

Genome Sequence of *Halomonas* sp. Strain A3H3, Isolated from Arsenic-Rich Marine Sediments

Sandrine Koechler,^a Frédéric Plewniak,^a Valérie Barbe,^b Fabienne Battaglia-Brunet,^c Bernard Jost,^d Catherine Jouliau,^c Muriel Philipps,^d Serge Vicaire,^d Stéphanie Vincent,^b Tao Ye,^d Philippe N. Bertin^a

UMR7156 Université de Strasbourg/CNRS, Génétique Moléculaire, Génomique, Microbiologie, Département Micro-organismes, Genome, Environnement, Strasbourg, France^a; Laboratoire de Finition, CEA-IG-Génoscope, Évry, France^b; BRGM, Orléans, France^c; Plateforme Biopuces et Séquençage, IGBMC, Illkirch, France^d

We report the genome sequence of *Halomonas* sp. strain A3H3, a bacterium with a high tolerance to arsenite, isolated from multicontaminated sediments of the l'Estaque harbor in Marseille, France. The genome is composed of a 5,489,893-bp chromosome and a 157,085-bp plasmid.

Received 12 September 2013 Accepted 16 September 2013 Published 10 October 2013

Citation Koechler S, Plewniak F, Barbe V, Battaglia-Brunet F, Jost B, Jouliau C, Philipps M, Vicaire S, Vincent S, Ye T, Bertin PN. 2013. Genome sequence of *Halomonas* sp. strain A3H3, isolated from arsenic-rich marine sediments. *Genome Announc.* 1(5):e00819-13. doi:10.1128/genomeA.00819-13.

Copyright © 2013 Koechler et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Sandrine Koechler, sandrine.koechler@unistra.fr.

Halomonas sp. strain A3H3, a slightly halophilic bacterium (0.5 to 9% NaCl) (1), was isolated from the harbor sediments of l'Estaque in the south of France. These sediments are highly contaminated with various metals, metalloids, and organic compounds, particularly with arsenic (653 μ g/liter in sediment interstitial water and 165 mg/kg in solid phase) (2, 3). A 16S rRNA-based phylogenetic analysis revealed close similarity to *Halomonas neptuniae* strain Eplume1 (GenBank accession number NR_027218.1).

The genome of *Halomonas* sp. A3H3 was sequenced from one paired-end and one 500-bp mate-paired library of 5-kb inserts. The resulting reads were assembled into 131 contigs using SOAPdenovo and GapCloser assemblers, achieving 150-fold coverage. The sequence assembly completed by optical mapping (4) resulted in two scaffolds representing a total of 5,646,978 bp. The genome consists of a 5,489,893-bp chromosome with a 54.59% average GC content and 5,143 coding sequences (CDS) and a 157,085-bp plasmid with a 61.97% average GC content, 221 CDS, and a coding density of 89.38%. The size of the plasmid was experimentally validated using Wheatcroft's method (5). A total of 5,568 CDS, 48 tRNAs, 13 miscellaneous RNAs, and 4 rRNA genes were predicted and annotated using the MicroScope platform (6). A total of 31.55% of CDS are of unknown function.

Best synteny and gene conservation were found with *Halomonas* sp. strain HAL1 (7), *Halomonas boliviensis* LC1 (8), and *Halomonas* sp. strain TD01 (9) genome sequences, with 64.7, 63.7, and 58.9% of genes in syntons, respectively. Remarkably, the genome of *Halomonas* sp. A3H3 is about 1.5 Mb larger, suggesting the acquisition of functions allowing better adaptation to its environment, e.g., genes coding for tripartite ATP-independent periplasmic (TRAP) transporters for substrate uptake (10) or salicylate degradation. In contrast, no *car* or *bph* operon was identified, although *Halomonas* sp. A3H3 uses aromatic compounds such as biphenyl and carbazole as sole carbon sources, which suggests the possible use of alternative degradation pathways. The presence of

numerous transposases or phage-related CDS throughout the genome sustains a large number of rearrangements.

Halomonas sp. A3H3 tolerates up to 29 mM As(III) and more than 106 mM As(V). Four *ars* operons (two on the chromosome and two on the plasmid) containing *arsD*, *arsA*, *acr3*, *arsC*, *arsH*, and/or *arsR* genes (11, 12, 13) were identified. Importantly, *aioBA* genes (14) located on the plasmid may also have been acquired by horizontal transfer. The bacterium oxidizes 100 mg/liter arsenite under aerobic conditions or under anaerobic conditions in the presence of nitrate used as an alternative final electron acceptor, as supported by the presence on the chromosome of the *narGHJI* operon (15). *Halomonas* sp. A3H3 is motile by means of peritrichous flagella, the genes of which are clustered in a large chromosomal region. The motility of the bacterium increases with arsenite, as shown by swarming assays, which further supports the use of arsenite oxidation in the energy metabolism of *Halomonas* sp. A3H3.

