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Isolation, Identification and Characterization of Heavy Metal Resistant Bacteria from Industrial Affected Soil in Central India

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ABSTRACT

Microorganisms related technologies may provide an alternative or addition to conventional method of metal recovery or metal removal. The present study deals with isolation, identification and characterization of heavy metal resistant bacteria was isolated from industrial affected soils collected from different places at a depth of 0-15 centimeter, in and around Mandideep industrial areas, Raisen district, Central India. Initially, a total of 175 isolates were screened from industrial affected soil. The five isolates were selected based on high level of heavy metal resistances. On the basis of morphology, biochemical revealed that, the isolates were identified as Proteus vulgaris (MR1), Bacillus cereus (MR2), Bacillus decolorationis (MR3), Pseudomonas fluorescence (SS4) and Pseudomonas fluorescence (SS5). The soil isolates showed optimum growth at pH 7.0 and 30°C. The identified isolates were resistant to cadmium (Cd), nickel (Ni), lead (Pb), arsenic (As), and chromium (Cr). The minimal inhibitory concentration (MIC) of soil isolates against Cd, Cr, Ni, Pb and As was determined in solid media. The identified heavy metal resistant bacteria could be effective and useful for the bioremediation of heavy metal contaminated soil.

Key words: *Contaminated Soil, Heavy metals, Soil Bacteria, and Bioremediation.*

INTRODUCTION

Rapid increase of population and the increased demand for industrial establishments to meet human needs have created problems such as over exploitation of available resources, increased pollution taking place on land, air and water environment. Heavy metal pollution of soil and wastewater is a significant environmental problem[1]. Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the environment[2]. According to EPA[3], bioremediation is defined as a managed of spontaneous process in which microbiological process are used to degrade, break down or transform hazardous contaminants to less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from environment media.

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Microorganisms used chemical contaminants as an energy source using their metabolic process throughout the microbiological process. However, the excessive amounts inorganic nutrients in soil cause microbial inhibition[4]. The microorganisms in particular have the abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds.

There are different sources of heavy metals in the environment such as: natural, agricultural, industrial, domestic effluent, atmospheric sources and other sources. Activities such as mining, electroplating, metallurgical, smelting operations and agriculture have contaminated extensive areas of world such as Japan, Indonesia and China mostly by heavy metals such as Cd, Cu and Zn[5]. Heavy metal (HM) pollution is a major environmental problem[6], that reduces crop production and food quality. Unlike organic contaminants, metals are not degradable and thus remain in the environment for long periods of time; when present at high concentrations, metals can negatively affect plant metabolism[7]. In our previous studies, we have studied the feasibility of remediation of soils contaminated with Cr using different floriculture plant species, i.e., tuberose (*Polianthes tuberosa*), chrysanthemum, calendula, aster and dahlia[8-9]. From these studies, it was found that majority of the plant species could tolerate at the most 10–15 mg Cr/kg soil. However, the contaminated sites would have very high levels of Cr and at times would even be unfit for cultivation of the crops.

During the last few decades, the Mandideep, State Madhya Pradesh, Central India, has undergone rapid social and economic development. It could face public health and ecological problems if heavy metal loads exceed a critical value. Little information is available on heavy metal concentrations on soils of Mandideep[10]. There is a need for innovative treatment technologies for the removal of heavy metal ions from soil, water bodies and wastewater. Different microbes have been proposed to be efficient and economical alternative in removal of heavy metals from soil and water[11]. The objective of this study is to determine heavy metals resistance of bacteria, MIC, growth studies, and biochemical characteristics were used to exploit these isolates for cleanup of industrial affected soil, wastewater and sewage.

MATERIALS AND METHODS

Soil Collection

Soil samples were collected randomly from industrial contaminated soil areas nearby pharmaceutical, textile, food and beverages, leather, steel and iron workshop, graphite, and tractor manufacturing industry of Mandideep District Raisen of Madhya Pradesh, Central India. Soil samples were collected in sterilized polythene bags and immediately bought to the Microbiology laboratory, Department of Microbiology Barkatullah University Bhopal, Madhya Pradesh, for bacteriological analysis.

Isolation of bacteria

The microbial strains were isolated from the collected soil samples by serial dilution technique. Selective isolation of bacterial spp. was performed by spreading the samples on their individual media.

Identification and characterization of bacteria from soil sample

Individual distinct colonies were further undergone repeated sub-culturing. Selected colonies were grown on nutrient agar media (Himedia, India). Identification of microbes by using morphological, cultural and biochemical characteristics for the activities of motility, oxidase, catalase, VP test, MR test, starch and gelatin hydrolysis, indole production and citrate utilization according to Bergey's manual of systemic bacteriology[12] .

