Biology 1510 Fall 2012

Read: Wolfe-Simon, F. et al. 2011 A bacterium that can grow by using arsenic instead of phosphorus Science 332:1163-1166 http://www.sciencemag.org/content/332/6034/1163.full at least up through the "Geomicrobiology of GFAJ-1". This paper created much excitement and controversy, as the first report that a living cell may be able to use an element other than the canonical six elements that comprise organic molecules.

Work in groups of 3-5 members. Divide and conquer; assign different questions to each group member, then discuss the answers together. Some of the answers are in the Wolfe-Simon et al. paper; others you may have to look up in the textbook or other sources.

1. What are the six major elements that comprise living organisms?

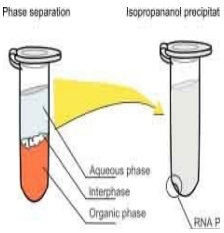
2. What cellular macromolecules contain the element P?

3. Why is arsenate (AsO_4^{3-}) toxic to living organisms?

4. Do you think GFAJ-1 existed in the original inoculum from Mono Lake sediment, or did it evolve during culture?

5. What do you conclude from Figures 1A and 1B? Do these look like logistic growth curves?

Wolfe-Simon et al. used a radioactive isotope of As to determine whether As became incorporated into macromolecules (Table 2) in GFAJ-1 cells.



After feeding their cells with radioactively labeled arsenate, they lysed the cells with detergent and performed an extraction with phenol and chloroform. In this extraction process, phenol, a much less polar solvent than water, emulsifies with water, then separates into an organic solvent layer. Detergent molecules unfold water-soluble proteins and bind to exposed hydrophobic amino acid side chains. These exposed hydrophobic side chains interact with non-polar solvents like phenol, causing the unfolded proteins to aggregate at the interphase, with the hydrophobic side chains interacting with phenol, and the hydrophilic side chains interacting with water.

6. What will happen to lipids, carbohydrates and nucleic acids exposed to phenol? When the phenol and water separate into organic and aqueous phases, which of these molecules will be in the organic phase, and which will be in the aqueous phase?

7. If the GFAJ-1 cells could use arsenate in place of phosphate for all cellular processes, which molecules would you expect to have the most labeled arsenate? Table 2 shows that 80% of the radioactive arsenate ended up in the phenol phase. Does this agree with your expectations?

8. The basal culture medium used by Wolfe-Simon et al. contained 3×10^{-6} mole/L phosphate even with no phosphate added. Marine bacteria grown in P-limiting conditions contain about 2 fg (femtogram = 10^{-15} gram) per cell. Wolfe-Simon estimates that 0.4 fg of phosphate are needed for each copy of the genome (DNA) of GFAJ-1. How much cell growth will 3 micromolar phosphate support, if GFAJ-1 has the same phosphate requirements as the marine bacteria? What if GFAJ-1 used the phosphate just for replicating DNA? State your answers in terms of cells/mL. Does this change your interpretation of Figs. 1A and 1B? The molar mass of phosphate is 95 g/mole; you can approximate it as 100 g/mole.