



Metagenome-assembled bacterial genomes from benthic microbial mats in ice-covered Lake Vanda, Antarctica

Tyler Powell,^{1,2} Dawn Y. Sumner,¹ Anne D. Jungblut,³ Ian Hawes,⁴ Tyler Mackey,⁵ Christen Grettenberger^{1,6}

AUTHOR AFFILIATIONS See affiliation list on p. 8.

ABSTRACT We recovered 57 bacterial metagenome-assembled genomes (MAGs) from benthic microbial mat pinnacles from Lake Vanda, Antarctica. These MAGs provide access to genomes from polar environments and can assist in culturing and utilizing these Antarctic bacteria.

KEYWORDS Antarctica, microbial mat, metagenome-assembled genomes

Most microorganisms, especially extremophilic organisms cannot be cultured easily under *in vitro* laboratory conditions (1, 2). Molecular methodologies like metagenomic shotgun sequencing now provide a culture-independent method of examining extremophilic taxa and the adaptations that they have for life in these extreme environments. They provide a helpful alternative approach to answer questions about the organisms and their community when culturing attempts are unsuccessful. Here, we retrieved 57 metagenome-assembled genomes (MAGs) from microbial pinnacles that were subsampled from benthic mats that grew on the floor of the perennially ice-covered Lake Vanda, Antarctica.

Samples were collected as described previously (3). Briefly, scientific divers sampled microbial mat pinnacles in Lake Vanda in the McMurdo Dry Valleys, Antarctica (–77.529, 161.578) between 8th and 18th December 2013. Microbial mats were subsampled based on color into photosynthetically active green (G) or purple (P) areas and aphotic beige (IB) areas within pinnacles (4). DNA was extracted in the field from multiple green, purple, and inner beige samples and one un-subsampled “bulk mat” sample. DNA was extracted using the Zymo Soil/Fecal miniprep kit (Zymo Research, USA). Libraries were prepared using the KAPA HyperPrep kit (KAPA Biosystems, USA) and DNA was sequenced using the Illumina HiSeq-2500 1TB platform with 2 × 151 bp paired-end sequencing and quality controlled using the standard Joint Genome Institute protocol as described previously (3). Briefly, reads with four or more “N” bases with average quality less than three or minimum length ≤51 bp or 33% of the full read length and those with 93% identity to masked human, dog, cat, mouse, and common contaminants were removed using BBDuk (version 37.36) within BBTools (5). Metagenomes were assembled using MEGAHIT v1.9.6 with a minimum contig length of 1500 (6). Reads were mapped to the assembly using Bowtie2 v1.2.2 and samtools v1.7 (7, 8). A depth file was generated using the `jgi_summarize_bam_contig_depths` command in MetaBAT v2.12.1 (9). Bins were generated using metaBAT using a minimum contig size of 2,500 bp. The completeness and contamination of the bins were calculated using CheckM v1.0.7 (10). Bins > 90% complete with less than 5% contamination were retained. Average nucleotide identity (ANI) between bins was calculated using the `anvi-compute-genome-similarity` command in Anvi’o v6.2 using fastANI (11, 12). Bins sharing >98% ANI was considered to be the same taxon and the bin with the highest completeness was selected as the representative MAG for that taxon. Representative MAGs were classified using GTDB-tk v1.7.0 (13). Previously published MAGs were removed (3, 14, 15). Genome coverage was calculated

Editor Elinne Becket, California State University San Marcos, San Marcos, California, USA

Address correspondence to Christen Grettenberger, clgrett@ucdavis.edu.

The authors declare no conflict of interest.

See the funding table on p. 8.

