



Draft Genome Sequences of Four Alkaliphilic Bacteria Belonging to the *Anaerobacillus* Genus

 Naji M. Bassil, Jonathan R. Lloyd

Research Centre for Radwaste and Decommissioning and Williamson Research Centre for Molecular Environmental Science, School of Earth and Environmental Sciences, University of Manchester, Manchester, United Kingdom

ABSTRACT The draft genomes of the alkaliphilic, anaerobic bacteria, *Anaerobacillus arseniciselenatis*, *A. alkalidiazotrophicus*, and *A. alkalilacustris*, and a novel closely related isolate of the *Anaerobacillus* genus are reported here. These assembled genomes will help identify, at the molecular level, the phenotypic differences between the species of this poorly characterized genus.

The species *Bacillus arseniciselenatis* (1), *B. alkalidiazotrophicus* (2), and *B. macyae* (3) were transferred in 2009 to the newly established genus *Anaerobacillus* as *A. arseniciselenatis* comb. nov., *A. alkalidiazotrophicus* comb. nov., and *A. macyae* comb. nov., respectively (4). Furthermore, *A. alkalilacustris* was also added to the genus *Anaerobacillus* (4). Species belonging to this genus are anaerobic or aerotolerant, Gram-positive rods that grow under obligate or moderately alkaliphilic and halophilic conditions, through fermentative or anaerobic respiration (4). The genome of the moderately alkalitolerant *A. macyae* DSM 16346 was reported recently and has the GenBank accession number LELK00000000 (5); however, genome sequences have not been reported to date for the obligate alkaliphilic bacteria assigned to this genus.

Isosaccharinic acid (ISA) is a polyhydroxycarboxylic acid that is important in the geologic disposal of radioactive waste, as it is the product of the abiotic, alkaliphilic hydrolysis of cellulose (6) and has the potential to mobilize radionuclides in the geosphere (7). A novel bacterium, *Anaerobacillus* sp. NB2006, whose 16S rRNA gene sequence aligned with species of the genus *Anaerobacillus*, was isolated in a study of the microbial degradation of ISA at high pH (8).

Here, we report the draft genomes of the obligate alkaliphiles *A. arseniciselenatis* DSM 15340, *A. alkalidiazotrophicus* DSM 22531, and *A. alkalilacustris* DSM 18345, and the novel ISA-degrading bacterium *Anaerobacillus* sp. NB2006. Cells were harvested at the late log phase by centrifugation at $4,000 \times g$ for 10 min, and gDNA was extracted using the All-in-One purification kit (Norgen), following the protocol for Gram-positive bacteria. The DNA from each bacterium was sheared to 200 to 1,000 bp using NEBNext dsDNA Fragmentase (New England BioLabs), and barcoded libraries were prepared using the NEBNext Ultra DNA library prep kit for Illumina (New England BioLabs). Whole-genome sequencing of the pooled libraries was performed on a MiSeq platform (Illumina, San Diego, CA, USA), using V2 reagents to produce 250-bp paired-end reads. The barcode-separated raw reads were quality trimmed, and the sequencing adaptors were removed using Trimmomatic version 0.36 (9); then the PhiX sequences were removed and the sequence quality was checked using FaQCs version 1.34 (10). Overlapping reads were then joined into longer sequences using FLASH version 1.2.11 (11), and the resulting joined and unjoined sequences were *de novo* assembled using A5-miseq version 2015 (12), SOAPdenovo version 2.04 (13), ABySS version 2.0 (14), and SPAdes version 3.9 (15). The resulting assemblies were combined into a consensus assembly for each genome using CISA (contigs less than 1 kb

Received 9 November 2016 Accepted 14 November 2016 Published 19 January 2017

Citation Bassil NM, Lloyd JR. 2017. Draft genome sequences of four alkaliphilic bacteria belonging to the *Anaerobacillus* genus. *Genome Announc* 5:e01493-16. <https://doi.org/10.1128/genomeA.01493-16>.

Copyright © 2017 Bassil and Lloyd. 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/).

Address correspondence to Naji M. Bassil, naji.bassil@manchester.ac.uk, or Jonathan R. Lloyd, jon.lloyd@manchester.ac.uk.

TABLE 1 Genome features and GenBank accession numbers of four *Anaerobacillus* spp.

