Genome Sequence of *Nitrosomonas* sp. Strain AL212, an Ammonia-Oxidizing Bacterium Sensitive to High Levels of Ammonia


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Received 10 June 2011/Accepted 29 June 2011

*Nitrosomonas* sp. strain AL212 is an obligate chemolithotrophic ammonia-oxidizing bacterium (AOB) that was originally isolated in 1997 by Yuichi Suwa and colleagues. This organism belongs to *Nitrosomonas* cluster 6A, which is characterized by sensitivity to high ammonia concentrations, higher substrate affinity (lower *Km*), and lower maximum growth rates than strains in *Nitrosomonas* cluster 7, which includes *Nitrosomonas europaea* and *Nitrosomonas europa*. Genome-informed studies of this ammonia-sensitive cohort of AOB are needed, as these bacteria are found in freshwater environments, drinking water supplies, wastewater treatment systems, and soils worldwide.

*Nitrosomonas* sp. strain AL212 belongs to cluster 6A nitrosomonads (20), a group characterized by higher substrate affinity (low *Km*), lower maximum growth rates, and increased sensitivity to high ammonia/ammonium compared to *Nitrosomonas* strains in cluster 7 (13), including the genome-sequenced species *Nitrosomonas europaea* (4) and *Nitrosomonas europa* (18). These characteristics improve their ability to grow at low substrate concentrations (<1.0 mM), and this has been key to their isolation and enrichment (3, 19). Investigations into the molecular underpinnings of ammonia sensitivity in ammonia-oxidizing bacteria (AOB) continue, as related organisms are commonly detected in drinking water supply and wastewater treatment systems (16, 17), freshwater environments (2, 11), and soils (13) worldwide.

The *Nitrosomonas* sp. strain AL212 culture was revived from a frozen stock and cultivated, and genomic DNA (>60 μg) was isolated in the laboratory of Yuichi Suwa as previously described (20). *Nitrosomonas* sp. strain AL212 grew at 11 mM but was suppressed at 36 mM (NH₄)₂SO₄ (20). The draft genome of strain AL212 was generated at the DOE Joint Genome Institute using Illumina (1) and 454 (12) technologies. The Illumina GAii shotgun library produced 13,571,840 reads (488 Mb), which were assembled with VELVET, version 0.7.63 (21). The 454 Titanium standard and paired-end libraries produced 305,716 reads and 18,574 reads, respectively; data were assembled with Newbler, version 2.3. Reads were reassembled after computational shedding using parallel Phrap (version SPS-4.24; High Performance Software, LLC). Consed software (6–8) was used for finishing. Potential base errors and consensus quality were corrected using Illumina data and Polisher software (A. Lapidus, unpublished data). Possible misassemblies were corrected using gapResolution (C. Han, unpublished data), Dupfinisher (9), or sequencing of subcloned bridging PCR fragments. Gaps were closed by PCR or Bubble PCR primer walks (1,095 reactions) using Consed. The final assembly is based on 119 Mb of 454 data (34.6% coverage) and 486 Mb of Illumina data (140.8% coverage). The finished genome (5) consists of a chromosome (3.18 Mb) and two plasmids (92 kb and 64 kb), totaling 3,337,023 bp with a 44.7% GC content, and contains a single rRNA operon and a full complement of tRNA genes.

In silico analysis predicted 2,983 candidate protein-encoding gene models. The taxonomic distribution of top KEGG hits identified 2,172 with proteobacteria, with the highest single-organism hits being those for *Nitrosospira multiformis* (15). Previously reported or predicted genes encoding enzymes and proteins involved in ammonia and hydroxylamine catabolism and urea utilization were identified (10, 14, 19). Complete *amo* and *hao* gene clusters are present in three nearly identical copies on the chromosome. Two distinct gene clusters encoding the Calvin-Benson-Bassham cycle, including ribulose-bisphosphate carboxylase/oxygenase, were identified. Genes encoding inventory implicated in nitrogen oxide metabolism include copper nitrite reductase (*nirK*, singleton), nitric oxide reductase (*norCBQD*), several cytochromes (three *cytL* and *cytS*) and a NO-responsive regulator (*nrrS*). Genes encoding the nitric oxide reductase heme-copperoxidase (*norSY*) present in all other genome-sequenced AOB (17) were not found. Further sequence annotation and genome comparisons with other AOB are under way.

**Nucleotide sequence accession numbers.** The complete *Nitrosomonas* sp. strain AL212 genome sequence is available in GenBank under accession numbers NC_015223 (plasmid pNAL21201), NC_015221 (plasmid pNAL21202), and NC_015222 (chromosome). The technical support of Yoko Ohto and Yukie Kakiyama in the production of DNA is gratefully acknowledged.
This collaborative project was supported by NSF Research Coordination Network grant 0541797 from the National Science Foundation. Y. Suwa was supported by internal funds. J. M. Norton was supported by the Utah Agricultural Experiment Station, project number UTA00371. M.G. Klotz received incentive funds from the University of Louisville. L.Y. Stein was supported by a grant from NSERC. The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231.

REFERENCES

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Volume 193, no. 18, pages 5047–5048, 2011. Page 5047: The article byline should read as given above.