

Draft Genome Sequences of Supercritical CO₂-Tolerant Bacteria *Bacillus subterraneus* MITOT1 and *Bacillus cereus* MIT0214

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We report draft genome sequences of *Bacillus subterraneus* MITOT1 and *Bacillus cereus* MIT0214 isolated through enrichment of samples from geologic sequestration sites in pressurized bioreactors containing a supercritical (sc) CO₂ headspace. Their genome sequences expand the phylogenetic range of sequenced bacilli and allow characterization of molecular mechanisms of scCO₂ tolerance.

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During geologic carbon sequestration (GCS), large quantities of CO₂ are captured, compressed to supercritical (sc) state, and injected underground. Whether microbial activities transform injected CO₂ is not well understood due to toxic effects of scCO₂ (1–5). Samples from GCS sites at Otway Basin, Australia and Frio-2, Texas, were used as inocula for serial enrichment cultures in bioreactors containing scCO₂, yielding strains *Bacillus subterraneus* MITOT1 and *Bacillus cereus* MIT0214, respectively (6). Tolerance of scCO₂ was confirmed by growth of spores in pure cultures and was time and inocula density dependent. To investigate mechanisms of growth under scCO₂, genomic DNA was sequenced.

MITOT1 was sequenced on the Illumina HiSeq 2000 platform (Beijing Genomics Institute). MIT0214 was sequenced on the Illumina GAIIx platform (MIT Biomicrocenter). Paired-end 100 bp reads were quality trimmed (removing 10 starting and 20 trailing bases) and assembled *de novo* with CLC Genomic Workbench with automatic k-mer sizes of 23 and 21, yielding 185 and 238 contigs of >500 bp, respectively. The draft genome of MITOT1 is 3.9 Mbp with 42.1% G+C content, while the MIT0214 draft genome is 5.6 Mbp with 34.9% G+C content. Annotation using the RAST server (7) predicted 4,021 (with 1,235 hypothetical) and 5,640 (with 1,399 hypothetical) coding sequences in MITOT1 and MIT0214.

Phylogenetic analysis of the 16S rRNA gene placed MITOT1 within a clade of bacilli isolated from diverse environments including deep subsurface, soil, manufacturing effluent, and fermented seafood (8–12), some of which are capable of anaerobic reduction of Fe(III), Mn(IV), Se(VI), and As(V) (8, 10). The closest relative by BLASTn of the 16S rRNA gene was *B. subterraneus* HWG-A11 (98.6% identity). The nearest genome sequenced strain (98.1% 16S rRNA identity) was *B. boroniphilus* DSM17376, isolated from boron-contaminated soil (13) and sharing 83.3% average nucleotide identity (ANI) (14) with 2,600 sequence homologs (>60% identity). RAST functional comparison of the MITOT1 and *B. boroniphilus* DSM17376 genomes with closely related bacilli (strain 1NLA3E, *B. infantis* NRRL B-14911,

B. megaterium DSM319, and *B. coagulans* 36D1) predicted multiple anaerobic respiratory reductases and terminal cytochrome C oxidases unique to MITOT1 and *B. boroniphilus*, pointing to diverse catabolic potential for this group (15, 16).

Strain MIT0214 was most similar to *B. cereus* ATCC 14579 by BLASTn of 16S rRNA (99.8% identity), sharing 98.5% ANI and 4,858 sequence homologs (>60% identity). *B. cereus* strains have been isolated from diverse environments, including strain Q1 (92.5% ANI; 4,617 sequence homologs) from an oil reservoir (17). Comparisons among genomes of MITOT1, MIT0214, and the closely related sequenced genomes did not reveal clear signatures associated with scCO₂ tolerance, which is unsurprising in light of recent observations that tolerance is widespread among bacilli (6). Availability of draft genome sequences for *B. subterraneus* MITOT1 and *B. cereus* MIT0214 from two GCS sites will facilitate future work targeting gene/protein expression to advance mechanistic insights into scCO₂ tolerance.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers [JXIQ00000000](https://www.ncbi.nlm.nih.gov/nuccore/JXIQ00000000) and [JXDH00000000](https://www.ncbi.nlm.nih.gov/nuccore/JXDH00000000). The versions described in this paper are the first versions.

