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Genome Sequences for Six *Rhodanobacter* Strains, Isolated from Soils and the Terrestrial Subsurface, with Variable Denitrification Capabilities

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We report the first genome sequences for six strains of *Rhodanobacter* species isolated from a variety of soil and subsurface environments. Three of these strains are capable of complete denitrification and three others are not. However, all six strains contain most of the genes required for the respiration of nitrate to gaseous nitrogen. The nondenitrifying members of the genus lack only the gene for nitrate reduction, the first step in the full denitrification pathway. The data suggest that the environmental role of bacteria from the genus *Rhodanobacter* should be reevaluated.

The genus *Rhodanobacter* contains 11 described species of Gram-negative, non-spore-forming, rod-shaped bacteria belonging to the family *Xanthomonadaceae* and the class *Gamma-proteobacteria* of the phylum *Proteobacteria*. Described species have been isolated mainly under aerobic conditions from surficial soils (1, 4, 5, 9, 12, 15, 16). Denitrification has not been considered a property of this genus. Recently, two strains of a new species, *Rhodanobacter denitrificans*, were isolated from a contaminated terrestrial subsurface environment and shown to denitrify (7, 13). Furthermore, nitrate-reducing isolates were recently recovered from sewage sludge (17), and we and others determined that *Rhodanobacter thiooxydans* is capable of denitrification (13, 14). In some acidic and nitrate-rich environments, *Rhodanobacter* species dominate bacterial communities (8, 14).

To explore the genetic basis of phenotypes leading to bacterial community dominance in such environments, genome sequences were acquired for three denitrifying strains (*R. denitrificans* 2APBS1 and 116-2 and *R. thiooxydans*) and three strains incapable of denitrification (*Rhodanobacter fulvus*, *Rhodanobacter spathiphylli*, and *Rhodanobacter* sp. 115). A complete *R. denitrificans* 2APBS1^T genome sequence was generated using paired-end Illumina and Roche 454 mate-pair sequencing and manual finishing steps, essentially as described previously (3, 6). Four draft genomes (*R. denitrificans* 116-2, *R. thiooxydans*, *R. fulvus*, and *R. spathiphylli*) were generated by *de novo* assembly of paired-end Illumina sequence data (~5.7 to 9.5 million paired-end reads/genome, yielding ~1.1 to 1.9 Gb of total output/genome) (CLC Genomics Workbench 5.0; CLC bio A/S, Denmark). DNA from each strain was prepared for sequencing using the Nextera library preparation kit (Epicentre, Madison, WI). DNA from *Rhodanobacter* sp. 115 was prepared for sequencing using the Ion Xpress fragment library kit (Life Technologies, Grand Island, NY) and sequenced using a Personal Genome Machine (Ion Torrent, San Francisco, CA), yielding approximately 1.4 Mb of reads (~138 Mb of total output). For *Rhodanobacter* sp. 115, genome assembly was performed as described previously (10) using CG-Pipeline modules (11), yielding 453 contigs and 4.2 Mb of genomic sequence data.

The complete genome of *R. denitrificans* 2APBS1 is 4.23 Mb. Annotation was performed in RAST (2) and in the CG-Pipeline before being submitted to NCBI.

Denitrification is a strain-specific trait, and the high sequence divergence observed in genetic markers for denitrification challenges our ability to understand the fundamental ecological principles and environmental parameters controlling nitrate attenuation in terrestrial environments (7). Thus, whole-genome sequencing of closely related denitrifying and nondenitrifying taxa is essential to improve detection of denitrifying bacteria in the environment and to develop hypotheses regarding the distribution and acquisition of denitrification genes. Comparative analysis of the six genomes revealed that all strains contained genes coding for complete or nearly complete denitrification pathways. The three nondenitrifying lineages lacked only genes for nitrate reduction. These organisms may still be capable of denitrification, however. Nitrate to nitrite reduction is a widespread physiological capability in the bacterial domain, and in complex environments, such as soil, nitrite will be available for organisms capable of nitrite reduction to gaseous nitrogen end products. These data indicate that the environmental role of bacteria from the genus *Rhodanobacter* should be reevaluated.

