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Genome Sequence of *Anoxybacillus geothermalis* Strain GSSed3, a Novel Thermophilic Endospore-Forming Species

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***Anoxybacillus geothermalis* strain GSSed3 is an endospore-forming thermophilic bacterium isolated from filter deposits in a geothermal site. This novel species has a larger genome size (7.2 Mb) than that of any other *Anoxybacillus* species, and it possesses genes that support its phenotypic metabolic characterization and suggest an intriguing link to metals.**

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From 2013 to date, 10 genome sequences of a total of 23 species that belong to the *Anoxybacillus* genus have been announced. GSSed3 (also known as ATCC BAA-2555) is the type strain of the novel species *Anoxybacillus geothermalis*, isolated in 2011 from above-ground filter deposits of the geothermal site in Gross Schönebeck, in the North German Basin (52°54'13.15"N 13°36'5.43"E) (S. Filippidou, M. Jaussi, N. Jeanneret, L. Roussel-Delif, T. Wunderlin, A. Vieth-Hillebrand, A. Vetter, P. Chain, S. Regenspurg, and P. Junier, unpublished data). All *Anoxybacillus* species are moderately thermophilic, and most of them have been isolated from geothermal sites. According to its 16S rRNA gene sequence, *A. geothermalis* strain GSSed3 is closely related to *Anoxybacillus rupiensis* (1), whose full genome sequence is not yet available. The genome of GSSed3 was sequenced and annotated, and its metabolic capabilities were revealed.

Genomic DNA was extracted from an overnight culture using the QIAamp DNA minikit (Qiagen GmbH, Germany). The draft genome of *A. geothermalis* strain GSSed3 was generated by the Los Alamos National Laboratory (LANL) Genome Science Group using Illumina (2) technology. For this genome, an Illumina short-insert paired-end library was constructed and sequenced, generating 21,691,466 reads totaling 2,191 Mbp. The Illumina draft data were assembled with Velvet version 1.2.08 (3). The estimated size of the genome is 7.2 Mb, and the final assembly is based on 1,330 Mbp of Illumina draft data, which provide 185× coverage of the genome. The genomic DNA G+C content is estimated to be 46.8 mol%. Genome annotation was performed using an Ergatis-based (4) workflow with minor manual curation and visualized with the Artemis genome browser and annotation tool (5).

The complete genome sequence contained 7,003 genes, 7 rRNAs (5S, 16S, and 23S), 72 tRNAs, and 2 noncoding RNAs (ncRNAs) predicted. This strain possesses a large genome compared to those of other *Anoxybacillus* species, whose genomes do not exceed 3.3 Mb. In addition to its size, the genome contains more than double the number of genes in other published ge-

nomes of this genus (6–12). Among the annotated genes, 143 genes encoding proteins related to sporulation and nine genes related to dipicolinic acid synthesis were found. GSSed3 was biochemically tested and found to hydrolyze urea and reduce nitrates to nitrites. Genome annotation supports these findings, since it revealed the presence of the loci *ureE* and *ureC* for urease activity (13) and two copies of a putative nitrate reductase. It assimilates mannitol as an alternative carbon source to glucose. Six proteins encoding mannitol transporters and dehydrogenases were found. Similarly, ribose ABC transporter permeases have been found, which explains the assimilation of ribose. Interestingly, this *Anoxybacillus* species is found to weakly assimilate xylan, and its genome contains genes for 1,4-β-xylanase, a typical characteristic of the genus *Geobacillus* (14) rarely observed in *Anoxybacillus* (Filippidou et al., unpublished data). Finally, binding proteins and transporters of copper, manganese, cadmium, iron, and zinc were found, as well as an arsenic resistance protein (ArsB). Copper oxidase and manganese catalase genes are also present.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [JYCG00000000](https://www.ncbi.nlm.nih.gov/nuccore/JYCG00000000). The version described here is JYCG00000000.1.

