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Bioremediation of Heavy Metals by Employing Resistant Microbial isolates from Agricultural Soil Irrigated with Industrial Waste Water

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ABSTRACT

A total of 14 microbial isolates werecharacterized and out of 14, IS1 and IS14 were observed to be most effective because of their high relative growth and resistance against heavy metals. Further, these two isolates were assessed for their ability to remove Zinc and Lead from medium amended with heavy metals. IS1, *Bacillus thuringiensis* strain *"Simi"* (Accession number KF 916618.1) was found to be more effective as compared to IS14, *Bacillus subtilis* strainPSB (Accession number KF 279045.1) for the remediation of heavy metals. IS1 showed mean of 54% biodegradation efficacy in the first three days and from day 4 onwards the mean percentage of biodegradation efficacy decreased to around 31%. The results of the present study showed that the metal resistant bacteria can be used for heavy metal bioaccumulation.

Key words: ICP-AES, Phylogenetic tree, waste water, Hydrocarbons.

INTRODUCTION

The presence of heavy metals and pesticides in the environment has been a subject of great concern due to their toxicity, non-biodegradable nature and the long biological half-lives for their elimination from biological tissues[1-5]. Pollutants deteriorates the quality of soil and crops produced. Excessive metal concentrations and pesticides in contaminated soils can result in decreased soil microbial activity and soil fertility, and yield losses[2-3]. Agricultural irrigation with wastewater is common in arid areas but has possible public health and environmental side

effects, as effluent may contain pathogens, high level of salts, detergents and toxic metals[2-5]. There is a need for monitoring of toxic effects of wastewaters and such irrigation practices should be carried out only after treatment of wastewater. Numerous methods have been proposed to remove heavy metals from sewage sludge, including chlorination, use of chelating agents and acid treatments at high temperatures[6-7]. However, those methods are generally ineffective in practical applications due to high cost, operational difficulties and low metal leaching efficiency. An alternative way to replace chemical methods in removing heavy metals is bioremediation through microbial isolates.

The bioremediation techniques are effective and efficient for remediation of pollutants so as the bioremediation technology from laboratory to field to clean up the environment can be taken up[8]. For bioremediation to be effective, microorganisms must enzymatic ally attack the pollutants and convert them to harmless products[9]. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. These factors include the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors[10].Therefore, this study was designed with the objective to isolate and characterize metal (Zinc and lead) resistant bacteria from heavy metal contaminated soil to determine their feasibility on the removal of metals through bio-accumulation.

Methodology

Collection and Physicochemical Analysis of soil samples

Soil samples were collected from the top 15 cm from industrial effluents of the Ludhiana (Punjab) region (30.91°N75.85°E). These samples were collected in sterile zip lock bags with the help of sterilized spatula, properly sealed and labeled, and sent to the laboratory within 24 hours. Heavy metals in soil were analyzed by inductively coupled plasma-atomic emission spectrometry at P.A.U., Ludhiana (ICP-AES).

Isolation&Identification of the microorganism

Isolation was done by the method as described by Jyothi[11].Isolated microbes were identified through morphological, biochemical& molecular characterization[12].

Determination of comparative growth and growth pattern

Extent of heavy metal resistance of selected microbial isolates was evaluated in bacillus cereus broth (bacteria) containing 25, 100, 250 and 300 ppm of Lead nitrate $Pb(NO_3)_2$, Copper sulphate $CuSO_4$, Zinc nitrate $Zn(NO_3)_2$ and Ferrous sulphate $FeSO_4$ and growth is determined by measuring Optical density (O.D.)at 540nm with un-inoculated broth as control. To check the growth pattern of the isolates, cultures were inoculated into broth, treated with 0 (control), 25, 100, 250 and 300 ppm of $Pb(NO_3)_2$, $CuSO_4$, $Zn(NO_3)_2$ and $FeSO_4$, and incubated at 37°C[13].

Molecular analysis

Isolates showing high resistant against heavy metals were sent for 16S rRNA analysis to Ahmedabad, Gujarat for molecular analysis[14-15].

Minimum Inhibitory Concentration (MIC) determination of the isolated strains

Bacillus cereus agar plates and yeast peptone dextrose agar plates were inoculated with 100µl aliquots of 24hr culture with all isolates with different concentrations of $Pb(NO_3)_2$, $CuSO_4$, $Zn(NO_3)_2$ and $FeSO_4$ (250, 500,750,1000,1250 and 1500 mg/l).Diameter of inhibition zones were measured (in mm) in order to determine the MIC[16].

Efficacy of isolated strains on bioaccumulation of heavy metals

80µl of the bacterial inoculum was inoculated into test tubes containing 8.0 mL of broth supplemented with 100ppm of zinc & lead. The control treatment was prepared by mixing 9.0 ml of broth with 1.0 ml of bacterial suspension. All the tubes were sealed with parafilm and kept at 27 ± 2 °C for 7 days. To determine degradation efficacy, the heavy metal was first extracted with sequential extraction method[17] diluted 10^{-4} times and the absorbance was recorded at 225 nm[18]. The biodegradation efficacy (BE) was calculated using the following formula BE (%) = 100 – (As/Aac × 100)[19,20].

