Antibiotic Resistance Genes and Taxa Analysis from Mat and Planktonic Microbiomes of Antarctic Lake Fryxell and Lake Bonney, and a Mat-forming Culture of *Rhodoferax antarcticus*

By Sheetal Tallada,[1†] Grant Hall,[1†] Daniel Barich,[1] and Joan L. Slonczewski[1*]

Address:

[1]Department of Biology, Kenyon College, Gambier, Ohio, 43022, USA

[†]These two authors contributed equally.

*Corresponding Author:

Joan L. Slonczewski, slonczewski@kenyon.edu

ABSTRACT

The Antarctic Taylor Valley Lakes Fryxell and Bonney harbor oligotrophic microbial communities that are separated geographically from other aquatic systems. Their microbiomes include planktonic as well as lift-off mat communities that float to the underside of the perennial ice cover and eventually emerge at the surface. We investigated the antibiotic resistance genes (ARGs) from metagenomes of lift-off mats emerging from ice, from filtered water samples of Lake Fryxell, and from filtered water samples of Lake Bonney. ARG sequence markers were designed by ShortBRED-Identify using the Comprehensive Antibiotic Resistance Database (CARD). The overall proportion of ARG hits in the metagenomes was found to be similar to that found in temperate-zone rural water bodies with moderate human inputs (0.0002-0.0007%). The specific ARGs found showed distinct distributions for the two lakes, and for mat versus planktonic sources. Metagenomic taxa distributions showed that mat phototrophs were mainly cyanobacteria or betaproteobacteria, whereas the water column phototrophs were mainly protists. An enrichment culture of the betaproteobacterium *Rhodoferax antarcticus* from a Lake Fryxell mat sample showed a mat-forming phenotype not previously reported for this species. Its genome showed no ARGs associated with betaproteobacteria, but had ARGs consistent with a minor *Pseudomonas* component. The Antarctic lake mats and water showed specific ARGs distinctive to the mat and water sources, but overall ARG levels were similar to those of temperate water bodies with minimal human inputs.

INTRODUCTION

One of Earth's coldest dry deserts, the Antarctic Taylor Valley possesses a string of stratified lakes with unique geochemistry and microbial communities (1–4). The microbial genomic analysis of these lakes remains limited (5–7). There are a number of reports on antibiotic resistance genes (ARGs) in Antarctic soil or marine bacteria (8–11) but few studies of ARGs in Antarctic lake water or their mat communities (12). The prevalence of ARGs in relatively pristine habitats is of compelling importance for human health and environment, as it provides a baseline for assessment of human-associated ARG contamination (13, 14). Antimicrobial resistance is a leading cause of death worldwide (15).

We report on the taxonomic composition and ARG prevalence in the water of Lake Fryxell and Lake Bonney, and in the phototrophic mat communities of Lake Fryxell (**Fig. 1A**). Lake Fryxell extends along the Taylor Valley, 10 km downstream of Lake Bonney, 80 km across the Ross Sea from McMurdo Station. No permanent stream connects the two lakes; their main hydrology involves glacial meltwater and an underground brine aquifer (16). Their water ecosystems are entirely microscopic, with no fauna larger than nematodes. The lakes and their ecosystems are studied as models for ancient Mars (17) and as indicators of climate change (18).

Lakes Fryxell and Bonney have permanent stratified layers in the water column that give rise to oligotrophic microbial communities (2, 19, 20). The lack of turnover allows for the growth of benthic microbial mats that form flame-shaped towers several centimeters tall (21, 22). The microbial mats have been studied with primary emphasis on the role of cyanobacteria such as *Microcoleus* and *Oscillatoria* (21, 23) although metagenomes reveal a high abundance of proteobacteria (5) and protists (19, 24). Known as "lift-off" mats, the benthic biofilms reach upward, buoyed by oxygen bubbles, until pieces break off and float upward to the undersurface

of the ice cover (25, 26). During winter, water freezes beneath the mat fragments, adding to the ice layer, but above the lake the valley's dry winds ablate the ice. Ice sections reveal 6-8 annual layers of freezing below followed by ablation above (25) leading to exposure of the dessicated mats (**Fig. 1B**). Surviving several years trapped in ice, the mat material remains viable, and psychrophiles may continue growing (27). When mat organisms reach the surface of ablated ice, they can blow off and enter "moats" of melted ice that occur in summer, surrounding the permanent ice in the main part of the lake. The mat material carries communities that include microscopic arthropods and nematodes; thus, the mats can act as vectors for transport of entire ecosystems (5, 25, 28).

Molecular genetic data on Dry Valley microbes remained limited until recently (20, 23, 29). In December, 2014, the austral summer, we obtained water samples from Fryxell and Bonney as well as desiccated mat samples emerging from the ice cover of Fryxell. For the present study, we applied tools of metagenome analysis to compare the taxonomic diversity and ARG abundance of mat and lake samples. We also sequenced the genome of a novel form of *Rhodoferax antarcticus* from a mat-forming enrichment culture, whose phenotype differs markedly from planktonic isolates of this species (30–32).

Our study addressed the important question of ARG prevalence in relatively pristine Antarctic lakes. A modest level of antibiotic resistance is an ancient, widespread phenomenon naturally and historically occurring in all environments (13, 14). Non-anthropogenic processes can select for ARGs in pristine habitats; for example, cyanobacterial blooms drive increases in bacterial ARG prevalence and diversity (33). But inputs of human origin can add substantially to the native ARG pool as well as add additional types of ARGs (8, 10, 12). We surveyed our mat

and water metagenomes for the presence of ARGs, referenced to the Comprehensive Antibiotic Resistance Database (CARD) (34).

RESULTS

Taxa abundance of mat and water samples.

We characterized the taxa abundance from three microbial sources: the water columns of Lake Fryxell (FRY-01, 02, 03) and Lake Bonney (BON-01, 02, 03), and the lift-off mat from the ice surface of Lake Fryxell (Mat 01 – 06) (**Fig. 2A**). Metagenomic DNA was sequenced by Illumina MiSeq. The 150-kb sequence reads were assigned to taxonomic classes using the Kraken 2 classifier pipeline (35, 36) with taxa percentages calculated via the Bracken abundance estimator (37) (described below under Methods).

The ice-surface mat samples (Mat-01 through Mat-06) showed substantial amounts of cyanobacteria, ranging from 9-60% of the total (**Fig. 2A**). The predominant orders of cyanobacteria were Oscillatoriales and Nostocales (**Fig. 2B**). High abundance of Oscillatoriales and Nostocales is consistent with previous reports for Fryxell mats obtained from benthic samples (5). For Mat-01, 02, 03, 06 the cyanobacterial assignments were primarily Oscillatoriales and Nostocales. Mat-04 and Mat-05 however showed depletion of Oscillatoriales, with predominant abundance of Betaproteobacteria. Throughout the six mat samples, other taxa with significant abundance included Actinobacteria, Bacteroidetes, and Alphaproteobacteria, with smaller abundance of Planctomycetes, Bacteroidetes and Firmicutes. Thus the ice-trapped, air-exposed community showed a composition remarkably similar to that of benthic mat samples (5).

The filtered water from both lakes Fryxell and Bonney contained abundant bacterial taxa of Actinobacteria, Bacteroides, Betaproteobacteria, and Alphaproteobacteria, similar to previous reports of Fryxell water (20) and other glacier-associated water bodies (27). The water column showed almost no cyanobacteria but a high proportion of eukaryotes, in some cases more than 50% of the reads classified by Kraken2/Bracken. By contrast, the mat samples showed virtually no detectable eukaryotic DNA. The one-sided Mann-Whitney U test confirms that water from each lake contains more eukaryotic DNA than the mat samples ($p = 0.01$) and that the mat samples contain more cyanobacteria than either lake ($p = 0.01$).

To classify the eukaryotes, we used EukDetect (38) a pipeline that matches short read data to marker genes from fungal, protist and invertebrate genomes (**Fig. 3**; full output provided in **Table S1**). EukDetect identified the protist species *Geminigera cryophila* (39), *Mesodinium rubrum* (40), *Nannochloropsis limnetica* (41), and *Chlamydomonas* sp. ICE-L (42). The two lakes showed similar taxa profiles, except that *Chlamydomonas* was found only in Lake Bonney. Mat samples had too few eukaryotic read counts to classify.

The validity of taxa classifier pipelines is highly dependent on their algorithm and taxa database. For comparison with Kraken2/Braken output, our mat and water reads were mapped to core taxonomic markers using an alternative pipeline, MetaPhlAn2 (43, 44) (**Fig. 4**). Four of the six mat samples showed mainly cyanobacteria, consistent with longstanding reports of cyanobacterial mats (21–23). Consistent with the Kraken analysis, Mat-04 and Mat-05 samples showed major amounts of Betaproteobacteria and Alphaproteobacteria, with depletion of cyanobacteria. For the filtered water samples, MetaPhlAn2 markers include limited content of Eukarya, and the pipeline did not find the eukaryotic taxa predicted by Kraken2 (**Fig. 2**). The

filtered water from both lakes showed mainly Actinobacteria, with some Betaproteobacteria. Overall MetaPhlAn2 appeared less effective than Kraken 2/Bracken at classifying our samples.

