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Abstract

The contamination of soil, sediment, and water by toxic metals is a major problem worldwide, both environmentally, and economically. Microorganisms that are resistant to lead are rare in nature. Lead is toxic to most microorganisms and only a few have developed mechanisms to survive in this toxic environment. In this study, bacteria found in soil and water with high concentrations of soluble lead are isolated and characterized. I grew and isolated bacteria that are resistant to the lead found in the solid medium. A gram stain was used to characteristically determine the bacteria that were isolated. Four isolated bacteria were found. Three were found to be cocci in shape, and one was bacillus shaped. After continuation with this research, I hope to identify the four isolated bacteria and to further characterize the bacteria for future studies.

Introduction

Lead is mined every day from the environment and some lead gets deposited back into the soil or water. Lead and other residue separated in the preparation of mining is deposited back into the soil forming piles that can stretch for miles called tailings piles. St. Joseph State Park is found in the old "Lead Belt" of southeast Missouri where much of the nation's lead ore was extracted for more than a century. In 1972, the St. Joe Minerals Corporation ceased operations and subsequently donated the land to the state in 1976. The by-products of the lead mine at St. Joe State Park in Missouri were deposited in valleys causing the formations of many small lakes. The leftover lead from the tailings piles can react with the acidic rain and become soluble in the water. The soluble lead can run-off into the lakes causing lead poisoning to many animals, plants, and microorganisms. Lead poisoning is also a problem for humans. Lead poisoning attacks the nervous system causing vomiting, periods of stupor, higher blood pressure, being delirious, seizures and coma (2). The U.S. Environmental Protection Agency regulates drinking water for lead at 15 micro g/l, but this does not include the excess lead that is deposited into the water when soft, acidic drinking water wear down lead connectors, pipes, joints, and other fixtures that hold water that may contain lead (2). With the help of bacteria that can detoxify the lead, water treatment may become easier and more efficient.

Lead, the most common metal found at contamination sites, is the least studied of the metals (1). When lead and other organic compounds are released into the soil, the soil is considered cocontaminated. Cocontaminated soil is difficult to remediate because the metal is toxic, inhibiting the activity of the organic-degrading microorganisms (3). Most bacteria cannot perform this task because lead and other metals can inhibit enzymatic activities, disrupting membrane functions, and damaging nucleic acids (1). One reason may be that the energy requirements to maintain metal resistance and the organic degradation are too high (4). The microorganism cannot perform both activities along with environmental stress. The issue of cocontamination is serious. Approximately 37% of all contaminated sites in the United States contain metal and organic contaminants (4). By studying these microbes that have ability to resist and detoxify metals, environmental microbiologist can identify ways of cleaning up metal-contaminated systems (Roane, personal communication). Using these microbes to enhance the biological activity of the existing populations within a contaminated site is called bioaugmentation (3). Bioaugmentation of these wastes has been investigated in recent years as a safe and more environmental friendly alternative to chemical removal methods.

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Materials and Methods

Field site. The sampling site for the water sample was taken from a well that near Monsanto Lake. The well contained groundwater that was surrounded by tailings. The water was monitored with an average pH of 8.12. The average temperature of the water was 11.98°C. The soil sample was collected from the center of the tailings pile with no lake nearby. A hole was dug about a foot or two down into the soil and I collected the sample from the lower, damper tailings. I gathered the samples at the sample site in sterile and cold conditions. The samples remained in an ice chest until further use in the lab.

I then mixed up a minimal broth containing .7g K_2HPO_4, .2g KH_2PO_4, .1g $(NH_4)_2SO_4$, .1g Glucose, .05g Sodium Citrate, .01g $MgSO_4 \cdot 7H_20$, and .01g $Pb(OAc)_2$ to produce 100mL of broth. I then added HCl until the pH was neutral (7.0). The broth was autoclaved for 15 minutes at 15 psi and 121°C. I made two different broths to grow separate soil and water samples. The broths were cooled to room temperature. I then added 1mL of the water sample to one of the broth flask. I added one scoop of the soil sample to the other broth flask. I allowed the bacteria to set for a week in room temperature to allow the bacteria incubation time.

I also grew the bacteria in a separate broth flask using Dr. Timberly Roane's recipe for the medium that she uses in her laboratory. Her recipe to make 200 mL of broth contained .1g Sodium Citrate, .2g Magnesium Sulfate, .2g Ammonium Sulfate, .2g Glucose, and .02g Sodium tripolyphosphate. I also added 2.04g of Potassium Hydrogen Pthalate as a buffer and .02g of $Pb(OAc)_2$ to introduce the lead into the broth. I then added NaOH until the medium has a pH of 6.5. The broth was autoclaved for 15 minutes at 15 psi and 121°C Using this same recipe with the addition of 3.0g of Agar, I made another 200mL for a solid medium that was used to isolate the colonies.

