Characterization of Cultivatable Arsenic Resistant Bacteria from Black Mountain Open Space Park

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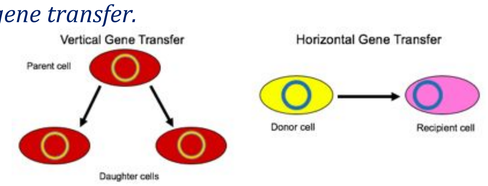
Introduction

Arsenic is a metalloid that is toxic to most organisms because it disrupts ATP synthesis. However, some bacteria are able to survive and even thrive in arsenic-contaminated environments (Pepi et al. 2007). Black Mountain Park, located in East County San Diego, was mined for arsenic in the 1920's. The resulting mine tailings liberated copious amounts of arsenic oxide and created a challenging evolutionary pressure for the local microbial community. The goal of this study is to characterize bacteria living in the soil at the Black Mountain Park site and to determine whether transfer of genetic material is occurring both through vertical and horizontal gene transfer. The hypothesis for the project is that *extreme exposure to an arsenic-rich environment has contributed to the evolution of arsenic resistance in the bacterial community, inherited both via vertical and horizontal gene transfer.*



Objectives

1) What species live in the soil?
2) Are they resistant to As and to what extent?
3) How do they use arsenic to their own advantage?
4) Is horizontal gene transfer happening?

Methods

1) DNA ISOLATION & SEQUENCING FOR SPECIES IDENTIFICATION

DNA was extracted from each of the 61 visibly different isolates. Extraction was performed using E.Z.N.A. Bacterial DNA Kit. 50µL PCR reactions were prepared according to Qiagen'sTaq PCR Master Mix kit with the universal 16S rRNA gene primers 27F and 1492R. The reactions were sent to Eton Bioscience for sequencing and the resulting sequences were run through EZ BioCloud databases. Phase-contrast microscopy was completed on the 37 unique species identified to analyze cell morphology.

2) MINIMUM INHIBITORY CONCENTRATIONS

Assessment of MICs were performed by spotting strains onto R2A agar plates mixed with different concnetrations of As(III) and As(V), respectively of 0, 2.5, 7, 14, 28, 56, 112 mM As(III) and 0, 20, 40, 80, 160, 320 mM As(V). Plates were incubated for 72 hr at 30˚C.

3) COLORIMETRIC ASSAY

To measure the ability of bacteria to oxidize arsenite or reduce arsenate, the assay was performed as in the Shade paper. The 96-well plate was incubated for 72h at 25˚C. Standard curves of As(V) to As(III) (0:100, 10:90, 25:75, 50:50, 75:25, 90:10, 100:0) were plated alongside the cells. To test for cell viability after the 72h, cells were spotted on plates before triggering the color reaction with AgNO34.

ArsC SCREENING

All 37 identified species were screened for presence of arsC gene. 50µL PCR reactions were prepared according to Qiagen'sTaq PCR Master Mix kit with the arsC gene primers P52F and P323R. PCR products were directly extracted and purified from gel using the MinElute Gel Extraction Kit (50) by Qiagen.

Results

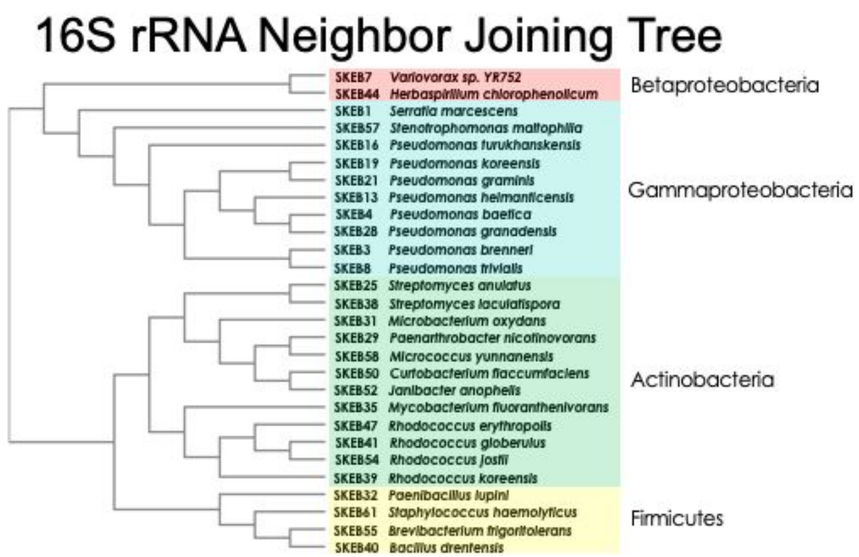


Figure 1. Identified Species from Black Mountain Open Space Park Soil Samples. 37 species were identified spanning 17 different genera.

