INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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ISOLATION AND IDENTIFICATION OF HEAVY METAL (LEAD, ZINC AND COPPER) RESISTANT BACTERIA FROM OIL FIELD SOIL COLLECTED FROM MORAN, DIBRUGARH DISTRICT, ASSAM

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ABSTRACT
The present study deals with isolation and identification of heavy metal resistant bacteria from oil field soil collected from Kordoiguri, Moran of Dibrugarh district, Assam. A total of three strains could be isolated from the oil field samples. The isolated strains were *Klebsellia* sp., *Staphylococcus* sp. and *Bacillus* sp. on the basis of their biochemical and morphological characters. Minimum inhibitory concentration (MIC) and antibiotic resistance pattern of the isolates was studied. From all three strains isolated, the maximum resistance was shown for Lead (800 µg/ml for the strain *Klebsellia* sp.) and Zinc (800 µg/ml for the strain *Klebsellia* sp.). The minimum resistance was shown for Lead (200 µg/ml for the strain *Bacillus* sp.). For the antibiotic tolerance tests, maximum resistance was shown for cefotaxime (140 µg/ml for the strain *Klebsellia* sp.) and minimum for Ampicillin (40µg/ml for the strain *Staphylococcus* sp.). The present study reveals that the metal resistant bacteria can be explored in the field of bioremediation.

KEYWORDS: Heavy Metal, Kordoiguri (Moran), Minimum inhibitory concentration, Antibiotics.

INTRODUCTION

Pollutants are among the main factors that affect our environment day by day. Among pollutants the heavy metals are the sources of contamination that are observed from the oil wells, petroleum plants etc. as trace heavy metals are common constituents of crude oil, petroleum derivatives (leaded gasoline, lubricating oils or greases etc). Moreover, metals play a vital role in biological systems as a living cell cannot exist without metal ions. Trace amounts of heavy metals are also required by living organism including copper, cobalt, iron but excessive levels of essential metals however can be toxic to the organism (Franke *et al.*, 2003). With rapid industrialization, pollution is also in rapid increase. Pollution of soil with heavy metals is becoming one of the most severe environmental and human health hazards. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (Mclaughlin *et al.*, 1999). Heavy metals like Zinc, Cadmium, Copper, Lead, Nickel and mercury have been reported as the most toxic pollutants (Cameron, 1992). Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and microbes (Low *et al.,* 2000). Excessive levels of heavy metals can be damaging to the organism. Some of them are dangerous to health or environment (*e.g.* mercury, cadmium, lead,chromium) and some may cause corrosion (*e.g.* zinc, lead (Hogan, 2010). Heavy metals contaminate the environment because of their known accumulation in the food chain and persistence in nature. Heavy metal contamination is one of the most important environmental concerns from mine tailings. Metals are significant toxic factor to biota in the environment. For example, heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities (Giller *et al.,* 1998). Each heavy metal has unique toxicity or function. Zinc and copper can enhance microbial growth at low concentrations but suppresses growth at high concentrations (Ge *et al.,* 2009). Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Wuertz and Mergeay *et al.*, 1997). To survive under metal-stressed conditions, bacteria have themselves evolved up to a several types of adaptation mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, bioaccumulation and complexation of the metal ions inside the cell, and the reduction of the heavy metal ions to a less toxic state (Montuelle *et al.,* 1994). Bacteria have adapted to heavy metals through a variety of chromosomal-, transposon-, and plasmid-mediated resistance systems (Said *et al.,* 1991). For treating heavy metal contaminated tailing and soils, bioremediation is the most efficient and least costly method (Ge *et al.,* 2009). Some microorganisms have the resistance against the heavy metal and they can grow in the heavy metal rich environment also. Although most organisms have detoxification abilities (*i.e*., mineralization, transformation and/or immobilization of pollutants), particularly bacteria play a crucial role in biogeochemical cycles and in sustainable development of the biosphere (Diaz, 2004). Bioremediation may be employed in order to attack specific contaminants such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken, such as oil spills that are broken down using multiple techniques including the addition of fertilizer to facilitate the decomposition of crude oil by bacteria. Bioremediation can be considered as most useful technique for environmental cleanup and ecosystem service provider. The objective of the present study is to isolate and identify the bacteria from oil field soil of Kordoiguri, Moran of Dibrugarh district, Assam. The bacteria were biochemically identified and their potentiality to resist the heavy metals such as Zinc, Lead and Copper was determined.

