

1 **Genome sequence of *Halorhabdus tiamatea*, the first archaeon isolated from a deep-**  
2 **sea anoxic brine lake.**

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21 **Abstract**

22 We present the draft genome of *Halorhabdus tiamatea*, the first member of the *Archaea*  
23 ever isolated from a deep-sea anoxic brine. Genome comparison with *H. utahensis*,  
24 revealed some striking differences, including marked increase in genes associated with  
25 trans-membrane transport, and putative genes for a trehalose synthase and a lactate-  
26 dehydrogenase.

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28 **Main text**

29 *Halorhabdus tiamatea* is the first archaeon isolated from deep-sea brines (2, 3),  
30 specifically from Shaban Deep. The genome of the type species of this genus (11) has  
31 been recently sequenced (1).

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33 Cells were grown under optimal conditions (2). Genomic DNA was extracted with a  
34 Blood & Cell Culture DNA Mini Kit (Qiagen), following the manufacturer's instructions.

35 The *Halorhabdus tiamatea* genome was sequenced using the Roche 454 GS (FLX  
36 Titanium) and Illumina sequencing platforms (single and paired-end). A total of  
37 93,895,127 bp (mean read length: 306 bp) was obtained from Roche 454 providing  
38 approximately 22-fold genome coverage. Single and paired-end Illumina data provided  
39 5,816,168 bp (mean read length: 30 bp) and 5,220,362 bp (mean read length: 35 bp)  
40 corresponding to 420-fold coverage. Roche 454 sequencing data was assembled using  
41 Newbler Assembler version 2.5 (Roche), while Illumina data was assembled with  
42 SOAPdenovo (<http://soap.genomics.org.cn/soapdenovo.html>). Resulting assemblies were

43 merged using AMOS Minimus2 (<http://sourceforge.net/apps/>  
44 [mediawiki/amos/index.php?title=Minimus2](http://mediawiki/amos/index.php?title=Minimus2)).

45 The sequences were assembled into 76 scaffolds, with an N50 contig size of  
46 approximately 88.6 kb. Genes were identified using Prodigal software  
47 (<http://compbio.ornl.gov/prodigal/>) followed by mpiBLAST (<http://www.mpiblast.org/>)  
48 and Interproscan (<http://www.ebi.ac.uk/InterProScan/>) annotation. This approach  
49 provided annotation for 89% of all 4034 predicted genes. Additional analysis was done  
50 using the RAST server (4). The draft genome has a G+C content of 62%.

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52 Genome comparison with *H. utahensis* (1, 12), revealed some striking differences,  
53 namely, a marked increase in genes associated with transport across the membrane,  
54 mainly transport and utilization of phosphonate, di- and oligopeptides, maltose and  
55 maltodextrin.

56 While phosphonate transport and utilization is frequent for *Bacteria*, it seems to be quite  
57 rare for *Archaea*, (6). Genes involved in phosphonate utilization are subjected to  
58 extensive lateral gene transfer (9) and are likely transferred in this manner from *Bacteria*  
59 to *Archaea*. The use of phosphonates is associated with adaptations to phosphate-limited  
60 environments, which is in agreement with data from Shaban Deep (11).

61 Genes related to transport and utilization of maltose and maltodextrins are associated  
62 with genes for transport of other sugars, and, most notably, with a trehalose synthase  
63 (likely using maltose as a substrate). Trehalose synthases have only been reported in few  
64 *Archaea* (e.g. *Sulfolobus*) and, to our knowledge, have never been detected in members of  
65 *Halobacteriaceae*. Trehalose has several possible functions in cells, namely structural or

66 protection against oxidic, thermal or osmotic stress (10). In halophilic microbes, trehalose  
67 is most often used as compatible solute for coping with osmotic stress. However,  
68 haloarchaea are traditionally associated with the "salt-in" strategy, which is thought to  
69 preclude the use of compatible solutes with few exceptions (7). Additional studies are  
70 necessary to clarify the role of trehalose in *H. tiamatea*.

71 An additional interesting feature of this genome is the presence of a gene coding for an L-  
72 Lactate dehydrogenase (LDH), which might provide a new fermentative pathway within  
73 *Halobacteriaceae*. Although LDH activity has previously been reported in  
74 *Halobacterium salinarum* cell extracts (5), no clear LDH homologues had been reported  
75 in any haloarchaeal genomes (8).

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77 **Nucleotide sequence accession numbers.** Nucleotide sequences are available in  
78 GenBank under accession number AFNT00000000.

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