafrost soil samples were obtained at the erosion front left of the snowpack, Citronen Fjord (b) and Cape Morris Jessup (c). d: Location of Peary Land within Greenland and sampling sites: Cape Morris Jessup (magenta diamond), Citronen Fjord (red triangle) and Kap København (dark green circle). Greenland image: NASA. Peary Land map: Blue Green Atlas, licensed under CC BY 4.0*

Due to its proximity to the Arctic Ocean, Cape Morris Jessup (**Fig. 1c**) harbours more dense vegetation (5-15 percent ground cover) compared to Citronen Fjord. The only weather station in Peary Land, WMO 04301 in Cape Morris Jessup, reported a mean temperature of -14ºC, mean annual precipitation of 234 mm, and temperature maxima of around 12ºC in the summer for the period 2020-2023 (Meteostat). The vegetation is dominated by Purple Saxifrage (*Saxifraga oppositifolia*) and Arctic Willow. At N83.657236, W33.368495, 5 m.a.s.l., we sampled biological soil crust (as described above) and soil from 0-4 cm depth by hammering sterile steel cores (4.5 cm diameter) into the soil. A subsample from each soil core was transferred to sterile plastic tubes.

115 **Strain isolation**

116 Samples were stored at 4ºC for a period between one and three months, which
117 included the travel time from the sample sites in Peary Land to Copenhagen, Denmark, and pre-
118 incubation of the samples, known as cold enrichment, which has been shown to increase the
119 number of isolated bacteria from permafrost (Vishnivetskaya et al. 2000).

120 After pre-incubation, the samples were plated on agar plates. Under sterile
121 conditions, approximately 1 gram of soil was suspended in 1 mL of phosphate-buffered saline
122 (PBS) solution supplemented with 10% NaCl. This buffer was chosen to initially select for
123 bacteria that were resistant to shock from high osmolarity. After gentle mixing for 5 minutes, the
124 samples were briefly centrifuged to remove solid particles from suspension. 100µL of the
125 supernatant were plated on agar plates (see below) and left to grow. For the permafrost samples,
126 the supernatant was diluted 100 and 1000 times prior to plating. The plates were incubated at
127 either 4ºC, 10ºC or 25ºC, and checked weekly and daily, respectively. New colonies were
128 randomly selected based on unique morphologies, then plated into fresh agar plates and
129 incubated again. After incubation, a single colony was picked, plated and incubated. This
130 process was repeated several times until only a single type of colony was observed, at which
131 point we considered that the culture was axenic. Single colonies from the axenic plates were
132 transferred into liquid medium and incubated overnight. 1mL of these cultures was mixed with a
133 glycerol stock (20% v/v final concentration) and frozen at -70ºC for long-term storage.

134 For isolation of halotolerant and halophilic bacteria, three saline media were used.
135 The first was a modified HM medium (Ventosa et al. 1982), referred to in this manuscript HM10
136 for its 10% w/v content of NaCl, and containing, in g L^{-1}: NaCl (100) $MgSO_4 \cdot 7H_2O$ (1), $CaCl_2 \cdot 2H_2O$
137 (0,36), KCl (2), KBr (0,27), $FeCl_3 \cdot 6H_2O$ (0,002), $NaHCO_3$ (0,06), Proteose-peptone no. 3 (5), yeast
138 extract (10), glucose (1) and Bacto agar (20, when applicable) adjusted to pH 7,8-8. The second
139 was a medium devised for this study named OHAM (Oligotrophic Halophilic Actinobacteria
140 Medium) containing, in g L^{-1}: soluble starch (20), KNO_3 (0.2), NaCl (100), $MgSO_4 \cdot 7H_2O$ (0.5),
141 K_2HPO_4 (0.5), $FeSO_4 \cdot 7H_2O$ (0.01), sodium pyruvate (0.05) and Bacto agar (15, when applicable),
142 and a final pH of 7.2. The third was R2A medium (Sigma Aldritch, Germany) supplemented with
143 10% NaCl.

144 In addition, a non-saline medium, Gauze Medium 1 (GM1), was also used,
145 containing in g/L: Soluble starch (20), KNO_3 (1), NaCl (0.5), $MgSO_4 \cdot 7H_2O$ (0.5), K_2HPO_4 (0.5),
146 $FeSO_4 \cdot 7H_2O$ (0.01) and bacto agar (15, when applicable). Final pH was adjusted to 7.2-7.4. This

147 medium has been used in previous works to isolate bacterial strains from permafrost (Wang et
148 al. 2018).

149 Premade marine agar (MarA, Sigma Aldritch, Germany) and Tryptic Soy Broth (TSA,
150 Sigma Aldritch, Germany) were used for routine culturing of the isolates after axenic cultures
151 were obtained.

152 Determination of the effect of NaCl on growth

153 To test the NaCl tolerance of the selected isolates, 96-well plates were prepared
154 with HM in different concentrations of NaCl, from 0% (w/v) to 20% in increments of 2.5%. The
155 plates were inoculated with overnight cultures to a constant initial OD_{600}, blanks were made for
156 each different NaCl concentration. The plates were then incubated at 25ºC in a BioTek Synergy
157 H1 Multimode Reader (Agilent, USA) for 5 days with constant rotation and OD measurements
158 every 30 minutes. Range of growth was determined based on the observed growth in the wells at
159 the end of the experiment. To calculate the effect of NaCl on growth, blanks were subtracted and
160 each replicate was fit to a Gompertz model using the R package *growthrates* (Petzoldt 2016)
161 version 0.8.4. The resulting parameters were used to calculate the area under the curve using the
162 R package *pracma* (Borchers 2011) version 2.4.4.

163 16S rRNA gene sequencing and analysis

164 Total DNA was isolated from overnight cultures using the DNeasy PowerSoil Pro Kit
165 (Qiagen, Germany). The 16S rRNA gene was then amplified with the PCRBio kit (PCR Biosystems,
166 UK) using the universal primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-ACG
167 GYT ACC TTG TTA CGA CTT-3') purchased from TAG Copenhagen (www.tagc. com). After the size
168 and quality of the amplicons was assessed with electrophoresis on agarose gel and ethidium
169 bromide staining, the amplified product was sent for Sanger sequencing to Eurofins (Germany)
170 with the same primers, to obtain both forward and reverse reads.

171 The forward and reverse reads were aligned in MEGA11 (Tamura et al. 2021) using
172 the MUSCLE algorithm (Edgar 2004) to produce the near full-length 16S rRNA gene sequences.
173 Then, BlastN (Sayers et al. 2022) was used to identify the strains to the genus level; this search
174 was restricted to type strain sequences. To study phylogenetic relationships, an alignment file
175 was produced in MEGA11 using the MUSCLE algorithm with the near full-length 16S rRNA gene
176 sequences and the full-length 16S rRNA gene sequences of related type strains. A phylogenetic
177 tree was constructed with this alignment file using IQ-TREE 2 (Minh et al. 2020) with ModelFinder
178 (Kalyaanamoorthy et al. 2017) and ultrafast bootstrap (Hoang et al. 2018). Lastly, the tree was

179 drawn with FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Inkscape
180 v 1.3.2 (https://inkscape.org/).

181 Genome sequencing and assembly

182 Total genomic DNA was isolated from overnight cultures using the DNeasy
183 PowerSoil Pro Kit (Qiagen, Germany). Short read libraries were prepared using the DNA-Seq Enz
184 kit for MagicPrep (Tecan, USA). The quality of the libraries was checked using a 5200 Fragment
185 Analyzer System (Agilent, USA) before sequencing of the DNA fragments using Miseq2 (Illumina,
186 Germany). The quality of the reads was examined with FastQC v.0.12.0
187 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Trimmomatic v0.5.1 (Bolger
188 et al. 2014) was used to remove adapter sequences and low quality reads. Unicycler v0.5.1 (Wick
189 et al. 2017) was used with SPAdes v4.0.0 (Prjibelski et al. 2020) to assemble the genomes. The
190 basic statistics and quality of the assemblies were determined with QUAST v5.2 (Mikheenko et
191 al. 2018), checkM v1.2.3 (Parks et al. 2015) and BUSCO v5.7.1 (Manni et al. 2021). To sequence
192 the genome of *Arthrobacter halodurans* DSM 21081^{T}, genomic DNA was directly ordered from the
193 German Collection of Microorganisms and Cell Cultures (DSMZ) and sequenced as described
194 above.

