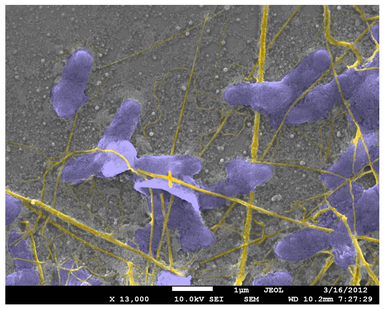
Direct *in vivo* Observation of Microbially Synthesized Nanostructured Materials

J. R. Lazzari-Dean, I. R. McFarlane, M. Y. El-Naggar
Department of Physics and Astronomy, University of Southern California

USC University of Southern California

Introduction

Microbes have a remarkable ability to synthesize nanostructures with precise control over their composition and functional properties. Understanding and exploiting these microbial activities will result in potentially transformative approaches to synthesizing nanomaterials for semiconductor, optical, and photoactive applications. Our laboratory primarily investigates *Shewanella* species, which are known to reduce a variety of terminal electron acceptors, including thiosulfate, arsenate, selenite, and tellurite[1]. Here we use diascopic, bright field, and fluorescence microscopy to investigate the mechanism through which *Shewanella* species produce arsenic sulfide nanomaterials.

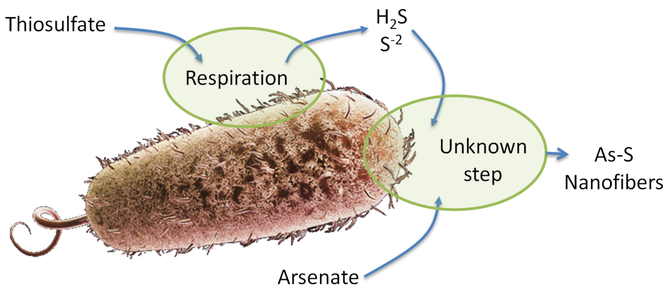


False color SEM image depicting cells (purple) and fibers (yellow). Fiber width ranges from 20 to 200 nm.

Method

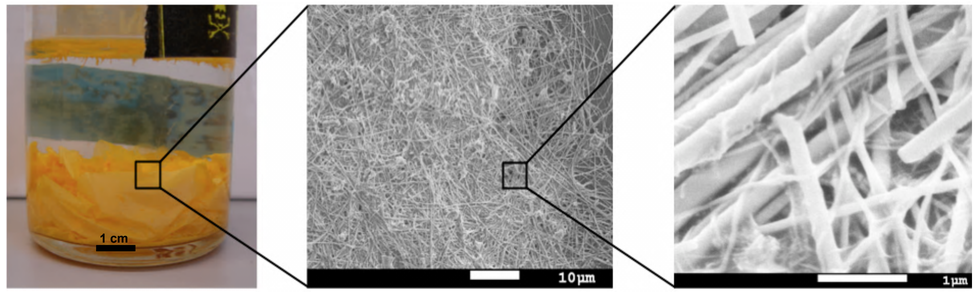
Shewanella sp. strain ANA-3 [2,3] is incubated under anaerobic conditions with 20 mM lactate as the electron donor (carbon source) and 10 mM thiosulfate as the electron acceptor for respiration (oxidant), in the presence of 5 mM sodium arsenate heptahydrate ($Na_2HAsO_4.7H_2O$). Fiber formation in the chamber is then monitored using *in situ* microscopy.

Proposed Pathway



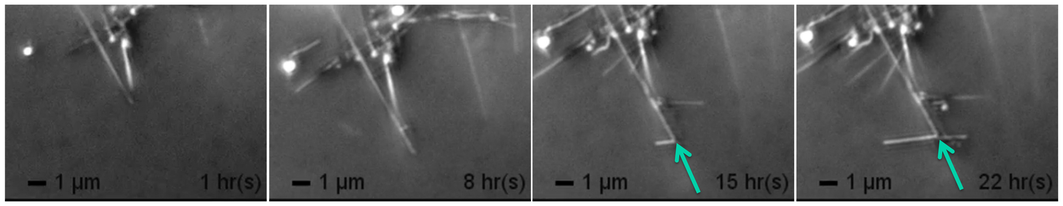
1 A. Klonowska *et. al., App. and Enviro. Microbio.* **71,** 5607 (2005)
2 C. Saltikov *et. al, App. and Enviro. Microbio.* **69**, 2800 (2003)
3 C. Saltikov *et. al, PNAS.* **100**, 10983 (2003)

