

Complete Genome Sequence of *Nitrosomonas ureae* Strain Nm10, an Oligotrophic Group 6a Nitrosomonad

Jessica A. Kozlowski, K. Dimitri Kits, Lisa Y. Stein

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

The complete genome of *Nitrosomonas ureae* strain Nm10, a mesophilic betaproteobacterial ammonia oxidizer isolated from Mediterranean soils in Sardinia, Italy, is reported here. This genome represents a cluster 6a nitrosomonad.

Received 22 January 2016 Accepted 28 January 2016 Published 10 March 2016

Citation Kozlowski JA, Kits KD, Stein LY. 2016. Complete genome sequence of *Nitrosomonas ureae* strain Nm10, an oligotrophic group 6a nitrosomonad. *Genome Announc* 4(2):00094-16. doi:10.1128/genomeA.00094-16.

Copyright © 2016 Kozlowski et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Lisa Y. Stein, lisa.stein@ualberta.ca.

The isolation and genome sequencing of ammonia-oxidizing bacteria (AOB) remain vital to our understanding of the potential roles these organisms play in the global nitrogen cycle. To complement physiological studies on AOB, complete-genome sequences provide insight into how inventory relates to metabolic capacity and environmental niche. The AOB *Nitrosomonas ureae* Nm10 was first isolated from soils in Sardinia, Italy (1), and is an oligotrophic aerobic betaproteobacterium belonging to *Nitrosomonas* cluster 6a (2).

The genome of *N. ureae* was sequenced at the University of Washington, WA, using the PacBio RSII platform; 300,584 raw reads resulted in 166,852 quality-filtered trimmed reads yielding 1,340 Mb, with a mean genome-wide coverage of 311×. The filtered reads were assembled at the University of Alberta, Alberta, Canada, using HGAP version 2.3 (3), and resulted in a 1-contig scaffold. Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (4). The genome is 3.3 Mbp, with a mean G+C content of 44.5% and 2,897 predicted protein-coding genes. The genome includes 40 tRNA genes and a single copy of the 16S-23S-5S rRNA operon. Gene prediction analysis and comparative genomics were performed with IMG (5). The closest neighbor of *N. ureae* is *Nitrosomonas* sp. strain AL212 (6), with an average nucleotide identity (ANI) (7) of 93.18%.

N. ureae oxidizes ammonia to nitrite as a sole source of energy and reductant. The genome contains 3 operons for ammonia monooxygenase (*amoCAB*), two of which are followed by the *orf4* and *orf5* genes that are often found in β-AOB (8). Two orphan *amoC* genes were also identified, along with a single copy of the AOB-specific red-copper protein nitrosocyanin (9). It is important to note that this is the first report of an AOB containing four complete operons for hydroxylamine dehydrogenase (*haoABCycAB*), as betaproteobacterial AOB usually contain 2 or 3 copies, and one copy often lacks the *cycB* gene (8).

N. ureae can utilize urea as an alternate nitrogen source (1) and contains both urea carboxylase (EC 6.3.4.6) and a putative allophanate hydrolase (EC 3.5.1.54) genes (10), as well as genes for a complete urease found in some *Nitrosospira* genomes (11). Carbon fixation genes, including two copies of form I RubisCO-

encoding genes, were identified with similarity to those of *Nitrosomonas* sp. strain Is79 (12).

Terrestrial AOB can contribute to nitrogen-oxide release, including the production of nitric and nitrous oxide through nitrifier denitrification (13, 14). The genes in *N. ureae* that are implicated in this process include a copper-containing nitrite reductase (*nirK*), NO-responsive regulator *NnrS*, cytochrome P460 (*cytL*), and cytochrome *c'* beta (*cytS*). Interestingly, no homologues for nitric oxide reductases were found in the genome, a featured shared by the closely related 6a AOB *Nitrosomonas* sp. Is79 (12).

The *N. ureae* genes for iron acquisition and storage include one copy of the ferric uptake regulation protein (FUR) (15), a *Streptococcus*-like ferric iron ABC transporter (16), two copies of TonB-associated ferric siderophore transporters (17), and two copies of bacterioferritin genes. Two copies of cyanophycin synthetase genes, utilized for nitrogen storage (18), were also identified.

Nucleotide sequence accession numbers. The genome sequence has been deposited in GenBank under the accession no. CP013341. The version described in this paper is the first version, CP013341.1.

ACKNOWLEDGMENTS

Support for this research was provided by Alberta Innovates Technology Futures (to J.A.K. and K.D.K.) and by a discovery grant from the Natural Sciences and Engineering Research Council of Canada (to L.Y.S.). We also thank Andreas Pommerening-Röser at the University of Hamburg for providing cultures of *N. ureae* strain Nm10.

FUNDING INFORMATION

This work, including the efforts of Lisa Y. Stein, was funded by Gouvernement du Canada | Natural Sciences and Engineering Research Council of Canada (NSERC). This work, including the efforts of Jessica Kozlowski, was funded by Alberta Innovates - Technology Futures. This work, including the efforts of Kerim Dimitri Kits, was funded by Alberta Innovates - Technology Futures.

