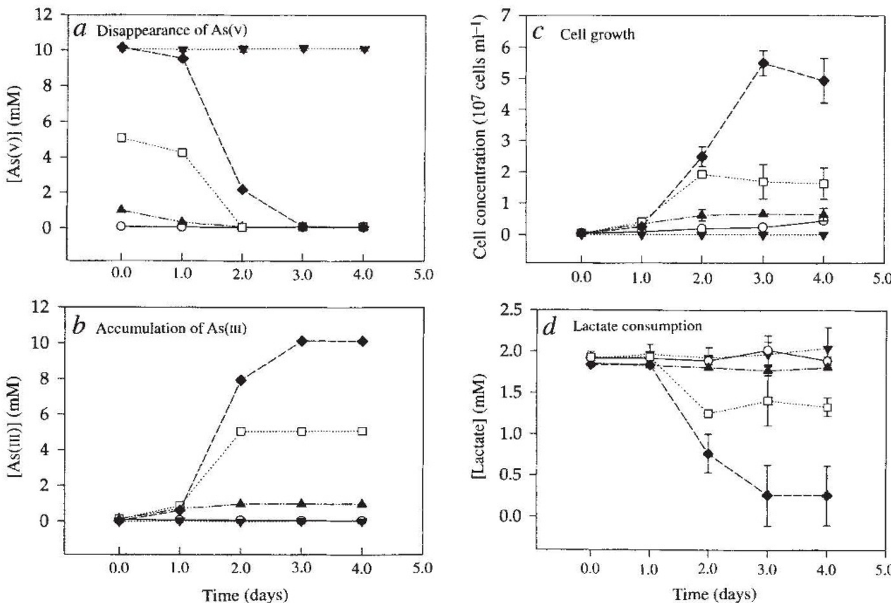
Microbe grows by reducing arsenic

SIR — Arsenic is a legendary poison, renowned in its effectiveness against heroines, gentlemen, rats and wood rot. Arsenic is lethal to microorganisms, yet certain bacteria and phytoplankton are known to survive arsenic exposure and transform arsenic species through oxidation[1], reduction[2] and methylation[3]; to date, these transformations are thought to occur either incidentally or through specific detoxification mechanisms[4]. There is an extraordinary diversity in microbial use of inorganic compounds as terminal electron acceptors in anaerobic respiration, from the common Fe(III) (ref. 5) and Mn(IV) (ref. 6) to the exotic U(VI) (ref. 7). Thermodynamic calculations show that dissimilatory arsenate reduction could also provide sufficient energy to sustain microbial growth, although the universal toxicity of arsenic militates against such an idea. We report here, however, the discovery and isolation of a microorganism, designated MIT-13, that reduces As(V) to As(III) and gains energy for growth from this reduction.

The isolate MIT-13 was obtained from arsenic-contaminated sediments of the Aberjona watershed in eastern Massachusetts. Anoxic sediments were inoculated into minimal medium to develop an enrichment culture, and a pure culture was obtained by successive colony isolations on solid medium.

To demonstrate the dependence of cell growth and substrate consumption on arsenate concentration, we inoculated MIT-13 into medium supplemented with 2 mM lactate and various concentrations of arsenate. Within 4 days, complete disappearance of As(V) (*a* in the figure) was accompanied by stoichiometric appearance of As(III) (*b*). Cells grew as As(V) was reduced (doubling time approximately 14 h with 10 mM arsenate) and reached final numbers that were proportional to initial As(V) concentrations (*c*). Lactate disappearance was observed in proportion to As(III) production (*d*). Uninoculated cultures showed no arsenic reduction, lactate consumption, or cell growth. Inoculated cultures deprived of As(V) showed minimal cell growth and lactate consumption. Addition of formaldehyde to a final concentration of 4% completely inhibited both cell growth and arsenate reduction.



a, As(V) concentration; *b*, As(III) concentration; *c*, cell growth; *d*, lactate consumption over a 4-day incubation of MIT-13 in anaerobic medium supplemented with lactate as substrate and AS(V) as electron acceptor. Cells were inoculated into duplicate cultures of arsenate-lactate medium containing 0 (O), 1(▲), 5(□), and 10 mM (◆) arsenate. An additional set of bottles with 10 mM arsenate was prepared, not inoculated, and treated exactly as the others (▼). At 24-h intervals, samples were taken from each bottle and analysed for cell concentration, lactate concentration, and arsenic concentration and speciation. Cell concentrations were determined by DAPI staining and direct counting with epifluorescence microscopy. Lactate concentrations were measured by HPLC with separation on a polysulphonate ion exclusion column (Hamilton PRP × 300) and detection by ultraviolet absorbance at 210 nm. Arsenic concentrations and speciation were measured by the molybdenum blue spectrophotometric assay[9]. Medium for enrichment and purification consisted of freshwater minimal salts medium buffered at pH 6.8 with $NaHCO_3$ and supplemented with 0.1 mM cysteine, 2 mM lactate and 10 mM sodium arsenate. Medium was reduced with $PdCl_2$ (0.005%) + H_2, and immediately after inoculation headspaces were changed to a N_2:CO_2 ratio of 80:20. All incubations were carried out under N_2:CO_2::80:20 atmosphere at 22 °C in darkness.

Microscopic examination of MIT-13 reveals a vibrio-shaped, highly motile microorganism of approximately 1 μm in length and 0.1 μm in width. Cells are unable to use acetate as a substrate, but using lactate they are able to grow on sulphate as well as arsenate. Arsenate reduction and cell growth are transiently inhibited by molybdate (50% increase in lag phase at 20 mM) but not by sulphate. Complete reduction of 10 mM arsenate in the presence of 2 mM lactate and 20 mM sulphate demonstrates that As(V) is the preferred electron acceptor in cultures previously grown on As(V). Oxygen completely inhibits both arsenate reduction and cell growth.

Before the isolation of MIT-13, we incubated sediment samples from the Aberjona watershed in anoxic freshwater medium supplemented with typical fermentation end-products (acetate, lactate and butyrate) and solid iron (III) arsenate, a compound representative of sedimentary arsenic[8]. The enrichment cultures did indeed cause dissolution and reduction of the arsenate to arsenite within several days. Autoclaved and formaldehyde-killed cultures did not dissolve nor reduce the ferric arsenate, showing that active metabolism is necessary to transform the arsenate (data not shown).

Our evidence shows that rapid, extensive arsenate reduction and dissolution can be accomplished by microbial activity, both in pure culture and in natural consortia, and suggests that microbial metabolism plays a greater, more direct role in arsenic cycling than previously suspected.

Dianne Ahmann
Department of Civil and Environmental Engineering,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139, USA
A. Lynn Roberts
Department of Geography and Environmental Engineering,
Johns Hopkins University,
Baltimore, Maryland 21218, USA
Lee R. Krumholz
Department of Botany and Microbiology,
University of Oklahoma,
Norman, Oklahoma 73019, USA
François M. M. Morel
Department of Geology,
Princeton University,
Princeton, New Jersey 08540, USA

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