Isolation and Identification of Lead and Cadmium Tolerant Bacteria from Blackbird Creek Marsh in Townsend, Delaware

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INTRODUCTION

Heavy metals are elements that can cause toxic effects in living organisms even at low levels of exposure. Their applications in domestic, agricultural, technological and industrial sources have increased the risk of environmental pollution. Climate change effects can aggravate the impact of heavy metals on the environment and the organisms living within these environments. Studies report that microorganisms can efficiently remove these toxic metals from soils and water.

The project studies the abundance and diversity of metal tolerant bacteria from the soils dominated by the invasive marsh grass, *Phragmites australis* and the native marsh grass, *Spartina alterniflora* from the Blackbird Creek Marsh.

The future goal of this research is to isolate bacteria that have bioremediation applications.

MATERIALS & METHODS

❑Soil samples were collected by Composite Sampling Method from the areas dominated by both *Phragmites australis* and *Spartina alterniflora* from the Blackbird Creek Marsh in May 2016.

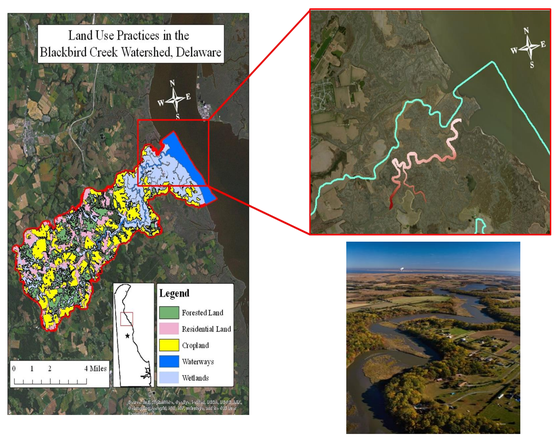

Fig.1. Blackbird Creek Sampling sites

❑1000ppm of lead and cadmium were used to separately enrich 100ml of Luria-Bertani Broth (LB) and inoculated with 10 grams of soil. The medium was incubated for 48hours at 37˚ C.

❑0.1 ml of this cultured suspension was then spread platted on Luria- Bertani agar plates with cadmium (100 to 1000ppm) and lead (1000 to 1400ppm). Analysis was performed in triplicates.

❑Colonies were counted as Colony Forming Units (CFU)
CFU/ 10 grams of soil = Number of Colonies X 0.1X 10

❑Isolated colonies from each heavy metal plate were inoculated into LB broth, and incubated overnight at 37◦ C.

❑Genomic DNA was isolated using the Phenol: Chloroform method (He, 2011) and PCR was performed using 27F and 1492R primers targeting the 16srRNA gene.

❑PCR products were identified by Sanger Sequencing at University of Delaware Institute of Biotechnology.

❑The Sanger sequences were aligned by CLUSTAL W alignment tool.
❑Phylogenetic tree of the aligned Sanger sequences was performed using MEGA 4.0.2 software.

RESULTS AND DISCUSSION

❑ Results indicate that bacteria from marsh soils were able to grow up to 1000ppm of lead and 500ppm of cadmium concentration

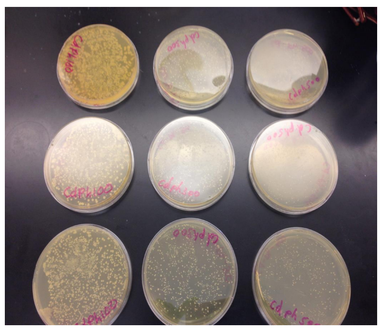

Fig.2. Colonies on LB plates with 100 to 500 ppm of Cadmium

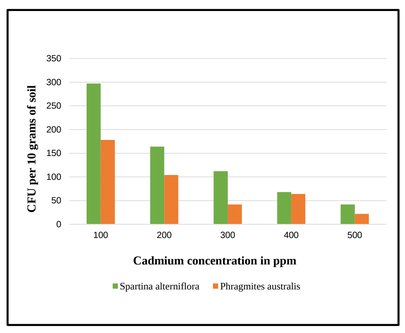

Fig.3. Comparison of Cadmium Tolerant Bacteria from *Spartina alterniflora* and *Phragmites australis* Soils

❑ Plates inoculated initially with lead concentrations of 100 to 1000 ppm had more colonies and were uncountable. Therefore the concentrations of lead were increased to 1600ppm to asses tolerance of bacteria to higher lead concentrations

❑ LB plates with concentrations 1100 to 1600 ppm were used, and uncountable colonies were observed on plates with 1600ppm of lead.

❑ Lead tolerant bacteria were in high numbers in soils isolated from *Spartina alterniflora* rich areas than in the *Phragmites australis* soil.