Received 18 December 2023

Accepted 10 March 2024

Published 8 April 2024

Copyright © 2024 Powell et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

TABLE 1 Summary of metagenome statistics and MAG properties^a

Site	Metagenome statistics					Genome statistics									
	Accession	Raw read pairs (millions)	Quality controlled read pairs (millions)	Contigs	N50	Accession	Genome name	Classification	Total length (Mb)	GC (%)	Contigs	Completeness (%)	Contamination (%)	Coverage (x)	Protein-encoding genes (total)
Bulk Mat	SRX3539175	208	207	179,859	4156	SAMN38186868	BulkMat.140	Bacteria; Bacteroidota; Bacteroidia; Chitinophagales; Saprospiraceae; JABWAJ01	5.56	49.33	409	97.78	1.01	50.0	4,494
						SAMN38186869	BulkMat.37	Bacteria; Pseudomonadota; Alphaproteobacteria; Caulobacterales; TH1-2; Terricaulis	3.7	62.78	136	98.62	1.54	23.1	3,852
						SAMN38186870	BulkMat.75	Bacteria; Zixibacteria; MSB-5A5; GN15; FEB-12; JACRAV01	2.7	48.5	82	98.84	0	44.0	2,246
						SAMN38186871	BulkMat.82	Bacteria; Chloroflexota; Anaerolineae; Aggregatilineales; A4b; GCA-2794515	3.9	47.52	509	96.18	1.82	15.4	3,595
MP5G1	SRX3539176	95	94	213,763	4255	SAMN38186872	MP5G1.132	Bacteria; Bacteroidota; Bacteroidia; Chitinophagales; Saprospiraceae; JABWAJ01	5.76	49.38	437	97.78	2.27	24.6	4,673
						SAMN38186873	MP5G1.138	Bacteria; Spirochaetota; Leptospirae; Leptospirales; Leptospiraceae; Leptospira_A	4.11	35.7	37	100	0	44.6	3,834
						SAMN38186874	MP5G1.179	Bacteria; Chloroflexota; Chloroflexia; Chloroflexales; Roseiflexaceae	6.13	54.9	757	95.91	1.89	12.1	5,489
						SAMN38186875	MP5G1.50	Bacteria; Bacteroidota; Bacteroidia; AKYH767; B-17BO; UBA2475	3.53	36.14	190	97.44	2.66	28.9	3,021
MP5IB2	SRX3539179	103	102	217,012	4652	SAMN38186876	MP5IB2.11	Bacteria; Bdellovibrionota; Bdellovibrionia; JABDDW01; JABDDW01	3	39.57	70	95.48	2.68	17.6	2,935
						SAMN38186877	MP5IB2.114	Bacteria; Bacteroidota; Bacteroidia; NS11-12g; SHWZ01	3.5	36.82	205	96.43	0.63	20.4	2,970

(Continued on next page)

TABLE 1 Summary of metagenome statistics and MAG properties^a (Continued)

Site	Metagenome statistics				Genome statistics										
	Accession	Raw read pairs (millions)	Quality controlled read pairs (millions)	Contigs	N50	Accession	Genome name	Classification	Total length (Mb)	GC (%)	Contigs	Completeness (%)	Contamination (%)	Coverage (x)	Protein-encoding genes (total)
MP6G1	SRX3539172	101	100	223,185	4583	SAMN38186878	MP5IB2.151	Bacteria; Planctomycetia; Pirellulales;	4.77	56.21	204	97.63	0	42.4	4,053
						SAMN38186879	MP5IB2.172	Lacipirellulaceae; Bythopirellula Bacteria; Bacteroidota; Bacteroidia; Chitinophagales; Saprospiraceae; JADKGY01	3.92	44.25	298	95.42	0.41	18.5	3,141
						SAMN38186880	MP5IB2.194	Bacteria; Bacteroidota; Rhodothermia; Rhodothermales; JADKGY01	5.14	62.43	304	95.9	2.19	21.4	4,229
						SAMN38186881	MP5IB2.201	Bacteria; Bdellovibrionota; Bdellovibrionia; Bdellovibrionales; SG-bin7	3.26	42.33	111	99	1.79	17.7	3,273
						SAMN38186882	MP5IB2.37	Bacteria; Bacteroidota; Bacteroidia; NS11-12g; UKL13-3; B1	3.59	37.51	149	95.24	1.59	23.9	3,020
						SAMN38186883	MP5IB2.67	Bacteria; Gemmatimonadota; Gemmatimonadales; Gemmatimonadales; GWC2-71-9; SZUA-320	3.29	67.18	419	95.6	2.2	27.9	3,077
						SAMN38186884	MP5IB2.78	Bacteria; Planctomycetota; Planctomycetia; Planctomycetales; Planctomycetaceae; DSVQ01	7.43	63.99	717	95.51	1.23	15.0	6,251
MP6G1	SRX3539172	101	100	223,185	4583	SAMN38186885	MP6G1.103	Bacteria; Chloroflexota; Anaerolineae; Aggregatilineales; A4b; OLB15	5.21	59.04	383	98.18	0.91	25.8	4,619
						SAMN38186886	MP6G1.12	Bacteria; Planctomycetota; Phycisphaerae; Phycisphaerales; SM1A02; JAEUIT01	4.3	64.28	281	95.8	2.84	20.6	3,691
						SAMN38186887	MP6G1.168	Bacteria;	4.44	61.39	335	97.27	0	28.5	4,031