ARG composition characterized using ShortBRED.

Gene sequences encoding various forms of antibiotic resistance, including genomic loci as well as mobile elements, are collected in the Comprehensive Antibiotic Resistance Database (CARD) (34). We used the ShortBRED pipeline to identify ARGs in our samples, based on a set of marker peptides matching CARD sequences. The percentage of reads from each sample that matched ARGs ranged from 0.0001-0.0006% (**Fig. 5**). The overall ARG abundances were similar amongst the three sample classes (Fryxell mat, Fryxell water, Bonney water).

The specific ARGs identified by ShortBRED were sorted by abundance across samples (**Table 1;** Supplementary file **Table S2**). Eight of the top 20 sorted ARGs showed a difference among the three sample classes (Kruskal-Wallis test, $P \leq 0.05$); of these results, 7 of 8 are significant. Of the top-ranked ARGs, BUT-1, and vancomycin-resistance genes *vanYA*, *vanTG* and *vanI* appeared mainly in the water column. BUT-1 is class-C beta-lactamase reported in *Buttiauxella agrestis*, an environmental Gammaproteobacterium found in environmental water sources (45, 46). The vancomycin resistance genes occur in *Enterococcus* and other Gram-positives (47). By contrast, the beta-lactamase AAC(3)-Ia (48) occurred only in the mat samples. AAC(3)-Ia is encoded on *Pseudomonas aeruginosa* integrons as well as in other Proteobacteria. Other ARGs showed possible differences in prevalence between Lake Bonney and Lake Fryxell water; for example, the Streptomyces-associated beta-lactamase AAC(3)-VIIa (49) was more abundant in Bonney water whereas *vanYA* was more abundant in Fryxell water.

Overall, the three sample classes each had distinctive ARG-abundance signatures. Ten of the top 20 ranked ARGs were associated with Proteobacteria, which roughly corresponds with the proportion of bacterial taxa abundances predicted by Kraken2/Bracken (**Fig. 2**).

Enrichment of Mat-forming *Rhodoferax antarcticus*

The relatively high proportions of Betaproteobacteria and Alphaproteobacteria in MAT-04 differed from that of other samples, and the color of the sample material was more red than green. We cultured the various samples in Harwood PM mineral salts medium with 10 mM succinate (50, 51), anaerobically (in closed Pyrex tubes) at 10°C with illumination (approximately 10% PAR). From MAT-04 red spots of biofilm were obtained. Approximately 0.05g portions were twice subcultured for two weeks. The organism grew entirely as a mat, forming large red blobs with a "red nose" appearance. In addition the culture extended as a red film-like mat growing along the inside of the culture tube (**Fig. 6A**). There was no evidence of planktonic growth (cloudiness) or cell motility within the culture medium. It was not possible to obtain isolated colonies, but serial culture of the "red noses" showed consistent biofilm formation and Gram-stain morphology (**Fig. 6B**). This finding is consistent with an earlier report that the Fryxell sedimentary biofilm includes a layer of phototrophic purple bacteria beneath the cyanobacterial layer (52). Ours is the first report of such biofilm cultured from material at the ice surface.

The MAT-04 biofilm culture was subjected to genome analysis. DNA was sequenced and short reads were used to BLAST the NCBI database. The predominant hits were to the genome of *Rhodoferax antarcticus* ANT.BRT, a Betaproteobacterium originally isolated from a saltwater pond at Cape Royds, Ross Island, Antarctica (30, 31). Kraken 2/Bracken analysis of sequenced

reads agreed with this classification, assigning the samples as 95% *R. antarcticus* with about 1% *Pseudomonas.*

We used the *breseq* pipeline (53) to assemble reads from two samples (7-RN1 and 8-RN2) whose DNA sequence reads were assembled to the *R. antarcticus* ANT.BRT reference genome (Supplementary file, **Table S3** and **S4**). These tables present "mutations," that is, all differences from the ANT.BR reference sequence that show 100% read coverage. The two samples, 7-RN1 and 8-RN2, showed 490 and 473 sequence differences respectively, out of the reference genome length 4,007,881. A large part of the observed sequence differences from reference were silent mutations, the same ones in both samples. These observations confirm the close relatedness of our culture to the published strain.

The *R. antarcticus* enrichment culture (designated *R. antarcticus* JLS) was tested for ARG abundance using ShortBRED (**Table 2**). More than thirty ARGs showed hits, but nearly all were associated with *Pseudomonas aeruginosa*. By contrast, no ARGs matched those from CARD database that were associated with *Rhodoferax.* This finding suggests that a wide range of naturally-occurring ARGs remain to be discovered in polar strains.

Characterization of the mat-forming *R. antarcticus* JLS

Despite the high sequence identity, the *R. antarcticus* JLS enrichment culture differed from the original reported *R. antarcticus* ANT.BR (30) in important respects. Unlike the highly motile *R. antarcticus* ANT.BR, our culture showed no motility and little sign of planktonic single-celled growth. Instead, the subcultured material grew entirely as a biofilm, in globular spots and as a film-like growth along the interior surface of the glass tube.

The *R. antarcticus* JLS biofilm was further tested for growth range of pH and salinity. The biofilm was subcultured anaerobically with illumination, in a malate-succinate *Rhodoferax* medium modified from that of references (30, 54) as described under Methods. The pH was maintained by inclusion of 100 mM of sulfonate buffers with pKa appropriate for each pH value: pH 6, pH 7, and pH 8, all adjusted with KOH (**Fig. 7A, B, C**). After 45 days, at pH 7, the bacteria formed globules as well as a red coating along the glass. At pH 6, spots of biofilm grew slowly, and at pH 8 little growth was seen. Growth was also tested for cultures buffered at pH 7 with NaCl amendment (**Fig. 7D, E, F**). The fullest growth was seen in the absence of NaCl amendment (the core medium contains approximately 10 mM Na^+ ions). Less growth was seen with added NaCl (23 mM or 46 mM).

DISCUSSION

Identification of antibiotic resistance genes from relatively pristine environments is of interest for several reasons. Long before human introduction of high-dose antibiotics, environmental bacteria evolved multidrug pumps to efflux toxic products of their own metabolism, as well as antimicrobial substances produced by their competitors (13, 55). Many antibiotics possess signalling capabilities and other unknown functions. Phylogeny dates the origin of beta-lactamases to hundreds of millions of years ago. Vancomycin resistance genes are found in 30,000-year-old permafrost (56). But the specific kinds of ARGs found in environmental sources may differ from those prevalent in human microbiomes, those conferring resistance to the drugs we depend on for therapy (57).

In polar regions, previous metagenomic studies reveal a range of naturally occurring ARGs. Surface soils of the Antarctic Mackay Glacier region show ARGs encoding multidrug pumps, beta-lactamases and aminoglycoside inactivators, largely associated with Gram-negative bacteria (58). A study of Tibetan soils showed a number of abundant ARGs, most notably encoding vancomycin resistance (59). Our study of ARGs from Antarctic lakes adds to this picture, showing that both water and microbial mat sources contain familiar ARGs, and likely contain others not yet discovered in Antarctic strains.

It was interesting to compare the total ARG abundance of our Antarctic lake samples with those of temperate-zone Ohio rural water bodies with moderate human inputs, in a study where samples were prepared by the same method and analyzed using the same ShortBRED marker set (60). The overall ARG abundance is about the same for the two environmental sources, except for river samples obtained just downstream of a wastewater effluent pipe, where the total ARG prevalence is increased approximately five-fold. This result is consistent with a

model that natural microbial communities generally harbor a small balanced prevalence of ARGs, which gets amplified by concentrated human input. Note however that a database such as CARD can only represent a fraction of the actual ARGs out there; new drug resistance families and related mobility agents are continually discovered.

The mat lift-off samples we obtained from the ice surface showed a range of DNA taxa consistent with those reported for samples obtained from benthic mats (5). Given that our collected mat organisms had survived several years within ice, followed by air dessication and prolonged UV radiation exposure, it is impressive how much of the Antarctic mat microbiomes retain viability with intact DNA. Even ciliated protists and invertebrate worms were obtained alive from samples cultured after months of storage at -80°C (not shown). It is likely that some of these organisms are psychrophiles that continue to grow within the ice (27).

Previous studies emphasize the cyanobacterial content of lift-off mats, primarily Oscillatoriales genera such as *Microcoleus*, as well as *Nostoc* (21, 23). While most of our lift-off samples showed abundance of cyanobacteria, one sample yielded cultures from which 80% of reads matched *R. antarcticus* using the *breseq* pipeline The finding of mat samples enriched for *R. antarcticus* indicates that portions of the lower layer of *Rhodoferax* mat (52), along with the cyanobacterial upper layer, can break off and form lift-off patches that emerge from the ice. Furthermore, our *R. antarcticus* JLS obtained from Lake Fryxell showed mat-forming morphology very different from the motile single cells of *R. antarcticus* ANT.BR isolated from Cape Royds (30, 31).

