





Complete Genome Sequence of *Bacillus altitudinis* Type Strain SGAir0031 Isolated from Tropical Air Collected in Singapore

Vineeth Kodengil Vettath,^a  Ana Carolina M. Junqueira,^{a,*} Akira Uchida,^a Rikky W. Purbojati,^a James N. I. Houghton,^a Caroline Chénard,^b Daniela I. Drautz-Moses,^a Anthony Wong,^a Sandra Kolundžija,^a Megan E. Clare,^a Kenny J. X. Lau,^a Nicolas E. Gaultier,^a Cassie E. Heinle,^a Balakrishnan N. V. Premkrishnan,^a Elena S. Gusareva,^a Enzo Acerbi,^a  Liang Yang,^a Stephan C. Schuster^a

Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore^a; Asian School of the Environment, Nanyang Technological University, Singapore^b

ABSTRACT *Bacillus altitudinis* strain SGAir0031 (*Firmicutes*) was isolated from tropical air samples collected in Singapore. Its genome was assembled using short reads and single-molecule real-time sequencing, comprising one chromosome with 3.81 Mb and one plasmid with 32 kb. The genome consists of 3,820 protein-coding genes, 81 tRNAs, and 24 rRNAs.

Bacillus altitudinis is a Gram-positive, rod-shaped aerobic bacterium classified in the phylum *Firmicutes*. It was first reported to be isolated from extreme UV-stressed air samples collected in the stratosphere (1). Since then, *B. altitudinis* has been reported in diverse habitats, including the southern Indian Ocean (2), deep freshwater of Manasbal Lake (3), soil (4), and silt (5).

B. altitudinis strain SGAir0031 was isolated from an air sample collected in Singapore (global position system [GPS] coordinates 1.346N, 103.680E) using the Andersen single-stage impactor (SKC BioStage) with a median cutoff diameter of 0.6 μm . The air was impacted onto Trypticase soy agar (TSA) (Becton Dickinson), and further isolation of colonies was carried out by culturing on TSA at 30°C and in Luria-Bertani (LB) broth overnight. Genomic DNA was purified using the Wizard genomic DNA purification kit (Promega), according to the manufacturer's protocol, with an additional RNase digestion followed by phenol-chloroform cleanup and a final precipitation with isopropanol. Single-molecule real-time (SMRT) sequencing was performed on a PacBio RSII (Pacific Biosciences), using a concentration of 0.15 nM of the 8-kb genomic library loaded into one SMRT cell (Pacific Biosciences). Short reads were generated on a MiSeq (Illumina) 300-bp paired-end run using whole-genome shotgun libraries constructed with the TruSeq Nano DNA library preparation kit.

A total of 110,325 subreads were used for *de novo* assembly with Hierarchical Genome Assembly Process (HGAP) version 3 (6) implemented in the PacBio SMRT Analysis 2.3.0 package. The assembly was polished using Quiver (6) and error corrected using Pilon version 1.16 (7) and 836,357 MiSeq paired-end reads. The consensus assembly generated two contigs, one chromosome with 3,812,576 bp (173.78-fold coverage) and one plasmid with 32,110 bp (244.41-fold coverage). The chromosomal contig showed a mean G+C content of 41.4%, while the plasmid pSGAir0031 had 37.5% G+C content. Both the genome size and G+C content were similar to those of other *B. altitudinis* assemblies available.

Received 6 October 2017 Accepted 10 October 2017 Published 9 November 2017

Citation Vettath VK, Junqueira ACM, Uchida A, Purbojati RW, Houghton JN, Chénard C, Drautz-Moses DI, Wong A, Kolundžija S, Clare ME, Lau KJX, Gaultier NE, Heinle CE, Premkrishnan BNV, Gusareva ES, Acerbi E, Yang L, Schuster SC. 2017. Complete genome sequence of *Bacillus altitudinis* type strain SGAir0031 isolated from tropical air collected in Singapore. *Genome Announc* 5:e01260-17. <https://doi.org/10.1128/genomeA.01260-17>.

