Complete genome sequence of *Halopiger xanaduensis* type strain (SH-6)1

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*Halopiger xanaduensis* is the type species of the genus *Halopiger* and belongs to the euryarchaeal family *Halobacteriaceae*. *H. xanaduensis* strain SH-6, which is designated as the type strain, was isolated from the sediment of a salt lake in Inner Mongolia, Lake Shangmatala. Like other members of the family *Halobacteriaceae*, it is an extreme halophile requiring at least 2.5 M salt for growth. We report here the sequencing and annotation of the 4,355,268 bp genome, which includes one chromosome and three plasmids. This genome is part of a Joint Genome Institute (JGI) Community Sequencing Program (CSP) project to sequence diverse haloarchaeal genomes.

**Introduction**

*Halopiger xanaduensis* is the type species of the genus *Halopiger*, and strain SH-6 is the type strain of the species. It was isolated from the sediment of a salt lake, Lake Shangmatala, in Inner Mongolia, China [1]. The name *Halopiger* refers to its slow growth in the laboratory. There is one other described species in the genus *Halopiger*, *H. aswanensis*, which was isolated from a saline soil in Egypt [2]. We report here the first genome sequence from the genus *Halopiger*.

**Classification and features**

In 16S rRNA trees the *Halopiger* species are most closely related to *Natronolimnobius* species [1,2]. Currently there are fifteen complete genomes of haloarchaea in GenBank. Figure 1 shows the relationship of *H. xanaduensis* to other haloarchaea for which complete genomes have been sequenced. For *Halobacterium salinarum* and *Haloquadratum walsbyi*, only one sequence is included in Figure 1, although for both of these species two genomes have been sequenced. *H. xanaduensis* was isolated from a sediment sample of Lake Shangmatala in Inner Mongolia, China. The sample was enriched in liquid medium containing salts and yeast extract; the culture was then plated on agar to obtain pure colonies [1]. At the time of sample collection, the salinity of the lake was 16.7%, the temperature was 21.8°C, and the pH was 8.5 [1]. The cells were pleomorphic with the most common shape being rods. Motility was not observed [1]. An electron micrograph is shown in Figure 2. Growth was observed between 28 and 45°C with an optimum at 37°C [1]. The pH range for growth was 6.0-11.0 with an optimal pH of 7.5-8.0 [1]. Growth occurred within a salinity range of 2.5 M to 5.0 M NaCl and was optimal at 4.3 M NaCl [1]. The organism is strictly aerobic but was able to reduce nitrate and nitrite with production of gas [1]. Several sugars and amino acids can serve as sole carbon and energy sources, and amino acids are not required in the growth medium [1]. The features of the organism are listed in Table 1.
**Figure 1.** Phylogenetic tree showing the relationships between haloarchaea with sequenced genomes. The sequences were aligned with the Ribosomal Database Project (RDP) aligner [3], which uses the Jukes-Cantor corrected distance model to construct a distance matrix based on alignment model positions without the use of alignment inserts, and uses a minimum comparable position of 200. The tree was generated with the Tree Builder from the RDP which uses Weighbor [4] with an alphabet size of 4 and length size of 1,000. The building of the tree also involves a bootstrapping process repeated 100 times to generate a majority consensus tree. *Methanosarcina acetivorans* was used as the outgroup.

**Figure 2.** Electron micrograph of *H. xanaduensis* SH-6T.
| MIGS ID | Property               | Term                        | Evidence code<sup>a</sup> |
|---------|------------------------|-----------------------------|---------------------------|
| Domain  | Archaea                | TAS [6]                     |
| Phylum  | Euryarchaeota          | TAS [7]                     |
| Class   | Halobacteria           | TAS [8, 9]                  |
| Current classification | Order Halobacteriales | TAS [10-12]                |
|         | Family Halobacteriaceae | TAS [13, 14]             |
|         | Genus Halopiger        | TAS [1]                     |
|         | Species Halopiger xanaduensis | TAS [1]                  |
|         | Type strain SH-6       | TAS [1]                     |
| Cell shape | pleomorphic, mostly rods | TAS [1]            |
| Motility | nonmotile              | TAS [1]                     |
| Sporulation | nonsporulating        | NAS                        |
| Temperature range | 28-45°C           | TAS [1]                     |
| Optimum temperature | 37°C               | TAS [1]                     |
| Salinity | 2.5-5.0 M NaCl (optimum 4.3M) | TAS [1]              |
| Oxygen requirement | aerobe              | TAS [1]                     |
| Carbon source | sugars or amino acids | TAS [1]               |
| Energy metabolism | heterotrophic        | TAS [1]                     |
| Habitat | salt lake sediment     | TAS [1]                     |
| Biotopic relationship | free-living       | TAS [1]                     |
| Pathogenicity | none                | NAS                         |
| Biosafety level | 1                    | NAS                         |
| Isolation | sediment of Lake Shangmatala | TAS [1]            |
| Geographic location | Inner Mongolia, China | TAS [1]                   |
| Isolation time | before 2007        | TAS [1]                     |
| Latitude | 43.2                   | TAS [1]                     |
| Longitude | 114.017               | TAS [1]                     |
| Depth   | not reported           |                             |
| Altitude | not reported           |                             |