MATERIALS & METHODS

Site Description and Soil Sampling: Soil samples were collected from Kordoiguri oil field, Moran of Dibrugurah District, Assam. Non contaminated soil sample was collected from Dibrugarh district as control. Soil samples from soil surface (0-5 cm) and at a depth of approximately 10 cm were taken in sterilized polyethylene bags using sterilized spatula and stored at 4 ºC until examination. To obtain the microbial examination, top soil was collected from the oil field samples. Isolation and identification of bacteria: The soil samples were diluted up to a dilution factor 10^{-4} and plated on Nutrient Agar. Series of dilutions were made to reduce the cells no in the samples. One ml of diluted sample was spread onto the surface of Nutrient Agar medium in the petri dishes and incubated at 37ºC and allowed to grow for 24 h. Single developed colony was picked on the Nutrient Agar plates and subcultured in nutrient broth and plated again on Nutrient Agar medium after dilution to obtain pure cultures. Pure bacterial strains were obtained after successive transfer of individual colony in Nutrient Agar plates and incubated for 24 h at 37ºC temperature. Colony forming units were determined after 2 days of incubation at 37ºC. The shape and colors of the colonies were examined under the microscope after Gram staining. Isolates were biochemically analyzed for the activities of Catalase, MR-VP test, Starch hydrolysis, Phenylalanine Agar test, Tryptone test, MSA test, Eosine Methylene blue test, Nitrate test, Indole production and Citrate utilization. The tests were used to identify the isolates according to Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986). Determination of Minimum Inhibitory Concentration (MIC): For testing heavy metal resistance, the metals Cu, Pb and Zn were used as CuSO4, $PbNO_3$ and $ZnCl_2$. Stocks of the metal salts were prepared in distilled water and stored at 4 °C. The metal salts were then added to sterilize nutrient agar medium in concentrations varying from range 200 to 800 µg/ml with an increment of 200 µg/ml. Plates were then spot inoculated and incubated at 37 °C for 2 days. The concentration of the metal which permitted growth and beyond which there was no growth was considered as the MIC of the metal against the strain tested. A concentration above the MIC value of the control strain was accepted as resistant range. All of the experiments were replicated 3 times. Determination of Antibiotic Resistance: The isolated strains were tested for antibiotic resistance against Ampicillin and Cefotaxime with concentration ranging from 20 to 200 µg/ml. Antibiotic sensitivity and resistance of the isolated heavy metal resistant isolates were assayed according to the Kirby- Bauer disc diffusion method (Bauer *et al.*, 1996).

RESULT & DISCUSSION

Morphological Characterization: A total of three strains were isolated from the sample collected from the oil field of Kordoiguri, Moran. The density of microorganisms was found to be 11 x 10^4 cfu/ml.

TABLE 1: Morphological Characterization of Bacterial Isolates

Sl No	Isolates/Strains	Gram Staining	Colony Color	Morphology
1	Isolate 1	Gram Negative	Cream White	Rod Shaped
2	Isolate 2	Gram Positive	Orange	Rod Shaped
3	Isolate 3	Gram Positive	Yellow	Cocci Shaped

TABLE 2: Biochemical Characterization of Oil Field Isolates

Biochemical Tests	Isolate 1	Isolate 2	Isolate 3
Starch Test	-	+	+
Catalase Test	-	+	+
MR-VP Test	+	-	-
VP Test	+	-	-
MSA Test	-	+	-
EMB Test	+	-	-
Indole Test	-	-	-
Citrate Test	-	-	-
Phenyl Alanine Agar Test	-	+	+
MacConkey Test	+	-	-

