

## Complete genome sequence of *Halorhodospira halophila* SL1

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*Halorhodospira halophila* is among the most halophilic organisms known. It is an obligately photosynthetic and anaerobic purple sulfur bacterium that exhibits autotrophic growth up to saturated NaCl concentrations. The type strain *H. halophila* SL1 was isolated from a hypersaline lake in Oregon. Here we report the determination of its entire genome in a single contig. This is the first genome of a phototrophic extreme halophile. The genome consists of 2,678,452 bp, encoding 2,493 predicted genes as determined by automated genome annotation. Of the 2,407 predicted proteins, 1,905 were assigned to a putative function. Future detailed analysis of this genome promises to yield insights into the halophilic adaptations of this organism, its ability for photoautotrophic growth under extreme conditions, and its characteristic sulfur metabolism.

Abbreviations: PYP- photoactive yellow protein

### Introduction

*Halorhodospira halophila* is an anoxygenic photosynthetic halophile that was isolated from salt-encrusted mud along the shore of Summer Lake in Oregon [1], and from the hypersaline Wadi Natrun lakes in Egypt [2]. The original name of this organism, *Ectothiorhodospira halophila*, was modified to *Halorhodospira halophila* when the genus *Ectothiorhodospira* was divided into two genera (*Ectothiorhodospira* and *Halorhodospira*), and *E. halophila* was reclassified as a member of the genus *Halorhodospira*, serving as the type species of the new genus [3]. Over the last decade, the genomes of a number of extremely halophilic

*Archaea* have been sequenced and analyzed, including *Halobacterium salinarum* [4,5], *Haloarcula marismortui* [6], *Natronomonas pharaonis* [7], and *Haloquadratum walsbyi* [8]. In addition, the genomes of three halophilic *Bacteria* have become available: *Salinibacter ruber* [9], *Halothermothrix orenii* [10], and 'Halanaerobium hydrogeniformans' [11]. All of these organisms are obligate chemotrophs. Thus, *H. halophila* is the first phototrophic extreme halophile to have its genome sequence determined and analyzed. In contrast to other extreme halophiles that grow well in saturated salt concentrations, *H. halophila*

has a high flexibility with respect to the salt concentrations that it tolerates, and grows optimally at all NaCl concentrations from 15% to 35%, with growth down to 3.5% NaCl [12]. In contrast, the above extremely halophilic archaea and *S. ruber* require 15% NaCl for growth.

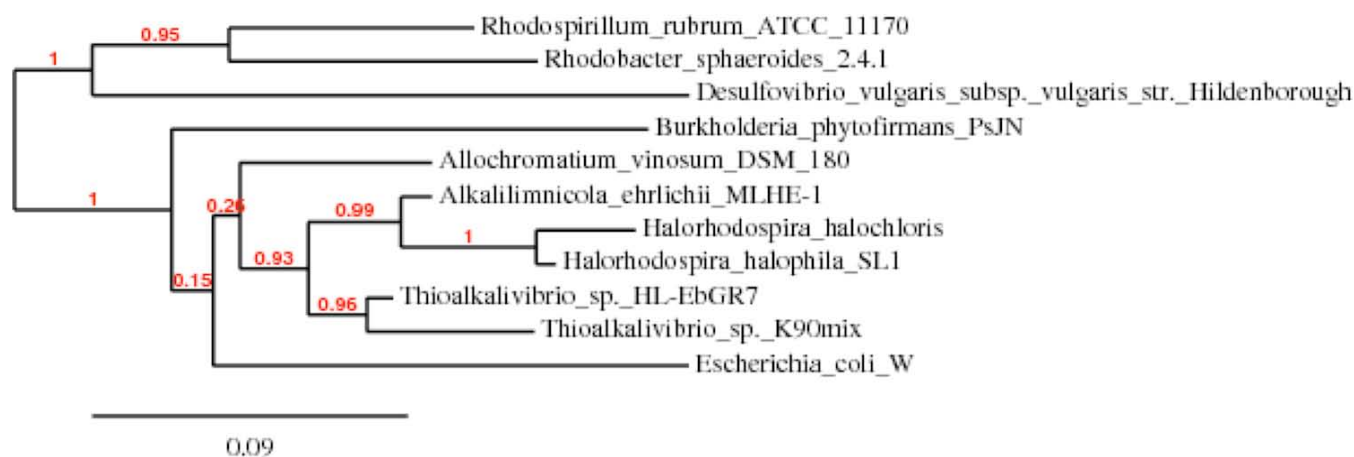
*H. halophila* is of significant interest because it is an obligately anaerobic purple sulfur bacterium, and among the most halophilic organisms known [13]. To date, genome sequences are available for two phototrophic purple sulfur bacteria, *Allochrochromatium vinosum* DSM 180 and the *H. halophila* SL1 genome reported here. *H. halophila* has very few growth requirements. However, it does need reduced sulfur compounds for growth, as does *A. vinosum* [14]. Its pathways for both photosynthetic electron transfer [15-17] and nitrogen fixation [18] have attracted attention. In addition, *H. halophila* contains photoactive yellow protein [19,20]. This is the first member of a novel class of blue light receptors, and triggers a negative phototaxis response in *H. halophila* [21]. The PYP from *H. halophila* has been studied extensively for its biophysical characteristics [22-24].