<sup>a</sup> Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [15].
Genome sequencing information

Genome project history

*Halopiger xanaduensis* was selected for sequencing as part of a JGI CSP project to sequence a representative from every genus of haloarchaea. The genome project is listed in the Genomes On Line Database [16], and the complete genome sequence has been deposited in GenBank. Sequencing was carried out at the JGI Production Genomics Facility (PGF). Finishing was done at Los Alamos National Laboratory. Annotation was done at both the PGF and Oak Ridge National Laboratory. Table 2 presents the project information and its association with MIGS version 2.0 compliance [5].

Growth conditions and DNA isolation

Cells were grown in DSMZ medium 372 (*Halobacteria* medium) [17] at 37°C. DNA was isolated from 1.0-1.5 g cell paste with the MasterPure Gram Positive DNA Purification Kit (Epicentre).

Genome sequencing and assembly

The draft genome of *Halopiger xanaduensis* SH-6 was generated at the DOE Joint genome Institute (JGI) using a combination of Illumina [18] and 454 technologies [19]. For this genome we constructed and sequenced an Illumina GAII shotgun library which generated 55,857,474 reads totaling 4,245.2 Mb, a 454 Titanium standard library which generated 159,242 reads, and 1 paired end 454 library with an average insert size of 8 kb which generated 341,165 reads totaling 141.8 Mb of 454 data. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website [20]. The initial draft assembly contained 15 contigs in 2 scaffolds. The 454 Titanium standard data and the 454 paired end data were assembled together with Newbler, version 2.3-PreRelease-6/30/2009. The Newbler consensus sequences were computationally shredded into 2 kb overlapping fake reads (shreds). Illumina sequencing data was assembled with VELVET, version 1.0.13 [21], and the consensus sequences were computationally shredded into 1.5 kb overlapping fake reads (shreds). We integrated the 454 Newbler consensus shreds, the Illumina VELVET consensus shreds and the read pairs in the 454 paired end library using parallel phrap, version 1.080812 (High Performance Software, LLC). The software Consed [22-24] was used in the following finishing process. Illumina data was used to correct potential base errors and increase consensus quality using the software Polisher developed at JGI (Alla Lapidus, unpublished). Possible mis-assemblies were corrected using gapResolution (Cliff Han, unpublished), Dupfinisher [25], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR (Jan-Fang Cheng, unpublished) primer walks. A total of 64 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. The total size of the genome is 4,355,268 bp and the final assembly is based on 117.9 Mb of 454 draft data which provides an average 26.8× coverage of the genome and 4,112.2 Mb of Illumina draft data which provides an average 934.6× coverage of the genome.

Table 2. Project information

| MIGS ID  | Property                  | Term                                      |
|---------|---------------------------|-------------------------------------------|
| MIGS-31 | Finishing quality         | Finished                                  |
| MIGS-28 | Libraries used            | Illumina standard library, 454 standard library, 454 paired end library |
| MIGS-29 | Sequencing platforms      | Illumina, 454                             |
| MIGS-31.2 | Fold coverage          | 454 26.8×, Illumina 934.6×                |
| MIGS-30 | Assemblers                | Newbler, Velvet, phrap                     |
| MIGS-32 | Gene calling method       | Prodigal, GenePRIMP                       |
|         | Genbank ID                | CP002839                                  |
|         | Genbank Date of Release   | June 9, 2011                              |
|         | GOLD ID                   | Gc01807                                   |
|         | NCBI Project ID           | 56049                                     |
| MIGS-13 | Source material identifier| DSM 18323                                 |
|         | Project relevance         | Phylogenetic diversity, biotechnology     |
Genome annotation
Genes were identified using Prodigal [26], followed by a round of manual curation using GenePRIMP [27]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. The tRNAscan-SE tool [28] was used to find tRNA genes, whereas ribosomal RNAs were found by using BLASTn against the ribosomal RNA databases. The RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL [29]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes (IMG) platform [30] developed by the JGI [31].