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REFERENCES

- Hong S-I, Pyun Y-R. 1999. Inactivation kinetics of *Lactobacillus plantarum* by high pressure carbon dioxide. *J Food Sci* 64:728–733. <http://dx.doi.org/10.1111/j.1365-2621.1999.tb15120.x>.
- Spilimbergo S, Mantoan D, Quaranta A, Mea GD. 2009. Real-time

- monitoring of cell membrane modification during supercritical CO₂ pasteurization. *J Supercrit Fluids* 48:93–97. <http://dx.doi.org/10.1016/j.supflu.2008.07.023>.
3. Zhang J, Davis TA, Matthews MA, Drews MJ, LaBerge M, An YH. 2006. Sterilization using high-pressure carbon dioxide. *J Supercrit Fluids* 38: 354–372. <http://dx.doi.org/10.1016/j.supflu.2005.05.005>.
 4. Bertoloni G, Bertucco A, De Cian V, Parton T. 2006. A study on the inactivation of micro-organisms and enzymes by high pressure CO₂. *Bio-technol Bioeng* 95:155–160. <http://dx.doi.org/10.1002/bit.21006>.
 5. Tamburini S, Anesi A, Ferrentino G, Spilimbergo S, Guella G, Jousson O. 2014. Supercritical CO₂ induces marked changes in membrane phospholipids composition in *Escherichia coli* K12. *J Membr Biol* 247: 469–477. <http://dx.doi.org/10.1007/s00232-014-9653-0>.
 6. Peet KC, Freedman AJE, Hernandez HH, Britto V, Boreham C, Ajo-Franklin JB, Thompson JR. 13 February 2015. Microbial growth under supercritical CO₂. *Appl Environ Microbiol* 81. <http://dx.doi.org/10.1128/AEM.03162-14>.
 7. 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. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 8. Kanso S, Greene AC, Patel BK. 2002. *Bacillus subterraneus* sp. nov., an iron- and manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. *Int J Syst Evol Microbiol* 52:869–874. <http://dx.doi.org/10.1099/ijs.0.01842-0>.
 9. Deepa CK, Dastager SG, Pandey A. 2010. Plant growth-promoting activity in newly isolated *Bacillus thioparus* (NII-0902) from western Ghat forest, India. *World J Microbiol Biotechnol* 26:2277–2283. <http://dx.doi.org/10.1007/s11274-010-0418-3>.
 10. Yamamura S, Yamashita M, Fujimoto N, Kuroda M, Kashiwa M, Sei K, Fujita M, Ike M. 2007. *Bacillus selenatarsenatis* sp. nov., a selenate- and arsenate-reducing bacterium isolated from the effluent drain of a glass-manufacturing plant. *Int J Syst Evol Microbiol* 57:1060–1064. <http://dx.doi.org/10.1099/ijs.0.64667-0>.
 11. Ahmed I, Yokota A, Fujiwara T. 2007. A novel highly boron tolerant bacterium, *Bacillus boroniphilus* sp. nov., isolated from soil, that requires boron for its growth. *Extremophiles* 11:217–224. <http://dx.doi.org/10.1007/s00792-006-0027-0>.
 12. Yoon JH, Kang SS, Lee KC, Kho YH, Choi SH, Kang KH, Park YH. 2001. *Bacillus jeotgali* sp. nov., isolated from jeotgal, Korean traditional fermented seafood. *Int J Syst Evol Microbiol* 51:1087–1092. <http://dx.doi.org/10.1099/00207713-51-3-1087>.
 13. Çöl B, Özkeserli Z, Kumar D, Özdağ H. 2014. Genome sequence of the boron-tolerant and-requiring bacterium *Bacillus boroniphilus*. *Genome Announc* 2(1):e00935-13. <http://dx.doi.org/10.1128/genomeA.00935-13>.
 14. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57: 81–91. <http://dx.doi.org/10.1099/ijs.0.64483-0>.
 15. Lovley DR. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol Rev* 55:259–287. [http://dx.doi.org/10.1016/S0065-2911\(04\)49005-5](http://dx.doi.org/10.1016/S0065-2911(04)49005-5).
 16. Lovley DR, Chappelle FH. 1995. Deep subsurface microbial processes. *Rev Geophys* 33:365–381. <http://dx.doi.org/10.1029/95RG01305>.
 17. Xiong Z, Jiang Y, Qi D, Lu H, Yang F, Yang J, Chen L, Sun L, Xu X, Xue Y, Zhu Y, Jin Q. 2009. Complete genome sequence of the extremophilic *Bacillus cereus* strain Q1 with industrial applications. *J Bacteriol* 191: 1120–1121. <http://dx.doi.org/10.1128/JB.01629-08>.