RESULTS AND DISCUSSION

Soil analysis reportand enumeration of bacteria

Soil analysis report shows the presence of various toxic heavy metals and sample contains Arsenic(0.756mg/kg), Calcium (327.9mg/kg), Cadmium (0.09mg/kg), Cobalt (0.02mg/kg), Chromium (0.053mg/kg), Copper (9.4mg/kg), Iron (53.71mg/kg), Potassium (118.6mg/kg), Magnesium (202.7mg/kg), Manganese (4.539mg/kg), Sodium (59.91mg/kg), Nickel (0.723mg/kg), Phosphorous (65.02mg/kg), Lead (8.387mg/kg), and Zinc (10.8mg/kg). A total of fourteen different isolates (IS1,IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS13, and IS14) were isolated andare biochemically and molecularly characterized and best two having high resistant against metals were further selected for bioaccumulation & heavy metal testi.e. IS1 and IS 14. The biochemical and isolated results were shown in fig. 1 and table 1.

Table 1: Biochemical tests

Test	IS1	IS14
Indole test	-	-
Methyl red	+	+
VogesProskauer	-	+
Citrate	+	-
Catalase	+	+
Coagulase	-	+
Motility	+	-
Nitrate reduction	+	+

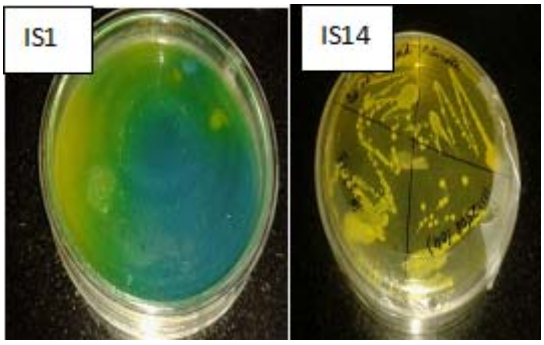
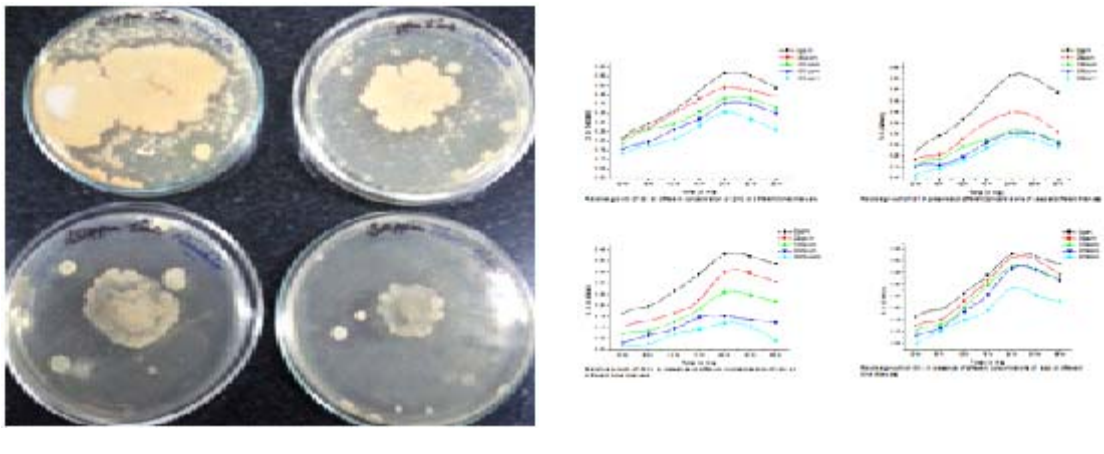


Fig. 1: Isolation of the microorganisms

Relative growth determination of isolates and their growth pattern

It was observed that the growth of the isolate decreases with the increase in metal concentration. IS1*Bacillus thuringiensis* strain *Simi*and IS14 was found to have maximum relative growth against Lead and Zinc solution. The relative growth rate was observed at different concentration of heavy metals. The results are consisted with Ahemad and Malik[15]. Thegrowth pattern of the microbial isolates at different concentration (25, 100, 250&300 μgml^{-1})areshown below:



Lead sulphite Zinc nitrate

Fig 2. Isolates amended with different concentrations of metals & Graph showing relative growth of isolates at different concentrations (Control, 25ppm, 100ppm 200ppm, 300ppm of metal solution) of Zinc and Lead

Heavy metal tolerancetest

All the isolates were tested for heavy metal tolerance test against Pb(NO3)2, and Zn (NO3)2. TheIS1 exhibited maximum tolerance for zinc and IS14exhibited maximum tolerance for lead and the results are shown in figure 3.