Genome properties
The genome includes one circular chromosome and three plasmids, for a total size of 4,355,268 bp (Table 3, Table 4). A map of the chromosome is shown in Figure 3 and maps of the plasmids are shown in Figures 4, 5, and 6. A total of 4,370 genes were predicted, 4,310 of which are protein-coding genes and 60 of which are RNA genes. There are three ribosomal RNA operons with one additional copy of 5S rRNA. Putative functions were assigned to 2,560 protein coding genes, with the remaining genes annotated as hypothetical proteins. There are 89 pseudogenes, accounting for 2.06% of protein-coding genes. Table 5 shows the distribution of genes in COG categories.

| Table 3. Summary of genome: one chromosome and three plasmids |
|-----------------|-----------------|-----------------|-----------------|
| **Label**       | **Size (bp)**   | **Topology**    | **INSDC identifier** | **RefSeq ID** |
| Chromosome      | 3,668,009       | circular        | CP002839.1          | NC_015666.1   |
| Plasmid pHALXA01| 436,718         | circular        | CP002840.1          | NC_015658.1   |
| Plasmid pHALXA02| 181,778         | circular        | CP002841.1          | NC_015667.1   |
| Plasmid pHALXA03| 68,763          | circular        | CP002842.1          | NC_015659.1   |

| Table 4. Nucleotide content and gene count levels of the genome |
|-----------------|-----------------|-----------------|
| **Attribute**   | **Value**       | **% of total**  |
| Genome size (bp)| 4,355,268       | 100.0%          |
| DNA Coding region (bp)| 3,724,648 | 85.5%          |
| DNA G+C content (bp)| 2,838,921 | 65.2%          |
| Total genes     | 4,370           |                |
| RNA genes       | 60              |                |
| rRNA operons    | 3               |                |
| Protein-coding genes | 4,310     | 100.0%          |
| Pseudogenes     | 89              | 2.1%            |
| Genes with function prediction | 2,560 | 58.6%          |
| Genes in paralog clusters | 469   | 10.9%          |
| Genes assigned to COGs | 2,877  | 66.8%          |
| Genes assigned Pfam domains | 2,735 | 63.5%          |
| Genes with signal peptides | 529   | 12.3%          |
| Genes with transmembrane helices | 1,023 | 23.7%          |
| CRISPR repeats  | 0               | 0               |

a) The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

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**Genome analysis**

*Halopiger xanaduensis* grows on only a few of the carbohydrates that were tested (glucose, galactose, and xylose) [1], but surprisingly it has 40 glycosyl hydrolases and 5 polysaccharide lyases [32]. It also has quite a large number of ABC transporters for carbohydrates: 10 full transporters and one additional substrate-binding protein. Among the sequenced haloarchaea, only *Haloferax volcanii* has a greater number of carbohydrate ABC transporters [33]. Taken together, these findings suggest that *H. xanaduensis* is capable of growth on other sugars that have not been tested.

While many of the glycosyl hydrolases have no characterized close homologs, for some of them, functions can be predicted. Halxa_0484 has 73% similarity to beta-galactosidase of *Haloferax luetentense* [34], while Halxa_3778 has 75% similarity to a xylanase from *Streptomyces sp. S27* [35]. Two of the polysaccharide lyases from family PL11 have greater than 65% similarity to rhamnogalacturonan lyases YesW and YesX from *Bacillus subtilis* [36], suggesting that *H. xanaduensis* may be capable of pectin degradation.
Degradation pathways for the three sugars that *H. xanaduensis* is known to utilize can be identified in the genome. Glucose is likely degraded by the semiphosphorylated Entner-Doudoroff pathway as in other haloarchaea [37]. Three enzymes of the pathway, glucose dehydrogenase, gluconate dehydratase, and 2-keto-3-deoxyphosphogluconate aldolase, are found in an operon (Halxa_4119-4121). The 2-keto-3-deoxygluconate kinase is found elsewhere in the genome (Halxa_2064). Galactose is probably metabolized via the De Ley-Doudoroff pathway as a galactonate dehydratase is present (Halxa_3608). Adjacent to this gene are a possible alpha-galactosidase (Halxa_3609) and a kinase and aldolase that may take part in this pathway (Halxa_3607, Halxa_3606). Xylose utilization appears to be via the pathway found in *H. volcanii* [38] which results in formation of 2-oxoglutarate. Again three enzymes of the pathway form an operon – xylonate dehydratase, 2-keto-3-deoxyxylonate dehydratase, and 2,5-dioxopentanoate dehydrogenase (Halxa_3763-3765).

