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Effect of Metal Ions on Naphthalene Degrading Ability of Bacteria Isolated from Oil Contaminated Soils

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Abstract: ***Polyaromatic hydrocarbons (PAHs) entering into the environment from various sources are highly toxic and deleterious to living beings. Naphthalene is one the sixteen toxic PAHs enlisted by EPA, US. Physical and chemical methods used for the removal of PAHs from polluted sites are not effective. Bioremediation which employs microbes to metabolize PAHs is an alternative method to physical and chemical processes. Naphthalene is considered as model compound for the biodegradation studies of PAHs. In the present study the effect of seven selected metal ions on naphthalene degrading ability of three bacterial species (viz., Bacillus, Pseudomonas and Micrococcus) isolated from oil contaminated soils of mechanical was studied. Five metal ions were reported to enhance the naphthalene degrading activity and two found to decrease the activity. These bacteria can be further improved and used for bioremediation of polluted sites.***
Keywords: Polyaromatic hydrocarbons, toxic, Naphthalene, Bioremediaton, bacteria, metal ions

I. INTRODUCTION

Nature is composed of soil, water and air. Soil is considered as an important component of nature as it provides nutrients to plants which provide food to humans and microbes which control nutrient cycles. Presently soil is getting accumulated with huge amounts of polyaromatic hydrocarbons (PAHs) due to partial oxidation of petroleum products and coal, burning of wastes, oil spill accidents and from petroleum refineries. PAHs contain two or more benzene rings and sparingly soluble in water. The PAHs are highly toxic and cause deleterious effects to humans. Environmental Protection Agency (EPA), US categorized 16 PAHs as highly carcinogenic and mutagenic to human beings. Naphthalene is one among those 16 PAHs. Naphthalene contains two fused benzene rings. PAHs can be removed by physical and chemical methods but they are ineffective and costly. Instead, microbes can be employed to degrade (metabolize) PAHs in the polluted soils. This process of application of microbes is regarded as bioremediation which is an economical process and ecofriendly. Naphthalene is widely present in the PAHs contaminated soils and easily soluble in water and hence, it is considered as model compound for biodegradation (metabolism) studies of PAHs. Bacteria which can degrade naphthalene include *Vibrio, Mycobacterium, Staphylococcus* etc. [1, 2, 3]. In the present work fourteen naphthalene degrading bacteria were isolated from oil contaminated soils of four mechanical workshops of Autonagar of Kaman region, Karimnagar, Telangana, India and identified till genus level. Eight bacterial isolates were identified as *Bacillus,* three were *Pseudomonas* and remaining three were identified as *Micrococcus* species. Three bacterial species viz., *Bacillus, Pseudomonas* and *Micrococcus* species designated as A3, D1 and D5 exhibiting enhanced naphthalene degradation activity were selected for the present study. In the present paper the effect of metal ions on the naphthalene degrading ability of selected three naphthalene degrading bacterial isolates was studied.

II. MATERIALS AND METHODS

A. Medium

Experiments were performed using basal salt medium (BSM) supplemented with naphthalene (0.1%) as the sole carbon and energy source. The composition of BSM is K_2HPO_4 - 0.38g, $MgSO_4.7H_2O$ - 02g, NH_4Cl - 1.0g, $FeCl_3$ - 0.05g, peptone - 1g, distilled water - 1000ml. Naphthalene is the sole source of carbon and energy in the BSM [2].

B. Metal Ions

Effect of seven metal ions viz., $Fe3^+$, Co^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, MoO_4^{2-} and Zn^{2+} on naphthalene degrading activity of bacteria was determined. Each metal ion was added to medium in the form of their salts (0.01 mM). BSM medium supplemented with naphthalene and all the sources (salts) of ions is treated as negative control and without any ions as positive control. Bacteria were grown in the absence of that ion whose effect has to be studied in the presence of remaining all ions in the medium [4]. The growth

of each bacterium in the absence of a particular ion and in the presence of remaining ions was compared with its growth observed in positive and negative controls. The effect of each ion was studied by observing growth pattern of bacteria in its absence and presence along with other ions.