Further studies will provide insights into the strategies evolved by *Halomonas* sp. A3H3 to deal with the toxic compounds present in marine anaerobic harbor sediments.

Nucleotide sequence accession numbers. The whole-genome sequence has been deposited at DDBL/EMBL/Genbank under the following accession numbers: contigs, [CBRE010000001](https://www.ncbi.nlm.nih.gov/assembly/100000001) through [CBRE010000131](https://www.ncbi.nlm.nih.gov/assembly/100000131); scaffolds, HG423310 through HG423342; and chromosomes, HG423343 and HG423344.

ACKNOWLEDGMENTS

This work was supported by the French National Research Agency, under the reference number "2008 CESA-003."

Sequencing was performed by the IGBMC microarray and sequencing platform, a member of the France Génomique program.

We thank Genoscope (Évry, France) for the use of MicroScope, the Microbial Genome Annotation & Analysis Platform (<http://www.genoscope.cns.fr/agg/microscope>).

REFERENCES

- Ollivier B, Caumette P, Garcia JL, Mah RA. 1994. Anaerobic bacteria from hypersaline environments. *Microbiol. Rev.* 58:27–38.

2. Plewniak F, Koechler S, Navet B, Dugat-Bony E, Bouchez O, Peyret P, Séby F, Battaglia-Brunet F, Bertin PN. 2013. Metagenomic insights into microbial metabolism affecting arsenic dispersion in Mediterranean marine sediments. *Mol. Ecol.* 22:4870–4883.
3. Mamindy-Pajany Y, Hurel C, Gérard F, Galgani F, Battaglia-Brunet F, Marmier N, Roméo M. 2013. Arsenic in marine sediments from French Mediterranean ports: geochemical partitioning, bioavailability and ecotoxicology. *Chemosphere* 90:2730–2736.
4. Aston C, Mishra B, Schwartz DC. 1999. Optical mapping and its potential for large-scale sequencing projects. *Trends Biotechnol.* 17:297–302.
5. Wheatcroft R. 1990. Changes in the *Rhizobium meliloti* genome and the ability to detect supercoiled plasmids during bacteroid development. *Mol. Plant Microbe Interact.* 3:9. doi:10.1094/MPMI-3-009.
6. Vallenet D, Engelen S, Mornico D, Cruveiller S, Fleury L, Lajus A, Rouy Z, Roche D, Salvignol G, Scarpelli C, Médigue C. 2009. MicroScope: a platform for microbial genome annotation and comparative genomics. *Database (Oxford)* 2009:bap021. doi:10.1093/database/bap021.
7. Lin Y, Fan H, Hao X, Johnstone L, Hu Y, Wei G, Alwathnani HA, Wang G, Rensing C. 2012. Draft genome sequence of *Halomonas* sp. strain HAL1, a moderately halophilic arsenite-oxidizing bacterium isolated from gold-mine soil. *J. Bacteriol.* 194:199–200.
8. Guzmán D, Balderrama-Subieta A, Cardona-Ortuño C, Guevara-Martínez M, Callisaya-Quispe N, Quillaguamán J. 2012. Evolutionary patterns of carbohydrate transport and metabolism in *Halomonas boliviensis* as derived from its genome sequence: influences on polyester production. *Aquat. Biosyst.* 8:9. doi:10.1186/2046-9063-8-9.
9. Cai L, Tan D, Aibaidula G, Dong XR, Chen JC, Tian WD, Chen GQ. 2011. Comparative genomics study of polyhydroxyalkanoates (PHA) and ectoine relevant genes from *Halomonas* sp. TD01 revealed extensive horizontal gene transfer events and co-evolutionary relationships. *Microb. Cell Fact.* 10:88.
10. Mulligan C, Fischer M, Thomas GH. 2011. Tripartite ATP-independent periplasmic (TRAP) transporters in bacteria and archaea. *FEMS Microbiol. Rev.* 35:68–86.
11. Rosen BP. 2002. Biochemistry of arsenic detoxification. *FEBS Lett.* 529:86–92.
12. Achour AR, Bauda P, Billard P. 2007. Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. *Res. Microbiol.* 158:128–137.
13. Hervás M, López-Maury L, León P, Sánchez-Riego AM, Florencio FJ, Navarro JA. 2012. ArsH from the cyanobacterium *Synechocystis* sp. PCC 6803 is an efficient NADPH-dependent quinone reductase. *Biochemistry* 51:1178–1187.
14. Muller D, Lièvreumont D, Simeonova DD, Hubert JC, Lett MC. 2003. Arsenite oxidase *aox* genes from a metal-resistant β -proteobacterium. *J. Bacteriol.* 185:135–141.
15. Zumft WG. 1997. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* 61:533–616.