JOURNAL OF BACTERIOLOGY, Sept. 2011, p. 5047–5048 Vol. 193, No. 18
0021-9193/11/$12.00 doi:10.1128/JB.05521-11

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

Suwa Yuichi,[1] Jeanette M. Norton,[2]* Annette Bollmann,[3] Martin G. Klotz,[4] Lisa Y. Stein,[5] Hendrikus J. Laanbroek,[6] Daniel J. Arp,[7] Lynne A. Goodwin,[8] Olga Chertkov,[8] Brittany Held,[8] David Bruce,[8] J. Chris Detter,[8] Janine C. Detter,[8] Roxanne Tapia,[8] and Cliff S. Han[8]

Chuo University, Tokyo, Japan[1]; Utah State University, Logan, Utah[2]; Miami University, Oxford, Ohio[3]; University of Louisville, Louisville, Kentucky[4]; University of Alberta, Edmonton, Alberta, Canada[5]; Netherlands Institute of Ecology, Wageningen, Netherlands[6]; Oregon State University, Corvallis, Oregon[7]; and DOE Joint Genome Institute, Los Alamos National Laboratories, Los Alamos, New Mexico[8]

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 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).

* Corresponding author. Mailing address: Utah State University, Department of Plants, Soils, and Climate, 4820 Old Main Hill, Logan, UT 84322. Phone (435) 797-2166. Fax: (435) 797-3376. E-mail: jeanette.norton@usu.edu.

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

1. **Bennett, S.** 2004. Solexa Ltd. Pharmacogenomics **5:**433–438.
2. **Bollmann, A., M. J. Bar-Gilissen, and H. J. Laanbroek.** 2002. Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria. Appl. Environ. Microbiol. **68:**4751–4757.
3. **Bollmann, A., and H. J. Laanbroek.** 2001. Continuous culture enrichments of ammonia-oxidizing bacteria at low ammonium concentrations. FEMS Microbiol. Ecol. **37:**211–221.
4. **Chain, P., et al.** 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J. Bacteriol. **185:**2759–2773.
5. **Chain, P. S. G., et al.** 2009. Genome project standards in a new era of sequencing. Science **326:**236–237.
6. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Res. **8:**186–194.
7. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res. **8:**175–185.
8. **Gordon, D., C. Abajian, and P. Green.** 1998. Consed: A graphical tool for sequence finishing. Genome Res. **8:**195–202.
9. **Han, C. S., and P. S. G. Chain.** 2006. Finishing repeat regions automatically with Dupfinisher, p. 141–146. *In* H. R. Arabnia and H. Valafar (ed.), Proceedings of the 2006 International Conference on Bioinformatics & Computational Biology. CSREA Press, Las Vegas, NV.
10. **Koper, T. E., A. F. El-Sheikh, J. M. Norton, and M. G. Klotz.** 2004. Urease-encoding genes in ammonia-oxidizing bacteria. Appl. Environ. Microbiol. **70:**2342–2348.
11. **Laanbroek, H. J., and A. Bollmann.** 2011. Nitrification in inland waters, p. 385–403. *In* B. B. Ward, M. G. Klotz, and D. J. Arp (ed.), Nitrification. ASM Press, Washington, DC.
12. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature **437:**376–380.
13. **Norton, J. M.** 2011. Diversity and environmental distribution of ammonia-oxidizing bacteria, p. 39–55. *In* B. B. Ward, M. G. Klotz, and D. J. Arp (ed.), Nitrification. ASM Press, Washington, DC.
14. **Norton, J. M., J. J. Alzerreca, Y. Suwa, and M. G. Klotz.** 2002. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. **177:**139–149.
15. **Norton, J. M., et al.** 2008. Complete genome sequence of *Nitrosospira multiformis*, an ammonia-oxidizing bacterium from the soil environment. Appl. Environ. Microbiol. **74:**3559–3572.
16. **Okabe, S., Y. Aoi, H. Satoh, and Y. Suwa.** 2011. Nitrification in wastewater treatment, p. 405–433. *In* B. B. Ward, M. G. Klotz, and D. J. Arp (ed.), Nitrification. ASM Press, Washington, DC.
17. **Regan, J. M., G. W. Harrington, H. Baribeau, R. De Leon, and D. R. Noguera.** 2003. Diversity of nitrifying bacteria in full-scale chloraminated distribution systems. Water Res. **37:**197–205.
18. **Stein, L. Y., et al.** 2007. Whole-genome analysis of the ammonia-oxidizing bacterium, Nitrosomonas eutropha C91: implications for niche adaptation. Environ. Microbiol. **9:**2993–3007.
19. **Suwa, Y., Y. Imamura, T. Suzuki, T. Tashiro, and Y. Urushigawa.** 1994. Ammonia-oxidizing bacteria with different sensitivities to $(NH_4)_2SO_4$ in activated sludges. Water Res. **28:**1523–1532.
20. **Suwa, Y., T. Sumino, and K. Noto.** 1997. Phylogenetic relationships of activated sludge isolates of ammonia oxidizers with different sensitivities to ammonium sulfate. J. Appl. Microbiol. **43:**373–379.
21. **Zerbino, D. R., and E. Birney.** 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. **18:**821–829.