Determination of optimal bacterial growth conditions

The optimal growth conditions with reference to temperature and pH were determined. The isolates were grown in nutrient agar medium with different temperature between 25^0C, 30^0C, 37^0C and 45^0C and incubation was carried out at pH values between (6.0, 7.0, 7.5, and 8.0). The optical density of the log phase growing cultures (12-14 h) conditions was noted at 600 nm using spectrophotometer to determine the growth.

Determination of Minimum Inhibitory Concentration (MIC)

The stock solutions of $K_2Cr_2O_7$, $CdCl_2$, Pb $(NO_3)_2$, $ZnSO_4.7H_2O$ $NiCl_2$, and $HgCl_2$ were prepared in sterile deionized water and sterilized by autoclaving at 121^0C for 15 min. The culture grow at a given concentration were subsequently transferred to the next concentration. Based on the evaluation, minimum inhibitory concentration (MIC) was determined at 30^0C for 6 days. Five bacterial strains showing higher MIC values for Cr, Cd, Pb, Zn, Ni, and Hg were selected for further studies.

Evaluation of Heavy Metal Tolerance

Tolerance of selected bacterial strains to various heavy metals was determined by agar dilution method[13]. Freshly grown agar cultures of these isolates were inoculated aseptically on nutrient agar plates supplemented individually with other heavy metals. The metal salts used were $K_2Cr_2O_7$, Pb $(NO_3)_2$, (Merck), $ZnSO_4.7H_2O$, $CdCl_2$, $NiCl_2$ and $HgCl_2$ (Qualigens). The metal ion concentration tested ranged from 10 to 150 mg/ L. Plates were incubated aerobically at different temperature between 25^0C, 30^0C, 37^0C and 45^0C for 6 days and observed for growth.

RESULTS

Isolation of heavy metal resistant bacteria

In the present study we identify and characterize heavy metals resistant bacteria isolated from industrial affected soil. 150 colonies were screened from initial level of heavy metal supplemented nutrient agar medium. 25 isolates were selected in the secondary screening from soil. Finally five strains were selected based on high degree of heavy metals resistances were used for further studies. The strains *Proteus vulgaris* (MR1), *Pseudomonas fluorescence* (SS4) and *Pseudomonas fluorescence (SS5)* was Gram-negative, rod shaped motile and *Bacillus cereus* (MR2), Bacillus decolorationis (MR3) was Gram-positive, rod shaped motile bacteria. The sewage isolates showed optimum growth at 30°C and pH 7.0. The biochemical characteristics of soil bacteria were shown in **(Table- 1, 2 and 3).**

Growth studies of bacteria and simultaneously resistant to heavy metals

Growth studies of MR1, MR2, MR3, SS4 and SS5 were carried out in nutrient agar medium with Cr, Cd, Pb, As, Ni (200 mg/l) and Hg (100 mg/l) were prepared from stock solutions. The measurements from the cultures incubated for 48 h were in good agreement according to bacterial resistance for each heavy metal. All isolates were found to be sensitive to for Cr and Hg. Thus, microorganisms having combined abilities of simultaneous resistance to heavy metals and their biotransformation to non-toxic form will be potentially useful for detoxification of heavy metals polluted soil.

MIC of heavy metal

Soil bacteria MR1, MR2, MR3, SS4 and SS5 showed high degree of resistance to all heavy metals, MIC values varying concentration from 0.35 - 17.5 mM. Among the heavy metals zinc and nickel was less toxic, where as chromium and mercury were highly toxic to all strains. MIC of heavy metal were shown in (**Table- 4 & Fig 1)**.

Table: 1 Morphological characterization of isolated bacterial species

S. No.	Morphological characteristics	Isolated bacterial colonies				
		MR1	MR2	MR3	SS4	SS5
1	Colour of the colony	White	White	White	White	White
2	Shape of the cell	Rod	Rod	Rod	Rod	Rod
3	Gram s Staining	Negative	Negative	Positive	Positive	Positive
4	Motility	Motile	Motile	Motile	Motile	Motile

Note: MR1 -*P. vulgaris,* MR2- *B. cereus,* MR3 -*B.decolorationis,* SS4- *P. fluorescence,* SS5- *P. fluorescence*

Table: 2 Biochemical characterization of isolated bacterial species

S. No.	Morphological characteristics	Isolated bacterial colonies				
		MR1	MR2	MR3	SS4	SS5
1	Indole	Positive	Negative	Negative	Negative	Negative
2	Methyl red	Positive	Negative	Negative	Negative	Negative
3	Voges-proskauer test	Negative	Negative	Positive	Negative	Negative
4	Citrate utilization test	Positive	Positive	Positive	Negative	Negative
5	Urease hydrolysis	Variable	Variable	Negative	Negative	Negative
6	Oxidase	Negative	Positive	Negative	Positive	Positive
7	Catalase	Positive	Positive	Positive	Positive	Positive