REFERENCES

1. Koops HP, Böttcher B, Möller UC, Pommerening-Röser A, Stehr G. 1991. Classification of eight new species of ammonia-oxidizing bacteria:

- Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. *J Gen Microbiol* 137:1689–1699.
2. Prosser JI, Head IM, Stein LY. 2014. The family *Nitrosomonadaceae*, p 901–918. *In* Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes*. Springer Verlag, Berlin Heidelberg, Heidelberg, Germany.
 3. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
 4. Tatusova T, Ciufu S, Federhen S, Fedorov B, McVeigh R, O'Neill K, Tolstoy I, Zaslavsky L. 2015. Update on RefSeq microbial genomes resources. *Nucleic Acids Res* 43:D599–D605. <http://dx.doi.org/10.1093/nar/gku1062>.
 5. Markowitz VM, Chen I-MA, Chu K, Szeto E, Palaniappan K, Pillay M, Ratner A, Huang J, Pagani I, Tringe S, Huntemann M, Billis K, Varghese N, Tennesen K, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC. 2014. IMG/M 4 version of the integrated metagenome comparative analysis system. *Nucleic Acids Res* 42:D568–D573. <http://dx.doi.org/10.1093/nar/gkt919>.
 6. Suwa Y, Yuichi S, Norton JM, Bollmann A, Klotz MG, Stein LY, Laanbroek HJ, Arp DJ, Goodwin LA, Chertkov O, Held B, Bruce D, Detter JC, Detter JC, Tapia R, Han CS. 2011. Genome sequence of *Nitrosomonas* sp. strain AL212, an ammonia-oxidizing bacterium sensitive to high levels of ammonia. *J Bacteriol* 193:5047–5048. <http://dx.doi.org/10.1128/JB.05521-11>.
 7. Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 102:2567–2572. <http://dx.doi.org/10.1073/pnas.0409727102>.
 8. Arp DJ, Chain PS, Klotz MG. 2007. The impact of genome analyses on our understanding of ammonia-oxidizing bacteria. *Annu Rev Microbiol* 61:503–528. <http://dx.doi.org/10.1146/annurev.micro.61.080706.093449>.
 9. Klotz MG, Stein LY. 2011. Genomics of ammonia-oxidizing bacteria and insights to their evolution, p 57–93. *In* Ward BB, Arp DJ, Klotz MG (ed), *Nitrification*. ASM Press, Washington, DC.
 10. Hausinger RP. 2004. Metabolic versatility of prokaryotes for urea decomposition. *J Bacteriol* 186:2520–2522. <http://dx.doi.org/10.1128/JB.186.9.2520-2522.2004>.
 11. Norton JM, Klotz MG, Stein LY, Arp DJ, Bottomley PJ, Chain PSG, Hauser LJ, Land ML, Larimer FW, Shin MW, Starkenburg SR. 2008. Complete genome sequence of *Nitrosospira multififormis*, an ammonia-oxidizing bacterium from the soil environment. *Appl Environ Microbiol* 74:3559–3572. <http://dx.doi.org/10.1128/AEM.02722-07>.
 12. Bollmann A, Sedlacek CJ, Norton J, Laanbroek HJ, Suwa Y, Stein LY, Klotz MG, Arp D, Sayavedra-Soto L, Lu M, Bruce D, Detter C, Tapia R, Han J, Woyke T, Lucas SM, Pitluck S, Pennacchio L, Nolan M, Land ML, Huntemann M, Deshpande S, Han C, Chen A, Kyrpides N, Mavromatis K, Markowitz V, Szeto E, Ivanova N, Mikhailova N, Pagani I, Pati A, Peters L, Ovchinnikova G, Goodwin L. 2013. Complete genome sequence of *Nitrosomonas* sp. Is79—an ammonia oxidizing bacterium adapted to low ammonium concentrations. *Stand Genomic Sci* 7:469–482. <http://dx.doi.org/10.4056/signs.3517166>.
 13. Stein LY. 2011. Heterotrophic nitrification and nitrifier denitrification, p 95–114. *In* Ward BB, Arp DJ, Klotz MG (ed), *Nitrification*. ASM Press, Washington, DC.
 14. Kozłowski JA, Price J, Stein LY. 2014. Revision of N₂O-producing pathways in the ammonia-oxidizing bacterium, *Nitrosomonas europaea* ATCC 19718. *Appl Environ Microbiol* 80:4930–4935. <http://dx.doi.org/10.1128/AEM.01061-14>.
 15. Fillat MF. 2014. The FUR (ferric uptake regulator) superfamily: diversity and versatility of key transcriptional regulators. *Arch Biochem Biophys* 546:41–52. <http://dx.doi.org/10.1016/j.abb.2014.01.029>.
 16. Chain P, Lamerdin J, Larimer F, Regala W, Lao V, Land M, Hauser L, Hooper A, Klotz M, Norton J, Sayavedra-Soto L, Arciero D, Hommes N, Whittaker M, Arp D. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. *J Bacteriol* 185:2759–2773. <http://dx.doi.org/10.1128/JB.185.9.2759-2773.2003>.
 17. Moeck GS, Coulton JW. 1998. TonB-dependent iron acquisition: mechanisms of siderophore-mediated active transport. *Mol Microbiol* 28:675–681.
 18. Obst M, Steinbüchel A. 2006. Cyanophycin—an ideal bacterial nitrogen storage material with unique chemical properties, p 167–193. *In* Shively JM (ed), *Inclusions in prokaryotes*. Springer Verlag, Berlin Heidelberg, Heidelberg, Germany.