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THE MICROBIAL ECOLOGY OF ANOXYGENIC ARSENITE OXIDIZING PHOTOAUTOTROPHS IN EXTREME ENVIRONMENTS

Project Report

Introduction

Although arsenic is commonly associated with murder and poison, it is less commonly recognized as an element ubiquitous in nature. An important contaminant of drinking water, arsenic is highly carcinogenic, with tens of millions of people exposed to unsafe levels of drinking water worldwide[2]. In the environment, inorganic arsenic cycles between two primary oxidation states: i) arsenite, which is the more mobile and toxic of the two, and ii) arsenate, which has a tendency to adhere to iron minerals. Microbial metabolism is the primary driving force behind the cycling of arsenic between these two forms in the environment[3].

Remarkably, one important reason behind this microbial cycling of arsenic is to gain energy. In other words, some bacteria and archaea are able to survive by "breathing" or "eating" arsenic. They gain the ability to do so by encoding at least one of several enzymes belonging to the DMSO reductase family of enzymes. A new and poorly understood member of this family of enzymes was recently discovered and is the focus of my research at the University of California, Santa Cruz. ArxA was first identified in *Alkalilimnicola ehrlichii* str. MLHE-1[1], a bacterium isolated from Mono Lake, an alkaline, hypersaline lake rich in arsenic located in Mono County, California[4]. Only one other arsenite-oxidizing organism has been shown to contain an *arxA*-like sequence: *Ectothiorhodospira sp.* str. PHS-1. An anaerobic, thermophilic, alkaliphilic and halotolerant phototroph, PHS-1 has the honor of being the first isolate shown capable of photosynthesis-linked arsenite oxidation, or arsenophototrophy[6].

Arsenophototrophy is interesting for a number of reasons. First of all, because it is a metabolism that allows for the conversion of the more toxic arsenite to the less toxic arsenate, it may be useful for bioremediation. Additionally, arsenophototrophy facilitates growth in extreme environments, making it a potential form a metabolism on other planets or satellites. Along the same lines, because anaerobic photosynthesis is one of the earliest known metabolisms on early Earth, arsenophototrophy is also potentially billions of years old[5]. On top of these reasons is the fact that so little is known about arsenophototrophy. ArxA has so far only been identified in two organisms from the same lake, and the distribution of the oxidase is unclear. Additionally, it is not even known for sure that the *arxA*-like sequence in PHS-1 encodes the arsenite oxidase responsible for arsenophototrophy. The two *arx* operons of MLHE-1 and PHS-1 are homologous and are found in the same order. Additionally, I have previously shown that *arxA* is strongly upregulated in the presence of arsenite as compared to other electron acceptors[7]. However despite this, all attempts to genetically modify PHS-1 and clearly demonstrate ArxA's role in this organism have failed.

Project Objectives

This project addresses two primary questions concerning arsenophototrophy. Firstly, what is the distribution and diversity of *arxA*-like sequences in environments rich in arsenic, and

secondly, do the *arxA*-like sequences in arsenophototrophs encode arsenite oxidases? These questions have been addressed by:

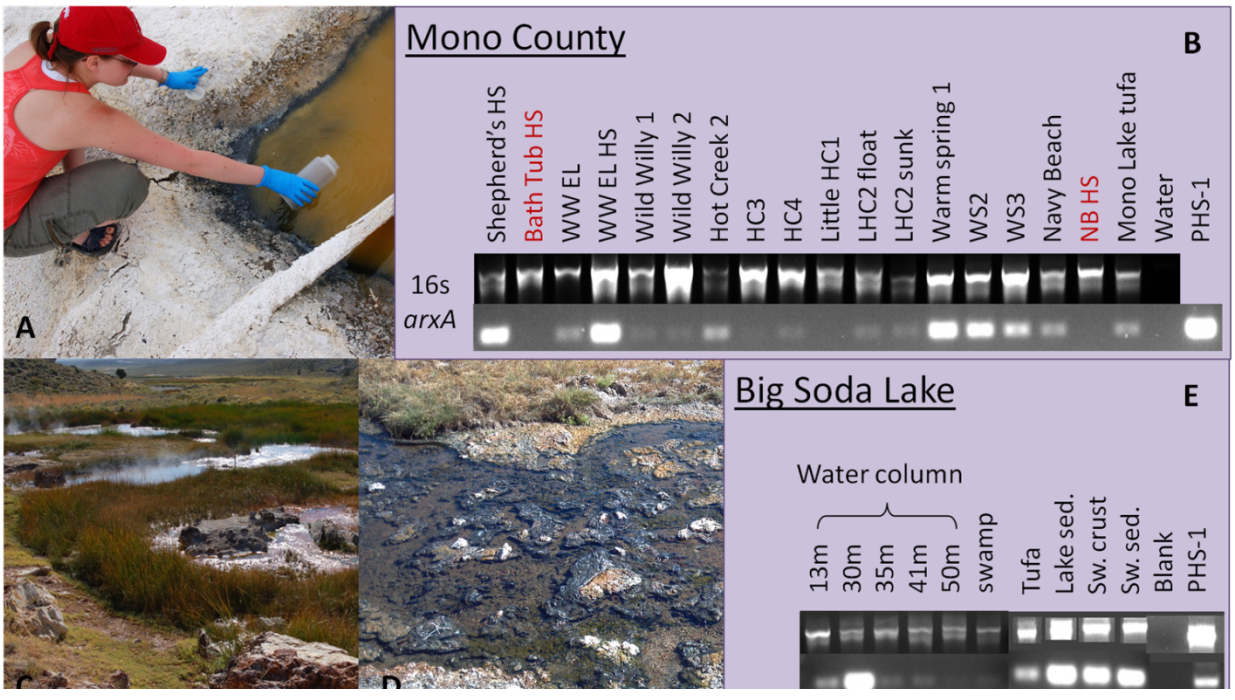
1. Collecting microbial mat material, sediment and water samples from Big Soda Lake, Mono Lake and surrounding hot springs
2. Culturing new photosynthetic arsenite oxidizing bacteria
3. Designing and utilizing PCR primers to screen samples and cultures for *arxA*

