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Genome of *Halomonas* Strain GFAJ-1, a Blueprint for Fame or Business as Usual

Eun-Hae Kim[a] and Christopher Rensing[b]

Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, Arizona, USA,[a] and Center for Agricultural and Environmental Biotechnology, RTI International, Research Triangle Park, North Carolina, USA[b]

Quae volumus, credimus libenter!
—What we desire, we readily believe.

"Evidence for arsenate in macromolecules that normally contain phosphate, most notably nucleic acids" (18). This extraordinary claim made in a research article in the December 2010 issue of Science *express* placed *Halomonas* sp. strain GFAJ-1 front and center of a fairly extensive discussion, quickly making this organism significantly interesting to a broad audience. It is apparent that the central question of whether arsenate can replace phosphate in DNA is still unresolved, as the 12 authors of the original paper hold to their conclusion as a reasonable interpretation of their data (17), while the majority of experts on arsenic microbiology and nucleic acid structure interpret the published data to be inconclusive (1, 2, 4–6, 9, 12–14). In early December 2011, 1 year after the *Science* paper appeared, the genome of GFAJ-1 was released in GenBank; now it is presented in a Genome Announcement by Phung et al. in this issue of the *Journal of Bacteriology* (10), providing some insight into the metabolic potential of this beguiling microorganism. However, there is agreement that the genome does not directly answer whether arsenate can substitute for phosphate in biomolecules, specifically DNA. Nevertheless, it helps contextualize an artist's canvas. Finishing a painting can be an emotional affair as an artist sees for the first time his or her vision made concrete. Scientists, as artists, long for this ephemeral moment. Over a year ago now, with publication of the original paper by Wolfe-Simon et al. (18), a few thought and hoped such a moment had arrived. Could poisonous arsenic also be the backbone of life, replacing phosphorus? Alas, the backlash was fierce, as can be expected for both scientific milestones and blunders (1, 2, 9, 12, 13, 15).

The recent release of the GFAJ-1 genome (GenBank accession no. AHBC00000000) provides gene content and thereby allows metabolic potential to be inferred, but robust linking of genome content to metabolism cannot be achieved with gene sequence alone. However, it does put the reported findings into perspective and, thus, can elicit necessary scientific explorations to answer testable hypotheses.

The genome of *Halomonas* sp. GFAJ-1 is most closely related to *Halomonas* sp. TD01 isolated from a salt lake (3), *Halomonas bolivienses* LC1 from a hypersaline lake (11), and *Halomonas* sp. HAL1 from soil of a gold mine (8). Upon investigation of arsenic-related genes, we found typical arsenic resistance determinants located on one contig (Table 1). Contig 69 contains a 3-gene operon with predicted gene products ArsH (gi:359787523), ACR3 (gi:359787524), and an NADPH-dependent flavin mononucleotide (FMN) reductase (gi:359787525). However, such news is not astonishing, as other *Halomonas* species, including TD01, LC1, and HAL1, all contain this operon, and indeed, these arsenic genes are distributed throughout the domains *Archaea* and *Bacteria*.

TABLE 1 Putative arsenic-related genes present on the *Halomonas* sp. GFAJ-1 genome

gi	Gene	Putative function	Contig
359787523	*arsH1*	NADPH:FMN-dependent reductase	69
359787524	*acr3*	Arsenical-resistance protein	69
359787525	*arsH2*	Flavoprotein	69
359787343	*mfs1*	Major facilitator superfamily permease	56
359787344	*mfs2*	Major facilitator superfamily transporter	56
359787345	G3P gene	Glyceraldehyde 3-phosphate dehydrogenase, type I	56
359787346	*pstB*	ABC-type phosphate transport system, ATPase component	56
359787347	*pstA*	ABC-type phosphate transport system, auxiliary component	56
359787348	*pstC*	ABC-type uncharacterized transport system, permease component	56
359787349	*pstS*	ABC-type phosphate transport system, periplasm-located phosphate binding protein	56
359787351	*mfs3*	Major facilitator superfamily transporter	56
359785681	*pstB*	ABC-type phosphate transport system, ATPase component	27
359785682	*pstA*	ABC-type phosphate transport system, auxiliary component	27
359785683	*pstC*	ABC-type uncharacterized transport system, permease component	27
359785684	*pstS*	ABC-type phosphate transport system, periplasm-located phosphate binding protein	27

The roles and interplay of these three players in arsenic resistance remain to be investigated. ACR3 is an inner membrane protein involved in the extrusion of As(III) and is widely distributed in bacteria, archaea, and fungi (7). The crystal structures of ArsH from *Shigella flexneri* (16) and *Sinorhizobium meliloti* (19) have been resolved, and the mechanism of function appears to be related to NADPH:FMN-dependent reduction of molecular O_2 to H_2O_2, though the specific substrate(s) and products within

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Address correspondence to Christopher Rensing, crensing@rti.org.

