

Complete Genome Sequence of the Denitrifying and N₂O-Reducing Bacterium *Pseudogulbenkiania* sp. Strain NH8B

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***Pseudogulbenkiania* sp. strain NH8B is a *Neisseriales* bacterium isolated from an agricultural field. This strain has strong denitrification and N₂O reduction activities. Here, we report the finished and annotated genome sequence of this organism.**

Pseudogulbenkiania sp. strain NH8B belongs to the order *Neisseriales* (*Betaproteobacteria*) and was originally isolated from an agricultural field in Niigata, Japan, where paddy rice and soybean were rotationally cultivated every 2 years (7). This strain has strong denitrifying as well as N₂O-reducing activities. When inoculated into fresh agricultural soils, this strain can reduce exogenous N₂O; therefore, it can be used to mitigate N₂O emission from agricultural fields. The N₂O reductase gene (*nosZ*) sequence could not be amplified by PCR, most likely due to a primer mismatch. Sequencing of the genome of this strain was conducted to obtain functional gene information as well as to identify other genomic features.

Pseudogulbenkiania sp. strain NH8B was grown under denitrifying conditions, and genomic DNA was extracted as described previously (7). The genome was sequenced using a combination of 3-kb and 10-kb Sanger libraries and Roche 454 pyrosequencing. We generated 33,624 and 12,554 reads from 3-kb and 10-kb insert libraries, respectively, by using ABI 3730xl sequencers (Applied Biosystems, Foster City, CA). A quarter plate processed using a Roche 454 GS FLX Titanium system generated 93,777,204-bp high-quality sequences (mean read length, 442 bp), providing approximately 22-fold genome coverage. The 454 pyrosequencing reads were first assembled using Newbler assembler software. A hybrid assembly of 454 and Sanger reads was performed using Phred/Phrap/Consed software (1). Remaining gaps between contigs were closed by direct sequencing of clones. The overall accuracy of the finished sequence was estimated to have an error rate of <1 per 10,000 bases (Phrap score of ≥ 40). Protein-coding sequences (CDSs) were predicted using MetaGeneAnnotator software (5) and annotated as described previously (6). tRNA and rRNA genes were predicted using tRNAscan-SE (3) and Rnammer (2) software, respectively.

Pseudogulbenkiania sp. strain NH8B has a circular chromosomal genome of 4,332,995 bp with an average GC content of

64.4% and no plasmids. The absence of plasmids was confirmed by pulse-field gel electrophoresis (PFGE). The genome comprises 4,015 predicted CDS, 88 tRNAs, and eight copies of rRNA genes. There are 3,201 (79.7%) CDS with predicted functions, 997 of which were assigned KO numbers (Kyoto Encyclopedia of Genes and Genomes Orthology). The genome has two complete (>40-kb) prophage regions, and their sequences were almost identical. The genome also has clustered regularly interspaced short palindromic repeats (CRISPR), which function as a mechanism of resistance to exogenous genetic elements (4).

The complete sets of functional denitrification genes were identified on the genome of the NH8B strain. Unlike other *Neisseriales* bacteria (e.g., *Neisseria* spp. and *Chromobacterium* spp.), this strain possesses a cytochrome *cd*₁-containing nitrite reductase gene (*nirS*). The N₂O reductase gene operon was also identified but was distantly located on the genome from the other functional denitrification genes. Genes encoding nitrogen fixation were not found. The genome contained genes encoding complete glycolysis, tricarboxylic acid (TCA) cycle, and pentose phosphate pathways, as well as a benzoate degradation pathway. These results suggested that strain NH8B may have adapted to soil environments, such as rice paddy fields, where nitrogen oxide and various C compounds are available.

Nucleotide sequence accession number. The sequence data for *Pseudogulbenkiania* sp. strain NH8B have been deposited in DDBJ/EMBL/GenBank databases under accession number AP012224.

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