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Despite the fact that it was isolated from lake sediment, *H. xanaduensis* has an operon of gas vesicle proteins (Halxa_0820-0830). It is lacking GvpC, GvpD, GvpE, and GvpH, but mutation studies have shown that these four proteins are not required for gas vesicle formation [39], so *H. xanaduensis* can probably form functional gas vesicles. This suggests that *H. xanaduensis* may spend part of its life close to the surface of the lake. 

*H. xanaduensis* has several genes involved in polysaccharide synthesis and transport that are not found in any other sequenced haloarchaea. It has two genes (Halxa_0209, Halxa_2361) belonging to the Capsular Polysaccharide Exporter family (TC 9.A.41). This is unusual as one of the members of this family is thought to transport polysaccharide across the outer membrane of Gram-negative bacteria [40]. Adjacent to these two exporter genes are two genes (Halxa_0208, Halxa_2362) belonging to COG1861, cytidyl transferases involved in polysaccharide biosynthesis. *H. xanaduensis* also has one gene (Halxa_2364) related to PseG, a UDP-sugar hydrolase involved in polysaccharide production [41]. The presence of these genes in *H. xanaduensis* suggests that it may be capable of extracellular polysaccharide synthesis using a process unlike any found in other haloarchaea.

Figure 5. Graphical circular map of plasmid pHALXA02. From outside to center: Genes on forward strand (colored by COG categories), genes on reverse strand (colored by COG categories), GC content, and GC skew.
### Table 5. Number of genes associated with the 25 general COG functional categories

| Code | Value | %age | Description                                      |
|------|-------|------|--------------------------------------------------|
| J    | 172   | 4.0% | Translation                                      |
| A    | 1     | 0.0% | RNA processing and modification                   |
| K    | 184   | 4.3% | Transcription                                    |
| L    | 134   | 3.1% | Replication, recombination and repair             |
| B    | 4     | 0.1% | Chromatin structure and dynamics                  |
| D    | 30    | 0.7% | Cell cycle control, mitosis and meiosis          |
| Y    | 0     | 0.0% | Nuclear structure                                |
| V    | 47    | 1.1% | Defense mechanisms                               |
| T    | 153   | 3.5% | Signal transduction mechanisms                    |
| M    | 120   | 2.8% | Cell wall/membrane biogenesis                    |
| N    | 46    | 1.1% | Cell motility                                   |
| Z    | 0     | 0.0% | Cytoskeleton                                    |
| W    | 47    | 1.1% | Extracellular structures                         |
| U    | 31    | 0.7% | Intracellular trafficking and secretion          |
| O    | 137   | 3.2% | Posttranslational modification, protein turnover, chaperones |
| C    | 194   | 4.5% | Energy production and conversion                 |
| G    | 194   | 4.5% | Carbohydrate transport and metabolism            |
| E    | 259   | 6.0% | Amino acid transport and metabolism              |
| F    | 79    | 1.8% | Nucleotide transport and metabolism              |
| H    | 158   | 3.7% | Coenzyme transport and metabolism                |
| I    | 80    | 1.9% | Lipid transport and metabolism                   |
| P    | 227   | 5.3% | Inorganic ion transport and metabolism           |
| Q    | 44    | 1.0% | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 554   | 12.9%| General function prediction only                 |
| S    | 314   | 7.3% | Function unknown                                |
| -    | 1433  | 33.2%| Not in COGs                                    |

a) The total is based on the total number of protein coding genes in the annotated genome.

![Graphical circular map of plasmid pHALXA03.](http://standardsingenomics.org)

**Figure 6.** Graphical circular map of plasmid pHALXA03. From outside to center: Genes on forward strand (colored by COG categories), genes on reverse strand (colored by COG categories), GC content, and GC skew.
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