(Continued on next page)

TABLE 1 Summary of metagenome statistics and MAG properties^a (Continued)

Site	Metagenome statistics				Genome statistics										
	Accession	Raw read pairs (millions)	Quality controlled read pairs (millions)	Contigs	N50	Accession	Genome name	Classification	Total length (Mb)	GC (%)	Contigs	Completeness (%)	Contamination (%)	Coverage (x)	Protein-encoding genes (total)
MP6IB1	SRX353917Z	101	100	182,767	5165	SAMN38186890	MP6IB1.110	Chloroflexota; Anaerolineae; Aggregatilineales; A4b; OLB15	5.23	59.6	389	95.81	1.31	19.8	4,590
								Bacteria; Myxococcota; Polyangia; Palsa-1104_A	3.7	41.41	269	95.61	0	16.1	2,907
								Bacteria; Bacteroidota; Bacteroidia; Flavobacteriales; UA16	3.61	37.22	262	95.36	1.09	31.4	3,123
								Bacteria; Bacteroidota; Ignavibacteria; SJA-28; B-1AR	4.75	42.75	325	95.17	0.99	23.7	3,981
								Bacteria; Bacteroidota; Bacteroidia; Chitinophagales; Saprospiraceae; JADJLQ01	3.61	64.33	253	97.65	2.03	40.7	3,409
								Bacteria; Pseudomonadales; Alphaproteobacteria; Caulobacterales; Hyphomondaceae; UBA7672	3.76	63.88	143	98.91	0.82	30.5	3,523
								Bacteria; Proteobacteria; Alphaproteobacteria; Dongiales; Rhodospirillaceae	3.46	50.16	52	99.45	0.55	18.2	3,064
								Bacteria; Bacteroidota; Bacteroidia; Cytophagales; Cyclobacteriaceae; ELB16-189	4.04	54.27	175	97.83	2.76	16.6	3,703
								Bacteria; Pseudomonadota; Gammaproteobacteria; Lysobacterales; SZUA-36; SHZL01	2.76	48.16	132	95.95	1.38	16.0	2,590
								Bacteria; Verrucomicrobiota; Verrucomicrobia	3.53	58.5	234	96.35	0	41.9	3,561
MP6IB1	SAMN38186897	101	100	182,767	5165	SAMN38186897	MP6IB1.66	Bacteria; Proteobacteria; Alphaproteobacteria;	3.53	58.5	234	96.35	0	41.9	3,561
								Alphaproteobacteria;	3.53	58.5	234	96.35	0	41.9	3,561

(Continued on next page)

TABLE 1 Summary of metagenome statistics and MAG properties^a (Continued)

Site	Metagenome statistics				Genome statistics										
	Accession	Raw read pairs (millions)	Quality controlled read pairs (millions)	Contigs	N50	Accession	Genome name	Classification	Total length (Mb)	GC (%)	Contigs	Completeness (%)	Contamination (%)	Coverage (x)	Protein-encoding genes (total)
MP7G1	SRX3539171	107	106	219,283	4526	SAMN381868902	MP7G1.125	Bacteria;	2.96	37.52	483	96.14	3.3	12.4	2,731
								Bdellovibrionota_C; UBA2361;							
								UBA2361							
								Bacteria; Planctomycetota;	4.49	64	287	97.85	4.48	26.6	3,736
								UBA1135; UBA1135;							
								GCA-002686595;							
								JAEUIA01							
								Bacteria; Nitrospirota; Nitrospiria; 4.32							
								Nitrospirales;							
								Nitrospiraceae;							
Palsa-1315															
Bacteria;	2.4	47.45	74	97.3	1.69	17.4	2,307								
Verrucomicrobiota; Verrucomicrobia; Methylacidiphilales;															
JAAUTS01; JAAUTS01															
Bacteria;	4.17	67.42	403	97.8	4.44	17.8	3,870								