The prevalence of eukaryotic sequences in the lake water metagenomes is consistent with previous reports that protists play important roles in the Taylor Valley lake communities (19, 24, 42, 61). Microbial eukaryotes including phototrophs and mixotrophs play important roles in the

lake ecology (19). In our data, the eukaryotic community of Lakes Fryxell and Bonney showed three major taxa in common: *Gemingera cryophila*, *Mesodinium rubrum*, and *Nannochloropsis limnetica*. *G. cryophila* is a mixotrophic cryptophyte that feeds on bacteria but also conducts photosynthesis as a secondary endosymbiont alga (39). *M. rubrum* is a ciliate that consumes cryptophytes but also uses the prey chloroplasts to conduct photosynthesis (kleptoplasty) (40). *N. limnetica* is a heterokont alga, with red alga-derived chloroplasts, primarily a phototroph (41). Our Lake Bonney samples also showed sequences from *Chlamydomonas*, a green alga that dominates some parts of the Lake Bonney water column (42).

We note that in the water samples, smaller aquatic phototrophs were likely missed by the 0.45-µm filter; 0.2-µm filters would have been preferable but were not available in the field at the time. Even 0.2-µm filters miss important microbial community members (62). The mat samples however had no filtration, so a broader spectrum of cell sizes was captured.

It is interesting that the Lake Fryxell water shows mainly eukaryotic phototrophs whereas the mat shows mainly cyanobacteria and proteobacterial phototrophs. The mat bacteria are likely to survive a wider range of light and temperature conditions than the eukaryotes. From the standpoint of drug resistance, cyanobacteria are more likely than eukaryotes to harbor and transfer ARGs of potential bacterial pathogens. Nevertheless, protists can regulate bacterial ARG composition in terrestrial communities (63), so this factor may be of interest when assessing the lake water ARG pools.

METHODS

Sample collection and culture.

Microbial communities were sampled in December, 2014 from two meromictic lakes of the Taylor Valley, Victoria Land, Antarctica. Lake Fryxell has a maximum depth of 20 m (20, 64), while Lake Bonney has a depth of 40 m (65). Both lakes are covered by a perpetual ice layer, approximately 5 m thick, although summer melting occurs near the shoreline.

Microbial lift-off mat samples were collected from independent mat tufts emerging separately from the Lake Fryxell ice surface, within the GPS area of: 77.60491, 163.16315; 77.60473, 163.16290; 77.60463, 163.16405; 77.60495, 163.16495. Each sample consisted of a separate tuft of dessicated microbial mat, collected with alcohol-sterilized forceps and stored at -20°C (4 weeks) then at -80°C (indefinitely).

Lake water was sampled from the permanent chemoclines of Lake Fryxell (77.605 S, 163.163 E, 9 m depth) and Lake Bonney, east lobe (77.719 S, 162.283 E, 15 m depth). Water samples were collected in 1-liter cubitainers pre-washed in 10% HCl. Each water sample was concentrated by filtration through 0.45 µm filter.

Enrichment culture was performed using Harwood PM medium supplemented with 10 mM succinate (50, 66). Screwcap pyrex tubes were filled with medium and inoculated with approximately 0.05 g dessicated material from sample Mat-04. Tubes were incubated under approximately 10% PAR at 10°C for five weeks. Portions of biofilm were serially subcultured for two-week periods, then frozen at -80°C.

Testing for growth range of pH and NaCl amendment was performed using a *Rhodoferax* medium modified from references (30, 54). The medium contained: yeast extract (0.5 g/l), EDTA (20 mg/l), malic acid (4 g/l), $(NH_4)_2SO_4$ (1 g/l), $MgSO_4 \cdot 7H_2O$ (200 mg/l), $FeSO_4 \cdot 7\ H_2O$ (12 mg/l), K_2HPO_4 (0.9 g/l), KH_2PO_4 (0.6 g/l), 10 mM sodium succinate, 100 mM MOPS (3-(N-morpholino)propanesulfonic acid) adjusted to pH 7 with KOH. In addition, 1ml/l of a trace

elements solution was added [H_3BO_3 (2.8 g/l), $MnSO_4 \cdot H_2O$ (1.6 g/l), $Na_2MoO_4 \cdot 2H_2O$ (0.76 g/l), $ZnSO_4 \cdot 7 H_2O$ (240 mg/l), $Cu(NO_3)_2 \cdot 3 H_2O$ (40 mg/l), $CoCl_2 \cdot 6 H_2O$ (200 mg/l)]. For pH 6, the MOPS buffer was replaced with 100 mM MES (2-(*N*-morpholino)ethanesulfonic acid) and for pH 8, the buffer was 100 mM TAPS ([tris(hydroxymethyl)methylamino]propanesulfonic acid).

DNA isolation and sequencing.

Mat DNA was extracted by the PowerBiofilm kit (MO BIO). From each sample, approximately 20 mg material was extracted, and 0.75-1.50 µg DNA (measured by Qubit) was sent for sequencing. From lake water filters, 300-400 ng DNA was obtained by extraction using an MP FastDNA SPIN DNA kit (MP Biomedicals, CA) (24). All metagenomic DNA was sequenced at DOE JGI (Joint Genome Institute Community Science Program award 1936). Shotgun metagenomic library construction and sequencing were carried out at JGI using standard protocols on the Illumina HiSeq 2500 platform. The raw reads were quality filtered using JGI standard protocols and 1.2 Tb total sequences were obtained.

Cultured *Rhodoferax* biofilm DNA was isolated by the PowerBiofilm kit (MO BIO). Sequencing was performed by the Michigan State University Genomics Core. Libraries were prepared using the Illumina TruSeq Nano DNA Library Preparation Kit. Sequencing was performed on an Illumina MiSeq done in a 2x250bp format using an Illumina 500 cycle v2 reagent cartridge. The raw reads were quality filtered and 1 Tb total sequences were obtained.

Identification of ARGs by ShortBRED.

ShortBRED (67) is a pipeline that identifies protein family sequences by generating specific peptide markers from a database of interest, then screening the markers for specificity against the UniProt universal database of protein sequences (68). The shortbred_identify command scans a reference database for protein family-specific peptide sequences. It was used to scan UniRef (the UniProt protein reference database) for sequences of the ARGs in version 3.0.7 of the Comprehensive Antibiotic Resistance Database (CARD) (34). The markers were then used by the shortbred_quantify command to assign ARGs in the 150-kb reads from lake water and mat samples.

Trimmomatic.

The Trimmomatic pipeline (69) removes adapters and low-quality sequence from Illumina reads. It counts the number of reads per FASTQ file, and was used to determine the total reads per sample metagenome.

Taxa classification of metagenomic reads.

The 150-kb sequence reads from mat and water metagenomes were assigned to taxonomic classes using the Kraken 2 classifier pipeline (35, 36). Kraken associates genomic *k*-mers (short sequence strings) in the reads with lowest common ancestor taxa. The read classifications were then used to calculate taxa percentages via the Bracken abundance estimator (37). The *k*-mer length was set at 150. For the Kraken 2-Bracken pipeline, the reference database used was the 5/17/2021 Standard Collection accessed at: https://benlangmead.github.io/aws-indexes/k2.

Eukaryotic taxa were further characterized using the pipeline EukDetect (38). This pipeline assigns reads to a database of 521,824 marker genes from 241 gene families, out of 3713 genomes and transcriptomes of fungi, protists and invertebrates.

For an alternative classifier, mat and water metagenomes were mapped to core taxonomic markers using the MetaPhlAn2 pipeline (43, 44). MetaPhlAn2 assigns short reads to taxa using a set of marker genes identified from approximately 17,000 microbial reference genomes, primarily bacteria and archaea.

Genome assembly by *breseq*.

The pipeline *breseq* 0.35.6 (53) was used to assemble reads from two samples of the enrichment culture obtained from Mat-04. The reads were mapped to the *Rhodoferax antarcticus* ANT.BRT reference genome (31).

Acknowledgments and Funding

For metagenome sequencing we thank the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, supported by the Office of Science of the U.S. Department of Energy Contract No. DE-AC02-05CH11231, JGI Metagenome award 1936 to JLS. Bioinformatic analysis and biofilm culture and sequencing were supported by the National Science Foundation award MCB-1923077. We thank Caroline Harwood for guidance regarding culture of the mat biofilm, and Emma Stuart-Bates for the biofilm culture micrograph. We thank Rachael Morgan-Kiss for directing the Taylor Valley field project and collecting the lake water samples.

Author Contributions Statement

ST designed the bioinformatic analysis of ARGs and drafted the manuscript. GH characterized the *Rhodoferax* enrichment culture and contributed to the manuscript. DB ran the bioinformatic pipelines and performed statistics. JLS conceived the central concept, collected the mat samples and isolated DNA, mentored students, and completed the manuscript.

Conflict of Interest Statement

The authors declare that the submitted work was carried out with no personal, professional or financial relationships that could potentially be construed as a conflict of interest.

Contribution to the Field Statement

Our study is the first to report metagenomic analysis of antibiotic resistance genes from the water column and from ice-surface lift-off mats of the Taylor Valley lakes, a major study site for Antarctic microbiology. We are the first to report a mat-forming enrichment culture of *Rhodoferax* species from an Antarctic lift-off mat.