Copyright © 2017 Vettath 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/).

Address correspondence to Stephan C. Schuster, scschuster@ntu.edu.sg.

* Present address: Ana Carolina M. Junqueira, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

V.K.V. and A.C.M.J. contributed equally to this work.

Taxonomic identification at the species level was performed using Phyla-AMPHORA (8). A total of 152 *Firmicutes*-specific marker genes were matched out of 168. In addition, average nucleotide identity (ANI) analysis, performed with MiSI (Microbial Species Identifier) (9), showed 98.81% identity to *B. altitudinis* strain DSM 26896.

The genome was annotated using NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) version 4.2 (10). A total of 3,982 genes were predicted with 3,820 protein-coding genes (PCGs), 24 rRNA operons (5S, 16S, and 23S rRNAs), 81 tRNAs, 5 noncoding RNAs, and 52 pseudogenes. The average G+C content of the plasmid pSGAir0031 is 37.5%, with 33 PCGs and no tRNA or rRNA genes. Functional annotation performed with Rapid Annotations using Subsystems Technology (RAST) (11–13) showed that most genes were associated with carbohydrate metabolism (439 genes) and amino acid and derivative metabolism (436 genes). The strain SGAir0031 potentially forms spores, since 120 genes were found to be related to dormancy and sporulation. Sporulation could be a potential mechanism for the dispersal and survival of *B. altitudinis* in tropical air.

Accession number(s). The complete genome sequences of *Bacillus altitudinis* SGAir0031 and the plasmid pSGAir0031 have been deposited in DDBJ/EMBL/GenBank under the accession numbers [CP022319](#) and [CP022320](#), respectively.

ACKNOWLEDGMENT

The work was supported by Singapore Ministry of Education Academic Research Fund tier 3 grant (grant MOE2013-T3-1-013).

REFERENCES

- Shivaji S, Chaturvedi P, Suresh K, Reddy GS, Dutt CB, Wainwright M, Narlikar JV, Bhargava PM. 2006. *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov. and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. *Int J Syst Evol Microbiol* 56:1465–1473. <https://doi.org/10.1099/ijso.0.64029-0>.
- Halder U, Banerjee A, Chaudhry V, Varshney RK, Mantri S, Bhandopadhyay R. 2017. Draft genome report of *Bacillus altitudinis* SORB11, isolated from the Indian sector of the Southern Ocean. *Genome Announc* 5(23): e00339-17. <https://doi.org/10.1128/genomeA.00339-17>.
- Shafi S, Kamili AN, Shah MA, Bandh SA, Dar R. 2017. Dynamics of bacterial class *Bacilli* in the deepest valley lake of Kashmir-the Manasbal Lake. *Microb Pathog* 104:78–83. <https://doi.org/10.1016/j.micpath.2017.01.018>.
- Vijay Kumar E, Srijana M, Kiran Kumar K, Harikrishna N, Reddy G. 2011. A novel serine alkaline protease from *Bacillus altitudinis* GVC11 and its application as a dehairing agent. *Bioprocess Biosyst Eng* 34:403–409. <https://doi.org/10.1007/s00449-010-0483-x>.
- Mao S, Lu Z, Zhang C, Lu F, Bie X. 2013. Purification, characterization, and heterologous expression of a thermostable β -1,3-1,4-glucanase from *Bacillus altitudinis* YC-9. *Appl Biochem Biotechnol* 169:960–975. <https://doi.org/10.1007/s12010-012-0064-3>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Wang Z, Wu M. 2013. A phylum-level bacterial phylogenetic marker database. *Mol Biol Evol* 30:1258–1262. <https://doi.org/10.1093/molbev/mst059>.
- Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati A. 2015. Microbial species delineation using whole genome sequences. *Nucleic Acids Res* 43:6761–6771. <https://doi.org/10.1093/nar/gkv657>.
- Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.