World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org

BIOREMEDIATION APPROACH OF HEAVY METAL TOLERANCE BY HYDROCARBON DEGRADING *PSEUDOMONAS* SP.

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Article Received on 10/07/2015 Article Revised on 02/08/2015 Article Accepted on 22/08/2015

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ABSTRACT

Organic pollutants and heavy metals are distributed throughout the environment and are highly recalcitrant to biodegradation processes. Indigenous microorganisms at contaminated sites have evolved for degradation of such contaminants. In this present study, indigenous *Pseudomonas* sp. were isolated, which was capable of utilizing petrol as carbon source. Total four heavy metal tolerance were studied namely, Cobalt, Iron, Zinc and Lead. The isolated *pseudomonas* Sp. were sensitive to Cobalt completely at all the concentration, resistant to Lead completely at all the concentration and for Iron & Zinc it is resistant to 1 µg/ml, 2 µg/ml and sensitive to 5 µg/ml, 7 µg/ml concentration. This work will spotlight the use of indigenous microorganism for bioremediation purpose.

KEYWORDS: bioremediation, heavy metals, Organic pollutants, *pseudomonas* Sp.

INTRODUCTION

In the face of speedy economic and industrial development, human actions have instigated extensive pollution of the natural universal environment.[1] About forty percent of hazardous waste sites on the Environmental Protection Agency's (EPA) National Priority List (NPL) are co-contaminated with organic pollutants and heavy metals which have cytotoxicity value.[2,3,4] Such issues provoked the need for innovative and cutting-edge bioremediation techniques to potentially remove organic contaminants from a variety of co-contaminated

environment.[5] Most of the important classes of pollutants (chemicals) have been shown to have carcinogenic activity in studied experimental animals, which increase study over the presence, disposition, and persistence of organic pollutants in the environment as it have potential human health risk.[6, 7] Organic contaminants such as, chlorinated organic solvents are more often encountered contaminant in water and soil, and most difficult contaminants to remediate.[8] Manufacture and practice of several chlorinated organic chemicals have been totally banned in several countries around the world, because of their latent toxicity to wildlife as well as humans.

The words "resistance" and "tolerance" are arbitrarily used as synonymous in literature. The term tolerance appears to be more suitable to state to the ability of a bacteria able to grow in the attendance of high concentrations of a metal.[9] Bacteria have capacity to survive in toxic heavy metals concentrations [10, 11] because they have specific genetic mechanisms of toxic metal resistance.[12, 13] Microbial degradation has been projected as a proficient strategy for organic waste elimination as it practices relatively low cost, low technology, and may be carried out on site for the complete degradation of organic pollutants deprived of obliteration of its indigenous flora and fauna.[14] Metal resistance is similar to the selection of antibiotic resistant strains as both resistance genes are frequently located on the same mobile genetic elements.[15, 16]

Even though plenty of research describes the action of heavy metals, inhibitory or deleterious effects on susceptible organisms, very scanty of studies on metal tolerant bacteria met.[17, 18] Biodegradation of organic contaminants to innocuous end products minimizes the environmental burden and residual contamination.[19, 20]

In our work we analyzed the presence of metal tolerance in bacteria. Present study may lead to broader investigation with the intentions to obtain data regarding metal tolerant bacteria and their potential use for bioremediation. Previously we have investigated the possible relationships between metal tolerance and the degradation of toxic aromatic compounds.

METHODS AND MATERIALS

Collection of Soil Sample

About 10 to 15 g of soil were aseptically collected in sterile polythene bags from different petroleum contaminated sites (petrol pumps) in and around Nagpur, (M.S) India. Immediately they were brought to the laboratory and stored at 4°C till its use.

Isolation of bacteria

1.0 g of soil sample was inoculated into the nutrient broth which was prepared by individual ingredient where carbon source is replaced by petrol. The petrol deficient media was sterilize by autoclaving and petrol (1 ml/100ml) was added after sterilization of medium under aseptic condition. The broth was then incubated at 37^0 C in orbital shaker incubator at 80 rpm for 48 hours. After 48 hrs broth culture was then streaked on nutrient agar plate as same composition as broth only 2% agar was added. For the preparation of nutrient agar plates nutrient media deficient with petrol was autoclaved and maintained at 45-48^0 C in water bath and then petrol (1 ml/100ml) were added and shaken vigorously for mixing of petrol as much as possible. Plates was then incubated at 37^0 C for 48 hrs. Colonies so appeared is then maintained as pure culture and stored at 4°C.