195 Genome analysis

196 The phylogeny of the assembled genomes was determined with the Type (Strain)
197 Genome Server (TYGS) at DSMZ (tygs.dsmz.de), with nomenclature provided by the List of
198 Prokaryotic Names with Standing in Nomenclature (LPSN) (Meier-Kolthoff and Göker 2019;
199 Meier-Kolthoff et al. 2022). A threshold of 70% digital DNA-DNA-Hybridation (dDDH), as
200 determined by the Genome-to-Genome Distance Calculator d_4 formula (GGDC formula 2), was
201 used to determine the species of the isolates. The genomes were annotated with Bakta
202 (Schwengers et al. 2021) and their metabolic pathways were explored by obtaining KEGG
203 Orthology (KO) numbers using the BlastKOALA and Reconstruct tools of the Kyoto Encyclopedia
204 of Genes and Genomes (KEGG) (Kanehisa et al. 2016). AntiSMASH (Blin et al. 2023) in relaxed
205 mode was used to find biosynthetic gene clusters (BGCs) in the assemblies.

206 RESULTS AND DISCUSSION

207 Bacterial isolates

208 A total of 49 bacterial isolates were obtained from the samples (**Table 1**). The soil
209 samples from Citronen Fjord (CF) and Kap København (KK) were the most prolific, while a lower
210 number of isolates was obtained from Cape Morris Jessup soil (CMS) and biological crust (CMC).

211 In addition to bacterial colonies, fungal colonies were observed in plates from all samples. We
212 focused on the bacterial isolates and fungal isolates were not further investigated.

213 The 17 strains isolated with saline media were capable of growth under a wide
214 range of salt concentrations and showed different responses to salt (**Fig. 2**). Three of these
215 isolates, KK5.5, CF3.5 and CF4.18 showed the highest growth at 0% w/v NaCl, while other
216 isolates such as CF4.1, CF4.2 or CMS1.3 showed almost or no growth in medium without NaCl.
217 The growth of all isolates was impaired at high salt concentrations, but eight isolates showed
218 some level of growth at the maximum concentration tested, 20% w/v NaCl. Interestingly, some
219 isolates belonging to the same genus, e.g., *Nesterenkonia*, showed similar responses to NaCl,
220 while both *Arthrobacter* isolates, KK5.5 and KK5.7, showed different growth patterns as NaCl
221 concentrations increased. Halophiles are commonly classified as such if their growth optimum
222 is above 2% w/v NaCl, while halotolerant organisms grow optimally below 2% and can tolerate a
223 wide range of salinities (Ollivier et al. 1994). Based on this classification, the tested isolates
224 consisted of a mixture of halotolerant (CF3.5, CF4.18 and KK5.5) and halophiles (the rest of the
225 strains).

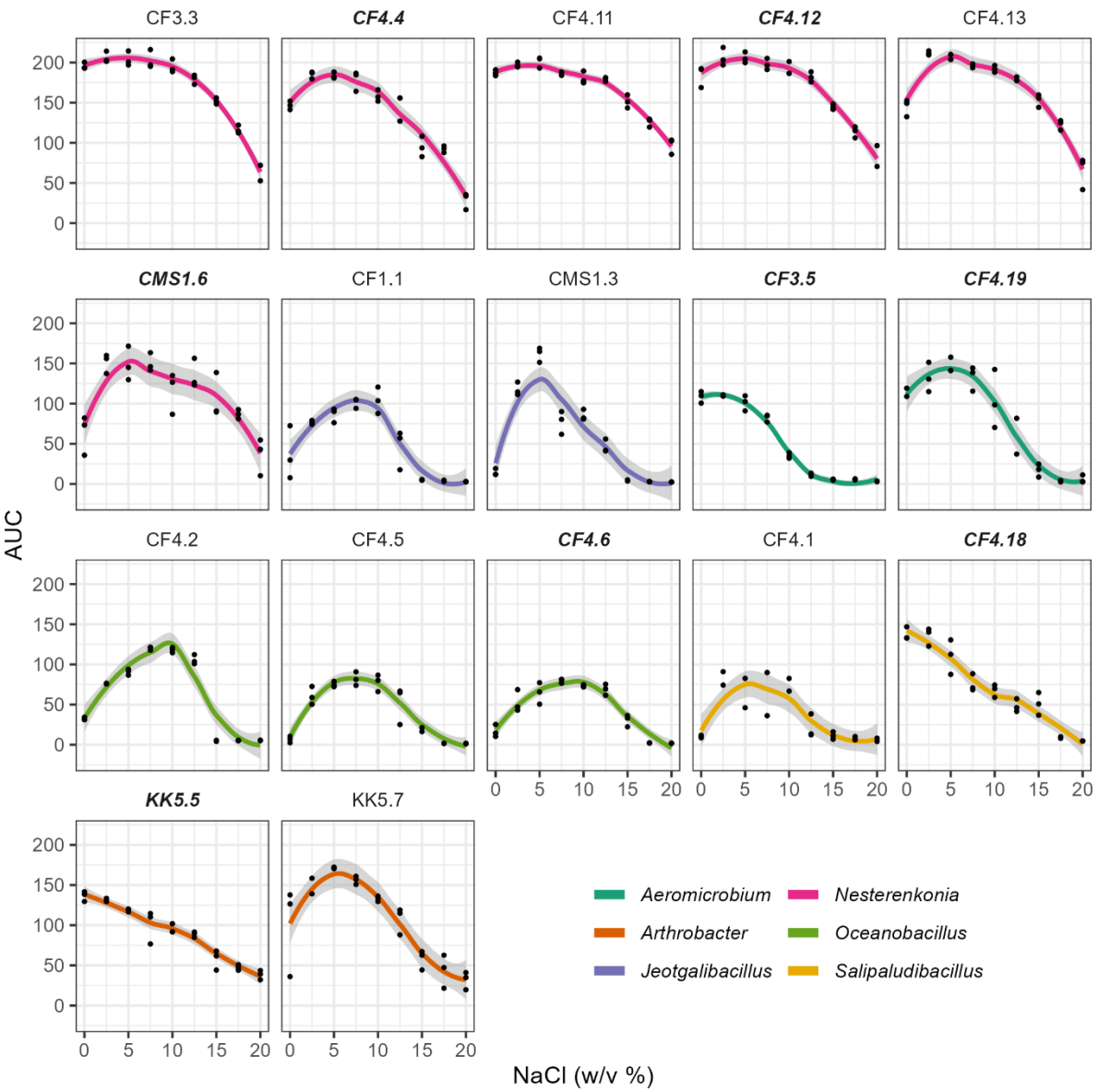


Fig. 2 *Growth of strains isolated in saline media at 0 to 20% w/v NaCl. Y axis is Area Under the Curve of a Gompertz model fitted to the data to indicate total growth during the 5-days incubation period. Black dots represent datapoints. Lines indicate LOESS fit to data with a span of 0.75. Shaded area is the 95% confidence interval. Colour of lines indicates genus. Isolates whose genomes were sequenced are highlighted in bold and italics*

236 ***Table 1*** *Number of bacterial isolates obtained from each site and medium. The total number of bacterial isolates was*
237 *49. Saline media are marked with an asterisk (*)*

Medium	**Citronen Fjord (biological crust)**	**Cape Morris Jessup (soil)**	**Cape Morris Jessup (biological crust)**	**Kap København (permafrost soil)**
GM1	10	4	2	16
HM10*	7	0	0	0
R2A+10% NaCl*	4	2	0	0
OHAM*	2	0	0	2
Total	23	6	2	18

238

239 ## 16S rRNA gene sequencing

240 Based on their 16S rRNA gene sequence, the affiliation of the isolates with known
241 type strains was visualized (**Fig. 3**). Full details of each isolate and accession numbers are
242 available in Table S1. The isolates mainly belonged to three phyla: Actinomycetota, Bacillota and
243 Pseudomonadota, while Bacteroidota was only represented by one isolate, *Acidovorax* sp.
244 CMS1.1 (Fig. 3B). All isolates demonstrated the ability to grow at 4ºC. Many of the strains were
245 isolated in saline media, thus demonstrating their halotolerance. These included all the
246 Bacillota, as well as isolates belonging to the genus *Nesterenkonia*. Some strains isolated in non-
247 saline media also proved to be halotolerant, as they grew on MarA (containing 1.945% w/v NaCl),
248 including most members of *Arthrobacter* and *Pseudomonas* sp. KK6.1. However, other isolates,
249 like *Massilia* sp. CMS3.1 and *Flavobacterium* sp. CMS1.2 were unable to grow in medium
250 supplemented with >1% NaCl (data not shown).