Methods

Sampling sites: Samples were collected from two areas: Big Soda Lake, NV in June of 2011, and Mono County, CA in September of 2011. At Big Soda Lake, samples were collected from the water column, tufas, lake sediment, and a connected swamp (Figure 1A). In Mono County, tufa and lake sediments were collected from Mono Lake, while sediment and microbial mat samples were collected from Hot Creek, Little Hot Creek, and various hot springs (Navy Beach, Warm Springs, Wild Willy's, Bath Tub, and Shepherd's, Figure 1C and D). Both Big Soda and Mono Lake are alkaline (pH 9.7), hypersaline (ML 90 g/L) and BSL 26-88 g/L) and contain high concentrations of inorganic arsenic, 20 and 200 uM respectively. Hot Creek, Little Hot Creek and the various hot springs sampled from Mono County were freshwater with neutral pH, and had inorganic arsenic concentrations between 1-13uM.

Environmental screening: DNA was extracted from environmental DNA using either MoBio's PowerSoil DNA Isolation Kit in combination with aluminum ammonium sulfate, or Promega's WizardGenomic DNA Purification Kit. Ribosomal 16s PCR products were amplified using the 8f and 1492r primers, while functional genes were amplified using the Malasarn ArrA primers[8], Inskeep AoxB primers[9], and ArxA primers developed within the Saltikov lab. PCR products were cloned using the TOPO TA Cloning Kit and sequenced by Sanger sequencing.

Isolation of arsenophototrophs: Anaerobic Balch tubes of arsenophototroph-selective minimal media were prepared prior to sampling. Water samplings were used to inoculate tubes at the time of sampling, while sediment and microbial mat samples were introduced to media within 1-48 hours. Cultures were incubated at either room temperature or at 37˚C, depending on temperature of sampling site, in the light. Cultures were streaked on plates and incubated anaerobically at room temperature in the light. Colonies were repeatedly picked, transferred to



liquid culture, and restreaked to ensure strains consisted of isolates. To determine genus of isolates, their DNA was extracted using Promega's WizardGenomic DNA Purification Kit and 16s PCR products were amplified using the 8f and 1492r primers and sequenced by Sanger sequencing.

Results and Discussion

At Big Soda Lake, all samples collected yielded PCR products when screened with *arxA*-specific primers (Figure 1E). At the time of writing, only the BSL tufa PCR product has been cloned and sequenced. These sequences all group within the ArxA clade of arsenite oxidases. In the Mono Lake area, all samples except for the Bath Tub hot spring and Navy Beach hot spring yielded PCR products when screened with *arxA*-specific primers (Figure 1B). In combination, these results demonstrate that *arxA*-like sequences can be found outside of the Mono Lake area and may have a widespread distribution.

