

# Comment on “A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus”

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Wolfe-Simon *et al.* (Research Articles, 3 June 2011, p. 1163; published online 2 December 2010) reported that bacterial strain GFAJ-1 can substitute arsenic for phosphorus in its biomolecules, including nucleic acids and proteins. Unfortunately, their study lacks crucial experimental evidence to support this claim and suffers from inadequate data and poor presentation and analysis.

The basic principles of life rest on the organization of different autocatalytic reaction systems (1), the exact chemical realization of which depends on the laws of chemistry, environmental conditions, and evolution. Finding that one element can be replaced by a similar one under certain conditions does not seem implausible, although finding such a system would be a major discovery. Wolfe-Simon *et al.* (2) reported that the bacterial strain GFAJ-1 can use arsenic (As) instead of phosphorus (P) in its basic biomolecules, but their conclusion is not well supported by the presented data.

To show the arsenate-dependent growth of strain GFAJ-1, Wolfe-Simon *et al.* measured the percentage of As and P in the dry weight of the bulk intracellular material of their samples. In table 1 in (2), they report that the mean intracellular As in +As/-P cells was  $0.19 \pm 0.25\%$  by dry weight, but only  $0.001 \pm 0.0005$  for cells grown in the -As/+P condition. To summarize the differences between the samples, the authors reported the As:P ratio for both the +As/-P (7.3) and the -As/+P (0.002) conditions. To arrive at these ratios, the authors calculated the ratios for each individual sample measurement and then averaged the ratios [see table S1 in (2)]. Averaging ratios in this way is incorrect. To illustrate the pitfall of this approach, take the hypothetical example of two experiments to determine the elemental profile of a bacterium. Experiment 1 yields As = 1, P = 10, As:P = 0.1, and P:As = 10. Experiment 2 yields As = 10, P = 1, As:P = 10, and P:As = 0.1. If we calculate the average As:P ratio

based on these values, we arrive at 5.05. From this value, we can calculate the P:As ratio that is the reciprocal of 5.05, namely 0.198, and conclude that on average, there is much more As than P in our samples. If we instead calculate the average P:As ratio first, it would be 5.05 and the As:P ratio 0.198, suggesting much more P than As. Wolfe-Simon *et al.* report a As:P ratio of 7.3 [shown in table 1 in (2)], but if calculated in reverse, this value would be a much less impressive 1.59.

Another concern with the data reported in table 1 (2) involves the calculated errors. The error for the As percent by dry weight ( $\pm 0.25\%$ ) is larger than the value itself (0.19%), so the null hypothesis that the +As/-P sample contains no As, cannot be excluded. Although the P concentration seems to be considerably smaller for the +As/-P grown sample (0.019%) than for the -As/+P (0.54%), the authors do not discuss whether this amount is sufficient to sustain the organism's cellular processes. However, closer inspection of table S1 in (2) reveals that this small amount of P may indeed be sufficient. In this table, the authors report two batches of measurements, one taken in June 2010 and the other in July 2010. Although the authors note that the June batch data are of poor quality in terms of correlation coefficients, they neither exclude these data nor attribute larger estimated errors to them, but rather average all of the data together. We can see that for the +As/-P sample in the July batch, the bacteria could survive with around 0.01% As and 0.01% P dry weight. Even if As can replace P, the sum of these values (0.02%) is not appreciably more than the amount of P in the +As/-P sample (0.019%). For the July batch, the measured As:P ratio is even smaller than 1 and does not prove that the bacteria use As instead of P. In the June batch, the amount of P is about twice this, and the measured amount of As is about 10 to 60 times as much.

This difference in As amount seems well beyond a reasonable standard fluctuation. Averaging together such bimodal data renders it difficult to draw meaningful conclusions from them.

Wolfe-Simon *et al.* used high-resolution secondary ion mass spectrometry to identify As in extracted, gel-purified DNA. Table S2 in (2) reports elemental concentrations and ion ratios for one +As/-P experiment, two -As/+P experiments, and one baseline measurement made on a blank agarose gel. The baseline value for P was  $820 \pm 143$  parts per billion (ppb), yet the P concentration in the +As/-P DNA sample was only  $299 \pm 36$  ppb. These two values alone indicate large fluctuations in the measurements and raise concerns about their accuracy. One possible solution is to use the reported baseline value in further calculations, because there is no reason a sample would contain less P than the blank gel itself. If we do so, we see that the amount of P is only slightly higher in the -As/+P samples. For the measurement of As concentration, the baseline value with estimated error is  $15 \pm 3$  ppb. However, this error estimate appears to be too optimistic: The As concentrations reported for the two -As/+P experiments are  $14 \pm 3$  ppb and  $5 \pm 1$  ppb, meaning that even in three measurements, the value could fluctuate from 15 ppb down to 5 ppb, which suggests that the correct error should be around 10 ppb. If we take this value, we see that the As concentration reported for +As/-P sample ( $27 \pm 10$  ppb) is not significantly larger than the As concentration for the -As/+P sample or the baseline ( $15 \pm 10$  ppb). In several other places in their Research Article, Wolfe-Simon *et al.* do not provide specific error estimates, but rather use the ad hoc value of 10% as standard deviation. In addition, some of their figures visually suggest just the opposite of the true data relations because of different color scales [figure 2, B to E in (2)] and orders of magnitude scaling difference of the axes [figure S2 in (1)].

Finally, the data presented by Wolfe-Simon *et al.* (2) do not show that As is biochemically incorporated into the DNA of GFAJ-1. To support such an extraordinary claim, additional chemical and structural analyses showing the replacement of phosphate by arsenate should have been provided.

## References and Notes

1. T. Gánti, *The Principles of Life* (Oxford University Press, Oxford, 2003).
2. F. Wolfe-Simon *et al.*, *Science* **332**, 1163 (2011); published online 2 December 2010; 10.1126/science.1197258.
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