251 The most abundant genera were *Arthrobacter*, predominantly isolated from
252 permafrost soil, and *Streptomyces* and *Nesterenkonia*, obtained from biological crust. Previous
253 studies have shown that these genera, as well as other genera isolated in this study, are
254 commonly found in polar environments. For example, Singh and colleagues (Singh et al. 2017)
255 reported that *Arthrobacter* and *Pseudomonas* were the most commonly isolated genera in
256 permafrost soil from Svalbard, Norway, where they also isolated strains belonging to
257 *Nesterenkonia, Bacillus*, and others. Interestingly, the authors also obtained isolates belonging
258 to *Streptomyces*, while none of our isolates in this genus were obtained from permafrost soil.
259 *Streptomyces* and other Actinomycetota are commonly found in arid and desert soils (Bull and
260 Asenjo 2013) as well as polar environments (Abirami et al. 2021), and extremophilic and
261 extremotolerant *Streptomyces* have drawn attention as potential producers of novel
262 antibacterial compounds and other compounds of interest (Sivalingam et al. 2019). In addition,

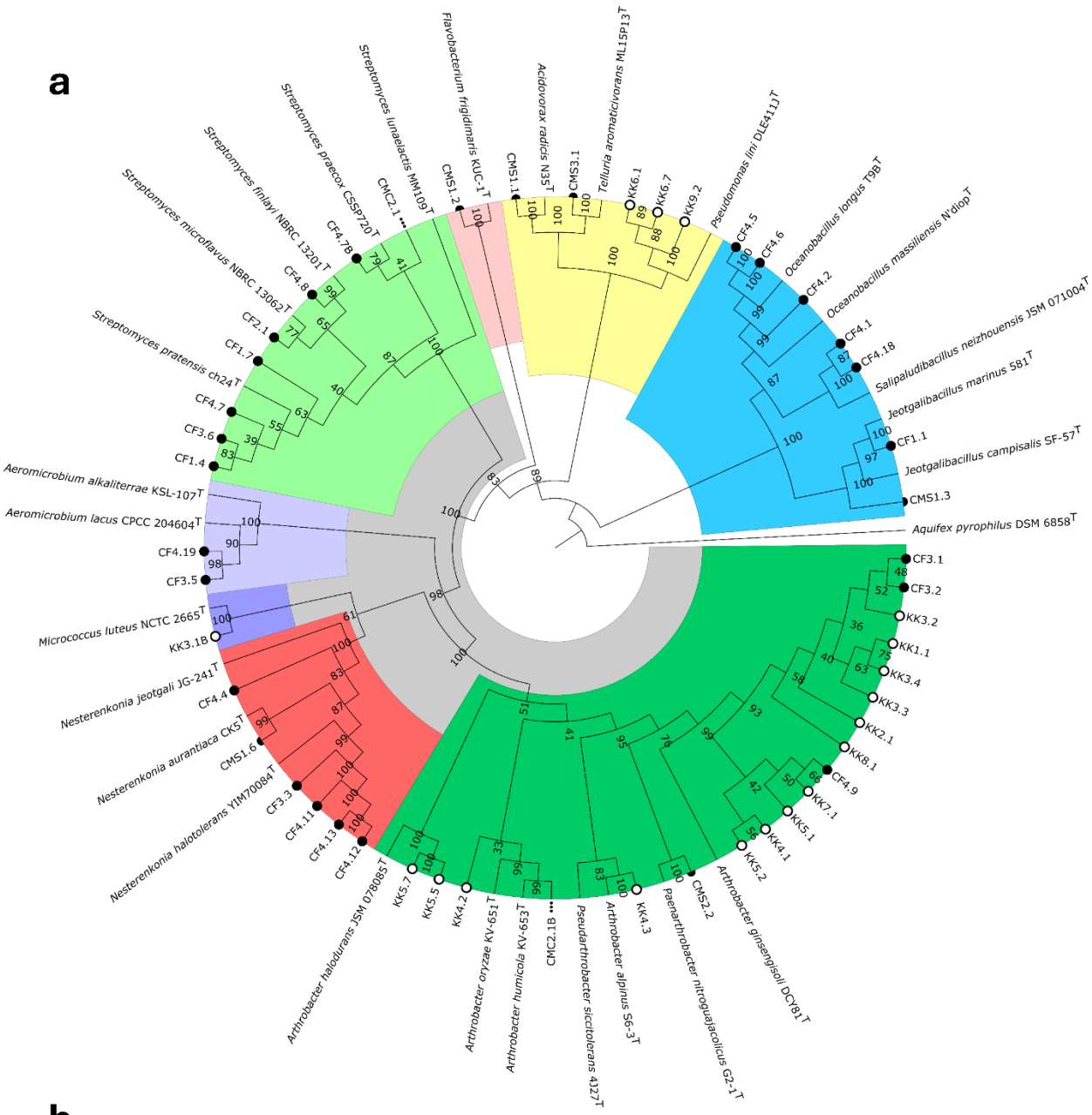
Nesterenkonia has been isolated from Arctic permafrost soil (Singh et al. 2021), as well as other cold environments, like soils of Antarctica and the Tibetan plateau (Finore et al. 2016; Dai et al. 2022).

Arthrobacter is commonly found in permafrost soil with both culture-dependent (Hinsa-Leasure et al. 2010; Singh et al. 2017) and culture-independent methods (Shen et al. 2021), the latter of which have shown that *Arthrobacter spp.* can constitute up to 4% of the total bacterial community (Dsouza et al. 2015). This suggests that either *Arthrobacter spp.* are especially adapted to survive in permafrost soil, or that they can quickly adapt to newly forming permafrost. The *Arthrobacter* pangenome (Gushgari-Doyle et al. 2022) revealed great potential adaptability, which could explain its prevalence in permafrost soil. The same study showed the halotolerance of several *Arthrobacter* strains, a similar observation to our *Arthrobacter* isolates, as many of them could grow on MarA or were directly isolated in saline medium. Based on its prevalence in a variety of environments, some authors have used *Arthrobacter* as a model to study bacterial adaptations in soil (Chauhan et al. 2018; Shen et al. 2021). In our study, the isolated *Arthrobacter* strains were initially isolated in either GM1 or OHAM, media with no nitrogen source other than nitrate, and with glycogen as the only carbon source (along with pyruvate in OHAM). Some members of this genus are known to be able to assimilate nitrate and nitrite for growth (He et al. 2020; Liu et al. 2023). Based on our results, GM1 may be an effective selective medium to isolate *Arthrobacter* strains from permafrost soil.

The diversity of isolates obtained from Kap København, Cape Morris Jessup and Citronen Fjord, and the displayed halotolerance of the isolates, show that the sampled sites are inhabited by a wide range of halotolerant bacteria, thus partially failing to reject our first hypothesis. In the next section, we discuss the novelty of the isolates.

Fig. 3 [next page] *Phylogenetic affiliation of isolates. a: Consensus cladogram based on the 16S rRNA gene sequences of the isolates and selected type strains calculated with TIM2+F+I+G4. Bootstrap values (as percentage of 10000 repetitions) are shown at the branch points. Phyla are highlighted with colours as indicated in panel b. Within Actinomycetota, genera are further highlighted with colours as indicated in panel b. The markers indicate the source of isolation: Citronen Fjord (black circle), Kap København (white circle), and Cape Morris Jessup soil (semicircle), and crust (dots). Type strains have no markers.* Aquifex pyrophilus *DSM 6858 was used as the outgroup.* ***b****: Number of isolates of each detected genus, and colours used to highlight groups in panel a*

a



b

Genus	Phylum	Isolates (saline media)	Isolates (non-saline media)
Aeromicrobium	Actinomycetota	2	-
Arthrobacter	Actinomycetota	2	13
Micrococcus	Actinomycetota	-	1
Nesterenkonia	Actinomycetota	6	-
Paenarthrobacter	Actinomycetota	-	1
Pseudarthrobacter	Actinomycetota	1	2
Streptomyces	Actinomycetota	-	8
Jeotgalibacillus	Bacillota	2	-
Oceanobacillus	Bacillota	3	-
Salipaludibacillus	Bacillota	2	-
Flavobacterium	Bacteroidota	-	1
Acidovorax	Pseudomonadota	-	1
Massilia	Pseudomonadota	-	1
Pseudomonas	Pseudomonadota	-	3

297 ## Genomes and functional analyses of selected isolates

298 From the 16S rRNA gene-based phylogeny, nine isolates were selected for whole
299 genome analysis (**Table 2**), of which eight were halotolerant. *Massilia* sp. CMS3.1 was
300 determined to not be halotolerant, as it was only able to grow in GM1 with <1% NaCl, but was
301 chosen for genome sequencing (a) as a Gram-negative and non-halotolerant representative and
302 (b) to investigate its adaptations to its natural environment as a non-halotolerant bacterium. In
303 the case of *Arthrobacter* sp. KK5.5, its closest strain was *A. halodurans* DSM 21081^{T}, whose
304 genome was not available in any database. To confirm the phylogeny of our isolate, we
305 sequenced the genome of *A. halodurans* DSM 21081^{T}. All genomes showed completeness above
306 98% and contamination below 2%, which was determined sufficient to proceed to the next part
307 of the analysis. Six of the nine sequenced isolates showed dDDH similarities to their closest
308 relatives below 30%, well below the established threshold of 70% to distinguish between
309 different species (**Table 2**). Following the phylogenetic analysis, we conducted a functional
310 analysis of the genomes using the KEGG database and KEGG Orthology numbers.