Identification of Bacteria

The isolated bacterial pathogens were identified on the basis of morphological, cultural and biochemical characteristics and results were compared with Bergey's Manual of Determinative Bacteriology 9th edition.

Heavy Metal Tolerance Range of Hydrocarbon Degrading Bacteria

Nutrient agar was prepared in different conical flasks. The molten agar medium is then maintained at 45-48^0 C in water bath and then various heavy metals were added as their compounds such as Cobalt as Cobalt Chloride, Iron as Ferrous Sulphate, Zinc as Zinc Sulphate and Lead as Lead acetate was studied. 1, 3, 5 and 7 µg/ml concentration of heavy metals was taken. The metal compound at various concentration was added and labeled accordingly. The molten agar plates are allowed to solidify and was then shifted to incubator at 37°C. After 24 hours of incubation, the plates were observed for growth. Nutrient Agar plates without heavy metals served as control.

RESULT AND DISCUSSION

In effective *in situ* bioremediation of contaminants, both metal and organic compounds, the microbes must be metal-resistant with degradative genes, or microbial consortia of metal-resistant microbes and desired catabolic capabilities.[21] Earlier, bioaugmentation practice concentrated on the inoculation of a microorganism with both metal resistant and capable of organic degradation. But, under environmental circumstances such methodology is often unsuccessful, due to the high energy requirements needed to maintain concurrent metal resistance and organic degradation. Current methods have validated the use of a dual-

bioaugmentation strategy and the part of cell bioaugmentation in the remediation systems.[22] Heavy metal remediation strategies depends on the detoxification and immobilization of the metal both to reduce the biological toxicity and to retard metal transport because metals cannot be degraded like organic compounds.[21]

In present study, in first half the research were intended for isolation of microorganism capable of degrading petrol from soil. The soil sample was indeed collected from petrol contaminated soil of petrol nearby region. The microorganism was isolated on basic nutrient media, carbon source replaced by petrol. Only three colonies were appeared on solid agar containing medium. These isolates were maintained on nutrient agar slant as pure culture, these were then proceed for further identification. The identification was done by following Bergey's Manual of Determinative Bacteriology 9th edition. On identification all three isolate displayed same cultural, biochemical and morphological character, these isolate were inferred to be of *pseudomonas* Sp. Results are displayed in Table 1.

Table 1: Cultural, Morphological and Biochemical characterization of bacterial isolates.

S. N.	Test		Isolate Character
1	Cultural Character	Colony Morphology	Small, Flat, Entire, Pigmented Circular dry colonies
2	Morphological Character	Gram's Staining	Gram negative
		Motility	Active Motile
3	Biochemical Character	Indole	+
		Methyl red	-
		VP Test	-
		Citrate Test	+
		Nitrate Test	+
		H2S production	+
		Catalase Test	+
		Oxidase disc Test	+

The only one isolate from the previous isolation procedure were carried for further study. Because all the three isolate displayed same character and it is supposed to have daughter of same cell appeared as different colony. In the second half of the study various heavy metals were added as their compounds such as Cobalt as Cobalt Chloride, Iron as Ferrous Sulphate, Zinc as Zinc Sulphate and Lead as Lead acetate was studied. The different concentration of heavy metals was taken, 1, 3, 5 and 7 µg/ml. Isolate was studied at mentioned four different concentration individually. Given in table 2 below.

Table 2. Heavy metal tolerance range of hydrocarbon degrading bacteria.