Table 2: Minimum inhibitory concentration (μgmL^{-1})

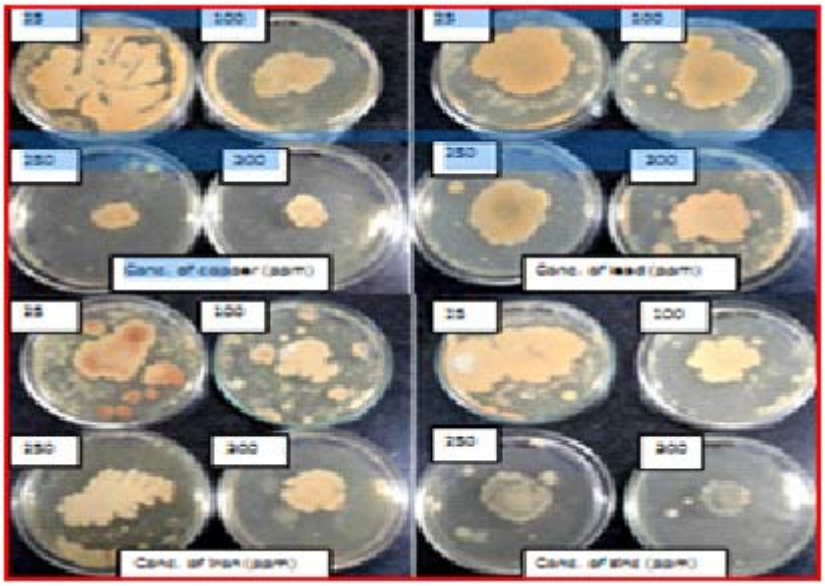
Metals	IS1	IS14
Zinc	960±10	100±10
Copper	100±10	500±10
Lead	250±10	1000±10
Iron	250±10	500±10

16srRNAsequenceanalysis

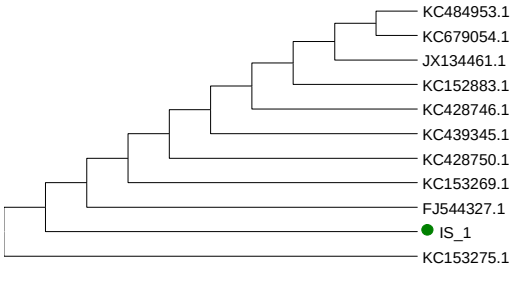
The isolates IS1 and IS14 were found similarto *Bacillus thuringiensis* strainsimi & *Bacillus subtilis* under accession number KF916618.1 & KJ489411.1, when submitted in NCBI. The phylogenetic tree shows relationship of isolated strains with other species.

Determinationof Minimum Inhibitory Concentration (MIC)

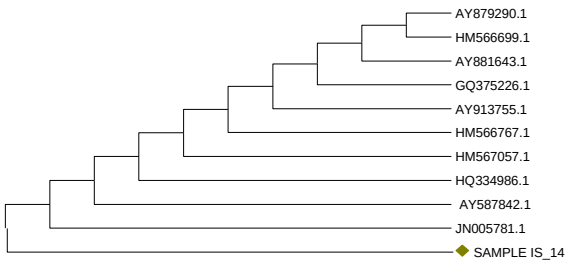
A great dealofvariation among the isolates against the different heavy metals was observed. The results in Table 8 reveal that MIC of Zinc for *Bacillus thuringiensis* strain *Simi* was at 1000 μg/ml where as *Bacillus subtilis* strain *PSB* had maximum MIC for lead.



Heavy metal tolerancetest for IS1& IS14



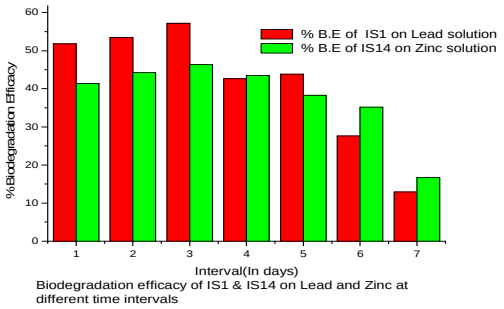
Phylogenetic tree IS1



Phylogenetic tree IS 14

Biodegradation efficacy on heavy metals

The degradation efficacy of *Bacillus thuringiensis* strain *Simi* on zinc showed rapid degradation in the first threedays, with mean of 54% biodegradation efficacyafter fourth day the degradation efficacy decreased and reached to the point of 31% and *Bacillus subtilis* strain *PSB* showed less degradation activity but the decrease in degradation efficacy started after fifth day. Similar work has already been reported against the hydrocarbon degradation by the isolate *P. lundensis* UTAR FPE2[18].



Efficacy of IS1 and IS14 on Biodegradation of lead and Zinc solution

CONCLUSION

The removal of heavy metals or breakdown into harmless state has become necessary. Thus bioremediation can be employed for the removal of such contaminants. This study of Zinc and lead accumulation by the isolates from heavy metal contaminated soils revealed a good and positive sign for its further use in bioremediation of zinc and lead in contaminated sites. The current study has illustrated some basic considerations that are important for the use of metal accumulating bacteria for bioremediation under field conditions.

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