Detection of Siderophore Producing Bacteria in Puerto Rican Soils

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Abstract

During the industrial revolution asbestos was a common material used in constructions due to its incredible properties. Unfortunately, long term inhalation of asbestos fibers has been linked with many lung diseases. Previous research has shown that trace amounts of iron in asbestos may be responsible for its toxicity since iron can promote reactive oxygen species to develop, which can lead to cellular damage. A possible way to treat asbestos-filled sites would be to remove the iron and to achieve this, a geomicrobiological solution using siderophores has been proposed. Siderophores are small chelating molecules produced by microorganisms capable of trapping iron in the environment. The focus of this research is to isolate and characterize siderophore-producing bacteria (SPB) from Puerto Rican soils. SPB have been identified using Blue CAS agar which turns from blue to orange in presence of siderophores. Soil samples were collected from the southwestern coastal municipality of Cabo Rojo including sample from a salt flat. To isolate SPB, serial dilutions until 10^{-6} were performed to the samples and grown on CAS media for 72 hours at 37°C. Colonies with orange halos were isolated for further molecular and microbiological characterization. Determination of UFC/g showed the samples ranged from 1.55 $\times 10^{-4}$UFC/g (where 9% SPB was identified) and 4×10^{-2} UFC/g (where 100% SBP was identified). Bacteria isolated from the salt flat had the most siderophore production compared to other soil samples indicated by halo size.). 16S rDNA has been amplified from SPB to further perform sequencing in future works. Spectrophotometry is currently being used to quantitatively determine siderophore production by measuring the absorbance of supernatant of bacteria grown on iron-limited media with CAS reactant. This research could help develop methods of utilizing bacteria as tools to decontaminate asbestos-filled sites.

Introduction

- Asbestos is a highly regulated silicate mineral which saw a widespread use during the Industrial Revolution.
- Long- term inhalation of asbestos fibers has been linked to various lung diseases such as mesothelioma.
- This toxicity can in part be atribuited to the presence of iron in the fibers which promotes radical oxygen species in lung cells leading to cell damage .
- A way to reduce this toxicity is by removing iron from asbestos by utilizing a bioremediation approach in the form of siderophores.
- Siderophores are small chelating molecules secreted by microorganisms in iron-scarce enviorments to fulfill their metabolic needs.
- This project focuses on identifying Siderophore Producing Biopropects (SPB) in Puerto Rican soils to be utilized in a posible bioremediation effort.

Objetives

- Isolate Siderophore Producing Bioprospects utilizing CAS Blue assay.
- Identify genes utilizing Siderophore synthesis specific primers

Methodology

Soil Sample Sites

Figure 1. 4 Soil samples were aseptically collected in the municipality of Cabo Rojo, Puerto Rico. Samples A, C1 and C2 were collected near roads while B was collected in Salts flats.

Serial Dilution

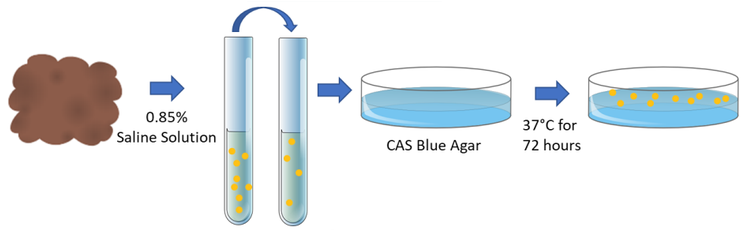

Figure 2. Serial dilutions were performed with soil samples utilizing 0.85% NaCl solution, then dilutions were grown on CAS Blue Agar media.

Identification of Siderophore production

- SPB where isolated using CAS Blue media which is colormetric assay. In the presence of siderophores the medium turn from blue to Orange

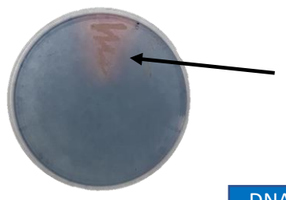

Figure 3. *P. aeruginosa 27853* grown on CAS blue agar plates producing siderophores indicated by the orange change of the medium

DNA Extraction

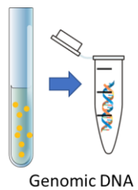

Figure 4. Genomic DNA was extracted from soil samples C2 and C3

Results

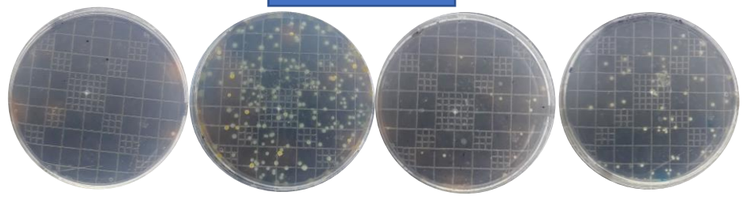

Fig. 5. Bacterial growth of soil samples A, B ,C1 and C2 in dilution 10^2. Sample C2 did not show siderophore production

Table 1. UFC per gram of soil samples percentage of siderophore producing bacteria

Soil sample	CFU per gram of soil	Siderophore producing percentage
1	4×10^2	100%
2	1.55×10^4	9%
3	1.2×10^3	100%
4	5.3×10^3	0%

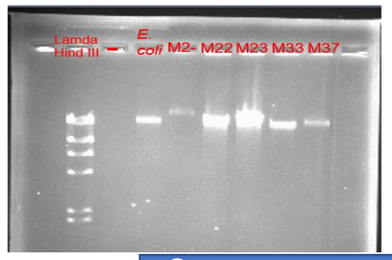

Figure 6. Electrophoresis of Genomic DNA of SPB from samples B and C1

Summary and Future work

- 3 out of 4 soil samples had the SPB which were isolated for further study.
- The SPB from the salt flats (sample B) appeared to have the most siderophore production indicated by halo size in CAS Blue assay.
- Genomic DNA was successfully extracted from isolates from samples B and C1 and is being studied by PCR utilizing siderophore specific primers.
- 16sRNA gene will be sequence to identify SPB genus.
- This experiment will be repeated utilizing a culture independent approach utilizing metagenomic libraries focusing on identifying novel siderophore genes.

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