The sulfur metabolism of *H. halophila* is unusual, resulting in the transient accumulation of extracel-

lular sulfur globules via metabolic pathways that are not yet fully resolved [14]. While purple non-sulfur phototrophs such as *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* use organic compounds like malate as electron donors, *H. halophila* obtains electrons from reduced sulfur compounds. The genome sequence of *H. halophila* promises to reveal insights into its adaptations to hypersaline environments, and to allow a better understanding of its unique combination of metabolic capabilities, combining properties from extreme halophiles, anoxygenic phototrophs, and purple sulfur bacteria.

## Classification and features

*H. halophila* belongs to the *Gammaproteobacteria* [3] (Table 1). The 16S rRNA gene sequence of *H. halophila* SL1 reveals closer relationships with *H. halochloris* and *Alkalilimnicola ehrlichii*, the other representatives of the *Ectothiorhodospiraceae* (Figure 1), than with *A. vinosum*, a purple sulfur bacterium in the *Chromatiaceae* family, and the haloalkaliphilic chemolithoautotrophic *Thioalkalivibrio* strains.



**Figure 1.** Phylogram representation of a phylogenetic tree highlighting the position of *Halorhodospira halophila* strain SL1 relative to other organisms of interest, including members of the *Ectothiorhodospiraceae*, as well as additional strains that were included for comparison purposes, based on environmental and functional considerations. The strains (type=) and their corresponding GenBank accession numbers (and coordinates) for 16S rRNA genes are: *H. halophila* strain SL1, CP00544:380025-381562; *Alkalilimnicola ehrlichii* strain MLHE-1, CP00453:369818-369894; *Thioalkalivibrio* sp. HL-EbGR7, CP001339:2548250-2549775; *Thioalkalivibrio* sp. K90mix, CP001905:423231-424758; *Allochrochromatium vinosum* DSM 180, CP001896:112452-113967; *Ectothiorhodospira halochloris* M59152; *Burkholderia phytofirmans* PsJN, CP001052:1541578-1543101; *Desulfovibrio vulgaris* subsp. *vulgaris* strain Hildenborough, AE017285:105921-107426; *Rhodobacter sphaeroides* 2.4.1, CP000143:1-1464; *Rhodospirillum rubrum* ATCC 11170, CP000230: 192528-194004; *Escherichia coli* B strain REL606, CP000819: 226609-228150. The 16S rRNA sequences were aligned by MUSCLE [37]. The tree was determined by the maximum likelihood model of PhyML [38] and rendered with TreeDyn [39], using the “one click” pipeline of the Phylogeny.fr web resource [40].

**Table 1.** Classification and general features of *H. halophila* SL1 according to the MIGS recommendations [25].

MIGS ID	Property	Term	Evidence code <sup>a</sup>
		Domain <i>Bacteria</i>	TAS [26]
		Phylum <i>Proteobacteria</i>	TAS [27]
		Class <i>Gammaproteobacteria</i>	TAS [28,29]
	Current classification	Order <i>Chromatiales</i>	TAS [28,30]
		Family <i>Ectothiorhodospiraceae</i>	TAS [31]
		Genus <i>Halorhodospira</i>	TAS [32-34]
		Species <i>Halorhodospira halophila</i>	TAS [32,33]
MIGS-7	Subspecific genetic lineage	DSM 244 <sup>T</sup>	
	Gram stain	negative	NAS
	Cell shape	spiral	TAS [1]
	Motility	motile	TAS [1]
	Sporulation	non-sporulating	NAS
	Temperature range	mesophilic	NAS
	Optimum temperature	47°C	TAS [1]
	Carbon source	CO <sub>2</sub> , succinate, acetate	TAS [35]
	Energy source	photosynthesis	TAS [1]
MIGS-6	Habitat	salt lake mud	TAS [1]
MIGS-6.3	Salinity	Extreme halophile	TAS [1]
MIGS-22	Oxygen	anaerobe	TAS [1]
MIGS-15	Biotic relationship	free living	NAS
MIGS-14	Pathogenicity	none	NAS
MIGS-4	Geographic location	Summer Lake, Lake County, OR	TAS [1]
MIGS-5	Sample collection time	about 1967	TAS [1]
MIGS-4.1	Latitude	not reported	
MIGS-4.2	Longitude	not reported	
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

<sup>a</sup>Evidence codes - IDA: Inferred from Direct Assay; 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 [36]. If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or an expert or reputable institution mentioned in the acknowledgements.