(Continued on next page)

TABLE 1 Summary of metagenome statistics and MAG properties^a (Continued)

Site	Metagenome statistics				Genome statistics										
	Accession	Raw read pairs (millions)	Quality controlled read pairs (millions)	Contigs	N50	Accession	Genome name	Classification	Total length (Mb)	GC (%)	Contigs	Completeness (%)	Contamination (%)	Coverage (x)	Protein-encoding genes (total)
MP8IB2	SRX3539178	94	92	205,304	4796	SAMN38186907	MP8IB2.145	Bacteria; Proteobacteria; Alphaproteobacteria; Micropepsales; Micropepsaceae; SZUA-430	5.07	63.43	186	99.78	1.74	30.5	4,930
MP8IB2	SAMN38186908	MP8IB2.79	Bacteria; Candidatus Eisenbacteria; RBG-16-71-46; JABDJR01	3.96	62.82	264	95.6	3.3	39.5	3,429					
MP9P1	SRX3539181	116	115	200,348	4604	SAMN38186909	MP9P1.103	Bacteria; Cyanobacteria; Vampirovibrionia; Obscuribacterales; Obscuribacteraceae	8.13	50.27	283	95.73	3.99	24.5	6,547
MP9P1	SAMN38186910	MP9P1.11	Bacteria; Bacteroidota; Bacteroidia;	4.53	44.3	137	96.55	0.33	40.0	3,885					
MP9P1	SAMN38186911	MP9P1.153	Chitinophagales; CAIOSU01	4.28	62.58	288	96.21	0.57	32.7	3,735					
MP9P1	SAMN38186912	MP9P1.20	Bacteria; Planctomycetota; Phycisphaerae; Phycisphaerales; SM1A02; JAEUIT01	2.98	47.26	138	97.26	1.35	17.3	2,763					
MP9P1	SAMN38186913	MP9P1.49	Bacteria; Verrucomicrobiota; Verrucomicrobiia	3.11	57.39	84	98.59	0.4	35.7	3,115					
P2IB	SAMN38186914	MP9P1.80	Bacteria; Bacteroidota; Bacteroidia; CAILMK01; CAILMK01	2.48	35.43	128	98.09	0	19.9	2,130					
P2IB	SRX3539180	98	97	198,359	5291	SAMN38186915	P2IB.100	Bacteria; Pseudomonadota; Gammaproteobacteria; Lysobacterales;	4.41	60.13	352	95.64	1.98	63.2	3,779
P2IB	SAMN38186916	P2IB.134	Ahniellaceae; 0-14-3-00-62-12	5.43	55.33	599	95.59	4.18	11.1	4,619					

(Continued on next page)

May 2024 Volume 13 Issue 5

²⁷Alternating grey and white bars indicate MAGs derived from the same metagenome.

using coverM (<https://github.com/wwood/CoverM>) and genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (16). Default parameters were used unless otherwise noted.

Metagenomes contained 177–229 million reads which assembled into 179,000–223,000 contigs (Table 1). We retrieved 57 MAGs from 20 bacterial phyla. The median contig number for MAGs was 264. The average size and GC content were 4.296 Mb and 52.828%, respectively. Average completeness was 96.41% and contamination was 1.38%. Coverage ranged from 11.1× to 111.3×. There were 2,130–6,778 protein-encoding genes (Table 1).

These Antarctic MAGs help to remove barriers to researchers interested in the Antarctic by expanding the catalog of Antarctic bacterial genomes. They provide information that can be used to inform culturing efforts and be used in parallel with *in vitro* studies to understand how these organisms adapt to their extreme environment and may respond to ongoing climate change.

ACKNOWLEDGMENTS

Samples used in this project were collected during a field season supported by the New Zealand Foundation for Research, Science, and Technology (grant CO1X0306) with field logistics provided by Antarctica New Zealand (project K-081). Sequencing was provided by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility under Contract No. CSP502867. The U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy and operated under Contract No. DE-AC02-05CH11231. Data analysis was supported by NSF grants OPP-1745341, OPP-1937748, and BII-2022126.