Availability of data and materials

Sequence read files from lake water and mat samples are deposited at NCBI under SRA accession numbers SRP104818, SRP104821, SRP098041, SRP098040, SRP098042, SRP104817, SRP098044, SRP104822, SRP098050, SRP104819, SRP104820, SRP104823. Sequence read files from cultured *Rhodoferax antarcticus* are deposited at NCBI under SRA accession number PRJNA736311.

REFERENCES

1. Spigel RH, Priscu JC. 1998. Physical limnology of the McMurdo Dry Valleys lakes, p. 153–187. *In* Ecosystem Dynamics in a Polar Desert: the McMurdo Dry Valleys, Antartica.

2. Roberts EC, Priscu JC, Wolf C, Lyons WB, Laybourn-Parry J. 2004. The distribution of microplankton in the McMurdo Dry Valley Lakes, Antarctica: response to ecosystem legacy or present-day climatic controls? Polar Biol 27:238–249.

3. Cavicchioli R. 2015. Microbial ecology of Antarctic aquatic systems. Nat Rev Microbiol 13:691–706.

4. Sohm JA, Niederberger TD, Parker AE, Tirindelli J, Gunderson T, Cary SC, Capone DG, Carpenter EJ. 2020. Microbial Mats of the McMurdo Dry Valleys, Antarctica: Oases of Biological Activity in a Very Cold Desert. Front Microbiol 11:1–12.

5. Dillon ML, Hawes I, Jungblut AD, Mackey TJ, Eisen JA, Doran PT, Sumner DY. 2020. Energetic and Environmental Constraints on the Community Structure of Benthic Microbial Mats in Lake Fryxell, Antarctica. FEMS Microbiol Ecol 96:1–10.

6. Koo H, Hakim JA, Morrow CD, Crowley MR, Andersen DT, Bej AK. 2018. Metagenomic analysis of microbial community compositions and cold-responsive stress genes in selected antarctic lacustrine and soil ecosystems. Life 8.

7. Li W, Dore JE, Steigmeyer AJ, Cho YJ, Kim OS, Liu Y, Morgan-Kiss RM, Skidmore ML, Priscu JC. 2020. Methane production in the oxygenated water column of a perennially ice-covered Antarctic lake. Limnol Oceanogr 65:143–156.

8. Antelo V, Giménez M, Azziz G, Valdespino-Castillo P, Falcón LI, Ruberto LAM, Mac Cormack WP, Mazel D, Batista S. 2021. Metagenomic strategies identify diverse integron-integrase and antibiotic resistance genes in the Antarctic environment. Microbiologyopen 10:1–14.

9. Hicks DB, Liu J, Fujisawa M, Krulwich TA. 2010. F_1F_0-ATP synthases of alkaliphilic bacteria: Lessons from their adaptations. Biochim Biophys Acta 1797:1362–1377.

10. Tam HK, Wong CMVL, Yong ST, Blamey J, González M. 2015. Multiple-antibiotic-resistant bacteria from the maritime Antarctic. Polar Biol 38:1129–1141.

11. Wang F, Stedtfeld RD, Kim OS, Chai B, Yang L, Stedtfeld TM, Hong SG, Kim D, Lim HS, Hashsham SA, Tiedje JM, Sul WJ. 2016. Influence of soil characteristics and proximity to antarctic research stations on abundance of antibiotic resistance genes in soils. Environ Sci Technol 50:12621–12629.

12. Jara D, Bello-Toledo H, Domínguez M, Cigarroa C, Fernández P, Vergara L, Quezada-Aguiluz M, Opazo-Capurro A, Lima CA, González-Rocha G. 2020. Antibiotic resistance in bacterial isolates from freshwater samples in Fildes Peninsula, King George Island, Antarctica. Sci Rep 10:3145.

13. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. 2010. Call

of the wild: Antibiotic resistance genes in natural environments. Nat Rev Microbiol 8:251–259.

14. D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. 2011. Antibiotic resistance is ancient. Nature 477:457–461.

15. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC, Browne AJ, Chipeta MG, Fell F, Hackett S, Haines-Woodhouse G, Kashef Hamadani BH, Kumaran EAP, McManigal B, Agarwal R, Akech S, Albertson S, Amuasi J, Andrews J, Aravkin A, Ashley E, Bailey F, Baker S, Basnyat B, Bekker A, Bender R, Bethou A, Bielicki J, Boonkasidecha S, Bukosia J, Carvalheiro C, Castañeda-Orjuela C, Chansamouth V, Chaurasia S, Chiurchiù S, Chowdhury F, Cook AJ, Cooper B, Cressey TR, Criollo-Mora E, Cunningham M, Darboe S, Day NPJ, De Luca M, Dokova K, Dramowski A, Dunachie SJ, Eckmanns T, Eibach D, Emami A, Feasey N, Fisher-Pearson N, Forrest K, Garrett D, Gastmeier P, Giref AZ, Greer RC, Gupta V, Haller S, Haselbeck A, Hay SI, Holm M, Hopkins S, Iregbu KC, Jacobs J, Jarovsky D, Javanmardi F, Khorana M, Kissoon N, Kobeissi E, Kostyanev T, Krapp F, Krumkamp R, Kumar A, Kyu HH, Lim C, Limmathurotsakul D, Loftus MJ, Lunn M, Ma J, Mturi N, Munera-Huertas T, Musicha P, Mussi-Pinhata MM, Nakamura T, Nanavati R, Nangia S, Newton P, Ngoun C, Novotney A, Nwakanma D, Obiero CW, Olivas-Martinez A, Olliaro P, Ooko E, Ortiz-Brizuela E, Peleg AY, Perrone C, Plakkal N, Ponce-de-Leon A, Raad M, Ramdin T, Riddell A, Roberts T, Robotham JV, Roca A, Rudd KE, Russell N, Schnall J, Scott JAG, Shivamallappa M, Sifuentes-Osornio J, Steenkeste N, Stewardson AJ, Stoeva T, Tasak N, Thaiprakong A, Thwaites G, Turner C, Turner P, van Doorn HR, Velaphi S, Vongpradith A, Vu H, Walsh T, Waner S, Wangrangsimakul T, Wozniak T, Zheng P, Sartorius B, Lopez AD, Stergachis A, Moore C, Dolecek C, Naghavi M. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 6736.

16. Mikucki JA, Auken E, Tulaczyk S, Virginia RA, Schamper C, Sørensen KI, Doran PT, Dugan H, Foley N. 2015. Deep groundwater and potential subsurface habitats beneath an Antarctic dry valley. Nat Commun 6:6831.

17. Head JW, Marchant DR. 2014. The climate history of early Mars: insights from the Antarctic McMurdo Dry Valleys hydrologic system. Antarct Sci 26:774–800.

18. Hall CM, Castro MC, Kenig F, Doran PT. 2017. Constraining the recent history of the perennially ice-covered Lake Bonney, East Antarctica using He, Kr and Xe concentrations. Geochim Cosmochim Acta 209:233–253.

19. Li W, Podar M, Morgan-Kiss RM. 2016. Ultrastructural and single-cell-level characterization reveals metabolic versatility in a microbial eukaryote community from an ice-covered Antarctic lake. Appl Environ Microbiol 82:3659–3670.

20. Kwon M, Kim M, Takacs-Vesbach C, Lee J, Hong SG, Kim SJ, Priscu JC, Kim OS. 2017. Niche specialization of bacteria in permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica. Environ Microbiol 19:2258–2271.

21. Jungblut AD, Hawes I, Mackey TJ, Krusor M, Doran PT, Sumner DY, Eisen JA, Hillman

C, Goroncy AK. 2016. Microbial mat communities along an oxygen gradient in a perennially ice-covered Antarctic lake. Appl Environ Microbiol 82:620–630.

22. Hawes I, Sumner D, Andersen D, Jungblut A, Mackey T. 2013. Timescales of growth response of microbial mats to environmental change in an ice-covered Antarctic lake. Biology (Basel) 2:151–176.

23. Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl Environ Microbiol 69:5157–5169.

24. Bielewicz S, Bell E, Kong W, Friedberg I, Priscu JC, Morgan-Kiss RM. 2011. Protist diversity in a permanently ice-covered Antarctic Lake during the polar night transition. ISME J 5:1559–1564.

25. Parker BC, Simmons GM, Wharton RA, Seaburg KG, Love FG. 1982. Removal of organic and inorganic matter from Anarctic lakes by aerial escape of bluegreen algal mats 18:72–78.

26. Moorehead DL, Doran PT, Fountain AG, Lyons WB, McNight DM, Priscu JC, Virginia RA, Wall DH. 1999. Ecological legacies: Impacts on ecosystems of the McMurdo Dry Valleys. Bioscience 49:1009–1019.

27. Boetius A, Anesio AM, Deming JW, Mikucki JA, Rapp JZ. 2015. Microbial ecology of the cryosphere: Sea ice and glacial habitats. Nat Rev Microbiol 13:677–690.