## Genome sequencing and annotation

### Genome project history

This organism was selected for sequencing to better understand its halophilic adaptations, its unusual sulfur metabolism, its photosynthetic pathways, and to provide a framework for better understanding signaling pathways for photoactive yellow protein. The complete genome sequence

has been deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). Table 2 presents the project information and its association with MIGS version 2.0 compliance [25].

**Table 2.** Project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	40kb, 8kb, 3kb
MIGS-29	Sequencing platforms	Sanger
MIGS-31.2	Fold coverage	12×
MIGS-30	Assemblers	phrap
MIGS-32	Gene calling method	Critica
	Genbank ID	CP000544
	Genbank Date of Release	January 12, 2012
	GOLD ID	Gc00492
	Project relevance	extremophile

### Growth conditions and DNA isolation

*H. halophila* SL1 strain DSM 44<sup>T</sup> was obtained from Deutsche Sammlung vor Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and were grown in DSMZ 253 medium. The cells were grown anaerobically and photosynthetically by placing them in 20 ml glass culture tubes completely filled with growth medium and sealed with screw caps. The tubes were kept at 42°C in a water bath and illuminated with 70 W tungsten light bulbs. Chromosomal DNA was purified from the resulting cell cultures using the CTAB procedure.

### Genome sequencing and assembly

The random shotgun method was used in sequencing the genome of *H. halophila* SL1. Large (40 kb), median (8 kb) and small (3 kb) insert random sequencing libraries were sequenced for this genome project with an average success rate of 88% and average high-quality read lengths of 750 nucleotides. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with Dupfinisher (unpublished, C. Han) or by transposon bombing of bridging clones (EZ-Tn5 <P6Kyori/KAN-2> Tnp Transposome kit, Epicentre Biotechnologies). Gaps between contigs were closed by editing, custom primer walks or PCR amplification. The completed genome sequence of *H. halophila* SL1 contains 36,035 reads,

achieving an average of 12-fold sequence coverage per base with error rate less than 1 in 100,000.

### Genome annotation

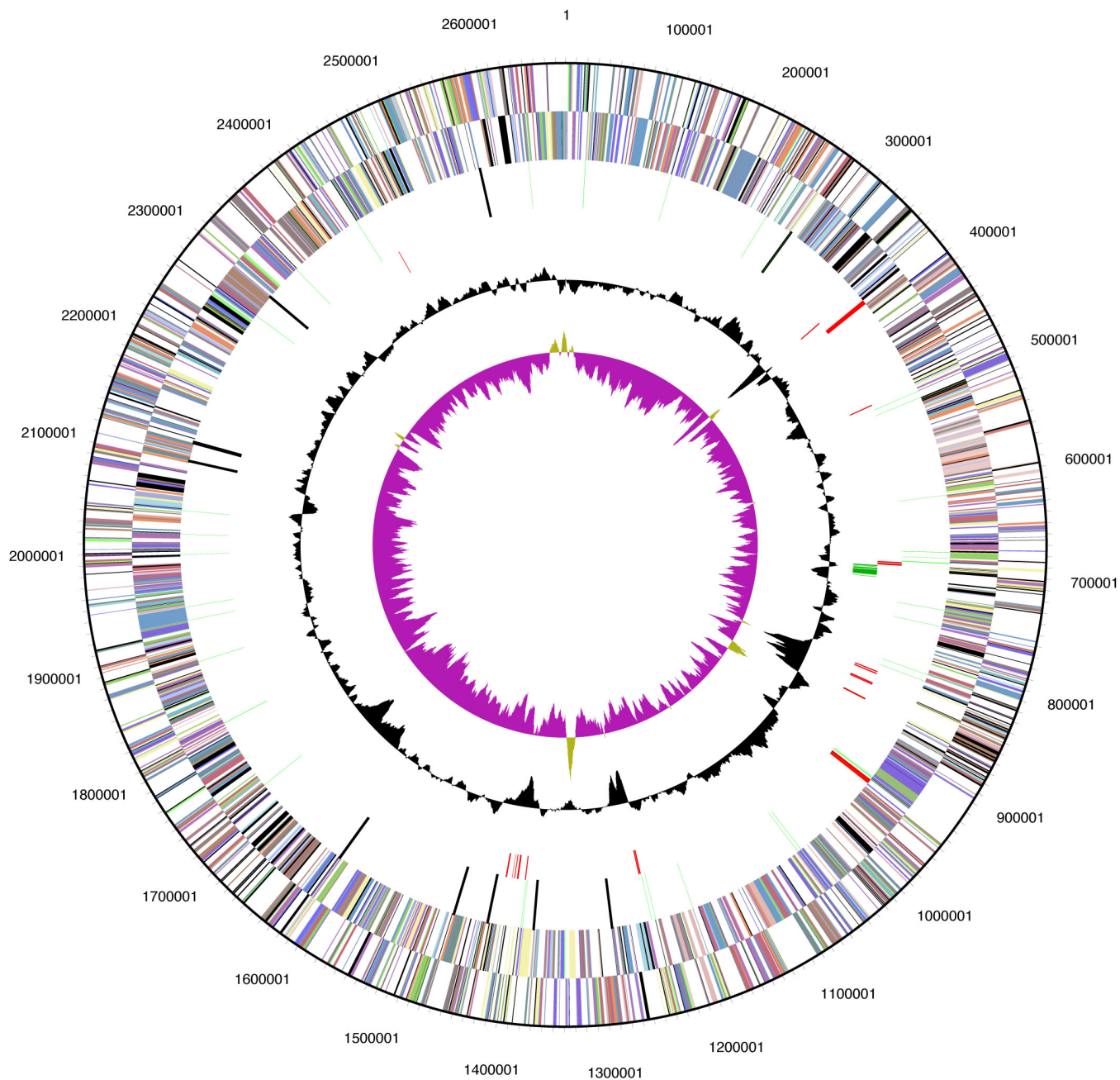
Identification of putative protein-encoding genes and initial automated annotation of the genome was performed by the Oak Ridge National Laboratory genome annotation pipeline. Additional gene prediction analysis and functional annotation was performed within the IMG platform [41].

