AMERICAN SOCIETY FOR MICROBIOLOGY

genomeAnnouncements™

Draft Genome Sequence of *Nitrobacter vulgaris* Strain Ab₁, a Nitrite-Oxidizing Bacterium

Brett L. Mellbye,[a] Edward W. Davis II,[a,b] Eva Spieck,[c] Jeff H. Chang,[a,b]
Peter J. Bottomley,[d] Luis A. Sayavedra-Soto[a]

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA[a]; Molecular and Cellular Biology Program, Oregon State University, Corvallis, Oregon, USA[b]; Biocenter Klein Flottbek, Department of Microbiology & Biotechnology, University of Hamburg, Hamburg, Germany[c]; Department of Microbiology, Oregon State University, Corvallis, Oregon, USA[d]

ABSTRACT   Here, we present the 3.9-Mb draft genome sequence of *Nitrobacter vulgaris* strain Ab₁, which was isolated from a sewage system in Hamburg, Germany. The analysis of its genome sequence will contribute to our knowledge of nitrite-oxidizing bacteria and acyl-homoserine lactone quorum sensing in nitrifying bacteria.

Aerobic nitrification is generally a two-step process where ammonia is oxidized to nitrite, which is subsequently oxidized to nitrate (1). The second step is carried out by nitrite-oxidizing bacteria (NOB) (2, 3). NOB include both *r*-strategists, such as *Nitrobacter* spp., and *K*-strategists, such as *Nitrospira* spp., which coexist in a variety of environments (2–4). *Nitrobacter* spp. play a role in the response to large nitrogen fluctuations in soils and other systems (5–7). In addition, *Nitrobacter* spp. were the first NOB shown to produce and respond to acyl-homoserine lactone (AHL) quorum-sensing (QS) chemical signals (8, 9). *Nitrobacter vulgaris* strain Ab₁ is a well-studied nitrifier, yet it has no available genome sequence (5, 10, 11). To address this need, we sequenced the genome of *Nitrobacter vulgaris* strain Ab₁. Our primary goal was to identify loci corresponding to AHL autoinducer synthase and AHL-binding LuxR transcription factors.

Genomic DNA was isolated using the Wizard genomic DNA purification kit (Promega). A Nextera XT DNA sample preparation kit was used to construct the sequencing library. The instructions were followed, up to those for normalization of libraries. A Qubit double-stranded DNA high-sensitivity assay kit (Life Technologies, Inc.) and Agilent TapeStation 4200 high-sensitivity D5000 DNA ScreenTape (Agilent Technologies) were used to determine the concentration and average sizes of the library fragments. The library was then quantified by quantitative PCR on an ABI 7500 Fast real-time system (Life Technologies, Inc.) using the Kapa library quantification kit (Kapa Biosystems). Sequencing was completed on a MiSeq (Illumina) 250-bp paired-end nano flow cell.

There was a total of 2,436,208 reads, for an average coverage of 156×. Nextera XT adapter sequences were trimmed from the raw reads using the BBDuk software, as recommended in the manual (http://jgi.doe.gov/data-and-tools/bbtools/). Reads were error-corrected and assembled into contigs using SPAdes version 3.10.0, with the "--careful" flag and the k-mer setting of "-k 21,33,55,77,99" (12), and screened for contaminating sequences with the blobtools software (version 0.9.19.5) (13, 14). *De novo* assembly of the MiSeq reads resulted in 95 contigs that totaled 3,900,573 nucleotides in length, with a mean contig size of 41,059 nucleotides; the N_{50} contig length was 130,999 nucleotides. Genome annotation was completed using the NCBI Prokaryotic Genome Annotation Pipeline, resulting in 3,501 coding genes and 56

RNA-coding genes (15). The *N. vulgaris* genome sequence is 59.8% G+C and has pairwise average nucleotide identities (16) of 83.0% and 81.2% to *Nitrobacter winogradskyi* and *Nitrobacter hamburgensis*, respectively (17, 18). These low values suggest that *N. vulgaris* is too distant from comparators to be considered a member of their species.

The *N. vulgaris* genome has all the genes necessary for chemolithotrophic growth on nitrite. Interestingly, genes encoding a putative AHL autoinducer synthase and AHL-binding LuxR homolog were present, as well as putative nitric-oxide-forming *nirK* (*aniA*) and *nnrS* genes, possibly suggesting similar QS regulation of NO fluxes to *N. winogradskyi* (9).

Accession number(s). The genome of *N. vulgaris* strain AB$_1$ was deposited at DDBJ/EMBL/GenBank under the accession number MWPQ00000000. The version described in this paper is the first version.

REFERENCES

1. Ward BB. 2011. Nitrification: an introduction and overview of the state of the field, p 3–8. *In* Ward BB, Arp DJ, Klotz MG (ed), Nitrification. ASM Press, Washington, DC.

