High-Quality Draft Genome Sequence of “Candidatus Methanoperedens sp.” Strain BLZ2, a Nitrate-Reducing Anaerobic Methane-Oxidizing Archaeon Enriched in an Anoxic Bioreactor

Stefanie Berger,a Jeroen Frank,a Paula Dalcin Martins,b Mike S. M. Jetten,a Cornelia U. Weltea

Department of Microbiology, Institute for Water and Wetland Research, Radboud University, Nijmegen, The Netherlands; Department of Microbiology, The Ohio State University, Columbus, Ohio, USA

ABSTRACT The high-quality draft genome of “Candidatus Methanoperedens sp.” strain BLZ2, a nitrate-reducing archaeon anaerobically oxidizing methane, is presented. The genome was obtained from an enrichment culture and measures 3.74 Mb. It harbors two nitrate reductase gene clusters, an ammonium-forming nitrite reductase, and the complete reverse methanogenesis pathway. Methane that escapes to the atmosphere acts as a potent greenhouse gas. Global methane emissions are mitigated by methanotrophs, which oxidize methane to CO2. “Candidatus Methanoperedens spp.” are unique methanotrophic archaea that can perform nitrate-dependent anaerobic oxidation of methane. A high-quality draft genome sequence of only 85 contigs from this archaeon is presented here.

We extracted DNA from a bioreactor highly enriched in “Ca. Methanoperedens sp.” strain BLZ2 using the cetyltrimethylammonium bromide (CTAB) method as described previously (1) and the Power soil DNA extraction kit (Mo Bio Laboratories, Uden, The Netherlands). To shear genomic DNA and add adapters in the same step (“tagmentation”), the Illumina Nextera XT library prep kit (Illumina, San Diego, CA, USA) was used. The library was normalized to 4 nM and sequenced with a MiSeq instrument (Illumina) using the 300 paired-end sequencing protocol. Sequencing data were quality trimmed, yielding 11.8 million paired-end reads with an average length of 268 bp. Reads were assembled de novo (CLC Genomics Workbench v9.5.2 [CLC bio, Aarhus, Denmark]; word size, 55; maximum bubble size, 5,000). Contigs were binned using MetaBAT (2), yielding a draft genome composed of 85 contigs \( N_{50} = 74.304 \) bp. Afterward, quality was assessed using CheckM (3). Annotation was done using Prokka (4) and also as previously described (5). Based on the presence of 228 specific marker genes, the draft genome was 99.35% complete and only 4.58% contaminated. The final sequence had a GC content of 40.3% and was composed of 3.74 Mb with 4,041 putative open reading frames.

The results of this study were largely in accordance with previous data (6, 7). The 16S rRNA gene in this genome was 100% identical to that found in “Ca. Methanoperedens” BLZ1 and 95% identical to “Ca. Methanoperedens nitroreducens” ANME2d. All genes necessary for reverse methanogenesis were present in the genome, including mer (encoding \( F_{420} \)-dependent 5,10-methenyl-H\(_4\)MPT reductase).

Electron carriers reduced during reverse methanogenesis include coenzyme B (CoB), coenzyme M (CoM), \( F_{420} \), and ferredoxin (Fd). It has been suggested that HdrABC/FrhB catalyzes the confurcation of electrons from CoM-SH, CoB-SH, and \( F_{\text{red}} \) to \( F_{420} \) (7). Interestingly, in the present data set, four copies of \( hdra \) were identified, two of which...
were fused with mvhD (see also reference 8). Furthermore, a gene cluster was identified that contains hdrABC as well as frhB and two copies of mvhD, with mvhD possibly being involved in electron transfer.

Membrane-bound enzymes included Ech hydrogenase (echABCEF missing echD) and the membrane-bound heterodisulfide reductase (hdrDE). Furthermore, the complete \( \text{F}_{420} \text{quinone oxidoreductase (Fqo)} \), a Rieske/cyt\( \text{c} \) complex, and an ammonium-forming cytochrome \( \text{c} \) nitrite reductase (nrt\( \text{AH} \)) were present. Nitrate reductase was present as canonical (NarGHI) and noncanonical (NarGH, potential membrane anchors HCOIIa, HCOIIb, NapH, cytochrome subunit Orf7 and chaperone NarJ, being the similarity to “Ca. Methanoperedens” BLZ1 between 92 and 100%) versions, both encoded in two clusters present in close proximity to each other on the same 129 kb-long contig.

**Accession number(s)**. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NTMG00000000. The version described in this paper is version NTMG01000000.

**ACKNOWLEDGMENTS**

This work was financially supported by the Netherlands Organization for Scientific Research through the SIAM Gravitation grant 024.002.002. S.B. and C.U.W. were supported by ALWOP.293. M.S.M.J. was furthermore supported by the ERC AG EcoMoM 339880 and the NESSC Gravitation grant 024.002.001.

We thank Theo van Alen for technical assistance.

**REFERENCES**