### Genome properties

The genome is 2,678,452 bp long and comprises one circular chromosome with 67% GC content (Figure 2). For the main chromosome, 2,493 genes were predicted, 2,407 of which are protein-coding genes. A total of 1,905 of protein coding genes were assigned to a putative function, with the remaining annotated as hypothetical proteins. In addition, 31 pseudo genes were identified. The properties and the statistics of the genome are summarized in Tables 3-4.

### Conclusion

*H. halophila* is among the most halophilic eubacteria known. Further analysis and characterization of its genome will provide insights into the mechanisms it uses to adapt to hypersaline environments.



**Figure 2.** Graphical circular map of the genome. From outside to the center: Circle 1, genes on forward strand (colored by COG categories); Circle 2, genes on reverse strand (colored by COG categories); Circle 3, RNA genes (tRNAs green, rRNAs red, other RNAs black); Circle 4, mobile element genes; Circle 5, CRISPR-associated protein genes; Circle 6, GC content; Circle 7, GC skew.



**Table 3.** Nucleotide content and gene count levels of the genome

Attribute	Value	% of total
Genome size (bp)	2,678,452	100.00%
DNA coding region (bp)	2,437,391	91%
DNA G+C content (bp)	1,794,562	67%
Total genes	2493	
RNA genes	63	2.65%
rRNA operons	2	
Protein-coding genes	2,407	96.55%
Pseudo genes	31	1.24%
Genes in paralog clusters	204	8.19%
Genes assigned to COGs	1,457	58.44%
Genes with signal peptides	499	20.02%
Genes with transmembrane helices	554	22.22%

**Table 4.** Number of genes associated with the 25 general COG functional categories

Code	Value	%age <sup>a</sup>	Description
J	147	5.9	Translation
A	1	0.0	RNA processing and modification
K	86	3.5	Transcription
L	125	5.0	Replication, recombination and repair
B	1	0.0	Chromatin structure and dynamics
D	36	1.4	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	29	1.2	Defense mechanisms
T	156	6.3	Signal transduction mechanisms
M	144	5.8	Cell wall/membrane biogenesis
N	93	3.7	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	80	3.2	Intracellular trafficking and secretion
O	103	4.1	Posttranslational modification, protein turnover, chaperones
C	168	6.7	Energy production and conversion
G	76	3.1	Carbohydrate transport and metabolism
E	158	6.3	Amino acid transport and metabolism
F	46	1.9	Nucleotide transport and metabolism
H	152	6.1	Coenzyme transport and metabolism
I	72	2.9	Lipid transport and metabolism
P	122	4.9	Inorganic ion transport and metabolism
Q	37	1.5	Secondary metabolites biosynthesis, transport and catabolism
R	222	8.9	General function prediction only
S	167	6.7	Function unknown
-	493	19.8	Not in COGs

<sup>a</sup> The total is based on the total number of protein coding genes in the annotated genome.

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