C. Inoculum

The bacterial cultures were grown in 10 ml of BSM supplemented with naphthalene at 30^0C for one day. Then bacterial culture broths were centrifuged at 3500 rpm to get cell pellet of each bacterial cell mass. The cell pellets were thoroughly washed and transferred to fresh BSM broth supplemented with naphthalene and incubated at 30^0C till the optical density (OD) of the cell biomass in the culture broth was read 1.0 at 600 nm in spectrophotometer [5]. Each such 1 ml of bacterial culture (whose biomass is 1.0 at 600 nm) was used as inoculum source for 10 ml of specified media for all experiments conducted in triplicates. For each result of experiment standard deviation (SD) was calculated.

D. Determination Of Naphthalene Degradation Activity

The bacteria cultured in the medium metabolize (degrade) naphthalene to derive energy. Hence, growth of the bacteria in medium (in terms of OD of cell biomass) is a direct measure of naphthalene degradation by bacteria.

III. RESULTS AND DISCUSSION

In the present study effect of seven metal ions ($Fe3^+$, Co^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, MoO_4^{2-} and Zn^{2+}) on the naphthalene degradation (metabolism) ability of three bacterial species viz., *Bacillus*, *Pseudomonas* and *Micrococcus* was determined. Five metal ions viz., Co^{2+}, Cu^{2+}, Ca^{2+}, MoO_4^{2-} and Zn^{2+} are required for the enhancement of naphthalene degradation activity in all the three bacteria. This was evident by decrease of bacterial growth in their absence and their growth pattern observed in negative and positive controls (Table-1). For the functioning of many enzymes metals are required as activators. Enzymes in turn regulate various metabolic reactions in living cells [6]. On the other hand some metal ions may inhibit the activity of certain enzymes [7]. Two metal ions viz., $Fe3^+$ and Mn^{2+} found to decrease the naphthalene degradation activity of all the three bacteria. The absence of these two metal ions increased the growth of bacteria indicating their inhibitory effect on naphthalene degradation ability (Table-1). The $Fe3^+$and Mn^{2+} ions inhibited the activity of malic dehyrogenase and alkaline phosphatase respectively [8, 9].

Table-1: Effect of metal ions naphthalene degrading bacteria

Metal ions	Growth (OD) of naphthalene degrading bacteria at 600 nm		
	A3 (Bacillus)	D1 (Pseudomonas)	D5 (Micrococcus)
Negative control (all ions present)	4.72 ± 0.05	4.25 ± 0.05	3.54 ± 0.08
Positive control (No ions)	2.24 ± 0.05	2.12 ± 0.05	1.43 ± 0.05
All ions present except Fe^{3+}	4.82 ± 0.14	4.32 ± 0.12	3.70 ± 0.08
All ions present except Co^2	3.76 ± 0.12	3.59 ± 0.08	2.87 ± 0.05
All ions present except Cu^{2+}	3.92 ± 0.05	3.79 ± 0.16	2.37 ± 0.17
All ions present except Mn^{2+}	3.46 ± 0.12	3.36 ± 0.05	3.43 ± 0.09
All ions present except MoO_4^{2-}	4.96 ± 0.05	4.39± 0.17	3.89 ± 0.05
All ions present except Zn^{2+}	2.82 ± 0.12	2.66 ± 0.05	2.23 ± 0.05
All ions present except Ca^{2+}	3.79 ± 0.08	3.66 ± 0.12	2.60 ± 0.14

IV. CONCLUSION

Effect of seven metal ions on naphthalene degradation ability of three bacteria isolated from oil contaminated soils of mechanical workshops was studied. Five metals were reported to enhance the naphthalene degradation activity of all the three bacteria whereas two metal ions found to inhibit the naphthalene degradation ability. These bacteria can be genetically improved to enhance the naphthalene degradation ability in them and employed for bioremediation process.

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