311

312

313

314

315

316 ***Table 2*** *Isolates selected for genome sequencing, information on the assemblies, and similarity to their closest relatives bases on digital DNA-DNA hybridization (dDDH).*
317 *Completeness and contamination were calculated with CheckM. Completeness calculated by BUSCO is available in Table S2. GGDC: Genome-to-Genome Distance Calculator.*

318 **[DISCLAIMER: We have received permission from the Greenlandic authorities to deposit our genome-sequenced strains at DMSZ and are currently in the process of doing so. DSMZ*
319 *numbers will be added to this table.]*

Isolate	**NaCl range (% w/v) (optimum)**	**Genome size (Mbp)**	**No. of contigs**	**N50**	**GC content (%)**	**Completeness (%)**	**Contamination (%)**	**Number of CDS**	**Closest match (dDDH)**	**Similarity to closest dDDH match (GGDC formula 2, %)**	**DSMZ strain number***	**Genome accession number**
CF4.18	0-17.5 (0)	5.27	159	76384	37.32	99.34	1.77	5087	*Salipaludibacillus neizhouensis* DSM 19794	74.7	TBD	JBIMEV000000000
CF4.6	0-15 (7.5)	4.07	39	241120	37.48	99.33	1.22	4050	*Oceanobacillus rekensis* PT-11^T	27.1	TBD	JBIMEX000000000
CMS3.1	0-1 (0)	5.37	85	300246	62.92	100	0.95	4727	*Massilia litorea* LPB0304^T	29.4	TBD	JBIMES000000000
CF4.12	0-20 (5)	2.94	13	734929	66.33	99.66	0.43	2687	*Nesterenkonia halotolerans* DSM 15474^T	81.8	TBD	JBIMEW000000000
CF4.4	0-20 (5)	3.31	19	389302	66.90	99.24	0.61	3018	*Nesterenkonia aurantiaca* DSM 27373^T	31.8	TBD	JBIMEY000000000
CMS1.6	0-20 (5)	3.05	37	330592	67.29	99.11	0.46	2775	*Nesterenkonia aurantiaca* DSM 27373^T	82.6	TBD	JBIMET000000000
KK5.5	0-20 (0)	3.34	40	190007	68.49	99.77	0	3053	*Arthrobacter halodurans* DSM 21081^T	22.6	TBD	JBIMER000000000
CF3.5	0-10 (2.5)	3.40	29	432807	68.19	99.74	0	3303	*Aeromicrobium fastidiosum* NRBC 14897^T	20.3	TBD	JBIMEZ000000000
CF4.19	0-15 (5)	3.49	21	441989	70.42	98.45	0.52	3442	*Aeromicrobium halocynthiae* JCM15749^T	21.2	TBD	JBIMEU000000000

320

321 **Adaptations to high salinity and low water activity**

322 All sequenced isolates, except for *Massilia* sp. CMS3.1, possessed orthologs of
323 genes encoding importers of osmoprotectants like proline, ectoine and betaine choline (**Fig. 4**
324 and **Table S3**). Osmoprotectants are small, neutral organic solutes that do not interfere with
325 cellular processes, and can thus be accumulated to mitigate osmotic and low-temperature
326 stress (Pathania et al. 2022). The ability to import osmoprotectants from the environment offers
327 flexibility, allowing bacteria to adapt to fluctuating salinity. This strategy reduces the energy cost
328 associated with de novo synthesis, providing a competitive advantage. Interestingly, the non-
329 halotolerant *Massilia* sp. CMS3.1 possessed only one ortholog of osmoprotectant transporters
330 (*yaaJ*), whereas its close relative, *Telluria aromaticivorans*, has 16 transporters and 9 synthesis-
331 related genes, despite its low salt tolerance (Son et al. 2021). Conversely, *Salipaludibacillus*
332 *neizhouensis* CF4.18 had 33 orthologues related to osmoprotectant transport, almost twice that
333 of the type strain of its species, revealing large intra-species variation. These findings suggests
334 that halotolerance may not be directly corelated with the copy number of genes related to
335 osmoprotectant synthesis and import.

336 Other abundant groups were orthologues related to ion transport (including *trkA*,
337 *kdpABC*, *tlyC*, and *nhaAC*). Ion transporters are very common in bacteria and are not necessarily
338 indicative of halotolerance, as evidenced by similar numbers of ion transporter orthologues in
339 non-halotolerant strains (**Fig. 4**). However, ion transporters may have specific adaptations that
340 aid with halotolerance. For example, the cyanobacterium *Aphanothece halophytica* has ion
341 transporters with different ion specificities that allow it to tolerate high salinity and pH
342 (Wutipraditkul et al. 2005). In the halophilic bacterium *Halomonas beimenensis*, the genes *nqrA*,
343 *trkA*, *atpC*, *nadA*, and *gdhB* — related to ion transport — along with *spoT*, *prkA*, *mtnN*, *rsbV*, *lon*,
344 *smpB*, *rfbC*, *rfbP*, *tatB*, *acrR1*, and *lacA* — related to cellular signalling, quorum sensing,
345 transcription/translation, and cell motility — are critical for halotolerance, and their deletion
346 impairs growth at high salinity (Chen et al. 2017). Similarly, the genes *trkA2*, *smpB*, *nadA*, *mtnN2*,
347 *rfbP*, *lon*, and *atpC* are upregulated in high salinity in *Virgibacillus chiguensis* (Chen et al. 2018).
348 Orthologues of many of these genes were found in varying numbers in the sequenced isolates
349 (**Table S3**), suggesting their potential role in the observed halotolerance across the bacteria
350 isolated in this study.

351 Cold temperatures can lead to similar stress variables than high salinity. For
352 example, water activity is reduced as ice crystals form, leading to osmotic stress, and brines can
353 form as salt ions are excluded from ice crystals (Iwahana et al. 2021). Thus, the halotolerance-
354 related genes discussed may also aid the isolates against the low temperatures in their native

Northern Greenland. In addition, the genomes also contained several orthologues of cold shock proteins (*cspA*). These proteins have a protective effect against the stresses associated with low temperatures by binding to single stranded nucleic acids and regulating transcription and translation (Horn et al. 2007).