Pure cultures were obtained in Nutrient Broth and they were checked for the Gram staining reactions and the morphology noted and the results are tabulated (Table 1). The genera of the bacterial strains were identified by Biochemical Method and following the handbook of Bergey's Manual of Systematic Bacteriology (Table 2). The isolates were identified as *Klebsiella* sp. (Isolate 1), *Staphylococcus* sp. (Isolate 2) and *Bacillus* sp. (Isolate 3). Metal Tolerance: *Klebsiella sp*. was found to be having high resistance pattern against Lead (800 µg/ml) and Zinc (800 µg/ml) as compared to control treated with different concentration of heavy metals. *Staphylococcus sp.* also showed highest MIC value against Copper (800 µg/ml) as compared to control. However *Bacillus sp.* showed highest resistance only against Copper (600 µg/ml) as compared to control (Table 3 & 4).

TABLE 3: Minimum Inhibitory Concentration of metals in µg/ml of Control tested

		MIC for various metals in µg/ml		
Sl No	CONTROL	Lead (Pb)	Zinc (Zn)	Copper (Cu)
1	*Klebsiella sp.*	600	600	400
2	*Staphylococcus sp.*	400	400	600
3	*Bacillus sp.*	200	400	400

TABLE 4: Minimum Inhibitory Concentration the isolates against heavy metals (µg/ml)

		MIC for various metals in µg/ml		
Sl No	ISOLATES	Lead (Pb)	Zinc (Zn)	Copper (Cu)
1	*Klebsiella sp.*	800	800	600
2	*Staphylococcus sp.*	400	600	800
3	*Bacillus sp.*	200	400	600

Antibiotic Tolerance: The Minimum Inhibitory Concentration of Ampicillin and Cefotaxime against *Klebsiella sp.* were found to be 120 µg/ml and 140 µg/ml respectively. Similarly the MIC of Ampicillin and Cefotaxime against *Staphylococcus sp.* were found to be 40 µg/ml and 80 µg/ml and against *Bacillus sp.* were found to be 60 µg/ml and 90 µg/ml (Table 4).

TABLE 5: Minimum Inhibitory Concentration of the isolates against the standard antibiotics (µg/ml).

		MIC for various antibiotics in µg/ml	
Sl No	ISOLATES	Ampicillin	Cefotaxime
1	*Klebsiella sp.*	120	140
2	*Staphylococcus sp.*	40	80
3	*Bacillus sp.*	60	90

Photographs of the isolates after metal treatment with different concentrations (Lead, Zinc and Copper)