Compound	Heavy Metal	Concentration (µg/ml)	Response for tolerance
Cobalt Chloride	Cobalt	1	-
		3	-
		5	-
		7	-
Ferrous Sulphate	Iron	1	+
		3	+
		5	-
		7	-
Zinc Sulphate	Zinc	1	+
		3	+
		5	-
		7	-
Lead acetate	Lead	1	+
		3	+
		5	+
		7	+

Where, - = Sensitive, + = Resistant

Total four heavy metal tolerance were studied namely, Cobalt, Iron, Zinc and Lead. The metals used in the study was in compound form. Concentrations studied was selected with the difference of 2 µg/ml so as to acquire satisfactory data about wide range of tolerance. Table 2 showing the results of the study. The isolated *pseudomonas* Sp. were sensitive to Cobalt completely at all the concentration. Contrastingly, surprisingly, *pseudomonas* Sp. were resistant to Lead completely at all the concentration and for the metals Iron & Zinc it showed same results, i.e. it is resistant to 1 µg/ml, 2 µg/ml and sensitive to 5 µg/ml, 7 µg/ml concentration. These results disclosed that the incidence of heavy metals such as cobalt even in 1 µg/ml concentration was highly toxic to the bacteria isolated from petrol contaminated sites and they will ensure serious threat to their metabolism in the environment. But they have shown resistance towards iron, zinc and lead at various metal concentration. Heavy metals can exist in a diverse chemical and physical forms, as, soil adsorbed species, soluble complex species or as an ionic solutes which raise difficulties in treatment of on organic pollutant biodegradation. Additional obstructions arise owing to the influence of environmental state of affairs on the physical and chemical state of the metals such as pH, redox potential of the water phase as well as soil properties and organic matter content.[23] Heavy metal ions form unspecific complex compounds within the cell, at higher concentrations, which leads to toxic effects, making them too dangerous for any physiological function. Nonessential metals

displaces essential metals from native binding sites or through ligand interactions which confers toxicity. Heavy metals inhibit the activity of sensitive enzymes. Hg^{2+}, Cd^{2+} and Ag^{2+} binds to sulfhydryl (–SH) groups of essential enzymes of microbial metabolism.[24, 25]

If the redox potential of a given heavy metal should be within the range of −421 mV to +808 mV i.e. physiological redox range of aerobic cells. The report suggested redox potential heavy metals like Hg^{2+}, arsenate, and Cu^{2+} with redox potential +430 mV, +139 mV, −268 mV respectively can be reduced by the aerobic cell, but Zn^{2+}, Cd^{2+}, and Ni^{2+} with redox potential −1.18 V, −824 mV, −678 mV respectively may not reduce.[25] pJP4 gene is present in plasmid of *Escherichia coli* D11 but absence of the chromosomal genes necessary for the transformation of 2-chloromaleylacetate to succinic acid, was used for gene bioaugmentation. Thus this finding recommends indigenous transconjugant population generated from *E. coli* D11 inoculation was better suited for subsequent 2, 4-D degradation.[21]

Biosurfactant-enhanced bioremediation such as rhamnolipids of *Pseudomonas aeruginosa*.[26] Moreover apart from biodegradation of hydrocarbon, biosurfactants can also be used to remove heavy metals. Chromium removed by rhamnolipid produced from *Pseudomonas* spp.[27] It complex favorably with Cd and Pb than Ca and Mg.[28] A washing of copper, cadmium and soil with the saponin (biosurfactant) increase the efficiency of metal removal.[29] Biosurfactants is an eco-friendly remediation method for soil contaminated with organic and inorganic contaminants including hydrocarbons and metals.[30] Biosurfactant, rhamnolipid produced by *P. aeruginosa* strains capable of increasing the bioavailability of substrates with limited aqueous solubilities, and by increasing cell surface hydrophobicity.[31,32]

CONCLUSION

So far, there is a scarcity of knowledge on the biodegradation of aromatic organic pollutant compound in co-contaminated environments of heavy metal by microorganism. Therefore, in this present work, we tried to solve current difficulties related with metal toxicity in co-contaminated environments, and focused light on promising development approaches for operational bioremediation of sites co-contaminated with aromatic organic chemicals and heavy metals. This work precisely addresses, bioremediation of toxic metals with the assistance of microorganisms. The finding in the research suggests use of indigenous *Pseudomonas* sp. for bioremediation of heavy metals and the isolate is also helpful in treatment of site contaminated with petrol. This research helpful in designing improvement

strategies aimed at increasing biodegradation in co-contaminated environments. This also provides scope and hope for further study over other metals, aromatic compounds, biosurfactants and molecular studies.

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