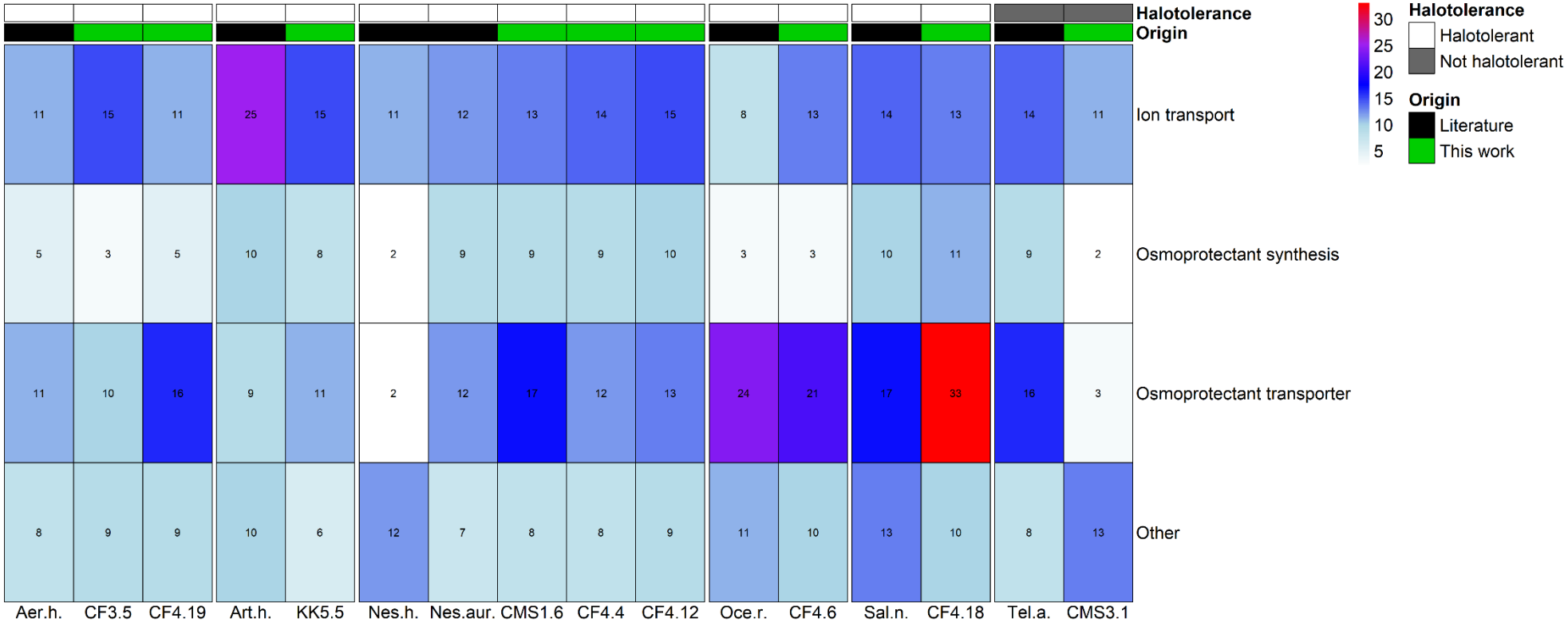


Fig. 4 KEGG Orthology count numbers of groups of genes that have been described to be important for halotolerance in the sequenced genomes and in genomes of close relatives. The complete dataset is available in Table S3. The close relatives are as follows, from left to right: *Aeromicrobium halocynthiae* JCM 15749^{T}, *Arthrobacter halodurans* DSM 21081^{T}, *Nesterenkonia halotolerans* DSM 15474^{T}, *Nesterenkonia aurantiaca* DSM 27373^{T}, *Oceanobacillus rekensis* PT11^{T}, *Salipaludibacillus neizhouensis* DSM 19794^{T}, and *Telluria aromaticivorans* ML15P13^{T}

364 **Other metabolic pathways**

365 Some of the sequenced genomes contained genes encoding enzymes related to
366 nitrogen and sulphur cycling. *S. neizhouensis CF*4.18 and *Oceanobacillus* sp. CF4.6 had a
367 complete assimilatory sulfate reduction pathway, while *N. aurantiaca* CMS1.6 and *N.*
368 *halotolerans* CF4.12 lacked this pathway completely. The rest of the sequenced isolates had an
369 incomplete pathway, lacking the enzymes to reduce sulphate to sulphite. However, all genomes
370 contained transporters for alkanesulfonates, and most genomes contained genes encoding for
371 alkanesulfonate monooxigenases. This alternative source of sulphur may aid against sulphur
372 starvation (Ellis 2011), as the low amount of organic carbon in Peary Land soils could limit the
373 amount of sulphur available from organic matter (Stimmler et al. 2022). However, sulphur may
374 not be a limiting nutrient in the permafrost at Kap København, as it consists mostly of marine
375 deposits, which are typically enriched in sulphur (Funder et al. 1984; Francois 1987).

376 *Nesterenkonia* sp. CF4.4, *Aeromicrobium* sp. CF3.5, *Arthrobacter* sp. KK5.5, *S.*
377 *neizhouensis* CF4.18 and *Massilia* sp. CMS3.1 had genes encoding for both nitrate and nitrate
378 reductases, while these were not found in the other isolates. Interestingly, *Oceanobacillus* sp.
379 CF4.6 was the only isolate whose genome contained *nirK*, which encodes for a nitric oxide-
380 producing nitrite reductase. The reduction of nitrite to nitric oxide (NO) is the second step in
381 denitrification after the reduction of nitrate to nitrite. NO is then reduced to nitrous oxide (N_2O),
382 which is finally reduced to N_2. NO and N_2O have significant impact on atmospheric chemistry, as
383 they can induce the formation of tropospheric ozone and form NO_X; furthermore, N_2O is a potent
384 greenhouse gas. In arid areas, especially in the polar regions, nitrification and denitrification
385 remain poorly understood (Ramond et al. 2022). There are no available studies on the nitrification
386 or denitrification rates, or on the emission of NO and N_2O, from Northern Greenland, but
387 ecosystem N losses from Arctic soils through N_2O emission are generally small, which may be
388 linked to the N limitation of these soils (Tamm 1991; Elser et al. 2007). *Oceanobacillus* sp. CF4.6
389 may play a role in denitrification and ecosystem loss of nitrogen in its native environment,
390 although environmental studies are needed to characterise the soil nitrogen cycle in the High
391 Arctic desert of Northern Greenland.

392 All the sequenced genomes encoded pathways for the synthesis of terpenoid
393 backbones. Apart from *Oceanobacillus* sp. CF4.6, whose genome encoded for the mevalonate
394 pathway, the sequenced genomes encoded for the MEP/DOXP pathway for isoprenoid backbone
395 synthesis. Interestingly, the pathway in *N. halotolerans* CF4.12 is incomplete, as it is missing 2-
396 C-methyl-D-erythritol 4-phosphate cytidylyltransferase (EC 2.7.7.60) encoded by *IspD*, and 2-C-
397 methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12), encoded by *IspF* (Phillips et al.

398 2008). *N. halotolerants* DSM 15474^{T} shows the same missing enzymes in its draft genome. These
399 findings suggest that *N. halotolerans* may contain undescribed isoforms of these enzymes. The
400 synthesis of terpenoid backbones is an important pathway to produce essential and secondary
401 metabolites like undecaprenol phosphate and carotenoids. In fact, all isolates apart from
402 *Oceanobacillus* sp. CF4.6 and the two *Aeromicrobium* sp. had BGCs encoding for terpene-
403 related products, with many of them encoding for carotenoids (see next section). This pathway
404 may be of particular relevance in *Nesterenkonia*, as all isolates belonging to this genus had a
405 bright red or orange colour, suggesting a significant content of carotenoids.

406 The adaptations discussed in this section allow the isolates to sustain activity in
407 their cold, nutrient-poor, and water-scarce environment. Furthermore, most of the sequenced
408 genomes showed very low dDDH percentages towards their closest known relative, suggesting
409 that they represent previously undescribed species. Thus, we fail to reject hypotheses i and ii.

410 Biosynthetic gene clusters in the genomes of the sequenced isolates

411 The sequenced genomes were found to contain several BGCs (**Fig. 5** and **Tables**
412 **S4-S12**), including several with no hits to known BGCs. 32% of the identified BGCs putatively
413 encoded for antimicrobial compounds, such as the lassopeptide paeninodin found in *S.*
414 *neizhouensis* CF4.18 and *Oceanobacillus* sp. CF4.6, ε-Poly-L-lysine synthesis clusters found in
415 *N. halotolerans* CF4.12 and *Nesterenkonia* sp. CF4.4, and other BGCs encoding for lactones
416 found in several isolates. Most of these putatively antimicrobial clusters encoded for polyketide
417 synthases (PKS) and non-ribosomal peptide synthases (NRPS). Antimicrobial compounds and
418 antimicrobial resistance are widely distributed in soils, where competition for available space
419 and resources exerts significant selective pressure (Mullis et al. 2019). The production of
420 antimicrobial compounds typically incurs a high metabolic cost; for example, Schlatter and
421 Kinkel (Schlatter and Kinkel 2015) found trade-offs between efficient growth, antimicrobial
422 resistance and antimicrobial production in *Streptomyces spp*., and suggested that a "super-
423 killer" phenotype results in a reduction in niche width. The most common strategy in
424 *Streptomyces spp*. was the simultaneous production of different kinds of antimicrobial
425 compounds to increase their synergistic effect and slow down the evolution of resistance.
426 However, in carbon-poor soils, as is the case in Peary Land, nutrient limitation may present an
427 obstacle for this strategy. On the other hand, nutrient scarcity may increase evolutionary
428 pressure to inhibit potential competitors. Many studies have demonstrated the production of
429 antibacterial and antifungal compounds by bacteria isolated from arid soils (e.g., Saadoun et al.
430 2008; Nasfi et al. 2018), and even the hyper-arid Atacama desert is a potential source of novel

431 antimicrobials (Bull and Asenjo 2013). Polar desert soils, although not as explored, have also
432 demonstrated this potential (van Dorst et al. 2017). In permafrost, where biomass can be higher
433 and environmental conditions are stable, antibiotic resistance is widespread, even in ancient
434 permafrost (Mindlin and Petrova 2017; Gomathinayagam and Muthukaliannan 2024). Although
435 measuring the production of antimicrobial compounds of our isolates was beyond the scope of
436 this study, the diversity of BGCs encoding for antimicrobial compounds found in the sequenced
437 genomes suggest a competitive environment where it may be advantageous to have different
438 antimicrobial strategies available.

