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GENOME ANNOUNCEMENT

Complete and Draft Genome Sequences of Six Members of the *Aquificales*[▽]

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The *Aquificales* are widespread in marine and terrestrial hydrothermal environments. Here, we report the complete and draft genome sequences of six new members of the *Aquificales*: two marine species, *Persephonella marina* strain EX-H1 and *Hydrogenivirga* strain 128-5-R1 (from the East Pacific Rise, 9°50.3′N, 104°17.5′W, and the Eastern Lau Spreading Center, 176°11.5′W, 20°45.8′S, respectively), and four terrestrial isolates, *Sulfurihydrogenibium azorense* strain Az-Fu1, *Sulfurihydrogenibium yellowstonense* strain SS-5, and *Sulfurihydrogenibium* strain Y03AOP1 (from Furnas, Azores, Portugal, and Calcite Springs and Obsidian Pool in Yellowstone National Park, United States, respectively), and the only thermoacidophilic isolate, *Hydrogenobaculum* strain Y04AAS1 (from a stream adjacent to Obsidian Pool). Significant differences among the different species exist that include nitrogen metabolism, hydrogen utilization, chemotaxis, and signal transduction, providing insights into their ecological niche adaptations.

The *Aquificales* are marine and terrestrial chemolithoautotrophic thermophiles. Like *Aquifex aeolicus* (2), *Hydrogenivirga* strain 128-5-R1 and *Persephonella marina* Ex-H1 (4) are marine isolates, and *Sulfurihydrogenibium azorense* Az-Fu1 (1), *Sulfurihydrogenibium yellowstonense* SS-5 (9), and *Sulfurihydrogenibium* strain Y03AOP1 were isolated from near-neutral-pH terrestrial hot springs whereas *Hydrogenobaculum* strain Y04AAS1 is a thermoacidophile. The terrestrial *Aquificales* are found in high abundance in hot spring outflow channels and play an important role in the biogeochemical cycling in these hot springs (10).

The complete (*S. azorense*, *P. marina*, *Sulfurihydrogenibium* strain Y03AOP1, and *Hydrogenobaculum* strain Y04AAS1) and draft (*S. yellowstonense* and *Hydrogenivirga*) genome sequences were generated by whole-genome random shotgun sequencing at The Institute for Genomic Research (*S. azorense*, *P. marina*, and *S. yellowstonense*), The Venter Institute (*Hydrogenivirga*), and the DOE Joint Genome Institute (*Sulfurihydrogenibium* strain Y03AOP1 and *Hydrogenobaculum* strain Y04AAS1). The genomes were sequenced and initially annotated as described in references 6 and 3 and on the DOE Joint Genome Institute Sequencing website (http://www.jgi.doe.gov/sequencing/index.html), respectively. Comparative gene prediction analysis was done using the Integrated Microbial Genomes (IMG; http://img.jgi.doe.gov/) platform (8).

The *S. azorense*, *P. marina*, *Sulfurihydrogenibium* strain Y03AOP1, and *Hydrogenobaculum* strain Y04AAS1 chromosome sequences contain about 1.64, 1.98, 1.84, and 1.56 Mbp with 1,708, 2,048, 1,774, and 1,640 putative open reading frames, respectively. *P. marina* has a megaplasmid (53,682 bp, 74 genes), the G+C content of the genomes varies from 31.9% (Y03AOP1) to 43% (*Hydrogenivirga*), and most of the genomes have two rRNA operons.

Although some members of the *Aquificales* can grow heterotrophically, all members are able to fix CO_2 using the reductive tricarboxylic acid (TCA) cycle. Like all *Aquificaceae*, *Hydrogenivirga* and *Hydrogenobaculum* have the “B-type” reductive TCA cycle (7), whereas all other isolates have the “A-type” reductive TCA cycle. Although hydrogen oxidation (“knallgas” reaction) was thought to be universal in this group, several isolates such as *S. yellowstonense* and *Sulfurihydrogenibium* strain Y03AOP1 are unable to oxidize hydrogen, and consequently no hydrogenases are detected in their genomes. What seems more diagnostic of the *Aquificales* is their ability to oxidize sulfur, and all genomes have a suite of *sox* genes and a sulfide:quinone oxidoreductase (*sqr*), which catalyzes the oxidation of sulfide to elemental sulfur. A homolog of SreABC, a sulfur reductase in *A. aeolicus* (5), was found in *Hydrogenivirga*.

The marine isolates, *P. marina* and *Hydrogenivirga*, are also

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autotrophic nitrate reducers and have cytoplasmic membrane-bound nitrate reductases (Nar) and nitrite reductases (Nir), nitric oxide reductases (NOR), and nitrous oxide reductases (Nos). However, although *Hydrogenobaculum* strain Y04AAS1 is unable to reduce nitrate in the laboratory, it contains Nir and Nap genes. Curiously, *S. azorense*, *Sulfurihydrogenibium* strain Y03AOP1, and *Hydrogenobaculum* strain Y04AAS1 all code for the quinone NOR, thought to be exclusively involved in denitrification. Given their redox reactive environment in deep-sea hydrothermal sulfide deposits or along iron-sulfide chemical gradients in terrestrial hot springs, it is no surprise that all the genomes contain a large number of different cytochromes. Additionally, unlike *Aquifex aeolicus*, whose genome lacks chemotaxis/motility genes (2), all the genomes reported here have a suite of the bacterium-like chemotaxis genes, numerous monocyte chemoattractant proteins, and FliM. Further comparative analysis of these genomes will enable future studies of the physiological ecology and phylogenetic position of the *Aquificales*.