2. Starkenburg SR, Spieck E, Bottomley PJ. 2011. Metabolism and genomics of nitrite-oxidizing bacteria: emphasis on studies of Pure cultures and of *Nitrobacter* species, p 267–293. *In* Ward BB, Arp DJ, Klotz MG (e), Nitrification. ASM Press, Washington, DC.

3. Daims H, Lucker S, Paslier DL, Wagner M. 2011. Diversity, environmental genomics, and ecophysiology of nitrite-oxidizing bacteria, p 295–322. *In* Ward BB, Arp DJ, Klotz MG (ed), Nitrification. ASM Press, Washington, DC.

4. Andrews JH, Harris RF. 1986. *R*-selection and *K*-selection and microbial ecology. Adv Microb Ecol 9:99–147. https://doi.org/10.1007/978-1-4757-0611-6_3.

5. Vanparys B, Spieck E, Heylen K, Wittebolle L, Geets J, Boon N, De Vos P. 2007. The phylogeny of the genus *Nitrobacter* based on comparative rep-PCR, 16S rRNA and nitrite oxidoreductase gene sequence analysis. Syst Appl Microbiol 30:297–308. https://doi.org/10.1016/j.syapm.2006.11.006.

6. Attard E, Poly F, Commeaux C, Laurent F, Terada A, Smets BF, Recous S, Roux XL. 2010. Shifts between *Nitrospira*- and *Nitrobacter*-like nitrite oxidizers underlie the response of soil potential nitrite oxidation to changes in tillage practices. Environ Microbiol 12:315–326. https://doi.org/10.1111/j.1462-2920.2009.02070.x.

7. Huang Z, Gedalanga PB, Asvapathanagul P, Olson BH. 2010. Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. Water Res 44:4351–4358. https://doi.org/10.1016/j.watres.2010.05.037.

8. Mellbye BL, Bottomley PJ, Sayavedra-Soto LA. 2015. Nitrite-oxidizing bacterium *Nitrobacter winogradskyi* produces *N*-acyl-homoserine lactone autoinducers. Appl Environ Microbiol 81:5917–5926. https://doi.org/10.1128/AEM.01103-15.

9. Mellbye BL, Giguere AT, Bottomley PJ, Sayavedra-Soto LA. 2016. Quorum quenching of *Nitrobacter winogradskyi* suggests that quorum sensing regulates fluxes of nitrogen oxide(s) during nitrification. mBio 7(5): e01753-16. https://doi.org/10.1128/mBio.01753-16.

10. Bock E, Koops HP, Möller UC, Rudert M. 1990. A new facultatively nitrite oxidizing bacterium, *Nitrobacter-vulgaris* sp.-nov. Arch Microbiol 153:105–110. https://doi.org/10.1007/BF00247805.

11. Nowka B, Daims H, Spieck E. 2015. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: nitrite availability as a key factor in niche differentiation. Appl Environ Microbiol 81:745–753. https://doi.org/10.1128/AEM.02734-14.

12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

13. Kumar S, Jones M, Koutsovoulos G, Clarke M, Blaxter M. 2013. Blobology: exploring raw genome data for contaminants, symbionts, and parasites using taxon-annotated GC-coverage plots. Front Genet 4:237. https://doi.org/10.3389/fgene.2013.00237.

14. Laetsch DR, Koutsovoulos G, Stajich J, Kumar S. 2016. DRL/blobtools: blobtools v 0.9.19.5. Zenodo. https://github.com/DRL/blobtools.

15. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. Omics 12:137–141. https://doi.org/10.1089/omi.2008.0017.

16. Davis EW, II, Weisberg AJ, Tabima JF, Grunwald NJ, Chang JH. 2016. Gall-ID: tools for genotyping gall-causing phytopathogenic bacteria. PeerJ 4:e2222. https://doi.org/10.7717/peerj.2222.

17. Starkenburg SR, Chain PS, Sayavedra-Soto LA, Hauser L, Land ML, Larimer FW, Malfatti SA, Klotz MG, Bottomley PJ, Arp DJ, Hickey WJ. 2006. Genome sequence of the chemolithoautotrophic nitrite-oxidizing bacterium *Nitrobacter winogradskyi* Nb-255. Appl Environ Microbiol 72:2050–2063. https://doi.org/10.1128/AEM.72.3.2050-2063.2006.

18. Starkenburg SR, Larimer FW, Stein LY, Klotz MG, Chain PS, Sayavedra-Soto LA, Poret-Peterson AT, Gentry ME, Arp DJ, Ward B, Bottomley PJ. 2008. Complete genome sequence of *Nitrobacter hamburgensis* X14 and comparative genomic analysis of species within the genus *Nitrobacter*. Appl Environ Microbiol 74:2852–2863. https://doi.org/10.1128/AEM.02311-07.