AUTHOR AFFILIATIONS

- ¹Department of Earth and Planetary Sciences, University of California, Davis, USA
- ²Microbiology Graduate Group, University of California, Davis, USA
- ³Department of Sciences, The Natural History Museum, London, United Kingdom
- ⁴Coastal Marine Field Station, University of Waikato, Tauranga, New Zealand
- ⁵Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico, USA
- ⁶Department of Environmental Toxicology, University of California, Davis, USA

AUTHOR ORCIDs

Dawn Y. Sumner  <https://orcid.org/0000-0002-7343-2061>
Christen Grettenberger  <http://orcid.org/0000-0002-6323-5179>

FUNDING

Funder	Grant(s)	Author(s)
New Zealand Foundation for Research, Science, and Technology	CO1X0306	Ian Hawes
Antarctic New Zealand	K-081	Ian Hawes
Joint Genome Institute (JGI)	CSP502867	Dawn Y. Sumner Christen Grettenberger
National Science Foundation (NSF)	OPP-1745341	Dawn Y. Sumner
National Science Foundation (NSF)	OPP-1937748	Dawn Y. Sumner
National Science Foundation (NSF)	BII-2022126	Dawn Y. Sumner Christen Grettenberger

AUTHOR CONTRIBUTIONS

Tyler Powell, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review and editing | Dawn Y. Sumner, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing | Anne D. Jungblut, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review and editing | Ian Hawes, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review and editing | Tyler Mackey, Data curation, Investigation, Methodology, Writing – review and editing | Christen Grettenberger, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Quality-controlled reads are available in the NCBI Sequence Read Archive under accession numbers [SRR6448204–SRR6448219](https://doi.org/10.1177/014107680209500207). Draft genomes are available under Genbank accession numbers [JAWXAL01–JAWXCP01](https://doi.org/10.1177/014107680209500207). Raw reads are available in IMG under project number [Gs0127369](https://doi.org/10.1177/014107680209500207).

REFERENCES

1. Wade W. 2002. Unculturable bacteria—the uncharacterized organisms that cause oral infections. *J R Soc Med* 95:81–83. <https://doi.org/10.1177/014107680209500207>
2. Zhu D, Adebisi WA, Ahmad F, Sethupathy S, Danso B, Sun J. 2020. Recent development of extremophilic bacteria and their application in biorefinery. *Front Bioeng Biotechnol* 8:483. <https://doi.org/10.3389/fbioe.2020.00483>
3. Grettenberger CL, Sumner DY, Wall K, Brown CT, Eisen JA, Mackey TJ, Hawes I, Jospin G, Jungblut AD. 2020. A phylogenetically novel cyanobacterium most closely related to *Gloeobacter*. *ISME J* 14:2142–2152. <https://doi.org/10.1038/s41396-020-0668-5>
4. Sumner DY, Jungblut AD, Hawes I, Andersen DT, Mackey TJ, Wall K. 2016. Growth of elaborate microbial pinnacles in Lake Vanda, Antarctica. *Geobiology* 14:556–574. <https://doi.org/10.1111/gbi.12188>
5. Bushnell B. 2018. BBtools. Sourceforge.Net/projects/Bbmap/
6. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31:1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
7. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>
8. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinform Oxf Engl* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
9. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. *PeerJ* 3:e1165. <https://doi.org/10.7717/peerj.1165>
10. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 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>
11. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9:5114. <https://doi.org/10.1038/s41467-018-07641-9>
12. Eren AM, Esen OC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO. 2015. Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* 3:e1319. <https://doi.org/10.7717/peerj.1319>
13. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-TK: a toolkit to classify genomes with the genome taxonomy database. *Bioinform Oxf Engl* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
14. Lumian J, Sumner D, Grettenberger C, Jungblut AD, Irber L, Pierce-Ward NT, Brown CT. 2022. Biogeographic distribution of five *Antarctic cyanobacteria* using large-scale k-mer searching with sourmash branchwater. *Biorxiv*. <https://doi.org/10.1101/2022.10.27.514113>
15. Grettenberger C, Sumner DY, Eisen JA, Jungblut AD, Mackey TJ. 2021. Phylogeny and evolutionary history of respiratory complex I proteins in melanobacteria. *Genes* 12:929. <https://doi.org/10.3390/genes12060929>
16. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the prokaryotic genome annotation pipeline reach with protein family model curation. *Nucleic Acids Res* 49:D1020–D1028. <https://doi.org/10.1093/nar/gkaa1105>