28. Brambilla E, Hippe H, Hagelstein A, Tindall BJ, Stackebrandt E, Fryxell L, Valleys MD. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample. Int J 23–33.

29. Vick-Majors TJ, Priscu JC, Amaral-Zettler LA. 2014. Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. ISME J 8:778–789.

30. Madigan MT, Jung DO, Woese CR, Achenbach LA. 2000. Rhodoferax antarcticus sp. nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an Antarctic microbial mat. Arch Microbiol 173:269–277.

31. Baker J, Riester C, Skinner B, Newell A, Swingley W, Madigan M, Jung D, Asao M, Chen M, Loughlin P, Pan H, Lin Y, Li Y, Shaw J, Prado M, Sherman C, Tang J, Blankenship R, Zhao T, Touchman J, Sattley W. 2017. Genome Sequence of Rhodoferax antarcticus ANT.BRT; A Psychrophilic Purple Nonsulfur Bacterium from an Antarctic Microbial Mat. Microorganisms 5:8.

32. Jung DO, Achenbach LA, Karr EA, Takaichi S, Madigan MT. 2004. A gas vesiculate planktonic strain of the purple non-sulfur bacterium Rhodoferax antarcticus isolated from lake Fryxell, Dry Valleys, Antarctica. Arch Microbiol 182:236–243.

33. Zhang Q, Zhang Z, Lu T, Peijnenburg WJGM, Gillings M, Yang X, Chen J, Penuelas J, Zhu YG, Zhou NY, Su J, Qian H. 2020. Cyanobacterial blooms contribute to the diversity of antibiotic-resistance genes in aquatic ecosystems. Commun Biol 3:1–10.

34. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen AL V., Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran HK, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar G V., McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525.

35. Wood DE, Salzberg SL. 2014. Kraken: Ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15.

36. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. bioRxiv 1–13.

37. Lu J, Breitwieser FP, Thielen P, Salzberg SL. 2017. Bracken: Estimating species abundance in metagenomics data. PeerJ Comput Sci 2017:1–17.

38. Lind AL, Pollard KS. 2021. Accurate and sensitive detection of microbial eukaryotes from whole metagenome shotgun sequencing. Microbiome 9:1–18.

39. van den Hoff J, Bell E, Whittock L. 2020. Dimorphism in the Antarctic cryptophyte Geminigera cryophila (Cryptophyceae). J Phycol 56:1028–1038.

40. Yih W, Hyung SK, Hae JJ, Myung G, Young GK. 2004. Ingestion of cryptophyte cells by the marine photosynthetic ciliate Mesodinium rubrum. Aquat Microb Ecol 36:165–170.

41. Kong W, Ream DC, Priscu JC, Morgan-Kiss RM. 2012. Diversity and expression of RubisCO genes in a perennially ice-covered antarctic lake during the polar night transition. Appl Environ Microbiol 78:4358–4366.

42. Lizotte MP, Sharp TR, Priscu JC. 1996. Phytoplankton dynamics in the stratified water column of Lake Bonney, Antarctica: I. Biomass and productivity during the winter-spring transition. Polar Biol 16:155–162.

43. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods 9:811–814.

44. Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, Tett A, Huttenhower C, Segata N. 2015. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12:902–903.

45. Fihman V, Rottman M, Benzerara Y, Delisle F, Labia R, Philippon A, Arlet G. 2002. BUT-1: A new member in the chromosomal inducible class C β-lactamases family from a clinical isolate of Buttiauxella sp. FEMS Microbiol Lett 213:103–111.

46. Nakamichi N, Moriuchi R, Dohra H, Futamata H, Tashiro Y. 2021. Complete Genome Sequence of Buttiauxella agrestis DSM 9389. Microbiol Resour Announc 10:20–22.

47. Courvalin P. 2006. Vancomycin resistance in Gram-positive cocci. Clin Infect Dis 42:S25–S34.

48. Riccio ML, Docquier JD, Dell'Amico E, Luzzaro F, Amicosante G, Rossolini GM. 2003. Novel 3-N-aminoglycoside acetyltransferase gene, aac(3)-Ic, from a Pseudomonas aeruginosa integron. Antimicrob Agents Chemother 47:1746–1748.

49. López-Cabrera M, Pérez-González JA, Heinzel P, Piepersberg W, Jiménez A. 1989. Isolation and nucleotide sequencing of an aminocyclitol acetyltransferase gene from Streptomyces rimosus forma paromomycinus. J Bacteriol 171:321–328.

50. Kim MK, Harwood CS. 1991. Regulation of benzoate-CoA ligase in Rhodopseudomonas palustris. FEMS Microbiol Lett 83:199–203.

51. Rey FE, Oda Y, Harwood CS. 2006. Regulation of uptake hydrogenase and effects of hydrogen utilization on gene expression in Rhodopseudomonas palustris. J Bacteriol 188:6143–6152.

52. Buffan-Dubau E, Pringault O, De Wit R. 2001. Artificial cold-adapted microbial mats cultured from Antarctic lake samples. 1. Formation and structure. Aquat Microb Ecol 26:115–125.

53. Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using *breseq*. Methods Mol Biol 1151:165–188.

54. Tayeh MA, Madigan MT. 1987. Malate dehydrogenase in phototrophic purple bacteria: purification, molecular weight, and quaternary structure. J Bacteriol 169:4196–4202.

55. Wright GD. 2019. Environmental and clinical antibiotic resistomes, same only different. Curr Opin Microbiol 51:57–63.

56. Dcosta VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. 2011. Antibiotic resistance is ancient. Nature 477:457–461.

57. Zeng J, Pan Y, Yang J, Hou M, Zeng Z, Xiong W. 2019. Metagenomic insights into the distribution of antibiotic resistome between the gut-associated environments and the pristine environments. Environ Int 126:346–354.

58. Van Goethem MW, Pierneef R, Bezuidt OKI, Van De Peer Y, Cowan DA, Makhalanyane TP. 2018. A reservoir of "historical" antibiotic resistance genes in remote pristine Antarctic soils. Microbiome 6:1–12.

59. Li B, Chen Z, Zhang F, Liu Y, Yan T. 2020. Abundance, diversity and mobility potential of antibiotic resistance genes in pristine Tibetan Plateau soil as revealed by soil metagenomics. FEMS Microbiol Ecol 96:1–10.

60. Murphy A, Barich D, Fennessy MS, Slonczewski JL. 2021. An Ohio State Scenic River Shows Elevated Antibiotic Resistance Genes, Including Acinetobacter Tetracycline and Macrolide Resistance, Downstream of Wastewater Treatment Plant Effluent. Microbiol Spectr 9:e00941.

61. Glatz RE, Lepp PW, Ward BB, Francis CA. 2006. Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-covered Lake

Bonney, Antarctica. Geobiology 4:53–67.

62. Brown CT, Hug LA, Thomas BC, Sharon I, Castelle CJ, Singh A, Wilkins MJ, Wrighton KC, Williams KH, Banfield JF. 2015. Unusual biology across a group comprising more than 15% of domain Bacteria. Nature 523:208–211.

63. Nguyen BAT, Chen QL, He JZ, Hu HW. 2020. Microbial regulation of natural antibiotic resistance: Understanding the protist-bacteria interactions for evolution of soil resistome. Sci Total Environ 705:135882.

64. Lawrence MJF, Hendy CH. 1985. Water column and sediment characteristics of lake fryxell, taylor valley, Antarctica. New Zeal J Geol Geophys 28:543–552.

65. Priscu JC, Spigel RH. 1996. Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. Hydrobiologia 321:177–190.

66. Fixen KR, Oda Y, Harwood CS. 2019. Redox regulation of a light-harvesting antenna complex in an anoxygenic phototroph. MBio 10.

67. Kaminski J, Gibson MK, Franzosa EA, Segata N, Dantas G, Huttenhower C. 2015. High-specificity targeted functional profiling in microbial communities with ShortBRED. PLoS Comput Biol 11.

68. Bateman A, Martin MJ, Orchard S, Magrane M, Agivetova R, Ahmad S, Alpi E, Bowler-Barnett EH, Britto R, Bursteinas B, Bye-A-Jee H, Coetzee R, Cukura A, Silva A Da, Denny P, Dogan T, Ebenezer TG, Fan J, Castro LG, Garmiri P, Georghiou G, Gonzales L, Hatton-Ellis E, Hussein A, Ignatchenko A, Insana G, Ishtiaq R, Jokinen P, Joshi V, Jyothi D, Lock A, Lopez R, Luciani A, Luo J, Lussi Y, MacDougall A, Madeira F, Mahmoudy M, Menchi M, Mishra A, Moulang K, Nightingale A, Oliveira CS, Pundir S, Qi G, Raj S, Rice D, Lopez MR, Saidi R, Sampson J, Sawford T, Speretta E, Turner E, Tyagi N, Vasudev P, Volynkin V, Warner K, Watkins X, Zaru R, Zellner H, Bridge A, Poux S, Redaschi N, Aimo L, Argoud-Puy G, Auchincloss A, Axelsen K, Bansal P, Baratin D, Blatter MC, Bolleman J, Boutet E, Breuza L, Casals-Casas C, de Castro E, Echioukh KC, Coudert E, Cuche B, Doche M, Dornevil D, Estreicher A, Famiglietti ML, Feuermann M, Gasteiger E, Gehant S, Gerritsen V, Gos A, Gruaz-Gumowski N, Hinz U, Hulo C, Hyka-Nouspikel N, Jungo F, Keller G, Kerhornou A, Lara V, Le Mercier P, Lieberherr D, Lombardot T, Martin X, Masson P, Morgat A, Neto TB, Paesano S, Pedruzzi I, Pilbout S, Pourcel L, Pozzato M, Pruess M, Rivoire C, Sigrist C, Sonesson K, Stutz A, Sundaram S, Tognolli M, Verbregue L, Wu CH, Arighi CN, Arminski L, Chen C, Chen Y, Garavelli JS, Huang H, Laiho K, McGarvey P, Natale DA, Ross K, Vinayaka CR, Wang Q, Wang Y, Yeh LS, Zhang J. 2021. UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Res 49:D480–D489.

69. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

TABLES

Table 1. Mat and water antibiotic resistance genes (ARGs) identified by ShortBRED marker peptides*

ARG Family	MAT-01	MAT-02	MAT-03	MAT-04	MAT-05	MAT-06	FRY-01	FRY-02	FRY-03	BON-01	BON-02	BON-03	TOTAL	P
ARO_3004294|BUT-1 *Buttiauxella agrestis*	0	1	2	0	0	0	554	1720	967	266	262	323	4095	0.027
ARO_3002541|AAC(3)-VIIa *Streptomyces rimosus*	89	235	114	173	152	54	44	31	31	323	313	231	1790	0.038
ARO_3002528|AAC(3)-Ia *Pseudomonas aeruginosa*	302	189	131	492	238	196	0	0	0	0	0	0	1548	0.022
ARO_3003167|CTX-M-159 Proteobacteria	36	9	25	1032	111	18	1	0	0	0	0	3	1235	0.052
ARO_3001461|OXA-156 *Pandoraea pulmonicola*	43	18	75	146	70	36	14	15	19	70	60	53	619	0.099
ARO_3002894|otrC *Streptomyces rimosus*	40	122	16	27	13	23	162	73	81	11	20	10	598	0.079
ARO_3003599|OXA-443 *Ralstonia mannitolilytica*	41	260	13	42	33	16	64	23	12	2	3	4	513	0.061
ARO_3001338|SHV-100 *Klebsiella pneumoniae*	58	1	49	144	56	44	4	1	5	10	6	11	389	0.241
ARO_3002955|vanYA *Enterococcus faecium*	0	3	2	1	1	0	101	59	47	19	29	31	293	0.027
ARO_3001855|ACT-35 *Enterobacter cloacae*	4	7	26	32	37	5	30	22	22	40	22	30	277	0.214
ARO_3000410|sul1 Proteobacteria	72	0	32	19	34	62	1	0	0	0	0	0	220	0.199
ARO_3003022|dfrB3 *Klebsiella oxytoca*	13	4	9	19	23	8	54	21	18	9	12	18	208	0.069
ARO_3002972|vanTG *Enterococcus faecalis*	1	0	1	0	2	0	10	9	13	50	53	55	194	0.027
ARO_3001517|OXA-329 *Acinetobacter calcoaceticus*	2	2	1	2	0	1	76	52	24	1	15	5	181	0.054
ARO_3002701|cmr *Rhodococcus fascians*	6	5	17	22	19	2	39	47	16	3	2	2	180	0.038
ARO_3003723|van *Desulfitobacterium hafniense*	0	0	0	0	0	0	53	50	40	9	16	10	178	0.024
ARO_3003942|abcA *Staphylococcus aureus*	31	75	2	9	17	20	0	0	0	7	4	5	170	0.055
ARO_3003583|basS *Pseudomonas aeruginosa*	12	1	30	8	7	12	1	0	0	47	32	19	169	0.046
ARO_3004652|Erm(O)-lrm *Streptomyces lividans*	28	8	16	37	35	10	8	7	4	5	2	5	165	0.061
ARO_3001299|tlrB_tylosin *Streptomyces fradiae*	18	8	10	30	26	8	2	2	0	18	18	22	162	0.034

*Class differences for ARG abundance were tested for significance ($P \leq 0.5$) using the Kruskal-Wallis test.

Table 2. *Rhodoferax* enrichment culture ARGs identified by ShortBRED*

A. *Rhodoferax* (95%), *Pseudomonas* (1.5%)	Hits	Marker Length	B. *Rhodoferax* (95%), *Pseudomonas* (0.7%)	Hits	Marker Length
ARO_3003679|TriA__Pseudomonas_aeruginosa_PAO1	8	242	ARO_3003680|TriB__Pseudomonas_aeruginosa_PAO1	3	144
ARO_3001796|OXA-50__Pseudomonas_aeruginosa	4	116	ARO_3003031|mexW__Pseudomonas_aeruginosa_PAO1	1	56
ARO_3003681|TriC__Pseudomonas_aeruginosa_PAO1	3	113	ARO_3002320|KPC-10__Acinetobacter_baumannii	1	66
ARO_3003710|mexL__Pseudomonas_aeruginosa_PAO1	3	134	ARO_3003583|basS__Pseudomonas_aeruginosa	1	290
ARO_3003030|mexV__Pseudomonas_aeruginosa_PAO1	3	176	ARO_3004777|CMH-1__Enterobacter_cloacae	1	25
ARO_3002507|PDC-8__Pseudomonas_aeruginosa	3	155	ARO_3003705|mexN__Pseudomonas_aeruginosa	1	63
ARO_3000802|OprJ__Pseudomonas_aeruginosa	2	36	ARO_3000808|mexI__Pseudomonas_aeruginosa_PAO1	1	118
ARO_3003680|TriB__Pseudomonas_aeruginosa_PAO1	2	144	ARO_3004054|CpxR__Pseudomonas_aeruginosa	1	26
ARO_3000379|OprM__Pseudomonas_aeruginosa_PAO1	2	35			
ARO_3000805|OprN__Pseudomonas_aeruginosa_PAO1	2	102			
ARO_3002985|arnA__Pseudomonas_aeruginosa_PAO1	2	102			
ARO_3003692|mexJ__Pseudomonas_aeruginosa_PAO1	2	120			
ARO_3003031|mexW__Pseudomonas_aeruginosa_PAO1	2	56			
ARO_3004038|emrE__Pseudomonas_aeruginosa_PAO1	2	51			
ARO_3003705|mexN__Pseudomonas_aeruginosa	2	63			
ARO_3003698|mexP__Pseudomonas_aeruginosa	2	85			
ARO_3002645|APH(3)-IIb__Pseudomonas_aeruginosa	2	153			
ARO_3000377|MexA__Pseudomonas_aeruginosa_PAO1	2	106			
ARO_3001214|mdtM__Escherichia_coli_str__K-12	1	71			
ARO_3004077|PmpM__Pseudomonas_aeruginosa_PAO	1	109			
ARO_3000804|MexF__Pseudomonas_aeruginosa_PAO1	1	102			
ARO_3004072|OpmB__Pseudomonas_aeruginosa_PAO1	1	111			
ARO_3004075|MuxC__Pseudomonas_aeruginosa_PAO1	1	177			
ARO_3003693|mexK__Pseudomonas_aeruginosa_PAO1	1	44			
ARO_3000809|opmD__Pseudomonas_aeruginosa_PAO1	1	116			
ARO_3003682|OpmH__Pseudomonas_aeruginosa_PAO	1	79			
ARO_3002695|cmlA5__uncultured_bacterium	1	99			
ARO_3003583|basS__Pseudomonas_aeruginosa	1	290			
ARO_3000795|mdtE__Escherichia_coli_str__K-12	1	54			
ARO_3004612|ampH__Escherichia_coli_DH1	1	68			

*Enrichment cultures A and B, taxa proportions were estimated by Kraken2/Bracken pipeline.