439 Desert soils often have higher pH compared to soils from forests and other
440 biomes. The high pH is caused by accumulation of minerals and salts that are not washed away
441 by rain. Peary Land soil is no exception, with soils at Cape Morris Jesup and Citronen Fjord having
442 pH of 7.8 and 9.2, respectively. At these pH values, the bioavailability of many metals is lowered.
443 Thus, bacteria in desert soils need effective metal-acquiring systems such as siderophores.
444 Siderophores allow for the solubilisation of iron essential for the production of, e.g., heme
445 proteins. Both *Aeromicrobium* isolates, as well as *Arthrobacter* sp. KK5.5 had BGCs encoding for
446 NRPS-independent siderophores; their genomes also contained orthologs of *fepBDG*, which
447 encode for Fe-siderophore complex transporters, and both *Aeromicrobium* isolates additionally
448 had *fepC*. Notably, *N. halotolerans* CF4.12 also had a complete set of *fepBCDG*, while the other
449 two *Nesterenkonia* isolates had orthologues of *fepCG*. This absence of siderophore synthesis
450 genes, while transport genes are present, may be due to a variety of causes. One possibility is
451 that these bacteria rely on siderophores produced by other bacteria for iron uptake (Kramer et al.
452 2020).

453 Lastly, *Massilia* sp. CMS3.1 had a BGC whose putative product is a homoserine
454 lactone. While it had no hits to known clusters, it contained, among others, a protein with an
455 acyltransferase domain; this suggests that this BGC may encode for an N-Acyl homoserine
456 lactone, a group of signalling molecules involved in quorum sensing and biofilm formation. This
457 aligns with the possible ability of *Massilia* sp. CMS3.1 to form biofilms. Indeed, the genome of
458 this isolate contained several genes involved in quorum sensing and biofilm formation, such as
459 orthologues of *lasIR* and *wspBEFR*, as well as *pilGHIJ*, which are involved in type IV pilus
460 formation. Young (<24h) colonies of *Massilia* sp. CMS3.1 were orange, soft and easily pickable,
461 but older (especially >48h) colonies became darker, hard, and difficult to disaggregate, which
462 suggests the formation of a hard extracellular matrix that may be mediated by these genes.
463 Biofilm formation has been shown to be a successful strategy to improve survivability against
464 extreme environmental factors like temperature and pH (high and low), aridity and high salinity

465 (Khan and Rao 2023). For example, biofilms are employed by epiphytic bacteria to survive periods
466 of extreme cold and freeze-thaw cycles (Wu et al. 2012).

467 Other types of BGCs found in the genomes included an ectoine synthesis cluster
468 and terpenoid synthesis clusters, which were discussed in the previous section; the terpenoid
469 clusters encoded mainly for carotenoids.

470 It is possible that some BGCs were missed by antiSMASH. Indeed, BGC detection
471 on contig-level assemblies can be challenging, as BGCs may be split into different contigs
472 (Meleshko et al. 2019). This is evidenced by a few BGCs that were found at the beginning or the
473 end of a contig, suggesting that these BGCs are incomplete. This, combined with the significant
474 amount of BGCs that had no hits with known BGCs, suggest that Peary Land is indeed a source
475 of potentially novel secondary metabolites, as per hypothesis iii; however, further investigation
476 *in vivo* will be necessary to fully characterise these secondary metabolites.

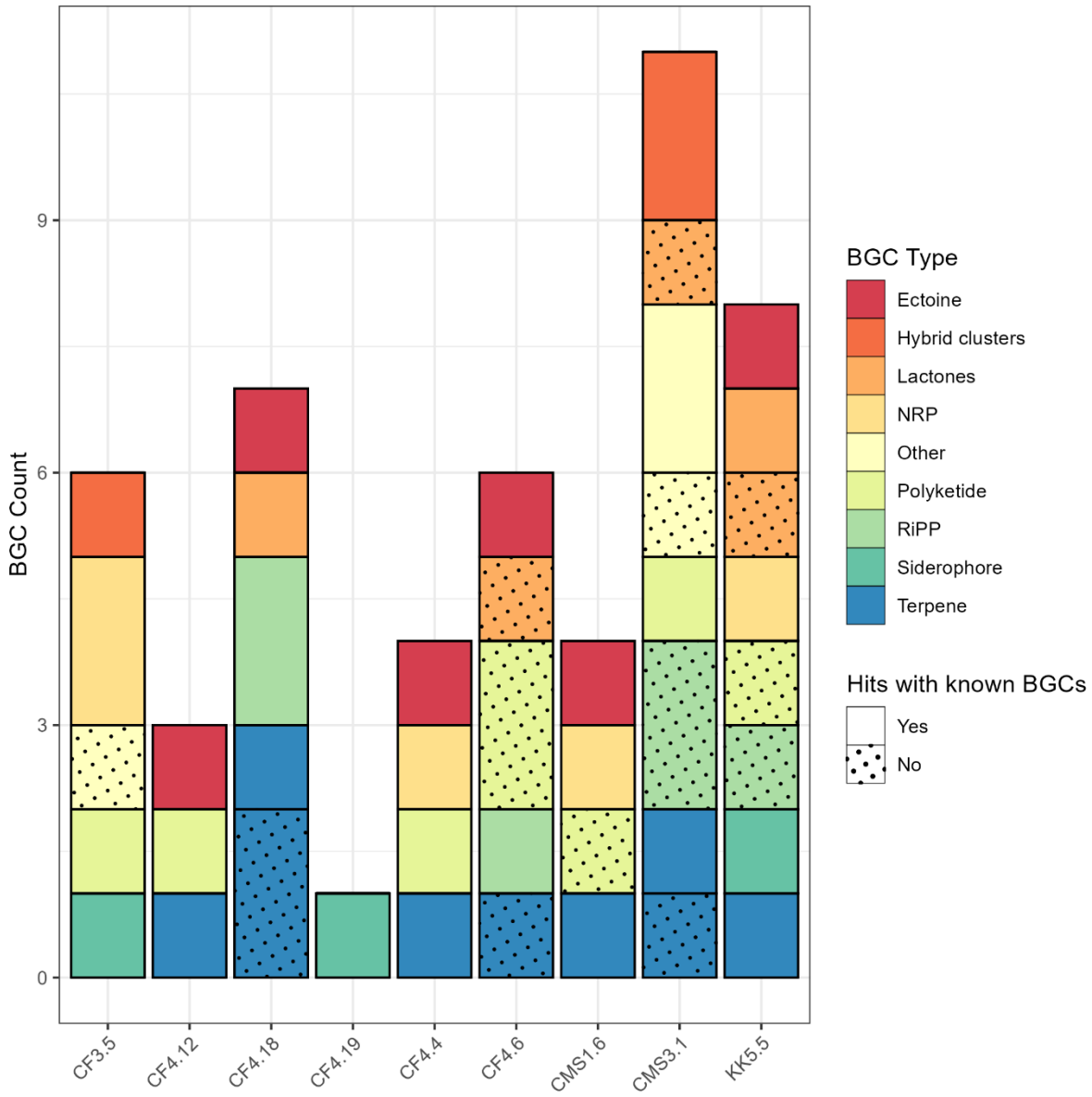


Fig. 5 *Biosynthetic Gene Clusters (BGCs) found in the assembled genomes. The dotted pattern represents BGCs with no hits to known BGCs. NRP: Non-Ribosomal Peptide. RiPP: Ribosomally synthesized and Post-translationally modified Peptide*

CONCLUSIONS

We isolated 49 bacteria from three different sites in Peary Land, northern Greenland, representing some of the most northernly bacterial isolates, and the first strains isolated from these areas. We showed the diversity of culturable halotolerant bacteria at the three sites and found potentially novel BGCs that suggest a competitive environment with many antimicrobial compounds present. The isolates showed adaptations to their cold and saline native environments, including a variety of ion transporters and genes related to osmoprotectant synthesis and transport. These findings underscore the importance of osmoprotectants in cold

and saline environments and expand our understanding of microbial life in extreme environments. Overall, this work not only provides the first insights into the diversity of culturable halotolerant bacteria of northern-most Greenland, but also suggests that the region harbours untapped microbial resources that may hold significant biotechnological value.

DATA AVAILABILITY

All sequences obtained and used in this study, including near-complete 16S rRNA gene sequences, genome assemblies, and short-read libraries, are available in the National Center for Biotechnology Information (NCBI) database under the BioProject accession number PRJNA1156031.

ACKNOWLEDGEMENTS

We would like to thank Lorrie Maccario for her help in the library preparation and sequencing of the genomes, and Anette Hørdum Løth and Ayoe Lüchau for their indispensable assistance in the lab. We would also like to thank Magnus Per Damsø Jeppesen for his help in the collection of some of the salt response data. We are grateful to the Greenlandic authorities for granting permission to sample in the Northeast Greenland National Park (Expedition Permit C-21-549) and to export the soil samples to Denmark for scientific work (Non-exclusive license number G21-031 for utilization of Greenlandic genetic resources).

