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Comment on "A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus"

Rosemary J. Redfield

University of British Columbia, Vancouver, BC, Canada. E-mail: redfield@zoology.ubc.ca

Wolfe-Simon *et al*. (*Science* Express Research Article, published online 2 December 2010; 10.1126/science. 1197258) reported that bacterium GFAJ-1 can grow by using arsenic instead of phosphorus. However, the presence of contaminating phosphate in the growth medium, as well as the omission of important DNA purification steps, cast doubt on the authors' conclusion that arsenic can substitute for phosphorus in the nucleic acids of this organism.

Wolfe-Simon *et al*. (*1*) reported that arsenic can substitute for phosphorous in the biomolecules of bacterial strain GFAJ-1. Although the researchers meticulously eliminated contamination of the reagents and equipment used in their elemental analyses, they made much less effort to eliminate contamination in their biological samples.

The reagents used for the culture media were not pure. The 3.1 μM phosphate present in the growth medium would have provided enough phosphorous (P) for all of the cell growth seen in this medium, using the authors' estimate of 7.5×10^6 atoms of P per genome and the generous assumption that phosphate-starved cells use 90% of their P for molecules other than DNA (*2*). This calculation (not done by the authors) obviates their hypothesis that the cells could only grow by replacing P with arsenic (As).

An independent contamination problem is the omission of standard DNA purification steps when testing for As in DNA (*3*). Contamination is typical in DNA/RNA pellets produced by ethanol precipitation of the aqueous phases from phenol:chloroform extractions. This is partly because this fraction contains most of the small molecules from the cytoplasm (contrary to the authors' assertion), which are often less soluble in 70% ethanol than in water. Pellets are also typically contaminated with small amounts of the ethanol supernatant. Yet, the usual step of washing the pellets was omitted, and the dried pellets were simply resuspended in water and loaded on an agarose gel.

Most surprisingly, the chromosomal DNA fractions [boxed in figure 2A in (*1*)] were not purified from the gel slices (a standard 10-minute procedure). Instead the authors simply dried the gel slices and assayed them. Not only does this bring in any contaminants present in the gel, but because each gel slice would have contained at least 1 mg of agarose (100 mg of 1% agarose gel), and each DNA band no more than 1 μg of DNA, at least 99.9% of the carbon in these samples would have come from the agarose, not the DNA. No correction can be made for the agarose-derived C because the actual amounts of DNA and agarose are not known. Omission of the gel-removal step for these critical samples is surprising because the authors did use it in preparing the rDNA fragments they sequenced for their phylogenetic analysis. Taken together, these methodological issues raise doubts about the validity of Wolfe-Simon *et al*.'s assertion that GFAJ-1 can vary the elemental composition of its biomolecules by substituting As for P.

References

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