FIGURES

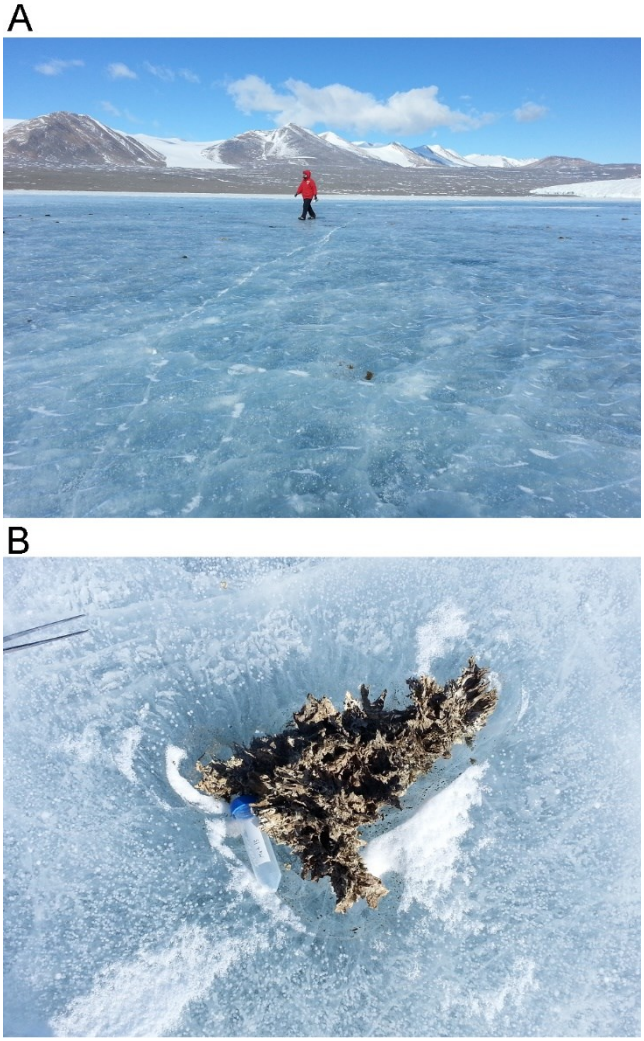

Figure 1. Lake Fryxell uplift mats.

(**A)** Lake Fryxell with permanent ice cover, weathered by katabatic winds; December 10, 2014.

(**B)** Source of MAT-4 DNA. Uplift mat emerges from the ice; approximately 40 cm across.

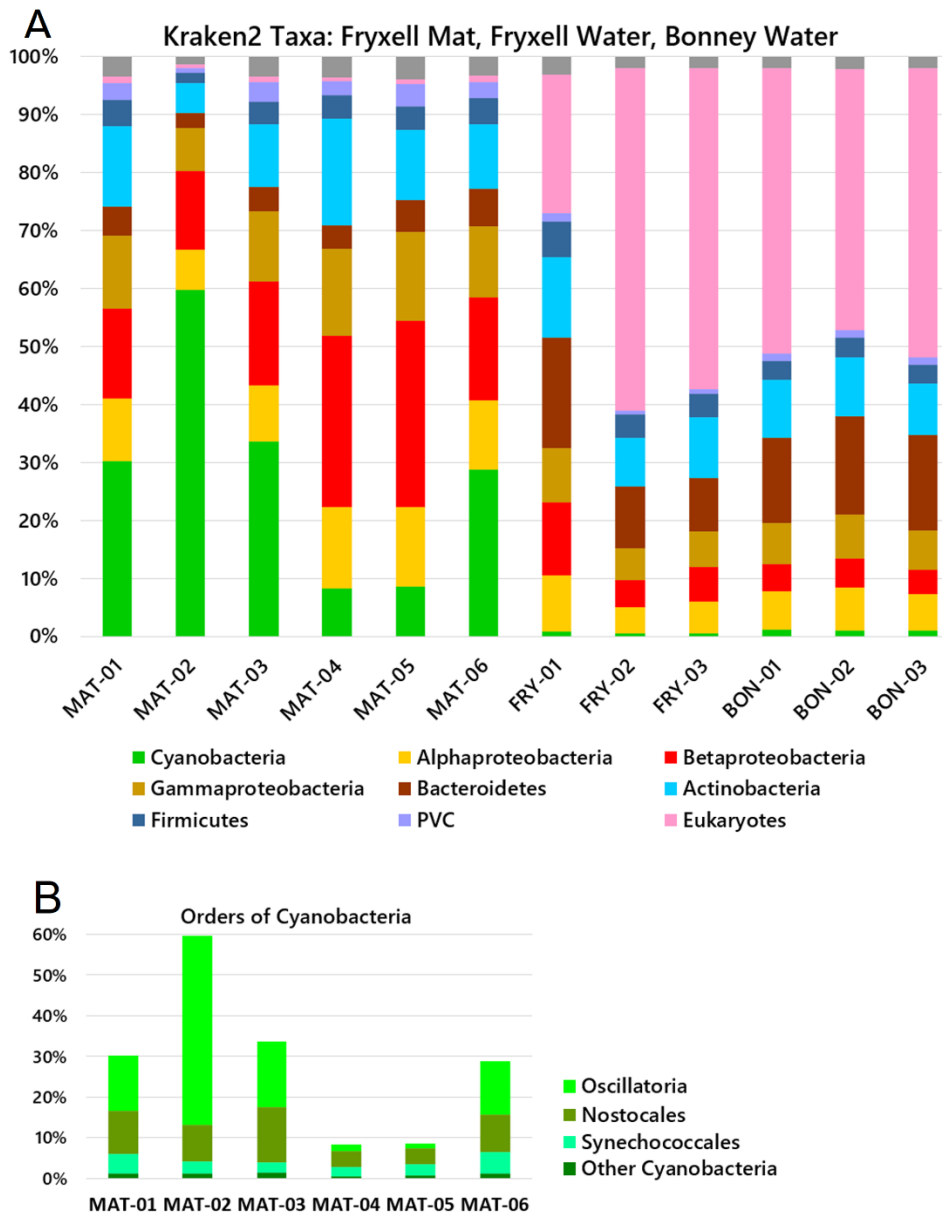

Figure 2. Microbial community composition of mat and water samples. Relative abundance of phyla was determined by read alignment to marker genes using Kraken2/Bracken pipeline. (**A**) Phyla and classes. (**B**) Orders of cyanobacteria in mat samples.

MAT (Lake Fryxell, mat samples); FRY (Lake Fryxell, water samples); BON (Lake Bonney, water samples). MAT samples were from ice surface, unfiltered. The water samples were collected on a 0.45-µm filter. PVC=Planctomycetes-Verrucomicrobia-Chlamydiae

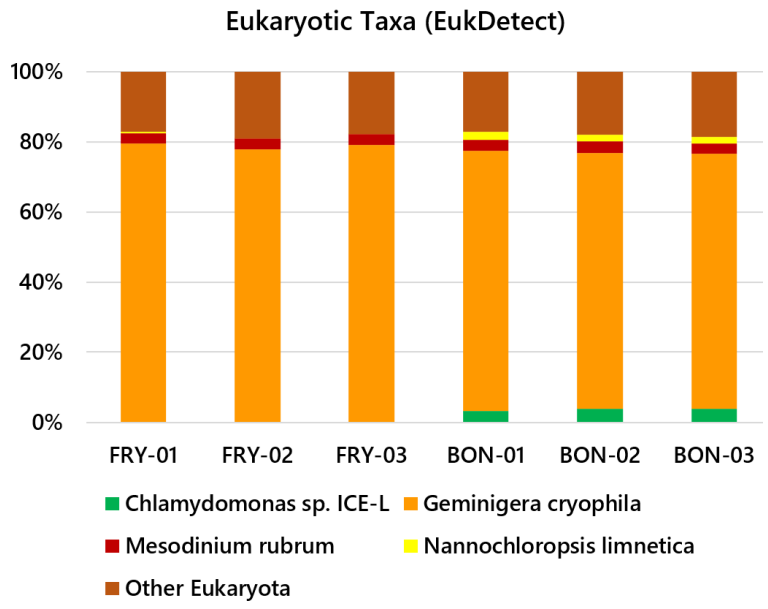

Figure 3. Eukaryotic taxa classified by EukDetect. Relative abundance of protist taxa were determined by read alignment to marker genes using EukDetect (38).

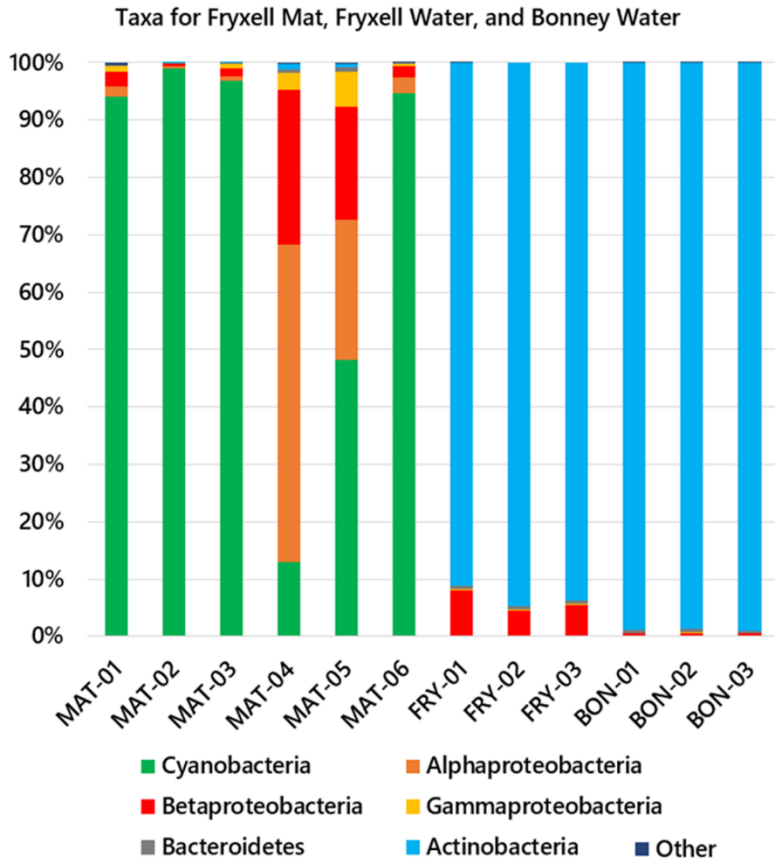

Figure 4. Microbial community composition via MetaPhlAn2. Relative abundance of class-level taxa was determined by read alignment to marker genes using MetaPhlAn2 (43, 44). Samples were as indicated for Figure 2.