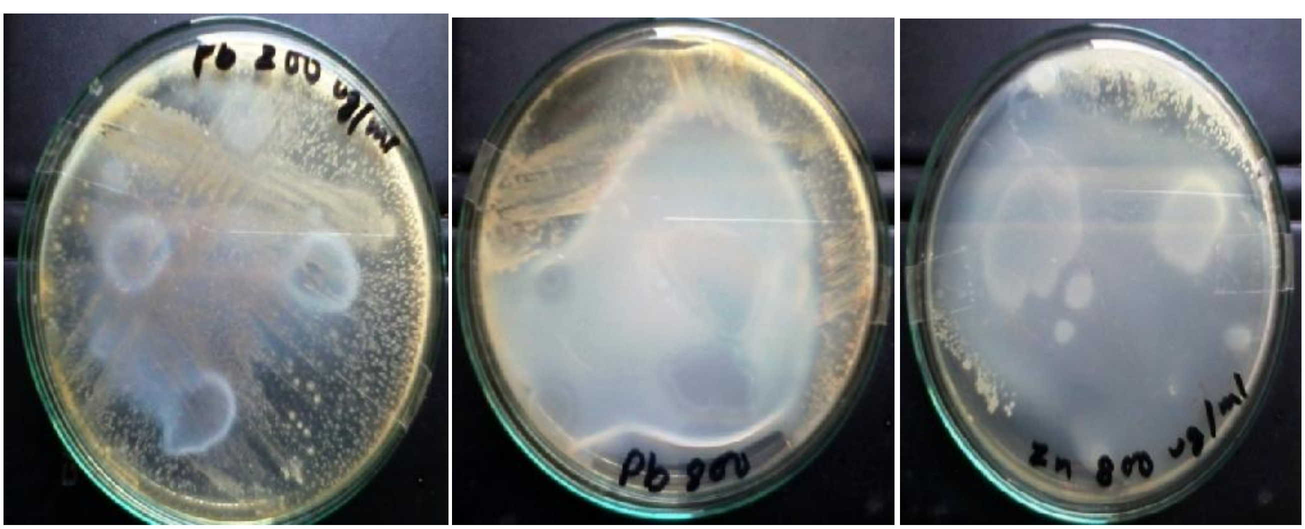

FIGURE1. *Klebsiella sp.* showing resistance against i) Lead and ii) Zinc both up to 800 µg/ml.

FIGURE 2. *Staphylococcus sp.* showing resistance against i) Copper at 800 µg/ml ii) Zinc at 600 µg/ml and iii) Lead at 400 µg/ml.

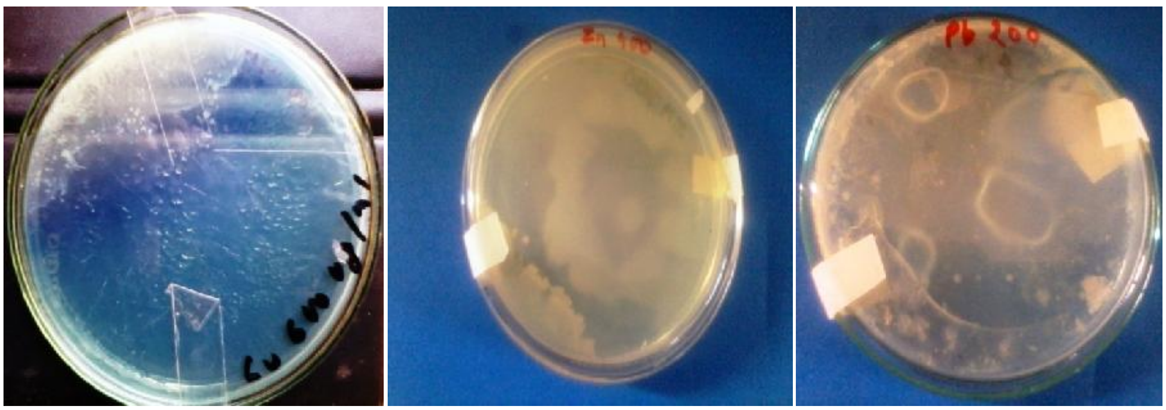

FIGURE 3. *Bacillus sp.* showing resistance against i) Copper at 600 µg/ml ii) Zinc at 400 µg/ml and iii) Lead at 200 µg/ml.

CONCLUSION

The bacterial strains isolated from the oil field soil of Kordoiguri, Moran were *Klebsiella sp.*, *Staphylococcus sp.* and *Bacillus sp*. The present study reveals that the bacterial species have the potentiality to reduce the heavy metals like Zn, Cu and Pb. These isolated strains can be used in the process of bioremediation by different techniques or methods. The future scope of this study mainly focuses on accumulation of toxic metals, reducing the growth of oil spill in particular areas, treating waste water etc. Thus from the present study it can be concluded that some of the organisms adapt themselves to protect or fight against high concentration of toxic metals and this property can be explored in the field of Bioremediation.

ACKNOWLEDGEMENT

The authors are thankful to DBT, Govt. of India for providing financial aid to establish Institutional Level Biotech Hub where the experiments were conducted.

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