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REFERENCES

1. Derekova A, Sjöholm C, Mandeva R, Kambourova M. 2007. *Anoxybacillus rupiensis* sp. nov., a novel thermophilic bacterium isolated from Rupi basin (Bulgaria). *Extremophiles* 11:577–583. <http://dx.doi.org/10.1007/s00792-007-0071-4>.
2. Bennett S. 2004. *Solexa Ltd. Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
3. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read

- assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
4. Hemmerich C, Buechlein A, Podicheti R, Revanna KV, Dong Q. 2010. An Ergatis-based prokaryotic genome annotation Web server. *Bioinformatics* 26:1122–1124. <http://dx.doi.org/10.1093/bioinformatics/btq090>.
 5. Carver TJ, Rutherford KM, Berriman M, Rajandream M-A, Barrell BG, Parkhill J. 2005. ACT: the Artemis comparison tool. *Bioinformatics* 21:3422–3423. <http://dx.doi.org/10.1093/bioinformatics/bti553>.
 6. Rozanov AS, Bryanskaya AV, Kotenko AV, Malup TK, Peltek SE. 2014. Draft genome sequence of *Anoxybacillus flavithermus* strain 25, isolated from the Garga Hot Spring in the Barguzin Valley, Baikal Region, Russian Federation. *Genome Announc* 2(6):e01258-14. <http://dx.doi.org/10.1128/genomeA.01258-14>.
 7. Caspers MP, Boekhorst J, Abee T, Siezen RJ, Kort R. 2013. Complete genome sequence of *Anoxybacillus flavithermus* TNO-09.006, a thermophilic sporeformer associated with a dairy-processing environment. *Genome Announc* 1(1):e00010-13. <http://dx.doi.org/10.1128/genomeA.00010-13>.
 8. Patel BK. 2015. Draft genome sequence of *Anoxybacillus* strain BCO1, isolated from a thermophilic microbial mat colonizing the outflow of a bore well of the Great Artesian Basin of Australia. *Genome Announc* 3(1):e01547-14. <http://dx.doi.org/10.1128/genomeA.01547-14>.
 9. Tatusova T, Ciufu S, Fedorov B, O'Neill K, Tolstoy I. 2014. RefSeq microbial genomes database: new representation and annotation strategy. *Nucleic Acids Res* 42:D553–D559. <http://dx.doi.org/10.1093/nar/gkt1274>.
 10. Goh KM, Gan HM, Chan K-G, Chan GF, Shahar S, Chong CS, Kahar UM, Chai KP. 2014. Analysis of *Anoxybacillus* genomes from the aspects of lifestyle adaptations, prophage diversity, and carbohydrate metabolism. *PLoS One* 9:e90549. <http://dx.doi.org/10.1371/journal.pone.0090549>.
 11. Kahar UM, Chan K-G, Salleh MM, Hii SM, Goh KM. 2013. A high molecular-mass *Anoxybacillus* sp. SK3-4 amylopullulanase: characterization and its relationship in carbohydrate utilization. *Int J Mol Sci* 14:11302–11318. <http://dx.doi.org/10.3390/ijms140611302>.
 12. Matsutani M, Shirakihara Y, Imada K, Yakushi T, Matsushita K. 2013. Draft genome sequence of a thermophilic member of the *Bacillaceae*, *Anoxybacillus flavithermus* strain Kn10, isolated from the Kan-nawa Hot Spring in Japan. *Genome Announc* 1(3):e00311-13. <http://dx.doi.org/10.1128/genomeA.00311-13>.
 13. Kakinuma Y, Iida H, Sekizuka T, Taneike I, Takamiya S, Moore JE, Millar BC, Matsuda M. 2008. Molecular characterisation of urease genes from urease-positive thermophilic campylobacters (UPTC). *Br J Biomed Sci* 65:148–152.
 14. Zeigler DR. 2014. The *Geobacillus* paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet? *Microbiology* 160:1–11. <http://dx.doi.org/10.1099/mic.0.071696-0>.