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MONITORING CHANGES IN MICROORGANISM'S PROTEIN CONTENT AND FUNCTIONAL GROUPS UPON TREATMENT WITH A HEAVY METAL

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Abstract

Microorganisms have been known to bioaccumulate heavy metals like mercury, nickel, copper, cadmium, and lead. This property allows the use of microorganisms in the bioremediation treatment of soil, sediment, and water contaminated with heavy metals. In this study, the ability of microorganisms to bind heavy metals was determined using instrumental analysis. A model microorganism, *Pseudomonas aeruginosa*, was cultured in a controlled and heavy metal (lead) treated environment. The biomass from the two cultures was collected and further mixed with lead metal. The turbidity and protein content of the different cultures before and after treatment of the metal was determined using absorbance and Folin-Lowry's method. Results showed higher absorbance reading and protein content in cultures exposed to the metal. In addition, the functional groups in the two cultures were identified using infrared (IR) spectroscopy. Peak changes can be observed, especially when lead was added with the bacterial culture.

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Introduction

Rapid urbanization and industrialization have resulted in the increased emission of toxic heavy metals such as lead, cadmium, chromium, mercury, and nickel. Heavy metal pollution of soil and wastewater is an important environmental problem that has gained significance.[1] Heavy metals constitute a global environmental hazard since they are not easily removable from the environment and are indestructible, unlike many other pollutants that can be chemically or biologically degraded.[2] Since they affect all groups of organisms, some bacteria were able to develop heavy-metal resistance. These bacteria possessing plasmids carry the genes for metal resistance, and since such traits are plasmid-borne, they can be easily transmitted from one bacterium to an entire population.

These bacteria have been utilized as remediating agents, and the use of microorganisms to remove heavy metals from environmental samples is an area of extensive research and development. One of these microorganisms is *Pseudomonas aeruginosa,* a gram-negative bacterium frequently found in soil, water, sewage, human, and plants. It plays an important role in the catabolism, transformation, degradation, and detoxification of various toxic wastes, including heavy metals. *P. aeruginosa* has been found capable of degrading aromatic compounds and providing resistance to heavy metals like lead. In this study, the interaction of *P. aeruginosa* with lead ions was monitored by looking at their growth, protein content, and changes in functional groups. These were performed using instrumental methods, particularly vibrational spectroscopy, for the functional group changes. Methods such as infrared (IR) spectroscopy are rapid, require little or no sample pretreatment, and permit the users to collect full spectra in less than a few seconds. FT-IR measures molecular vibrations of biochemical composition and structure, which provides characteristic biochemical "fingerprints",[3] and can easily distinguish structural features of bacteria.[4-5]

Materials and Methods

Pseudomonas aeruginosa culture (Carolina Biological Supply Company) was inoculated within 5.0 mL of tryptic soy broth (TSB). The culture was divided into two groups: one was treated with 3.0 mM of lead acetate (Pb-treated), and the other remained (control). The untreated (control) and Pb-treated cultures were then incubated in a shaking incubator for 24 hours at 37°C.

After 24 hours, both cultures were removed from the incubator and centrifuged, discarding the supernatant afterward. Half of the cultures in each treatment were set aside for analysis, while the other half were resuspended into 5.0 mL of TSB. Both cultures were treated with 3.0 M lead acetate and incubated within a shaking incubator for an additional 24 hours at 37°C. These cultures became known as control + Pb^{2+} and Pb-treated + Pb^{2+}.

The bacterial growth, protein content, and IR spectra were obtained for all four cultures (before and after lead acetate was added to both cultures). Turbidity representing bacterial growth was determined by obtaining the absorbance of the different bacterial cultures at 605 nm using GENESYS™ 30 visible spectrophotometer.

The protein content was determined using Folin Lowry's method (using BSA as standard). Bacterial pellet was collected after centrifugation and added with 1 mL lysis buffer (General lysis buffer: 50mM Tris HCl;100 mM NaCl;1mM Tween 20;5% Glycerol;1mM EDTA). The pellets were sonicated for a total of 2 minutes (4 times for 30 sec each time with a gap of 30 sec between successive sonications). The sonicated sample was centrifuged at 5000 rpm at 4°C for 2 min. 200 µL of this supernatant was incubated with chilled 1600 µL acetone for 30 min at 4°C. The precipitated protein was dried, and 100 µL PBS was added to it to bring it into solution. This was then analyzed for protein content using the method by Lowry et al. [6-7] modified in microplate level with bovine serum albumin as standard using Biotek Cytation 5 Image Reader.

Statistical analysis using student's t-test ($p < 0.05$) done in Mircosoft Excel was performed to compare the difference between the set of experimental data obtained in turbidity and protein content.

Lastly, IR analysis was done by placing the solid biomass ob-

tained after centrifuging the different bacterial cultures on a silver chloride window cell. The IR spectra were collected using a Nicolet 8700 equipped with a liquid N_2 cooled mercuric cadmium telluride (MCT) detector (Thermo Scientific, West Palm Beach, Fl). Each spectrum is an average of 256 scans with a resolution of 2 cm^{-1}.

