

## Complete Genome Sequence of the Thermophilic, Piezophilic, Heterotrophic Bacterium *Marinitoga piezophila* KA3

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*Marinitoga piezophila* KA3 is a thermophilic, anaerobic, chemoorganotrophic, sulfur-reducing bacterium isolated from the Grandbonum deep-sea hydrothermal vent site at the East Pacific Rise (13°N, 2,630-m depth). The genome of *M. piezophila* KA3 comprises a 2,231,407-bp circular chromosome and a 13,386-bp circular plasmid. This genome was sequenced within Department of Energy Joint Genome Institute CSP 2010.

*Arinitoga piezophila* strain KA3 is the first true thermopiezophilic bacterium, isolated in 2002 from enrichment cultures of deep-sea hydrothermal chimney samples in RC rich medium at 65°C under *in situ* hydrostatic pressure (30 MPa). This species grows optimally at 65°C and 40 MPa (1) and shows unusual morphological deformations as cells become very elongated and twisted when grown under atmospheric pressure conditions (1).

A phylogenetic analysis using the almost complete 16S rRNA gene sequence (1,443 bp) has shown that the gene sequences most similar to those of strain KA3 are those of *M. okinawensis* and *M. camini* (95% similarity), species that were isolated from the Yonaguni Knoll IV and Menez-Gwen sites, respectively, in the southern Okinawa Trough and on the Mid-Atlantic Ridge (MAR) (1, 4, 7). The next most closely related sequences were those of *M. hydrogenitolerans* and *M. littoralis* (94% similarity), species that were isolated from the Rainbow and Île Saint-Paul sites, respectively, on the MAR and in the southern Indian Ocean (5, 6).

*M. piezophila* KA3 was selected for sequencing on the basis of its ability to cope with the high hydrostatic pressure and high temperature conditions (1) that characterize the deep biosphere. Among the five *Marinitoga* species described above, only the *M. piezophila* KA3 genome sequence is currently available. It was sequenced by using a combination of the Illumina and 454 sequencing platforms with a genome coverage of  $30 \times$ . Detailed information about library construction and sequencing can be found at the Department of Energy (DOE) Joint Genome Institute (JGI) website (http://www.jgi.doe.gov/). Finishing and annotation were performed by the JGI.

The *M. piezophila* KA3 genome comprises a circular chromosome of 2,231,407 bp and a circular plasmid (pMARPI01) of 13,386 bp with 29.2% and 26.5% GC contents, respectively. The *M. piezophila* genome contains 2,131 genes, of which 2,070 were identified as protein-coding genes; 76.44% of these genes were assigned putative functions, while the rest were annotated as encoding hypothetical proteins. There were 24 pseudogenes and 61 RNA genes with 9 rRNA operons. The genome contains 5 CRISPR loci of CRISPR-associated *cas* genes, but no CRISPR spacers were found to match the putative *M. piezophila* prophage of  $\sim$ 44 kb (3).

KA3 has an impaired pentose phosphate pathway because of a lack of glucose-6-phosphate-1-dehydrogenase and 6-phosphogluconate dehydrogenase and also lacks the 6-phosphogluconate dehydratase and 2-dehydro-3-deoxy-phosphogluconate aldolase enzymes necessary for the Entner-Doudoroff pathway, but it possesses a complete Embden-Meyerhoff-Parnas pathway to convert glucose into pyruvate. The KA3 genome lacks cysteine synthase, serine acetyltransferase, and lactate dehydrogenase, and the trichloroacetic acid cycle is incomplete.

A phylogenetic assessment of the genes of *M. piezophila* shows that 1,297 (62.65%) of them are shared within the phylum *Thermotogae* and 304 (14.69%) of them have been potentially acquired by lateral gene transfer, while 261 (12.61%) of them are unassigned.

The *M. piezophila*-specific gene set, those genes having no homologues in the GenBank nr database, may provide clues to its adaptation to the high pressure that is ubiquitous in the deep biosphere.

**Nucleotide sequence accession numbers.** The genome sequence of *M. piezophila* KA3 is available in GenBank under accession numbers CP003257.1 (for the chromosome) and CP003258.1 (for the plasmid) and has been deposited in the Genomes On Line Database (GOLD ID Gc02084) (2).

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## REFERENCES

- 1. Alain K, et al. 2002. *Marinitoga piezophila* sp. nov., a rod-shaped, thermopiezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int. J. Syst. Evol. Microbiol. **52**:1331–1339.
- 2. Liolios K, et al. 2010. The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Res. 38:346–354.
- 3. Nesbø CL, Geslin C, Lossouarn J, Jebbar M, Zhaxybayeva O. 2012. Discovery and characterization of the first prophage in Thermotogales: a tailed bacteriophage predicted to infect Thermosipho and Thermotoga, with a bonus comparison to a related phage in Marinitoga, abstr D-1877.

Program Abstr. 112th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.

- 4. Nunoura T, et al. 2007. *Marinitoga okinawensis* sp. nov., a novel thermophilic and anaerobic heterotroph isolated from a deep-sea hydrothermal field, Southern Okinawa Trough. Int. J. Syst. Evol. Microbiol. 57:467–471.
- Postec A, et al. 2005. Marinitoga hydrogenitolerans sp. nov., a novel member of the order *Thermotogales* isolated from a black smoker chimney on the Mid-Atlantic Ridge. Int. J. Syst. Evol. Microbiol. 55:1217–1221.
- Postec A, et al. 2010. Marinitoga litoralis sp. nov., a thermophilic, heterotrophic bacterium isolated from a coastal thermal spring on Ile Saint-Paul, Southern Indian Ocean. Int. J. Syst. Evol. Microbiol. 60:1778–1782.
- Wery N, et al. 2001. Marinitoga camini gen. nov., sp. nov., a rod-shaped bacterium belonging to the order *Thermotogales*, isolated from a deep-sea hydrothermal vent. Int. J. Syst. Evol. Microbiol. 51:495–504.