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Draft genome of *Halomonas* strain GFAJ-1 (ATCC BAA-2256)

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Running title "Genome of *Halomonas* strain GFAJ-1"

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Abstract

***Halomonas* strain GFAJ-1 was reported in *Science* magazine to be a remarkable microbe for which there was "arsenate in macromolecules that normally contain phosphate, most notably nucleic acids." The draft genome of the bacterium was determined (NCBI accession numbers AHBC01000001 through AHBC01000103. It appears to be a typical gamma proteobacterium.**

***Halomonas* is a diverse genus of halophilic, alkalophilic gamma proteobacteria (2). Strain GFAJ-1 (13) has not been assigned a species name. The motivation for isolation of strain GFAJ-1 was to find "shadow life", which is defined in Wikipedia (http://en.wikipedia.org/wiki/Shadow_life) as life on earth "which has no evolutionary connection with life currently known to science" (perhaps cells without DNA or ribosomes). There is no evidence for "shadow life".**

When the initial report on strain GFAJ-1 was published (13), there was an immediate negative reaction (e.g. 4, 7, 10, 12) to the claim of "arsenate in macromolecules that normally contain phosphate, most notably nucleic acids", arguing about the inadequacy of the data supporting the claim and the expected instability of arsenate di-ester bonds. No progress has occurred on these matters since the initial publication. The Oremland laboratory has published on novel arsenic metabolism of other species (5, 6,11). The genome of strain GFAJ-1 was determined in an effort toward further understanding. The genome indicates that strain is a gamma proteobacterium, the same class that includes *Escherichia coli.* There is no indication in the genome of any unusual or unexpected metabolism. However, the genome does not directly address the basic problem.

Halomonas strain GFAJ-1was grown in the medium described (13) with 1.5 mM phosphate but no added vitamins or tungsten. The medium was supplemented with 0.2 g/l yeast extract, 10 mM KCl, and 10 mM potassium glutamate. The strain grows rapidly, with a doubling time of 2.5 hours and approximately 10^9 cells/ml after overnight incubation at 29 °C (data not shown). Cells in late log phase growth were harvested and lyzed by EDTA, lysozyme, and detergent treatment, followed by proteinase K and RNAse digestion. DNA isolation was by phenol/chloroform/isoamyl alcohol extraction and repeated isopropanol/ethanol precipitation (8). DNA purity was measured as A260nm/A280nm ratio and a single DNA band of size over 20 kbp was observed (data not shown) after agarose gel electrophoresis. The genome was sequenced using the Illumina Hiseq 2000 sequencing platform, with a random subset of 3.5 million paired-end reads (175x coverage) used for assembly with MIRA V3.4rc2 into 103 contigs that were submitted to GenBank.

Strain GFAJ-1 was initially (13) placed in genus *Halomonas* based on the sequence of its 16S rRNA gene; the genome sequence includes AHBC01000086.1, nte 475-2006, that is identical to that in ref. 13, with a single exception in the 5' "PCR primer" that was used (13). The *Halomonas* strain genome projects currently published are for *Halomonas* sp. strain TD01 (3) (NCBI accession AFQW00000000.1) and for *H. elongata* strain DSM 2581 (9) (NCBI sequence NC_014532.1); the genome of strain GFAJ-1 appears closely related to that of *Chromohalobacter salexigens strain* DSM 3043 (1). It is of interest to analyze potential genes involved in arsenic metabolism and resistance. The predicted protein-encoding genes do not include now-standard *ars*-gene operon of other proteobacteria, including *E. coli*. In particular, there appears to be an absence of genes for the ArsB arsenite efflux membrane protein and the ArsC arsenate reductase enzyme.

Nucleotide sequence accession numbers. The 3,624,896 nte in 103 contigs, 3341 CDS plus + 68 RNAs = 3409 genes draft genome of *Halomonas* strain GFAJ-1 was deposited in GenBank (http://www.ncbi.nlm.nih.gov/projects/WGS/WGSprojectlist.cgi) under accession numbers AHBC01000001 through AHBC01000103.

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