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1	cdsp3004	7/7/2016 10:34 AM	349.9	ng/ul	6.999	3.407	2.05	1.85
2	cdsp1006	7/7/2016 10:36 AM	126.9	ng/ul	2.539	1.261	2.01	2.03
3	cdsp3005	7/7/2016 10:36 AM	462.5	ng/ul	9.250	4.432	2.09	2.28
4	cdph5001	7/7/2016 10:37 AM	119.4	ng/ul	2.388	1.223	1.95	2.61
5	cdph5004	7/7/2016 10:40 AM	417.4	ng/ul	8.347	4.310	1.94	2.23
6	cdph5002	7/7/2016 10:41 AM	317.9	ng/ul	6.359	3.157	2.01	2.31
7	cdsp1002	7/7/2016 10:41 AM	172.2	ng/ul	3.444	1.801	1.91	2.09
8	cdsp1005	7/7/2016 10:42 AM	2245.4	ng/ul	44.909	21.231	2.12	2.32
9	cdph1006	7/7/2016 10:43 AM	519.8	ng/ul	10.397	5.112	2.03	2.16
10	cdph5005	7/7/2016 10:43 AM	917.2	ng/ul	18.344	8.959	2.05	1.95

Fig. 4. Nano Drop Concentrations of Genomic DNA

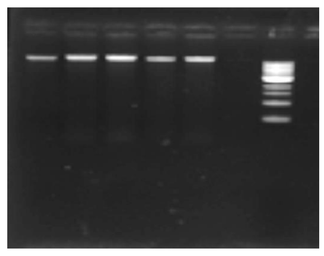

Fig.5. Genomic DNA of Soil on 1% Agarose gel

❑ 260/280 values for the genomic DNA ranged from 1.6 to 2.5ng and 260/230 values ranged from 2.0 to 2.3ng which indicate the integrity of the isolated DNA samples (Fig. 4).

❑ The isolated DNA concentrations ranged from100-1000ng/ul.

❑ PCR was performed by diluting the genomic DNA's to 100ng and gradient PCR was performed to optimize the annealing temperature (Fig.6).

❑ The primer sequences used in the PCR were:
27F: 5' AGAGTTTGATCCTGGCTCAG 3'
1492R: 5' TACCTTGTTACGACT 3'

❑ PCR results show that DNA optimum annealing temperature was at 44.9 ◦ C resulting in a product size of 1492 kb (Fig.7).

Fig.6. Gradient PCR

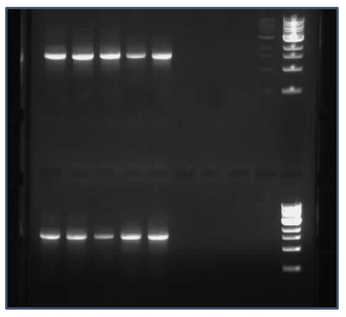

Fig.7. PCR Image for the Amplification of 16s gene

❑ Sanger sequencing results when blasted against the National Center for Biotechnology Information (NCBI) Data base identified the presence of larger numbers of *Bacillus* sp. and several other bacteria.

❑ As the lead tolerant bacteria were not countable, analysis is in progress to isolate well isolated colonies by increased the lead concentrations.

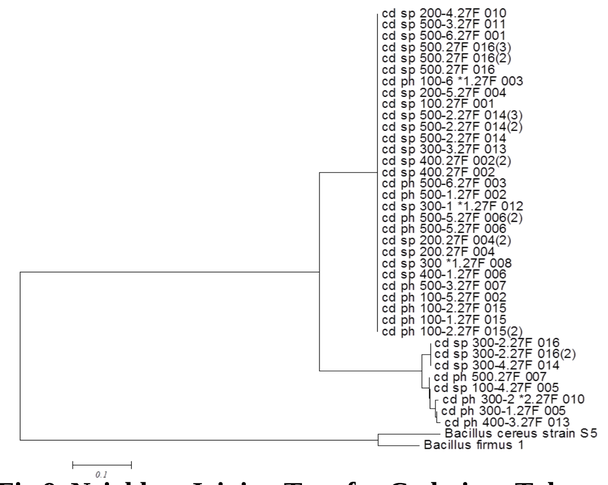

Fig.9. Neighbor Joining Tree for Cadmium Tolerant Bacteria using MEGA 4.0

CONCLUSION

❑ Most of the bacteria identified are 97% similar to cadmium tolerant *Bacillus cereus* and bacteria from *Phragmites* soil are much closely related to the reference heavy metal bacterial sequence.

❑ Higher abundance of lead tolerant bacteria present in the Blackbird Creek.

❑ Higher lead tolerance may be attributed to the hunting activities (lead bullets), paints and leaded gasoline from boats (Environmental Protection Agency sec.2). Human induced activities may be a major contributor for these bacteria to develop a greater resistance.

❑ Usage of herbicides may be one of the sources of cadmium in the creek. Cadmium resistant bacteria were less when compared to lead.

❑ Soils from areas dominated by the native marsh grass (*Spartina alterniflora*) showed a greater presence of bacteria tolerant to high concentrations of lead and cadmium when compared to the soils from the areas with the invasive marsh grass (*Phragmites australis*).

Future research
❑ Identify lead tolerant bacteria using Sanger sequencing.
❑ Bacteria that can tolerate higher concentrations of lead and cadmium will be selected basing on their phylogenetic details.
❑ Long term goal of this study is to perform bioremediation on metal treated soils.

LITERATURE CITED

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