

## Draft Genome Sequence of the Thermophilic Bacterium Anoxybacillus kamchatkensis G10

Sang-Jae Lee,<sup>a</sup> Yong-Jik Lee,<sup>b</sup> Naeun Ryu,<sup>c</sup> Seulki Park,<sup>c</sup> Haeyoung Jeong,<sup>b</sup> Sang Jun Lee,<sup>b</sup> Byoung-Chan Kim,<sup>d</sup> Dong-Woo Lee,<sup>a</sup> and Han-Seung Lee<sup>c</sup>

Division of Applied Biology and Chemistry, Kyungpook National University, Daegu, South Korea<sup>a</sup>; Systems and Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea<sup>b</sup>; Department of Bio-Food Materials, Silla University, Busan, South Korea<sup>c</sup>; and Biological Resource Center, KRIBB, Daejeon, South Korea<sup>d</sup>

Anoxybacillus kamchatkensis G10 is a spore-forming thermophilic bacterium isolated from a hot spring in Indonesia. Here, we report the draft genome sequence of *A. kamchatkensis* G10 that may reveal insights into aerobic/anaerobic metabolisms and carbon utilization in moderate thermophiles.

**G** ram-positive bacteria of the genus *Anoxybacillus* as facultative anaerobes are mainly isolated in hot springs, manure, and processed foods (5, 9, 11). The first-isolated *Anoxybacillus pushchinensis* K1<sup>T</sup> has been characterized as an obligate anaerobe (9). Subsequently, several *Anoxybacillus* strains were isolated (2, 4, 9, 11). However, *Anoxybacillus flavithermus*, *A. pushchinensis* K1<sup>T</sup> (8), *Anoxybacillus kamchatkensis* KG8<sup>T</sup> (5), and *Anoxybacillus rupiensis* DSM17127<sup>T</sup> (3) have been found to be aerobes. Moreover, their growth-optimal pH values are species dependent.

During the last decade, Anoxybacillus species grew either aerobically or anaerobically in different media and were found to be physiologically diverse with respect to growth-optimal temperatures and pH values (6). The facultative aerobe A. kamchatkensis JW/VK-KG4 (7) used various carbohydrates, including glucose, fructose, trehalose, and pectin, in aerobic conditions. Compared to other Anoxybacillus species, this bacterium also showed different growth phenotypes in terms of significantly broad pH optimum for growth, lack of catalase/oxidase activity, and lack of growth on starch/pectin/raffinose. In contrast, A. kamchatkensis KG8<sup>T</sup> was able to hydrolyze starch, but it was not able to utilize glucose for growth (5). Thus, such discrepancies in growth and carbon utilization led us to sequence the genome of A. kamchatkensis G10. The draft genome sequence of this strain will provide insight into the mechanism of metabolic and respiratory adaptation under various environments.

Genome sequencing was performed using an Illumina HiSeq 2000 system (101-nucleotide [nt] paired-end read sequencing from a 500-bp genomic library). Preprocessing of reads and de novo assembly were performed using CLC Genomics Workbench version 4.8. We assembled 29,122,382 reads (achieving ~990-fold coverage [2.83 Gb]) into 65 contigs over 200 bp. The total contig length, maximum contig size, average contig length, and N<sub>50</sub> were 2,858,657 bp, 547,056 bp, 43,979 bp, and 130,036 bp, respectively (G+C content, 41.35%). The draft genome sequence was subject to automatic annotation using the RAST server (1). A total of 3,045 putative protein-coding sequences were predicted, 52% of which were assigned a subsystem. While the 16S rRNA gene sequence of strain G10 showed high similarity (>99%) to those of A. kamchatkensis JW/VK-KG4 (7) and A. flavithermus DSM 2641 (9), RAST analysis suggested that A. flavithermus WK1 was actually the closest neighbor in terms of sequence similarity. ANI analysis using BLAST (10) showed that A. flavithermus WK1 was also

closest to strain G10 (88.3% sequence identity and 78.5% alignment when G10 draft sequences were used as the query).

Consequently, genes involved in carbohydrate metabolism, including sugar phosphotransferase systems for glucose, fructose, sucrose, and mannitol, were found. Genes involved in responses to oxygen, such as catalase, Mn-superoxide dismutase, Fe-superoxide dismutase, thiol peroxidase, and others, were also found. In contrast to *A. flavithermus*, strain G10 contains genes for nitrate/ nitrite reductases. However, it appears to lose a menaquinol-cytochrome *c* oxidoreductase found in *A. flavithermus*. Thus, genomebased understanding of such variable physiological phenotypes is of great importance and interest within thermophiles in terms of biodiversity, evolutionary ecology, and metabolic/respiratory adaptations.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number ALJT00000000. The version described in this paper is the first version, ALJT01000000.

## ACKNOWLEDGMENTS

This work was supported by grant 2010-0011707 from the National Research Foundation (NRF) of Korea, grant 311042-05-1-HD120 from the Korea Institute of Planning & Evaluation for Technology in Food, Agriculture, Forestry & Fisheries, and the KRIBB Innovative Research Program.

## REFERENCES

- 1. Aziz RK, et al. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- Belduz AO, Dulger S, Demirbag Z. 2003. Anoxybacillus gonensis sp. nov., a moderately thermophilic, xylose-utilizing, endospore-forming bacterium. Int. J. Syst. Evol. Microbiol. 53:1315–1320.
- Derekova A, Sjoholm C, Mandeva R, Kambourova M. 2007. Anoxybacillus rupiensis sp. nov., a novel thermophilic bacterium isolated from Rupi basin (Bulgaria). Extremophiles 11:577–583.

Received 27 September 2012 Accepted 28 September 2012 Address correspondence to Dong-Woo Lee, leehicam@knu.ac.kr, or Han-Seung Lee, hanslee@silla.ac.kr.

S.-J.L. and Y.-J.L. contributed equally to this work.

Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.01877-12

- 4. Dulger S, Demirbag Z, Belduz AO. 2004. *Anoxybacillus ayderensis* sp. nov. and *Anoxybacillus kestanbolensis* sp. nov. Int. J. Syst. Evol. Microbiol. 54:1499–1503.
- Gul-Guven R, Guven K, Poli A, Nicolaus B. 2008. Anoxybacillus kamchatkensis subsp. asaccharedens subsp. nov., a thermophilic bacterium isolated from a hot spring in Batman. J. Gen. Appl. Microbiol. 54:327–334.
- Heinen W, Lauwers AM, Mulders JW. 1982. Bacillus flavothermus, a newly isolated facultative thermophile. Antonie Van Leeuwenhoek 48: 265–272.
- Kevbrin VV, Zengler K, Lysenko AM, Wiegel J. 2005. Anoxybacillus kamchatkensis sp. nov., a novel thermophilic facultative aerobic bacterium with a broad pH optimum from the Geyser valley, Kamchatka. Extremophiles 9:391–398.
- Pikuta E, Cleland D, Tang J. 2003. Aerobic growth of Anoxybacillus pushchinoensis K1<sup>T</sup>: emended descriptions of A. pushchinoensis and the genus Anoxybacillus. Int. J. Syst. Evol. Microbiol. 53:1561–1562.
- Pikuta E, et al. 2000. Anoxybacillus pushchinensis gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of Anoxybacillus flavithermus comb. nov. Int. J. Syst. Evol. Microbiol. 50:2109–2117.
- Richter M, Rossello-Mora R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U. S. A. 106: 19126–19131.
- Saw JH, et al. 2008. Encapsulated in silica: genome, proteome and physiology of the thermophilic bacterium *Anoxybacillus flavithermus* WK1. Genome Biol. 9:R161.