After incubation, I inoculated three of the solid agar plates with the bacteria growing in the Roane's broth containing the lead tolerant bacteria from the soil sample. I then inoculated three other solid agar plates with the bacteria growing in the Roane's broth containing lead tolerant bacteria from the water sample. After the bacteria grew for a week, I isolated the colonies on a new set of agar plates. After the isolated plates were allowed to incubate for another three days, I did a gram stain to characterize the bacteria.

Results

Once the bacteria began to grow in the minimal broth, I observed the results. A white, creamy bacteria colony had begun to grow in the soil sample broth, but none could be observed in the water sample. Once the Roane recipe was ready, I inoculated the broth with 1mL of water sample for the water broth and a scoop of soil for the soil broth. After four days in room temperature, white, creamy bacteria was noted in the soil sample, but there was no visible sign of bacteria in the water sample. In order to observe the bacteria, a wet mount was made from each sample and I viewed the bacteria under a microscope. Seeing that both samples contained bacteria, I inoculated three plates with the bacteria from the soil. I also inoculated another three plates with the bacteria from the water sample. I allowed the plates to sit five days in a 37°C incubator. I observed minimal bacteria as small white dots in the water sample plates. In the soil sample plates, I observed what appeared to be four different bacteria. The bacteria that I observed were a small white dot, creamy, film-like bacteria, tan/brown dot, and a large white dot. After isolation of these bacteria, I observed the bacteria on the plate. There were no visible bacteria on the plate that was inoculated by the small white bacteria from the water plates. All four bacteria from the soil plates were growing isolated from one another. I then used the gram

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stain assay with the isolated bacteria for identification. The small white dot was gram positive and bacillus shaped. They were found clumped together or growing as individuals. The tan/brown dot was gram positive and cocci shaped. They were found in strains and clusters. The irregular, film-like bacteria were gram negative and cocci shaped. They were found growing in small clusters, chains, and as individual bacterium. What appeared to be an isolated colony of the large, white bacteria gave an incorrect result. Out of the three plates that the colonies were found, the soil plate 3 produced a gram positive while soil plate 1 and 2 produced a gram negative result. All of the stains showed a cocci shaped bacterium that was found in clusters. The bacteria had been growing for more than 48 hours which may have caused an invalid result. Further testing will be done to verify the results.

Soil			
Inoculated growth plates	Soil 1	Soil 2	Soil 3
Isolated plates	Soil 1	Soil 2	Soil 3
Bacteria growth	Brown, Large white, Film-like	Large White, Film-like	Brown, Large white, Small white, Film-like

Water			
Inoculated growth plates	Water 1-No growth	Water 2	Water 3-No growth
Isolated plates		Water 2-No growth	
Bacteria growth	None	None	none

Table 1. The table above gives the result of growth from beginning inoculation until isolation. After inoculation of the plates from the broth, the plates that contained water 1 and water 3 had no growth. Water 2 plate had minimal growth. After isolation, there were no bacteria colonies on the plate used to isolate the colony.

Gram Stain Results			
Bacteria	Soil 1	Soil 2	Soil 3
Brown-cocci	Gram Positive		Gram Positive
Small white dot-bacillus			Gram Positive
Large white dot-cocci	Gram Negative	Gram Negative	Gram Positive
Film-like-cocci	Gram Negative		Gram Negative

Table 2. The above gives the result of the gram stain assay in according to which plate the bacteria was isolated.

Discussion

While growing the bacteria, the pH was set slightly acidic. The acidity increases the lead bioavailability in the medium (1). Metals are generally considered biologically available and more toxic when they are soluble. Lead will readily precipitate out, especially when using inorganic phosphates (Roane, personal communication). Avoiding the inorganic phosphates helps to keep the lead more readily available.

After performing the gram stain test, I discovered that the large, white bacterium was not one but two different species, one gram negative and the other gram positive. The results of the gram stain assay may have formed an incorrect result because the bacteria were older than 48 hours from initial inoculation onto the plates. A bacterium's cell wall begins to break down after this time period causing opposite results of the true stain. In order for the gram stain to be considered correct, the bacterium must be stained no later than 48 hours after inoculation. I will try to re-isolate the bacteria again and perform another gram stain on the test to verify the results.

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If the results are the same, I then will try to isolate the two different large, white cocci bacterium from the three soil plates. I will also try to reisolate the colony from the water 2 plate. Once I have isolated all of the bacteria, I will further characterize my isolates using different staining assays. I will run DNA sequencing on the bacteria. I will also perform some biochemical assays. After characterization and hopeful identification, I will test the lead tolerance of the bacteria and monitor the effects of the growth of the organism on metal solubility. This will test the physiological stress on the bacteria. Overall, these results support the brighter outlook for future bioaugmentation using lead tolerant and other metal tolerant bacteria.

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