Results

Visual inspection of *P. aeruginosa* cultures grown in control and lead stressed environment showed additional biomass for the treated culture. Upon treating of lead acetate to both cultures, a distinct presence of biomass in both *P. aeruginosa* treated with lead ions was observed. Turbidity results support these observations. The turbidity of the treated bacteria is higher than the control (Figure 1). Treatment of lead ions results in an increase in turbidity in both cultures. There is a significant difference in terms of turbidity results among the four bacterial cultures analyzed.

Adding lead ions to both the control and treated bacteria increased protein content in terms of protein content (Figure 2). Although the increase is not dramatically similar to the turbidity results, the statistical analysis still showed a significant difference in protein content among the four bacterial cultures.

The IR spectra for the different conditions are shown in Figure 3. Table 1 shows the assignment for peaks observed in the IR spectra. For the changes in functional groups, one distinct change was observed around 3500 cm^{-1} where a broad band initially observed disappears when the bacteria were exposed to lead ions. Enlarging the IR region (Figure 3) showed changes in the functional groups present in the bacteria. One difference that can be observed between the control and lead treated culture is in the 1000 $cm^{-1,}$ where the control has two peaks in comparison to the treated samples that only have one. In the 1100 cm^{-1} region, a peak increase in the lower wavenumber was observed upon the addition of lead ions. The most obvious change in the treated sample upon adding lead ions is a peak shift. Another region where there is a difference is in the 1300 $cm^{-1,}$ where there is an upshift of a peak when lead ions are added. The same thing can be observed in the 1550 cm^{-1} region. At the 1600 cm^{-1} region, the broad peak observed in the control treatment downshifted as lead ions were added. Lastly, a peak at the 1750 cm^{-1} region disappeared when the microorganism

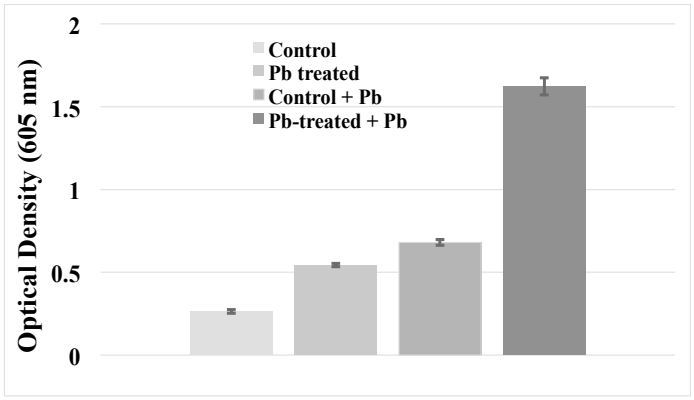

Figure 1. Turbidity of *P. aeruginosa* cultures exposed in different conditions.

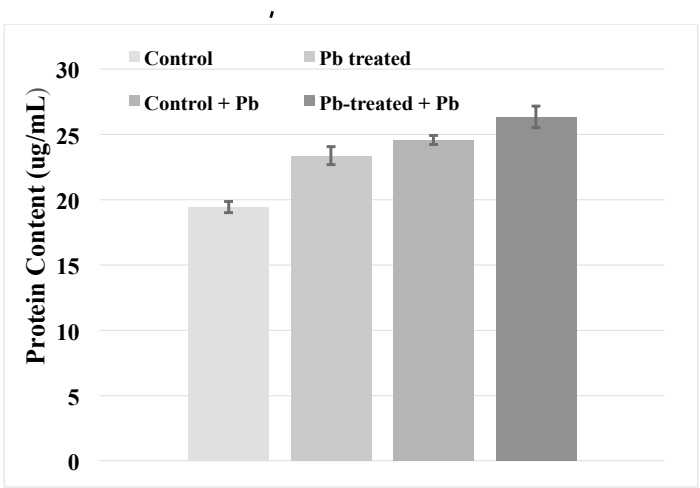

Figure 2. Protein content of *P. aeruginosa* cultures exposed in different conditions.