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the cell are still not clear. Lastly, the function of the putative NADPH:FMN-dependent reductase, which is different from ArsH, is not known, although the reoccurrence of this gene situated in the context of other genes encoding arsenic resistance determinants suggests an involvement in an arsenic detoxification process.

A reoccurring theme of "arsenic islands" comprising genes involved in arsenic tolerance, an additional *pst* operon (encoding PstA, PstB, PstC, and PstS) for phosphate-specific transport, transporters of the major facilitator family (MFS), and a putative glyceraldehyde-3-phosphate (G3P) occurs within the genomes of a variety of organisms, such as *Halomonas* sp. TD01 (GenBank accession no. AFQW01000000), *Halomonas* sp. GFAJ1 (GenBank accession no. AHBC01000000), *Herminiimonas arsenicoxydans* (NC_009138), and *Achromobacter* sp. SY8 (GenBank accession no. NZ_AGUF01000052). The roles and relationships among these arsenic-related and contextually affiliated genes are not yet clear, but this reoccurrence suggests some functional entanglement in the handling of arsenic. GFAJ-1 appears to have a similar tentative arsenic island such that the end of contig 69 contains three genes encoding arsenic-related determinants (ArsH, ACR3, and NADPH-dependent FMN reductase) and the beginning of contig 56 contains the remaining determinants of the arsenic island, including the MFS, G3P, and a Pst system (Table 1). Albeit these two regions are spread across two contigs, when linked together, this region highly resembles an arsenic island like those in other *Halomonas* spp.

A recent comment by Foster (6) suggests Wolfe-Simon and coworkers (18) may have selected for mutants employing an arsenate-stimulated high-affinity Pst system. Wolfe-Simon et al. (17) responded by pointing out that stimulation of the Pst system should result in (i) flooding of the cells with arsenate and therefore (ii) arsenate detoxification processes such as arsenate reduction. However, arsenate reduction, a requirement for subsequent transport by ACR3 out of the cytoplasm, was not observed under their tested growth conditions. Interestingly, upon mining the GFAJ-1 genome for genes encoding a putative arsenate reductase, ArsC, none were detected in the vicinity of the arsenic-related resistance determinants. However, a gene annotated *arsC* in a region unrelated to arsenic transformation or handling (*arsC*, gi:359295261) was identified but has no significant similarity to any known *arsC*-like genes. Subsequent experiments are necessary to determine whether arsenate reduction activity truly does not occur in strain GFAJ-1. If, however, future studies determine that this organism has no arsenate reductase activity, then this may argue that GFAJ-1 may more readily divert arsenate into macromolecules than many other organisms, which otherwise rapidly convert it to arsenite and thus remove it from the cell. At this stage, it is too early to draw any definitive conclusions. The exact function of an additional *pst*-like operon that is carried on many arsenic islands is as yet unknown and remains to be determined, whether it is another high-affinity phosphate transporter or somehow possibly interacts with arsenate.

The incorporation of arsenate in DNA may result in small alterations in the configurations of bases but not without consequence (13). Even the smallest differences in base stacking and pairing may result in a demand for accommodating replication and transcription enzymes. Most enzymes dealing with DNA replication or repair are closely related. For example, the DNA polymerase III α and β subunits of GFAJ-1 share 95% and 97% identity with those of TD01, respectively. The γ and τ subunits of DNA polymerase III, encoded by *dnaX*, display differences at the C terminus compared to similar proteins among *Halomonas* strains. The γ and τ subunits are specifically involved with the initiation of DNA replication, but the significance, if any, of these differences among strains remains to be investigated.

A superficial investigation of the *Halomonas* sp. GFAJ-1 genome reveals that it is not significantly different from the genomes of other related halomonads, with the exception of the potential lack of the capability to reduce arsenate. However, we must be reminded that the function of about half of the encoded gene products in projected arsenic resistance operons and how arsenic and phosphate metabolisms are connected are not comprehensively known. Based solely on the genome, it is therefore premature to draw any concrete conclusions regarding the central hypothesis of arsenate replacing phosphate in critical biomolecules, but it does allow us to speculate and draw alternative hypotheses for future explorations.

Perhaps an overlooked finding of the original paper is that many microorganisms living in "extreme" habitats of high arsenic concentrations can optimize their use of phosphate, as Schoepp-Cothenet et al. (14) call attention to: "GFAJ-1 appears to do all it can to harvest P atoms from the medium while drowning in As." For the time being, the debate continues whether the GFAJ-1 biomolecules contain significant amounts of arsenic. Understanding the molecular details of how the metabolisms of phosphate and arsenic are intertwined will take time, and we predict that these investigations will result in many unexpected findings and a turn of events.

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