Nucleotide sequence accession numbers. The sequences are deposited under the project identification numbers as follows (GenBank accession number): *Hydrogenivirga* strain 128-5-R1, 19313 (NZ_ABHJ00000000); *Hydrogenobaculum* strain Y04AAS1, 18891 (NC_011126); *Sulfurihydrogenibium* strain Y03AOP1, 18889 (NC_010730); *S. azorense*, 12529 (CP001229); *S. yellowstonense*, 19071 (ABZS00000000); *P. marina*, 12526 (CP001230 and CP001231).

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REFERENCES

1. **Aguiar, P., T. J. Beveridge, and A.-L. Reysenbach.** 2004. *Sulfurihydrogenibium azorense*, sp. nov., a thermophilic hydrogen-oxidizing microaerophile from terrestrial hot springs in the Azores. Int. J. Syst. Evol. Microbiol. **54:**33–39.
2. **Deckert, G., P. V. Warren, T. Gaasterland, W. G. Young, A. L. Lenox, D. E. Graham, R. Overbeek, M. A. Snead, M. Keller, M. Aujay, R. Huber, R. A. Feldman, J. M. Short, G. J. Olsen, and R. V. Swanson.** 1998. The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. Nature **392:** 353–358.
3. **Goldberg, S. M., J. Johnson, D. Busam, T. Feldblyum, S. Ferriera, R. Friedman, A. Halpern, H. Khouri, S. A. Kravitz, F. M. Lauro, K. Li, Y. H. Rogers, R. Strausberg, G. Sutton, L. Tallon, T. Thomas, E. Venter, M. Frazier, and J. C. Venter.** 2006. A Sanger/pyrosequencing hybrid approach for the generation of high-quality draft assemblies of marine microbial genomes. Proc. Natl. Acad. Sci. USA **103:**11240–112405.
4. **Gotz, D., A. Banta, T. J. Beveridge, A. I. Rushdi, B. R. T. Simoneit, and A.-L. Reysenbach.** 2002. *Persephonella marina* gen. nov., sp. nov. and *Persephonella guaymasensis* sp. nov., two novel, thermophilic, hydrogen-oxidizing microaerophiles from deep-sea hydrothermal vents. Int. J. Syst. Evol. Microbiol. **52:**1349–1359.
5. **Guiral, M., C. Aubert, and M. T. Giudici-Orticoni.** 2005. Hydrogen metabolism in the hyperthermophilic bacterium *Aquifex aeolicus*. Biochem. Soc. Trans. **33:**22–24.
6. **Heidelberg, J. F., R. Seshadri, S. A. Haveman, C. L. Hemme, I. T. Paulsen, J. F. Kolonay, J. A. Eisen, N. Ward, B. Methe, L. M. Brinkac, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, D. Fouts, D. H. Haft, J. Selengut, J. D. Peterson, T. M. Davidsen, N. Zafar, L. Zhou, D. Radune, G. Dimitrov, M. Hance, K. Tran, H. Khouri, J. Gill, T. R. Utterback, T. V. Feldblyum, J. D. Wall, G. Voordouw, and C. M. Fraser.** 2004. The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. Nat. Biotechnol. **22:**554–559.
7. **Hugler, M., H. Huber, S. J. Molyneaux, C. Vetriani, and S. M. Sievert.** 2007. Autotrophic CO_2 fixation via the reductive tricarboxylic acid cycle in different lineages within the phylum *Aquificae:* evidence for two ways of citrate cleavage. Environ. Microbiol. **9:**81–92.
8. **Markowitz, V. M., E. Szeto, K. Palaniappan, Y. Grechkin, K. Chu, I. M. Chen, I. Dubchak, I. Anderson, A. Lykidis, K. Mavromatis, N. N. Ivanova, and N. C. Kyrpides.** 2008. The integrated microbial genomes (IMG) system in 2007: data content and analysis tool extensions. Nucleic Acids Res. **36:** D528–D533.
9. **Nakagawa, S., Z. Shtaih, A. Banta, T. J. Beveridge, Y. Sako, and A.-L. Reysenbach.** 2005. *Sulfurihydrogenibium yellowstonense* sp. nov., an extremely thermophilic, facultatively heterotrophic, sulfur-oxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus *Sulfurihydrogenibium*, *Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense*. Int. J. Syst. Evol. Microbiol. **55:**2263–2268.
10. **Reysenbach, A.-L., A. Banta, S. Civello, J. Daly, K. Mitchel, S. Lalonde, K. O. Konhauser, A. Rodman, K. Rusterholtz, and C. Takacs-Vesbach.** 2005. The Aquificales of Yellowstone National Park, p. 129–142. *In* W. P. Inskeep and T. R. McDermott (ed.), Geothermal biology and geochemistry in Yellowstone National Park. Thermal Biology Institute, Montana State University, Bozeman.