Nucleotide sequence accession numbers. The *Rhodanobacter* genome assemblies and their annotations were deposited in GenBank under the accession numbers [AGIL00000000](#) (DSM 23569), [AJXS00000000](#) (*Rhodanobacter* strain 115), [AJXT00000000](#) (DSM 17631), [AJXU00000000](#) (DSM 18449), [AJXV00000000](#) (DSM 24678), and [AJXW00000000](#) (DSM 18863).

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REFERENCES

1. An DS, Lee HG, Lee ST, Im WT. 2009. *Rhodanobacter ginsenosidimutans* sp. nov., isolated from soil of a ginseng field in South Korea. *Int. J. Syst. Evol. Microbiol.* 59:691–694.
2. Aziz RK, et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
3. Bennett, S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438.
4. Bui TP, Kim YJ, Kim H, Yang DC. 2010. *Rhodanobacter soli* sp. nov., isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol.* 60:2935–2939.
5. De Clercq D, et al. 2006. *Rhodanobacter spathiphylli* sp. nov., a gamma-proteobacterium isolated from the roots of *Spathiphyllum* plants grown in a compost-amended potting mix. *Int. J. Syst. Evol. Microbiol.* 56:1755–1759.
6. Elkins JG, et al. 2010. Complete genome sequence of the cellulolytic thermophile *Caldicellulosiruptor obsidiansis* OB47T. *J. Bacteriol.* 192:6099–6100.
7. Green SJ, et al. 2010. Denitrifying bacteria isolated from terrestrial subsurface sediments exposed to mixed-waste contamination. *Appl. Environ. Microbiol.* 76:3244–3254.
8. Green SJ, et al. 2012. Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. *Appl. Environ. Microbiol.* 78:1039–1047.
9. Im WT, Lee ST, Yokota A. 2004. *Rhodanobacter fulvus* sp. nov., a β -galactosidase-producing gammaproteobacterium. *J. Gen. Appl. Microbiol.* 50:143–147.
10. Jordan IK, et al. 2011. Genome sequences for five strains of the emerging pathogen *Haemophilus haemolyticus*. *J. Bacteriol.* 193:5879–5880.
11. Kislyuk AO, et al. 2010. A computational genomics pipeline for prokaryotic sequencing projects. *Bioinformatics* 26:1819–1826.
12. Nalin R, Simonet P, Vogel TM, Normand P. 1999. *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. *Int. J. Syst. Bacteriol.* 49:19–23.
13. Prakash O, et al. 25 November 2011, posting date. Description of *Rhodanobacter denitrificans* sp. nov., isolated from uranium and nitrate contaminated subsurface sediment. *Int. J. Syst. Evol. Microbiol.* doi:10.1099/ijs.0.035840-0.
14. van den Heuvel RN, van der Biezen E, Jetten MSM, Hefting MM, Kartal B. 2010. Denitrification at pH 4 by a soil-derived *Rhodanobacter*-dominated community. *Environ. Microbiol.* 12:3264–3271.
15. Wang L, et al. 2011. *Rhodanobacter panaciterrae* sp. nov., a bacterium with ginsenoside-converting activity isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol.* 61:3028–3032.
16. Weon HY, et al. 2007. *Rhodanobacter ginsengisoli* sp. nov. and *Rhodanobacter terrae* sp. nov., isolated from soil cultivated with Korean ginseng. *Int. J. Syst. Evol. Microbiol.* 57:2810–2813.
17. Woo SG, Srinivasan S, Kim MK, Lee M. 6 January 2012, posting date. *Rhodanobacter caeni* sp. nov., a denitrifying bacterium isolated from sludge in a sewage disposal plant. *Int. J. Syst. Evol. Microbiol.* doi:10.1099/ijs.0.033365-0.