FUNDING

This project was funded by the Novo Nordisk Foundation under the NNF Interdisciplinary Synergy Program (grant number NNF19OC0057374): Effects of bacteria on atmospheres of Earth, Mars, and exoplanets -- adapting and identifying life in extraterrestrial environments. This project was also supported by The Danish National Research Foundation within the Center for Volatile Interactions (VOLT, DNRF168). The bacteria in this study were isolated from samples collected during the Leister Around North Greenland 2021 Expedition financed by the Leister Stiftung, Switzerland.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abirami B, Manigundan K, Radhakrishnan M, et al (2021) Diversity and Bioprospecting Potentials of Antarctic (Polar) Microbes. In: Microbiome-Host Interactions. CRC Press

Acker JG, Leptoukh G (2007) Online analysis enhances use of NASA Earth science data. EoS Transactions 88:14–17. https://doi.org/10.1029/2007eo020003

Blin K, Shaw S, Augustijn HE, et al (2023) antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research 51:W46–W50. https://doi.org/10.1093/nar/gkad344

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Borchers HW (2011) pracma: Practical Numerical Math Functions. 2.4.4

Bull AT, Asenjo JA (2013) Microbiology of hyper-arid environments: recent insights from the Atacama Desert, Chile. Antonie van Leeuwenhoek 103:1173–1179. https://doi.org/10.1007/s10482-013-9911-7

Charlier RH (1969) The Geographic Distribution of Polar Desert Soils in the Northern Hemisphere. Geol Soc America Bull 80:1985. https://doi.org/10.1130/0016-7606(1969)80[1985:TGDOPD]2.0.CO;2

Chauhan A, Pathak A, Jaswal R, et al (2018) Physiological and Comparative Genomic Analysis of Arthrobacter sp. SRS-W-1-2016 Provides Insights on Niche Adaptation for Survival in Uraniferous Soils. Genes 9:31. https://doi.org/10.3390/genes9010031

Chen Y-H, Lu C-W, Shyu Y-T, Lin S-S (2017) Revealing the Saline Adaptation Strategies of the Halophilic Bacterium Halomonas beimenensis through High-throughput Omics and Transposon Mutagenesis Approaches. Sci Rep 7:13037. https://doi.org/10.1038/s41598-017-13450-9

Chen Y-H, Shyu Y-T, Lin S-S (2018) Characterization of candidate genes involved in halotolerance using high-throughput omics in the halotolerant bacterium Virgibacillus chiguensis. PLOS ONE 13:e0201346. https://doi.org/10.1371/journal.pone.0201346

Dai D, Lu H, Xing P, Wu Q (2022) Comparative Genomic Analyses of the Genus Nesterenkonia Unravels the Genomic Adaptation to Polar Extreme Environments. Microorganisms 10:233. https://doi.org/10.3390/microorganisms10020233

Dsouza M, Taylor MW, Turner SJ, Aislabie J (2015) Genomic and phenotypic insights into the ecology of Arthrobacter from Antarctic soils. BMC Genomics 16:36. https://doi.org/10.1186/s12864-015-1220-2

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

Ellis HR (2011) Mechanism for sulfur acquisition by the alkanesulfonate monooxygenase system. Bioorganic Chemistry 39:178–184. https://doi.org/10.1016/j.bioorg.2011.08.001

Elser JJ, Bracken MES, Cleland EE, et al (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Fernandez-Guerra A, Borrel G, Delmont TO, et al (2023) A 2-million-year-old microbial and viral communities from the Kap København Formation in North Greenland

Finore I, Orlando P, Di Donato P, et al (2016) Nesterenkonia aurantiaca sp. nov., an alkaliphilic actinobacterium isolated from Antarctica. International Journal of Systematic and Evolutionary Microbiology 66:1554–1560. https://doi.org/10.1099/ijsem.0.000917

Flores-Gallegos AC, Delgado-García M, Ascacio-Valdés JA, et al (2019) Chapter 13 - Hydrolases of Halophilic Origin With Importance for the Food Industry. In: Kuddus M (ed) Enzymes in Food Biotechnology. Academic Press, pp 197–219

Francois R (1987) A study of sulphur enrichment in the humic fraction of marine sediments during early diagenesis. Geochimica et Cosmochimica Acta 51:17–27. https://doi.org/10.1016/0016-7037(87)90003-2

Funder S, Bennike E, Mogensen GS, et al (1984) The Kap København Formation, a late Cainozoic sedimentary sequence in North Greenland. Rapport Grønlands Geologiske Undersøgelse 120:9–18. https://doi.org/10.34194/rapggu.v120.7851

Gomathinayagam S, Muthukaliannan GK (2024) Profiling Antibiotic Resistance Determinants in Ancient Permafrost Microbiomes. 2024.05.20.595077

Guida G, Nicosia A, Settanni L, Ferro V (2023) A review on effects of biological soil crusts on hydrological processes. Earth-Science Reviews 243:104516. https://doi.org/10.1016/j.earscirev.2023.104516

Gushgari-Doyle S, Lui LM, Nielsen TN, et al (2022) Genotype to ecotype in niche environments: adaptation of Arthrobacter to carbon availability and environmental conditions. ISME Communications 2:32. https://doi.org/10.1038/s43705-022-00113-8

He T, Xie D, Ni J, et al (2020) Characteristics of nitrogen transformation and intracellular nitrite accumulation by the hypothermia bacterium *Arthrobacter arilaitensis*. Science of The Total Environment 701:134730. https://doi.org/10.1016/j.scitotenv.2019.134730

Hinsa-Leasure SM, Bhavaraju L, Rodrigues JLM, et al (2010) Characterization of a bacterial community from a Northeast Siberian seacoast permafrost sample. FEMS Microbiology Ecology 74:103–113. https://doi.org/10.1111/j.1574-6941.2010.00945.x

Hoang DT, Chernomor O, von Haeseler A, et al (2018) UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35:518–522. https://doi.org/10.1093/molbev/msx281

Horn G, Hofweber R, Kremer W, Kalbitzer HR (2007) Structure and function of bacterial cold shock proteins. Cell Mol Life Sci 64:1457. https://doi.org/10.1007/s00018-007-6388-4

Hovila J, Arola A, Tamminen J (2009) OMI/Aura Surface UVB Irradiance and Erythemal Dose Daily L3 Global 1.0x1.0 deg Grid

Iwahana G, Cooper ZS, Carpenter SD, et al (2021) Intra-ice and intra-sediment cryopeg brine occurrence in permafrost near Utqiaġvik (Barrow). Permafrost and Periglacial Processes 32:427–446. https://doi.org/10.1002/ppp.2101

Jaarsma AH, Zervas A, Sipes K, et al (2023) The undiscovered biosynthetic potential of the Greenland Ice Sheet microbiome. Front Microbiol 14:. https://doi.org/10.3389/fmicb.2023.1285791

Jansson JK, Taş N (2014) The microbial ecology of permafrost. Nat Rev Microbiol 12:414–425. https://doi.org/10.1038/nrmicro3262

Kalyaanamoorthy S, Minh BQ, Wong TKF, et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285

Kanehisa M, Sato Y, Morishima K (2016) BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. Journal of Molecular Biology 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006

Khan A, Rao TS (2023) Chapter 13 - Natural biofilms: Structure, development, and habitats. In: Das S, Kungwani NA (eds) Understanding Microbial Biofilms. Academic Press, pp 187–206

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Lebre PH, De Maayer P, Cowan DA (2017) Xerotolerant bacteria: surviving through a dry spell. Nat Rev Microbiol 15:285–296. https://doi.org/10.1038/nrmicro.2017.16

Liu Y, Zhang Y, Huang Y, et al (2023) Spatial and temporal conversion of nitrogen using Arthrobacter sp. 24S4–2, a strain obtained from Antarctica. Front Microbiol 14:. https://doi.org/10.3389/fmicb.2023.1040201

Manni M, Berkeley MR, Seppey M, et al (2021) BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Molecular Biology and Evolution 38:4647–4654. https://doi.org/10.1093/molbev/msab199

Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M (2022) TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Research 50:D801–D807. https://doi.org/10.1093/nar/gkab902

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3

Meleshko D, Mohimani H, Tracanna V, et al (2019) BiosyntheticSPAdes: reconstructing biosynthetic gene clusters from assembly graphs. Genome Res 29:1352–1362. https://doi.org/10.1101/gr.243477.118

Meteostat Kap Morris Jesup | Weather History & Climate. In: Meteostat. https://meteostat.net/en/station/04301?t=2020-01-01/2023-12-31. Accessed 26 Sep 2024

Mikheenko A, Prjibelski A, Saveliev V, et al (2018) Versatile genome assembly evaluation with QUAST-LG. Bioinformatics 34:i142–i150. https://doi.org/10.1093/bioinformatics/bty266

Mindlin SZ, Petrova MA (2017) On the Origin and Distribution of Antibiotic Resistance: Permafrost Bacteria Studies. Mol Genet Microbiol Virol 32:169–179. https://doi.org/10.3103/S0891416817040048

