“Artifactual” arsenate DNA

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The recent claim by Wolfe-Simon et al. that the *Halomonas* bacterial strain GFAJ-1 when grown in arsenate-containing medium with limiting phosphate is able to substitute phosphate with arsenate in biomolecules including nucleic acids and in particular DNA¹ arose much skepticism, primarily due to the very limited chemical stability of arsenate esters (see ref. 2 and references therein). A major part of the criticisms was concerned with the insufficient (bio)chemical evidence in the Wolfe-Simon study for the actual chemical incorporation of arsenate in DNA (and/or RNA). Redfield et al. now present evidence that the identification of arsenate DNA was artifactual.

In a new study, Redfield et al.³ have attempted to reproduce the growth conditions under which *Halomonas* strain GFAJ-1 was reported to utilize arsenate and build it into DNA instead of phosphate. First, Redfield et al. observed no growth of GFAJ-1 in pure AML60 medium, neither after addition of 40 mM arsenate, nor when adding 1.5 mM phosphate. However, AML60 medium supplemented with 1 mM glutamate yielded growth to 5×10^6 cells/ml, and an additional 3 μ M phosphate yielded 2×10^7 cells/ml (similar to the growth level observed in +As/-P medium by Wolfe-Simon et al.¹). Based on these growth level data, Redfield et al. conclude that their AML60 medium had a 1 μ M phosphate contamination, while the medium of Wolfe-Simon et al. most likely had a 4 μ M phosphate contamination, and that their arsenate supported growth was due to a contamination of glutamine [or other amino acid(s)] in the arsenate solution, and not due to the presence of the arsenate. More importantly, Redfield

et al. isolated DNA from GFAJ-1 grown under different conditions (no arsenate, 40 mM arsenate, 3 μ M phosphate and 1.5 mM phosphate) by extraction. The four DNA preparations were identical regarding size (by gel electrophoretic analysis) as well as stability showing no indications of breakage due to labile arsenate linkages, and based on the size of the DNA, it was concluded that less than one arsenate in 25 kb DNA could be present.³ Finally, the DNA was further purified by “old fashioned” and very efficient isopycnic density CsCl gradient centrifugation, nuclease digested to mono- and dinucleotides and free phosphate (arsenate), and was finally analyzed by high resolution LC-MS. As any arsenate present in the DNA would primarily be expected as free arsenate in the digest (due to the low arsenate ester stability) most efforts were devoted to this analysis. This showed that while a small amount (1 out of 300 phosphates) of arsenate could be detected in the initial DNA preparation, this practically disappeared after washing and in the CsCl purified DNA no arsenate was found at a detection limit of 5×10^{-8} M corresponding to an As:P ratio in the DNA of $< 0.1\%$, i.e., more than 50-fold lower than the content reported by Wolfe-Simon et al.¹ Finally, no arsenate containing mono- or di-nucleotides were detected either. Therefore, these results very strongly argue that even when grown in high arsenate and only trace (contaminating) amounts of phosphate, the *Halomonas* bacterial strain GFAJ-1 is able to selectively utilize the phosphate for synthesis of DNA (and presumably other biomolecules) with no (significant) arsenate (mis)incorporation, and that all living species as far we know thus use the same phosphate-linked backbone in their genetic material. Life retains its unity.

Keywords: arsenate, DNA, bacteria, life, genetic material

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