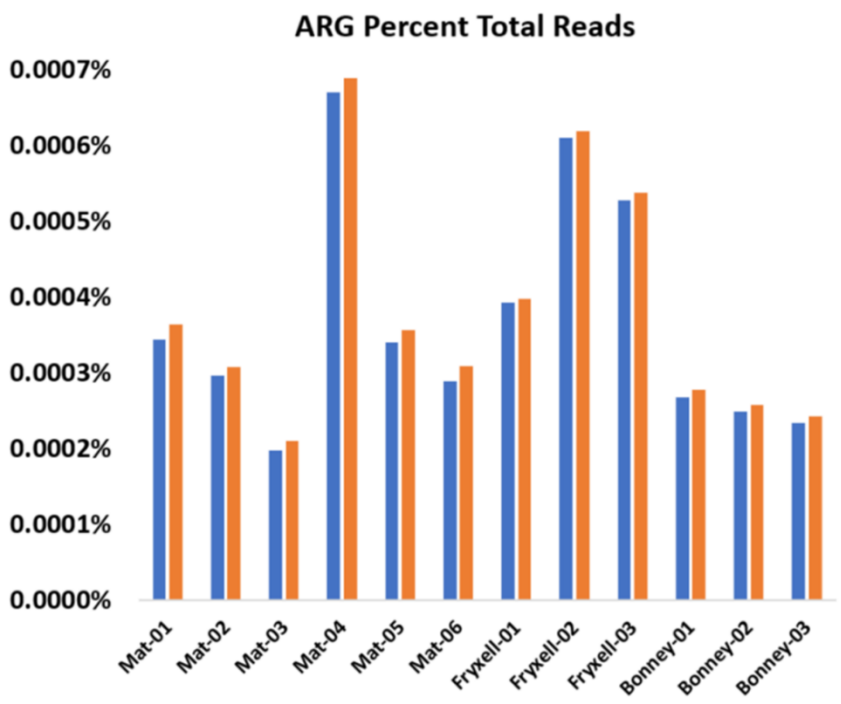

Figure 5. ARG abundance in lake samples. Percentage of reads matched to ShortBRED markers. Total reads were counted using Trimmomatic. Bars indicate total ARG hits per sample (blue) and top 60 ranked ARG hits (orange).

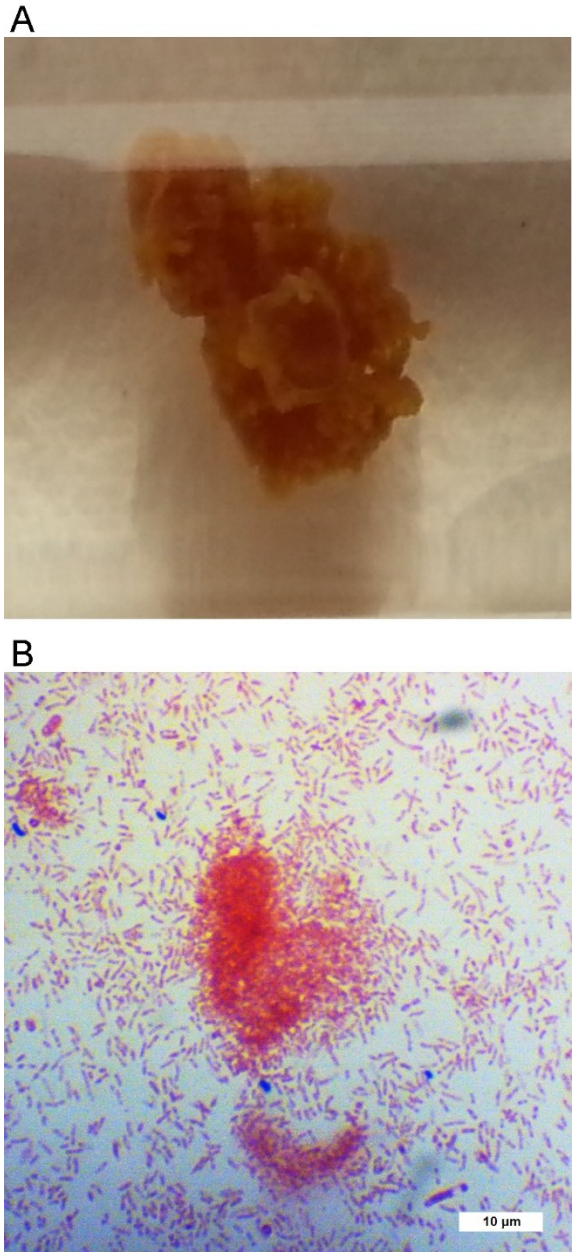

Figure 6. Mat-forming enrichment of Mat-04 *R. antarcticus* JLS.

(A) Biofilm of cultured *R. antarcticus* within closed tube of medium, showing globular form of growth. Initial Mat-04 sample was subcultured serially three times in PM medium, in horizontal closed tubes at 10°C with 30 umoles/m^2s illumination.

(B) Gram stain of culture, 1000X with oil immersion. Courtesy of Emma Stuart-Bates.

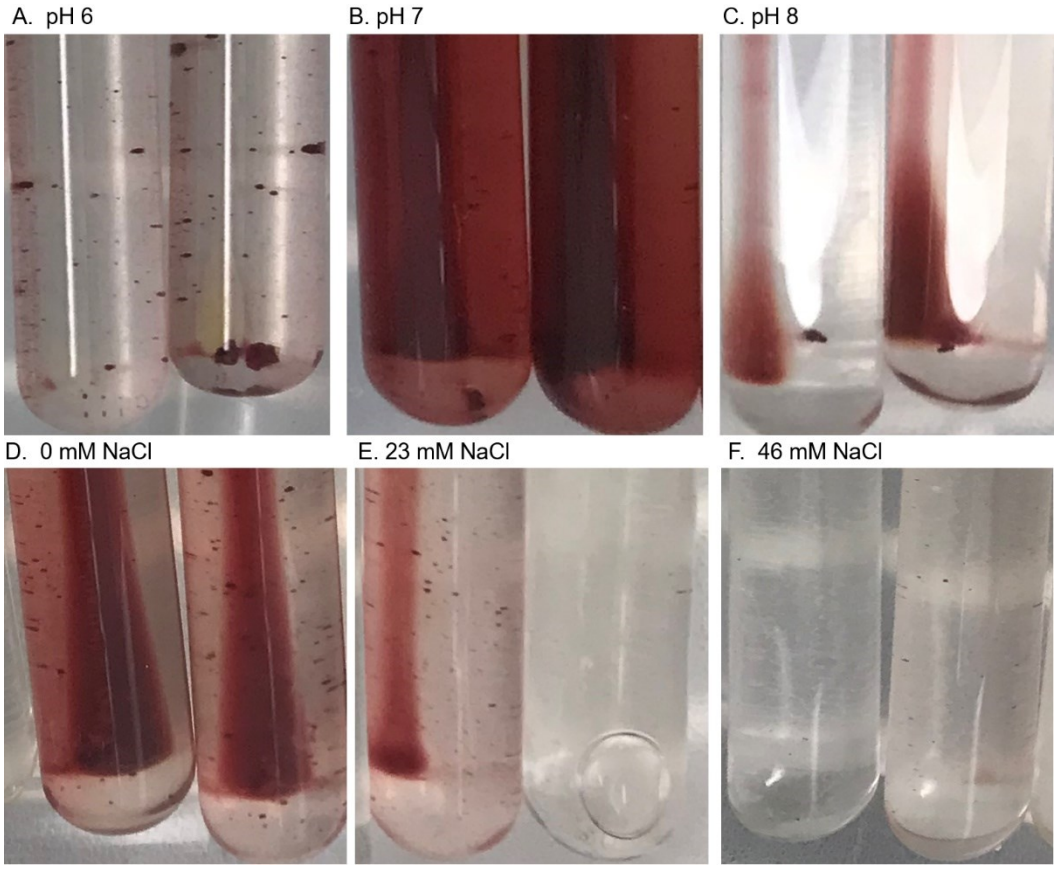

Figure 7. pH and NaCl dependence of *R. antarcticus* JLS.

(A, B, C) Medium PM contained **(A)** 100 mM MES pH 6; **(B)** 100 mM MOPS pH 7; **(C)** 100 mM TAPS pH 8. Cultures were incubated 45 days.

(**D, E, F)** Medium PM was amended with **(D)** no NaCl added; **(E)** 23 mM NaCl; **(F)** 46 mM NaCl. Cultures were incubated 45 days.