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

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***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 K_m), and lower maximum growth rates than strains in *Nitrosomonas* cluster 7, which includes *Nitrosomonas europaea* and *Nitrosomonas eutropha*. 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 K_m), 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 eutropha* (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 $(NH_4)_2SO_4$ (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 l0.7.63 (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 shredding 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 inventory of 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 (*nnrS*). 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).

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ERRATUM

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