Strain	Genome size (Mb)	No. of contigs	Longest contig (bp)	N_{50} contig length (bp)	No. of genes	Accession no.
<i>A. arseniciselenatis</i> DSM 15340	3.95	58	942,622	220,456	3,762	MLQQ00000000
<i>A. alkalidiazotrophicus</i> DSM 22531	4.61	36	1,531,730	641,971	4,263	MLQS00000000
<i>A. alkalilacustris</i> DSM 18345	4.05	50	818,671	262,426	3,798	MLQR00000000
<i>Anaerobacillus</i> sp. NB2006	4.95	211	256,216	56,969	4,984	LQXD00000000

were removed) (16), and this was later aligned to the previously published *A. macyae* DSM 16346 genome (GenBank accession no. LELK01000000) and scaffolded using Scaffold_ Builder version 2.2 (17). Annotation of the genomes was performed using the NCBI Prokaryotic Genome Annotation Pipeline after sequence submission to GenBank. Although the G+C mol% of the four genomes were slightly lower than the values acquired by high-pressure liquid chromatography, they were well within the values expected for genomes from the related genus *Bacillus* (Table 1).

Accession number(s). The assembled and annotated genome sequences were deposited in GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

We thank Stephen Eyre's group at the Institute of Inflammation and Repair at the University of Manchester for providing the Illumina MiSeq platform to perform the DNA library sequencing.

Funding from the European Community H2020 program "MIND" under "Euratom 2014–2015" and the call "NFRP-06-2014: Supporting the implementation of the first-of-a-kind geological repositories" is gratefully acknowledged, alongside funding from the NERC BIGRAD consortium under the UK Natural Environmental Research Council (NE/H007768/1).

REFERENCES

- Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS. 1998. *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30. <https://doi.org/10.1007/s002030050673>.
- Sorokin ID, Kravchenko IK, Tourova TP, Kolganova TV, Boulygina ES, Sorokin DY. 2008. *Bacillus alkalidiazotrophicus* sp. nov., a diazotrophic, low salt-tolerant alkaliphile isolated from Mongolian soda soil. Int J Syst Evol Microbiol 58:2459–2464. <https://doi.org/10.1099/ijs.0.65655-0>.
- Santini JM, Streimann ICA, vanden Hoven RN. 2004. *Bacillus macyae* sp. nov., an arsenate-respiring bacterium isolated from an Australian gold mine. Int J Syst Evol Microbiol 54:2241–2244. <https://doi.org/10.1099/ijs.0.63059-0>.
- Zavarzina DG, Tourova TP, Kolganova TV, Boulygina ES, Zhilina TN. 2009. Description of *Anaerobacillus alkalilacustris* gen. nov., sp. nov.—strictly anaerobic diazotrophic *Bacillus* isolated from soda lake and transfer of *Bacillus arseniciselenatis*, *Bacillus macyae*, and *Bacillus alkalidiazotrophicus* to *Anaerobacillus* as the new combinations *A. arseniciselenatis* comb. nov., *A. macyae* comb. nov., and *A. alkalidiazotrophicus* comb. nov. Microbiology 78:723–731. <https://doi.org/10.1134/S0026261709060095>.
- Wang JP, Liu B, Liu GH, Ge CB, Chen QQ, Zhu YJ, Chen Z. 2015. Genome sequence of *Anaerobacillus macyae* JMM-4^T (DSM 16346), the first genomic information of the newly established genus *Anaerobacillus*. Genome Announc 3(4):e00922-15. <https://doi.org/10.1128/genomeA.00922-15>.
- Glaus MA, van Loon LR, Achatz S, Chodura A, Fischer K. 1999. Degradation of cellulosic materials under the alkaline conditions of a cementitious repository for low and intermediate level radioactive waste. Part I: degradation products. Anal Chim Acta 398:111–122. [https://doi.org/10.1016/S0003-2670\(99\)00371-2](https://doi.org/10.1016/S0003-2670(99)00371-2).
- Keith-Roach MJ. 2008. The speciation, stability, solubility and biodegradation of organic co-contaminant radionuclide complexes: a review. Sci Total Environ 396:1–11. <https://doi.org/10.1016/j.scitotenv.2008.02.030>.
- Bassil NM, Bryan N, Lloyd JR. 2015. Microbial degradation of isosaccharinic acid at high pH. ISME J 9:310–320. <https://doi.org/10.1038/ismej.2014.125>.
- 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>.
- Lo CC, Chain PSG. 2014. Rapid evaluation and quality control of next generation sequencing data with FaQCs. BMC Bioinformatics 15:366. <https://doi.org/10.1186/s12859-014-0366-2>.
- Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. <https://doi.org/10.1093/bioinformatics/btu661>.
- Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaScience 1:18. <https://doi.org/10.1186/2047-217X-1-18>.
- Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. <https://doi.org/10.1101/gr.089532.108>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Lin SH, Liao YC. 2013. CISA: contig integrator for sequence assembly of bacterial genomes. PLoS One 8:e60843. <https://doi.org/10.1371/journal.pone.0060843>.
- Silva GG, Dutilh BE, Matthews TD, Elkins K, Schmieder R, Dinsdale EA, Edwards RA. 2013. Combining de novo and reference-guided assembly with scaffold_builder. Source Code Biol Med 8:23. <https://doi.org/10.1186/1751-0473-8-23>.