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.

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 Froio–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 839 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 relative by BLASTn of the 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 and JXDH00000000. The versions described in this paper are the first versions.

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**REFERENCES**