Note: MR1 -*P. vulgaris*, MR2- *B. cereus*, MR3 -*B.decolorationis*, SS4- *P. fluorescence*, SS5- *P. fluorescence*

Table: 3 Bacterial growth at different temperature

S. No.	Temperature (˚C)	Isolated bacterial colonies				
		MR1	MR2	MR3	SS4	SS5
1	4	-	-	-	-	-
2	25	-	+	+	+	+
3	30	+	+	+	+	+
4	37	+	+	+	+	+
5	45	-	-	-	+	+

Note: + positive, - negative

Table: 4- MIC of heavy metals against soil bacteria

S. No.	Heavy metals	Isolated bacterial colonies				
		MR1	MR2	MR3	SS4	SS5
1	Chromium	5.0	6.5	13	5.9	5.8
2	Cadmium	5.1	7.0	4.8	7.3	7.3
3	Lead	6.2	4.6	3.7	8.2	6.1
4	Zn	10.0	6.0	5.4	17.5	16.8
5	Nickel	8.8	0.79	0.35	13.2	12.5
6	Mercury	0.12	1.3	0.0	5.4	5.2

Note: Heavy metal concentration in mM.

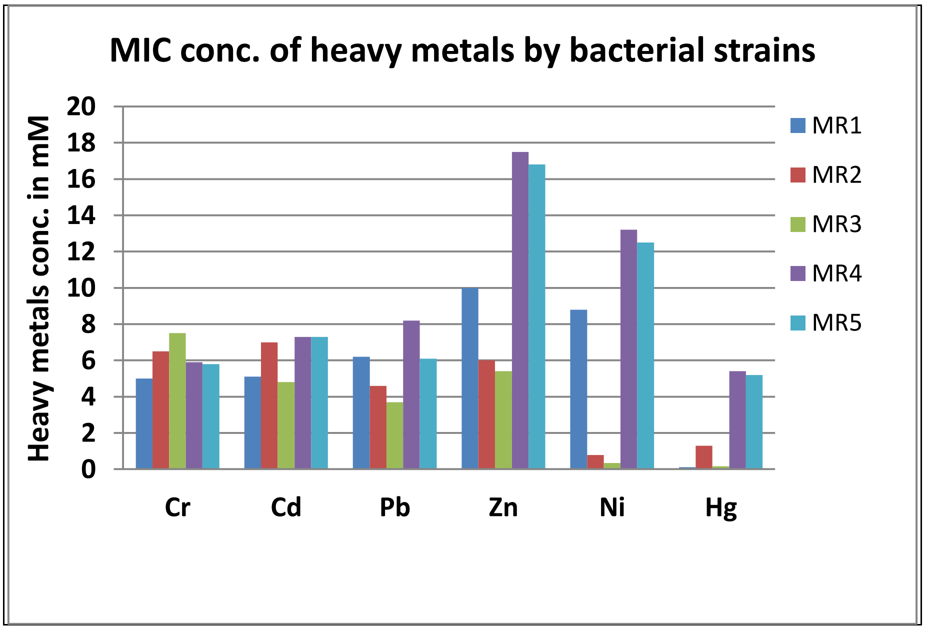

Fig. 1: Graphical representation of heavy metals resistance by isolated bacterial strains

CONCLUSION

The bioremediation process is influenced by various factors such as soil type, pH, temperature, nutrient, amendments and oxygen. The ability of microbial stains to grow in the presence of heavy metals would be helpful in the contaminated soil treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for soil treatment. In the present study high degree of heavy metals resistance associated with multiple heavy metals was detected in industrial affected soil bacteria. In the present study the order of resistance of Cr was: MR3(13mM) >MR2(6.5mM) >SS4 (5.9mM) >SS5(5.8mM) >MR1(5.0mM), resistance of Cd was: SS5(7.3mM) = SS4 (7.3mM) >MR2(7.0mM) >MR1(5.1mM) >MR3(4.8mM), resistance of Pb was: SS4(8.2mM) >MR1(6.2mM) >SS5(6.1mM) >MR2(4.6mM) >MR3(3.7mM), resistance of Zn was: SS4(17.5mM) >SS5(16.8mM) >MR1(10.0mM) >MR2(6.0mM) >MR3(5.4mM), resistance of Ni was: SS4(13.2mM) > SS5(12.5mM) > MR1(8.8mM) >MR2(0.79mM) >MR3(0.35mM) and the order of resistance of Hg was: SS4(5.4mM) > SS5(5.2mM) > MR2(1.3mM) > MR1(0.12mM) >MR3(0.0mM). Among the heavy metals zinc and nickel was less toxic, where as chromium and mercury were highly toxic to all bacterial isolates. The efforts must be directed towards faster and economic restoration of heavy metals and pesticide contaminated soil to secure the soil quality and food and health of mankind. The future research should investigate the heavy metals and pesticide bioremediation potential of recombinant as well as indigenous bacteria with and without supplements.

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