Screening *Geobacillus* Strains for Arsenic Resistance

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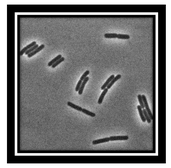
Abstract:
Arsenic contamination of groundwater is a high-profile problem causing serious arsenic poisoning to large numbers of people in many countries throughout the world, including the USA. Exposure to arsenic has shown to be associated with various forms of cancers and compromise the immune system. Many microorganisms have been shown to have high resistance to arsenic and may be used for bioremediation of arsenic in groundwater. One potential way to achieve this is to enhance the oxidation of As(III) to As(V) and/or limit As(V) reduction, since As(III) is more toxic than As(V). The poor understanding of the mechanism(s) of arsenic resistance significantly limits the application of microorganisms for arsenic bioremediation. In this study, we have screened a collection of thermophilic, spore-forming bacteria in the *Geobacillus* genus for their resistance to both As(III) and As(V). The ones with excellent resistance will be sequenced to identify the genes responsible for these features and elucidate the underlying pathways and mechanisms, which will provide the basis for engineering and improving these strains for arsenic bioremediation.

Introduction/Background:



Geobacillus is a genus of thermophilic bacteria that has been found from various geothermal environments of the Earth. They can survive harsh environments at temperatures as high as 75C and pH range of 2-12. Many are also capable of producing spores.
Arsenic is a naturally occurring element found in the Earth's crust. It is classified as a metalloid, which means it has properties of both metals and non-metals and is toxic to most living organisms. It is a great threat to our natural environment.

Methods:

- 64 strains of *Geobacillus* were used. They were grown on TBAB (Tryptose Blood Agar Base) Agar plates overnight at 60C.

- A colony was picked from the plate to inoculate the seed cultures in TGP in test tubes. These cultures were grown at 60 C for 6-7 hours.

- The OD of the seed cultures from different strains were checked to make sure the initial OD of the final culture is roughly the same after dilution in the 96 well plate.

Results

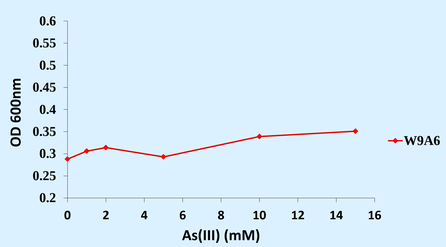
	As(III) Control	As(III) 1mM	As(III) 2 mM	As(III) 5 mM	As(III) 10 mM	As(III) 15 mM	As(V) Control	As(V) 5 mM	As(V) 10 mM	As(V) 20 mM	As(V) 50 mM	As(V) 100 mM
sample	0	1	2	5	10	15	0	5	10	20	50	100
W9A25	0.256	0.238	0.223	0.157	0.158	0.128	0.195	0.222	0.276	0.307	0.258	0.324
9A23	0.287	0.158	0.242	0.195	0.198	0.186	0.199	0.24	0.219	0.468	0.23	0.205
W9A107	0.169	0.133	0.173	0.178	0.132	0.153	0.191	0.188	0.183	0.215	0.226	0.281
W9A115	0.93	0.232	0.17	0.175	0.148	0.143	0.215	0.186	0.185	0.184	0.219	0.237
W9A4	0.42	0.187	0.181	0.16	0.15	0.152	0.922	0.633	0.295	0.232	0.236	0.267
W9A6	0.75	0.131	0.168	0.132	0.137	0.139	0.241	0.201	0.214	0.183	0.279	0.341
96A5	0.195	0.181	0.186	0.133	0.134	0.139	0.195	0.22	0.297	0.251	0.201	0.228
W9A86	0.391	0.348	0.293	0.125	0.128	0.13	0.304	0.254	1.073	0.225	0.203	0.272

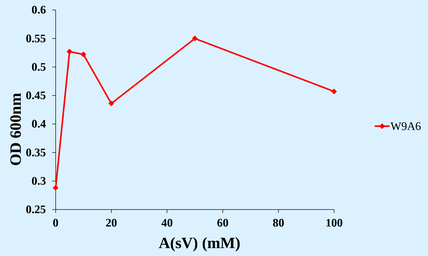
- Concentrations of Geobacillus need to be kept constant while the arsenic concentrations are varied in the well plates. A 96 well plate was set up with 1 mL of TGP, and then equal concentrations of 64 strains of Geobacillus were used. Sodium arsenite and Sodium dibasic arsenate were used.