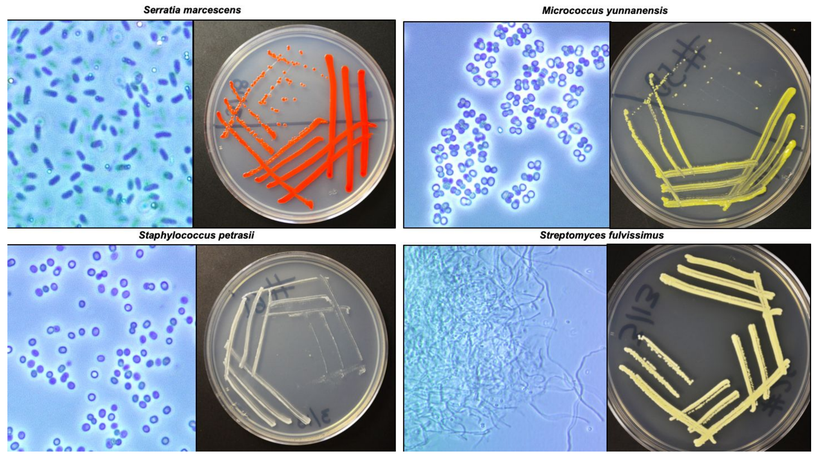


Figure 2. Microscopic and Morphological Diversity. Different cell-shape and different colony morphology were observed among the different species we managed to isolate.

MIMIMUM INHIBITORY CONCENTRATION RESULTS

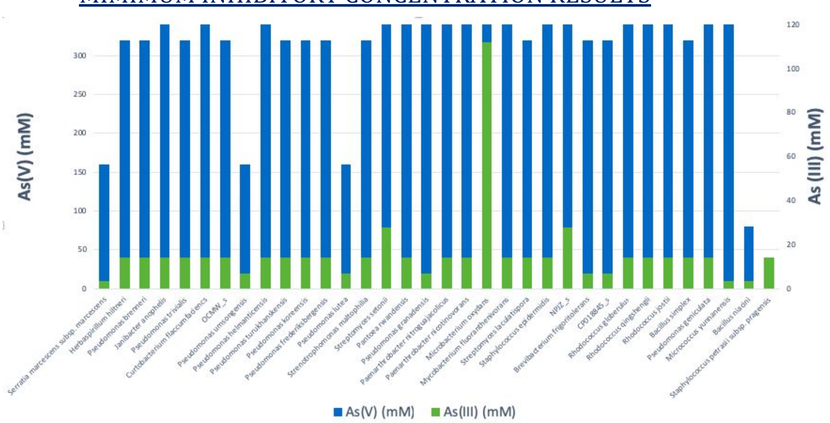


Figure 2. **MIC Results for Arsenite, the more toxic form of As**. Most of the species showed MICs around 14 mM As(III), with a maximum of 28 mM MICs. Most of the species showed MICs of 320 mM As(V), and even beyond that concentration.

ASSESSMENT OF As TRANSFORMING PROPERTIES

Isolates and closest relatives	Oxidizing	Reducing
Serratia marcescens subsp. marcescens	-	+
Herbaspirillum hiltneri	-	+
CP018845_s *(Herbaspirillum)*	++	+
Pseudomonas brenneri	-	+
Pseudomonas helmaticensis	-	+
Pseudomonas turukhanskensis	+	+
Pseudomonas koreensis	-	+
Pseudomonas frederiksbergensis	++	++
Pseudomonas granadensis	-	++
OCMW_s *(Pseudomonas)*	-	+
Strenotrophomonas maltophilia	+	++
Streptomyces setonii	-	++
Streptomyces laculatispora	-	++
Pantoea rwandensis	-	+
Microbacterium oxydans	-	+
Bacillus simplex	-	+
Rhodococcus globerulus	+	-
Rhodococcus qingshengii	++	-
Rhodococcus jostii	+	-
Paenibacillus lupini	++	-

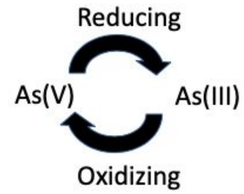


Table 1. **Transforming Capabilities.** A prevalence of reducing transforming capabilities is observed. Species within the same genus presented similar transforming capabilities. 17 reducing, 8 oxidizing.

arsC SCREENING

Species with *arsC* gene
Curtobacterium flaccumfaciens
Streptomyces setonii
Pantoea rwandensis
Rhodococcus globerulus
OCMW_s (Variovorax)
Streptomyces laculatispora
Staphylococcus epidermidis
Janibacter anopheles
FXWM_s (Bacillus)
Rhodococcus globerulus

Table 2. **Species detected with arsC gene.** Among all species screened for the *arsC* gene, 10 out of 37 presented the band corresponding to the *arsC* gene.

Discussion

Increased exposure to As-rich environment contributed to the evolution of bacteria with favorable traits towards arsenic resistance.

Future Directions

A phylogenetic tree for the *arsC* gene will be built using the neighbor-joining algorithm, similarly as for the 16S rRNA gene. Comparison of the trees will allow to determine whether horizontal gene transfer is occurring. We will build other phylogenetic trees for each of the known genes conferring arsenic-resistance properties to bacteria, including *arsB, arsH*, and *arsR* genes (Butcher et al., 2007).

Acknowledgements and References

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