

Draft Genome Sequence of an Anaerobic Ammonium-Oxidizing Bacterium, “*Candidatus Brocadia sinica*”

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A draft genome sequence of an anaerobic ammonium-oxidizing (anammox) bacterium, “*Candidatus Brocadia sinica*,” was determined by pyrosequencing and by screening a fosmid library. A 4.07-Mb genome sequence comprising 3 contigs was assembled, in which 3,912 gene-coding regions, 47 tRNAs, and a single *rrn* operon were annotated.

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Anaerobic ammonium oxidation (anammox) is a microbial process in which NH_4^+ and NO_2^- are converted to N_2 gas via N_2H_4 (1). The anammox process is mediated by monophyletic bacteria deeply branched in the phylum *Planctomycetes* (2). Anammox bacteria are affiliated with the order *Brocadiales*, which contains five candidate genera: “*Candidatus Kuenenia*,” “*Candidatus Brocadia*,” “*Candidatus Anammoxoglobus*,” “*Candidatus Jettenia*,” and “*Candidatus Scalindua*” (3). Draft genome sequences have been determined from anammox bacteria affiliated with “*Ca. Kuenenia*” (4, 5), “*Ca. Jettenia*” (6, 7), and “*Ca. Scalindua*” (8, 9). As for “*Ca. Brocadia*,” the draft genome sequence of “*Ca. Brocadia fulgida*” was previously determined (10); the genome comprised 2,786 contigs, in which large parts of the genes were fragmented.

We had initially enriched an anammox bacterium affiliated with “*Ca. Brocadia*,” namely, “*Ca. Brocadia sinica*,” from activated sludge by using a continuous up-flow column reactor (11). “*Ca. Brocadia sinica*” cells were further enriched in a membrane bioreactor (>94% of the total biomass as determined by fluorescence *in situ* hybridization analysis) (12). This anammox bacterium shares only 94% 16S rRNA gene sequence similarity with “*Ca. Brocadia fulgida*” and has a relatively high growth rate (i.e., $\mu_{\text{max}} = 0.0041 \text{ h}^{-1}$) (13). The objective of this study was to determine a high-quality “*Ca. Brocadia sinica*” genome by metagenomic sequencing and to provide a genetic platform for this anammox bacterium.

Genomic DNA of “*Ca. Brocadia sinica*” was extracted using a Genomic-tip 500/G kit (Qiagen KK, Japan, Tokyo Japan) and subjected to single and 3-kb paired-end pyrosequencing using a GS FLX titanium sequencer (Roche Diagnostics KK, Tokyo, Japan). The single and 3-kb paired-end pyrosequencing produced 628,274 (total 215,259,727 bp) and 576,054 reads (189,603,440 bp), respectively, which were assembled into 18 scaffolds using GS *De Novo* Assembler version 2.6 software (Roche Diagnostics KK, Tokyo, Japan). A 35-kb insert fosmid library was constructed using the genomic DNA and pCC1FOS vector (Illumina, Madison, WI, USA), and 1,632 clones were screened by PCR amplification. The PCR

products covering the regions of 15 missing links were obtained, and the gaps in the scaffolds were filled by standard primer-walking and Sanger sequencing techniques (14). A 4.07-Mb genome sequence comprising 3 contigs (42.41% G+C content) was obtained, and 3,912 gene-coding regions, 47 tRNAs, and a single *rrn* operon were annotated. Completeness was estimated to be 98.3% based on the presence/absence of 180 core genes (15).

The “*Ca. Brocadia sinica*” genome encodes gene clusters for core anammox metabolism, including 10 and 2 copies of *hao-hdh* and *hzs* encoding hydroxylamine dehydrogenase/hydrazine dehydrogenase and hydrazine synthase, respectively. Conversely, *nirS* and *nirK* encoding cytochrome *cd*₁-type or copper-containing NO-forming nitrite reductase were missing in the “*Ca. Brocadia sinica*” genome as well as the “*Ca. Brocadia fulgida*” genome (10), suggesting that “*Ca. Brocadia*” cells employ an unidentified nitrite reductase for NO_2^- reduction in the anammox process, which should be further investigated.

Nucleotide sequence accession numbers. The “*Ca. Brocadia sinica*” genome was deposited in the DDBJ nucleic acid sequence database under the accession numbers [BAFN01000001](https://www.ncbi.nlm.nih.gov/nuccore/BAFN01000001) through [BAFN01000003](https://www.ncbi.nlm.nih.gov/nuccore/BAFN01000003).

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