- The 96 well plates were placed in the 60C incubator for 16 hours on a shaker. After 16 hours they were taken out and transferred to a micro plate and place into the microplate reader to have OD measurements taken at 600nm. The data was then transferred to an excel spread sheet and graphed to look at OD vs. varying arsenic concentrations.

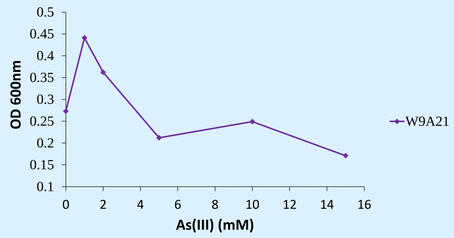
Results:

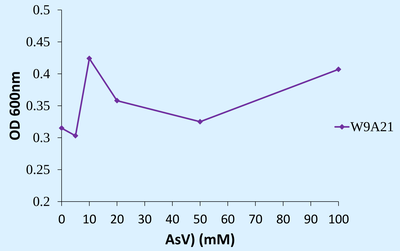
Graphs: Optical density after 16 hours of various *Geobacillus* strains at different arsenic concentration



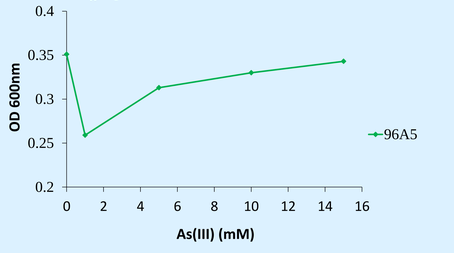


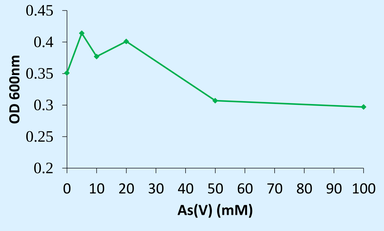
These graphs show a strain of *Geobacillus* with a high resistance to Arsenic(III) and Arsenic(V).



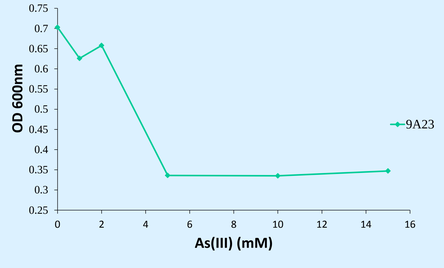


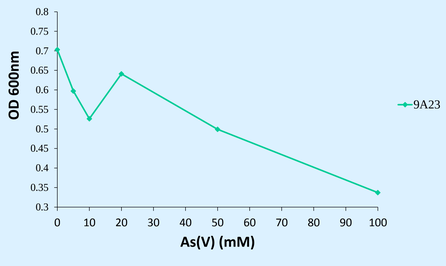
These graphs show a strain of *Geobacillus* with a low resistance to As(III) and a high resistance to As(V).





These graphs show a strain of *Geobacillus* with a high resistance to As(III) and a low resistance to As(V)





These graphs show a strain of *Geobacillus* with a low/moderate resistance to As(III) and a low/moderate resistance to As(V)

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Future Work & Discussion:

64 strains of *Geobacillus* have been tested. Four patterns have been identified based on their resistance to As(III) and As(V).

- 16 strains exhibit high resistance to both As(III) and As(V).
- 5 strains exhibit high resistance to As(III) but low to medium resistance to As(V).
- 15 strains exhibit high resistance to As(V) but low to medium resistance to As(III).

The genome of these strains will be sequenced and the genes/pathways rendering the arsenic resistance will be identified through a combination of computational and experimental methods.

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