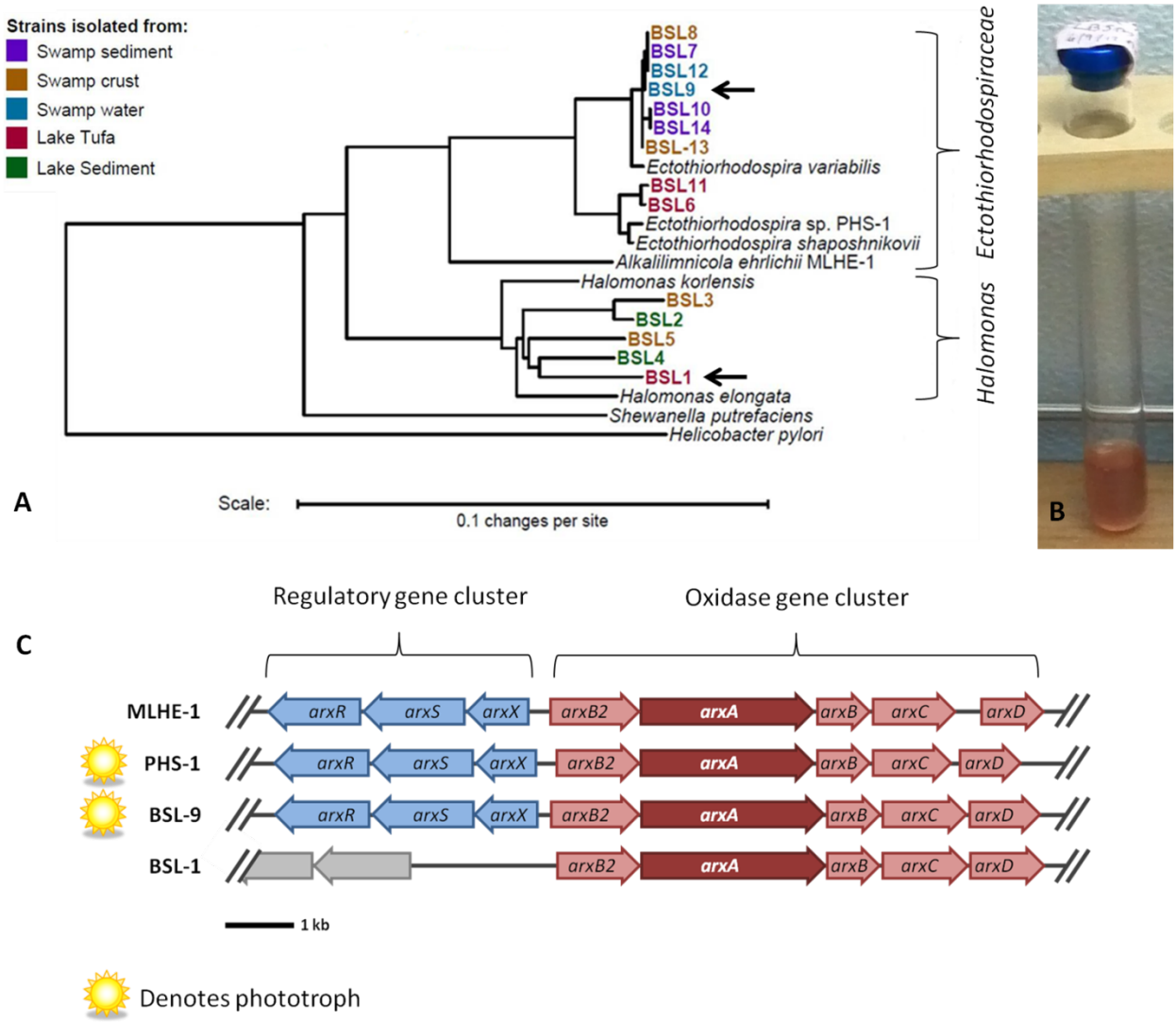


Figure 2: A) Phylogenetic tree of arsenite-oxidizing strains isolated from Big Soda Lake. B) *Ectothiorhodospira* strain BSL-9 in liquid culture. C) Layout of the *arx* operon of BSL-9 in comparison to other *arx* operon-containing bacteria.

Additionally, the samples collected were used to enrich for novel photoarsenotrophs. Photoarsenotrophs belonging to the genus *Ectothiorhodospira* were isolated from the Big Soda Lake samples (Figure 2A). These isolates are able to use arsenite as a sole source of electrons and oxidize it completely to arsenate. One isolate, BSL-9, was selected for further analysis (Figure 2B). Its genome was sequenced and it was found to contain an *arx* operon homologous to those found in both PHS-1 and MLHE-1 (Figure 2C). The finding of *arxA* in this photoarsenotroph further strengthens the hypothesis that ArxA is being used as an arsenite oxidase in these organisms. BSL-9 is relative easy to grow in the lab and will be used to further explore arsenophototrophy using genetic methods.

In addition to the research-related significance of this project, this project has resulted in research projects and the mentoring of 3 students. Two high school students, Kriti Lall and Hannah Meyers, assisted with field trips and sample collections. They along with an undergraduate student, Breanna Hoferer, helped analyze the samples by PCR in the lab and culture new bacterial strains. Also, video footage of the field trips was used to create an educational video on Big Soda Lake and arsenophototrophy. It can be found on youtube: http://www.youtube.com/watch?v=Ut_WNtKS5Yo.

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