Minh BQ, Schmidt HA, Chernomor O, et al (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37:1530–1534. https://doi.org/10.1093/molbev/msaa015

Mølgaard P (1982) Temperature Observations in High Arctic Plants in Relation to Microclimate in the Vegetation of Peary Land, North Greenland. Arctic and Alpine Research 14:105–115. https://doi.org/10.1080/00040851.1982.12004287

Mullis MM, Rambo IM, Baker BJ, Reese BK (2019) Diversity, Ecology, and Prevalence of Antimicrobials in Nature. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.02518

Nasfi Z, Busch H, Kehraus S, et al (2018) Soil Bacteria Isolated From Tunisian Arid Areas Show Promising Antimicrobial Activities Against Gram-Negatives. Front Microbiol 9:. https://doi.org/10.3389/fmicb.2018.02742

Nicholson WL, Krivushin K, Gilichinsky D, Schuerger AC (2013) Growth of Carnobacterium spp. from permafrost under low pressure, temperature, and anoxic atmosphere has implications for Earth microbes on Mars. PNAS 110:666–671. https://doi.org/10.1073/pnas.1209793110

Niederberger TD, Steven B, Charvet S, et al (2009) Virgibacillus arcticus sp. nov., a moderately halophilic, endospore-forming bacterium from permafrost in the Canadian high Arctic. International Journal of Systematic and Evolutionary Microbiology 59:2219–2225. https://doi.org/10.1099/ijs.0.002931-0

Ollivier B, Caumette P, Garcia JL, Mah RA (1994) Anaerobic bacteria from hypersaline environments. Microbiological Reviews 58:27. https://doi.org/10.1128/mr.58.1.27-38.1994

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 25:1043–1055. https://doi.org/10.1101/gr.186072.114

Parvizpour S, Hussin N, Shamsir MS, Razmara J (2021) Psychrophilic enzymes: structural adaptation, pharmaceutical and industrial applications. Appl Microbiol Biotechnol 105:899–907. https://doi.org/10.1007/s00253-020-11074-0

Pathania S, Solanki P, Putatunda C, et al (2022) Adaptation to Cold Environment: The Survival Strategy of Psychrophiles. In: Goel R, Soni R, Suyal DC, Khan M (eds) Survival Strategies in Cold-adapted Microorganisms. Springer, Singapore, pp 87–111

Petzoldt T (2016) growthrates: Estimate Growth Rates from Experimental Data. 0.8.4

Phillips MA, León P, Boronat A, Rodríguez-Concepción M (2008) The plastidial MEP pathway: unified nomenclature and resources. Trends in Plant Science 13:619–623. https://doi.org/10.1016/j.tplants.2008.09.003

Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes De Novo Assembler. Current Protocols in Bioinformatics 70:e102. https://doi.org/10.1002/cpbi.102

Ramond J-B, Jordaan K, Díez B, et al (2022) Microbial Biogeochemical Cycling of Nitrogen in Arid Ecosystems. Microbiol Mol Biol Rev 86:. https://doi.org/10.1128/mmbr.00109-21

Ruginescu R, Purcărea C, Dorador C, et al (2019) Exploring the hydrolytic potential of cultured halophilic bacteria isolated from the Atacama Desert. FEMS Microbiology Letters 366:fnz224. https://doi.org/10.1093/femsle/fnz224

Saadoun I, Wahiby L, Ababneh Q, et al (2008) Recovery of soil streptomycetes from arid habitats in Jordan and their potential to inhibit multi-drug resistant Pseudomonas aeruginosa pathogens. World J Microbiol Biotechnol 24:157–162. https://doi.org/10.1007/s11274-007-9451-2

Sarma J, Sengupta A, Laskar MK, et al (2023) Microbial adaptations in extreme environmental conditions. In: Bacterial Survival in the Hostile Environment. Elsevier, pp 193–206

Sayers EW, Bolton EE, Brister JR, et al (2022) Database resources of the national center for biotechnology information. Nucleic Acids Res 50:D20–D26. https://doi.org/10.1093/nar/gkab1112

Schlatter DC, Kinkel LL (2015) Do tradeoffs structure antibiotic inhibition, resistance, and resource use among soil-borne Streptomyces? BMC Evolutionary Biology 15:186. https://doi.org/10.1186/s12862-015-0470-6

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

Shcherbakova VA, Chuvil'skaya NA, Rivkina EM, et al (2009) Novel halotolerant bacterium from cryopeg in permafrost: Description of Psychrobacter muriicola sp. nov. Microbiology 78:84–91. https://doi.org/10.1134/S0026261709010111

Shen L, Liu Y, Allen MA, et al (2021) Linking genomic and physiological characteristics of psychrophilic Arthrobacter to metagenomic data to explain global environmental distribution. Microbiome 9:136. https://doi.org/10.1186/s40168-021-01084-z

Singh P, Kapse N, Gowdaman V, et al (2021) Comparative Genomic Analysis of Arctic Permafrost Bacterium Nesterenkonia sp. PF2B19 to Gain Insights into Its Cold Adaptation Tactic and Diverse Biotechnological Potential. Sustainability 13:4590. https://doi.org/10.3390/su13084590

Singh P, Singh SM, Singh RN, et al (2017) Bacterial communities in ancient permafrost profiles of Svalbard, Arctic. Journal of Basic Microbiology 57:1018–1036. https://doi.org/10.1002/jobm.201700061

Sivalingam P, Hong K, Pote J, Prabakar K (2019) Extreme Environment *Streptomyces* : Potential Sources for New Antibacterial and Anticancer Drug Leads? International Journal of Microbiology 2019:1–20. https://doi.org/10.1155/2019/5283948

Son J, Lee H, Kim M, et al (2021) Massilia aromaticivorans sp. nov., a BTEX Degrading Bacterium Isolated from Arctic Soil. Curr Microbiol 78:2143–2150. https://doi.org/10.1007/s00284-021-02379-y

Spirina EV, Durdenko EV, Demidov NE, et al (2017) Halophilic-psychrotrophic bacteria of an Alaskan cryopeg—a model for astrobiology. Paleontol J 51:1440–1452. https://doi.org/10.1134/S0031030117120036

Steven B, Léveillé R, Pollard WH, Whyte LG (2006) Microbial ecology and biodiversity in permafrost. Extremophiles 10:259–267. https://doi.org/10.1007/s00792-006-0506-3

Stevenson A, Cray JA, Williams JP, et al (2015) Is there a common water-activity limit for the three domains of life? ISME J 9:1333–1351. https://doi.org/10.1038/ismej.2014.219

Stimmler P, Priemé A, Elberling B, et al (2022) Arctic soil respiration and microbial community structure driven by silicon and calcium. Science of The Total Environment 838:156152. https://doi.org/10.1016/j.scitotenv.2022.156152

Tamm CO (1991) Nitrogen-Limited and Nitrogen-Depleted Terrestrial Ecosystems: Ecological Characteristics. In: Nitrogen in Terrestrial Ecosystems. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 34–49

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38:3022–3027. https://doi.org/10.1093/molbev/msab120

van Dorst J, Benaud N, Ferrari B (2017) New Insights into the Microbial Diversity of Polar Desert Soils: A Biotechnological Perspective. In: Chénard C, Lauro FM (eds) Microbial Ecology of Extreme Environments. Springer International Publishing, Cham, pp 169–183

Ventosa A, Quesada E, Rodriguez-Valera F, et al (1982) Numerical Taxonomy of Moderately Halophilic Gram-negative Rods. Microbiology 128:1959–1968. https://doi.org/10.1099/00221287-128-9-1959

Vishnivetskaya T, Kathariou S, McGrath J, et al (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4:165–173. https://doi.org/10.1007/s007920070031

Wang H, Zhang X, Wang S, et al (2018) Massilia violaceinigra sp. nov., a novel purple-pigmented bacterium isolated from glacier permafrost. International Journal of Systematic and Evolutionary Microbiology 68:2271–2278. https://doi.org/10.1099/ijsem.0.002826

Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595

Wu Z, Kan FWK, She Y-M, Walker VK (2012) Biofilm, ice recrystallization inhibition and freeze-thaw protection in an epiphyte community. Appl Biochem Microbiol 48:363–370. https://doi.org/10.1134/S0003683812040138

Wutipraditkul N, Waditee R, Incharoensakdi A, et al (2005) Halotolerant Cyanobacterium Aphanothece halophytica Contains NapA-Type Na+/H+ Antiporters with Novel Ion Specificity That Are Involved in Salt Tolerance at Alkaline pH. Applied and Environmental Microbiology 71:4176–4184. https://doi.org/10.1128/AEM.71.8.4176-4184.2005