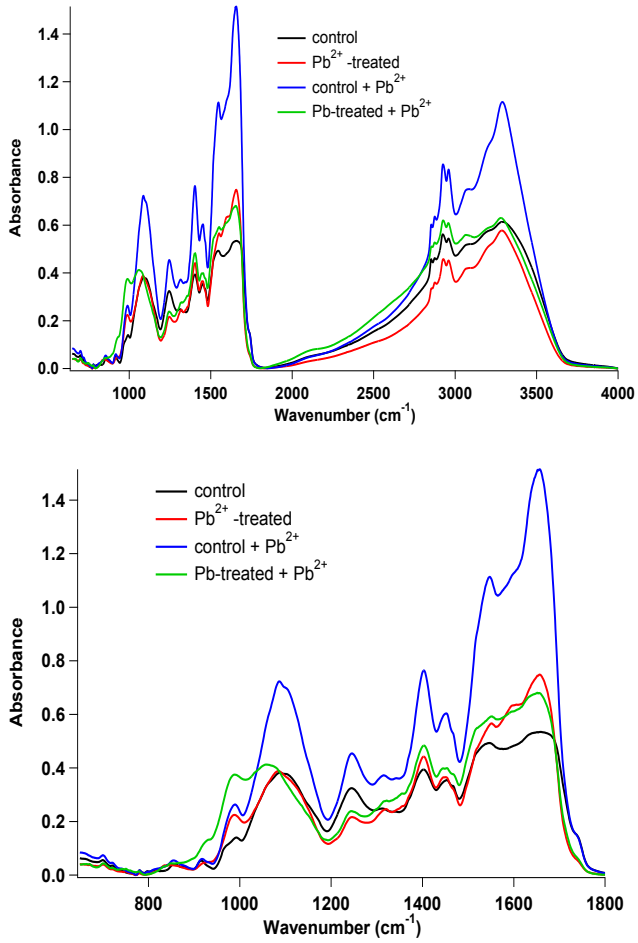

Figure 3. Differences in IR spectra of *P. aeruginosa* cultures exposed in different conditions.

Table 1. Some of the common peaks observed in the IR spectra of the four bacterial cultures.

Peak	Possible Assignments
3279	NH_2 stretching in adenine, cytosine, quinine; H-bonded OH groups
3073	Alipathic C-H stretching (fatty acid)
2960	C-H stretching in aliphatic cell wall
2931	C-H stretching in aliphatic cell wall
1655	NH_2 bending, C=O, C=N stretching (amide I band)
1453	Amide II band
1405	Amide II band
1246	Amide III band
1081	PO_2^1 very strong stretching (glycopeptides)
986	P-O-P stretching (phospholipids, ribose phosphate chain pypophosphate

was treated with lead ions.

Discussion

The increase in turbidity of *P. aeruginosa* upon treatment of lead ions can be traced to the acetate (lead acetate). Acetate can be a good source of carbon, giving the bacteria some nutrient source as it can be observed that the more the bacterial cultures are exposed to lead ions (coming from lead acetate), the higher is the absorbance pertaining to the increased population of bacteria. On the other hand, the increase in the protein content of lead-treated *P. aeruginosa* can be due to the formation of stress proteins upon treatment with the lead ions. This is necessary to counteract the harmful effect of lead ions. It has been reported that silver nanoparticles and several conventional antibiotics can induce a stress response in terms of the major chaperone protein (DnaK).[8] It has also been reported that some of the proteins that overexpressed when *Pseudomonas aeruginosa* is exposed to Cr(IV) included stress proteins increases.[9] There is also an increase and decrease of some proteins when *Pseudomonas putida* was exposed to different heavy metals, including lead.[10] Although stress proteins were not mentioned, some of the proteins that were induced are proteins involved in protein synthesis, degradation, and folding were induced along with enzymes that combat oxidative stress,[10] the same proteins that also increases when *P. aeruginosa* is exposed to Cr(IV).[9] FT-IR has been used to monitor *Bradyrhizobium japonicum* growth and its structural changes during growth.[5] Furthermore, FT-IR has been used to identify and classify *Bacillus cereus,*[11] *Listeria* spp.[12-13] *Staphylococcus* spp., *Clostridium* spp., and *Escherichia coli,*[14] and to investigate microbial colony heterogeneity.[3] IR methods can also be used to monitor microbial growth and quantify microbes responsible for spoilage in chicken muscles.[15-16]

There are four distinct regions where we can put the general or common features that the microorganisms have.[17] The regions are 3100-3400 cm^{-1} region is due to the nucleic acid, 2800-3100 cm^{-1} is due to cell wall constituents, 1200-1800 cm^{-1} is due to the proteinaceous structures, and 700-1200 cm^{-1} due to fingerprint region. In terms of the peaks obtained using IR, the most obvious is the one in the 3500 cm^{-1} region. This is the region for O-H and N-H stretching. The disappearance of the peak in this region could mean that the presence of lead ions perturb the O-H and N-H stretching. This can also be the reason for the changes in 1750 cm^{-1} that can be attributed to the carbonyl group (C=O). The disappearance of the peak at this region means that the C=O was affected by the introduction of lead ions. It has been reported that the bacterial cell wall functional groups (amino, carboxyl, hydroxyl, and phosphate groups) can interact with metals.[18-19] The other differences that can be observed are due to disturbances in the functional group when the lead ions bind with the microorganism. This only shows that IR could monitor the changes in the functional group upon the binding of lead ions.

Conclusions

The effect of a heavy metal added to a model microorganism, *Pseudomonas aeruginosa,* was monitored by looking at changes in the turbidity, protein content, and functional groups. There is an increase in the turbidity and protein content upon exposure of the microorganism to lead. IR spectroscopy also monitored peak changes pertaining to functional groups that can bind with lead.

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