Experimental Analysis

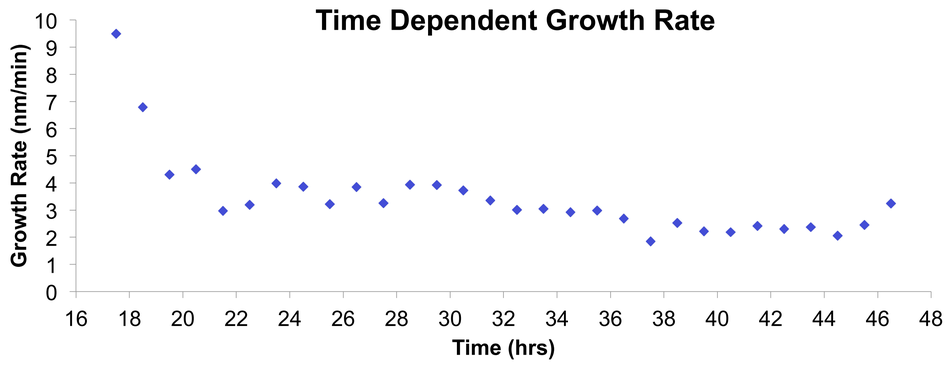


(A) Photograph of bright yellow macroscopic sheets of As_xS_y that precipitated from solution. (B) SEM image of the sheets composed of nanofibers. (C) A higher magnification SEM image showing individual nanofibers.

Bright Field Microscopy

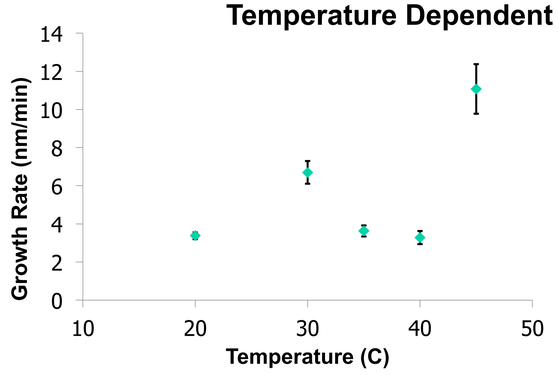


A series of images showing growth of arsenic sulfide nanofibers as observed with bright field microscopy at 45˚C. Elongation after the bend in the fiber indicates growth is from the fiber's tip. Fiber morphology remains consistent at different growth temperatures.



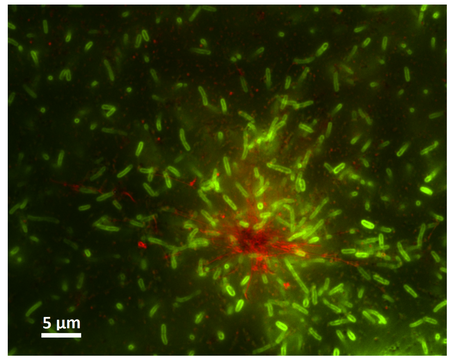
Binned averages of 20˚C growth rate of more than 50 arsenic sulfide nanofibers. Decay over time suggests dependence on arsenic and sulfur concentration. Growth rate remains roughly constant at later times, implying that arsenic and sulfur concentrations have become limiting.

Temperature Dependent Growth Rate



Average growth rates over the first 24 hours at various elevated temperatures. Preliminary data show an increased growth rate at higher temperatures, and further experiments will be conducted to verify. Error bars shown represent two standard errors.

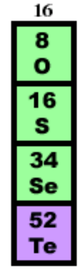
Fluorescence Microscopy

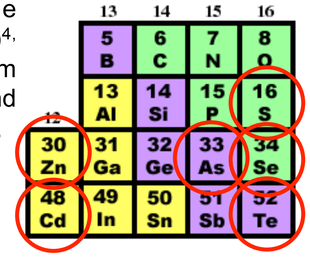


Combined bright field (red) and fluorescence (green) microscope images of room temperature growth with NanoOrange fluorescent protein stain. Stain was used to clarify the spatial relationship between cells and nanofibers and to determine if the nanofibers contain protein. The image above indicates that there is no protein component in the fibers and that local cell density is not significantly higher in the vicinity of fibers.

Future Applications

We are currently studying the ability of *Shewanella* strains (such as MR-1, which can respire selenite and tellurite)[1] to produce selenide or telluride nanostructures. Since our findings suggest that nanofiber production is biotically influenced but crystallization is abiotic, we may be able to replace the arsenic with other elements such as cadmium or zinc. This may provide a scalable method to efficiently produce a variety of semiconductor nanostructures, including arsenic selenide (optomechanical properties)[4], cadmium selenide (quantum dots and laser diodes), and cadmium telluride (solar cells).





Summary

Shewanella sp. strain ANA-3 can produce macroscopic quantities of As-S nanofibers. Nanofiber growth rates exhibit concentration dependence, and fluorescence images of cells and fibers suggest biotically influenced nucleation and abiotic elongation processes.

4 P. Krecmer *et al.*, *Science* **277**, 1799 (1997)
5 H Hur *et. al, PNAS.* **104**, 20410 (2007)