Isolation and Characterization of Cadmium and Lead Resistant Bacteria

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Thirty heavy metal resistant bacteria were isolated from sewage of industrial effluents, garages and petrol pumps of Barak Valley region of Assam, India, against cadmium and lead. Samples were streaked on selective media; the predominant and distinct colonies were identified as Pseudomonas sp., Klebsiella sp., Staphylococcus sp., Proteus sp. and Bacillus sp. on the basis of their biochemical and morphological characters. Minimum inhibitory concentration (MIC) and antibiotic resistance pattern of the potent isolates was also studied. Among all, six isolates exhibited high resistance to heavy metals. Bacillus sp. was found to have high resistance pattern against Cadmium (600 μg/ml) and Lead (1000 μg/ml). It was observed that the isolates having high MIC values for a set of metals exhibited high resistance pattern towards a group of antibiotics.

Keywords: heavy metal, Barak Valley, Minimum inhibitory concentration, antibiotics

INTRODUCTION

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil. Metals are directly or indirectly involve in all aspects of growth, metabolism and differentiation of the biota (Beveridge and Doyle, 1989). Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron manganese and zinc etc.) and are known as 'trace elements' (Bruins et al., 2000). Whereas some have no biological role and are detrimental to the organisms even at very low concentration (cadmium, copper, lead etc.). However, at high levels both of the essential and non-essential metals become toxic to the organisms.

Bacteria are among the most abundant organism that occurs everywhere on earth. Heavy metals are increasingly found in microbial habitats due to several natural and anthropogenic processes; therefore, microbes have evolved mechanisms to tolerate the presence of heavy metals by either efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Gadd, 1990). The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions (Huang et al., 1990; Avery and Tobin, 1993; Brady et al., 1994; Veglio et al., 1997).

Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of these metals to the environment, which cannot
be degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Igwe et al., 2005). These heavy metals not only influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity (Roane et al., 2000), but also plants and animals, but the degree of toxicity varies for different organisms. Heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities (Giller et al., 1998). Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals, and an increased metal uptake by food crops grown on such contaminated soils is often observed.

Heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from the native binding sites or through ligand interactions (Bruins et al., 2000). Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance (Porde et al., 1989). Metals can replace essential metals in pigments or enzymes disrupting their function (Henry, 2000). Thus, metals render the land unsuitable for plant growth and destroy the biodiversity.

The application of heavy metal tolerant microorganisms is a promising approach for increasing heavy metal bioavailability in heavy metal amended soils. The objectives of the study were to isolate and characterize Cadmium and Lead resistant bacteria from heavy metal-contaminated soils, and to select HMTB strains which might be useful in improving the Pb and Cd polluted soils under unfavorable environmental conditions.

**MATERIALS AND METHODS**

**Sample Collection**

The present study was conducted in the contaminated crop fields nearby petrol pumps, garages, industrial and garbage dumping sites of Barak Valley region of Assam, India. The rhizospheric soil samples were collected using a sterilized spatula and stored in sterile sealed plastic bags before being processed.

**Isolation and identification of heavy metal resistant bacteria**

The soil samples were mixed with 50 ml distilled water and then filtered. The filtrate was then inoculated in Nutrient Broth for 24 hrs for enrichment. 1ml of each sample was added to 5 ml of Nutrient Broth for sufficient enrichment and incubated at 37°C. Individual colonies of bacteria that varied in shape and color were selected and streaked on selective media with the help of calibrated loops and incubated at 37°C for 24 hrs for recovery of potent isolates. Biochemical and morphological characteristics of the predominant bacterial genera isolated were studied and finally characterized and identified by standard identification methods (Holt et al., 1994).

**Determination of minimum inhibitory concentration (MIC)**

All the six isolates were checked for metal tolerance. MIC was determined by the plate dilution method against respective heavy metals (Cd and Pb) by gradually increasing the concentration of the heavy metals on Nutrient Agar (NA) plates until the strains failed to give colonies on the plate. The initial concentration used was 50μg/ml and thereby gradual increasing the concentration each time on NA plates. The growth of cultures on last concentration was transferred to the higher concentration by streaking on the plate. The lowest concentration that prevented bacterial growth was considered the MIC.

**Antibiogram of the bacterial isolates**

Isolated heavy metal resistant isolates were tested for antibiotic sensitivity and resistance according to the Kirby-Bauer disc diffusion method (Bauer et al., 1996). After incubation, the organisms were classified as sensitive or resistant to an antibacterial according to the diameter of inhibition zone given in standard antibiotic disc chart.

**RESULTS AND DISCUSSION**

Thirty heavy metal resistant bacteria were isolated from sewage of industrial effluents, garages and petrol pumps of Barak Valley region of Assam, India, against cadmium and lead. They were identified as *Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp., *Proteus* sp. and *Bacillus* sp.. Among all, six isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 800μg/ml to 1800μg/ml (table-1). *Bacillus* sp. was found to be have high resistance pattern against Cadmium (1800 μg/ml) and Lead (1300 μg/ml). *Pseudomonas* sp. (PaP-4) isolated from petrol pump and *Klebsiella* sp. (K-G-1) from garage also showed high MIC values against cadmium as 1800 μg/ml and 1600 μg/ml. In case of lead, *Klebsiella* sp. (K-G-1) from garage and *Staphylococcus* sp. (StP-5) from petrol pump showed
Table 1. Resistance pattern of bacterial isolates to heavy metals (Cd and Pb).

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Strain Name</th>
<th>Minimum Inhibitory Concentration (MIC) against Cd (µg/ml)</th>
<th>Minimum Inhibitory Concentration (MIC) against Pb (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp.</td>
<td>Ps/G-1</td>
<td>1400</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Ps/P-4</td>
<td>1400</td>
<td>800</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>Ba/P-5</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>K/G-1</td>
<td>1600</td>
<td>1100</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>St/P-5</td>
<td>800</td>
<td>1100</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>Pr/G-2</td>
<td>800</td>
<td>800</td>
</tr>
</tbody>
</table>

Table 2. Antibiotic sensitivity of Cadmium and Lead resistant isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps/G-1</td>
<td>Ciprofloxacin, Amikacin, Gentamicin, Norfloxacin, Chloramphenicol, Cefoxitin</td>
<td>Methicillin, Cotrimoxazole, Cefalime, Bacitracin, Ampicillin, Amoxycillin, Ceftriaxone, Cefalaxin, Kanamycin, Tetracycline</td>
</tr>
<tr>
<td>Ps/P-4</td>
<td>Ampicillin, Cefoxitin, Ceftriaxone, Chloramphenicol</td>
<td>Cefoxitin, Cefalime, Methicillin, Cotrimoxazole, Norfloxacin, Kanamycin, Amoxycillin</td>
</tr>
<tr>
<td>Ba/P-5</td>
<td>Norfloxacin, Vancomycin, Bacitracin, Amikacin</td>
<td>Methicillin, Cotrimoxazole, Cefalime, Cefalaxin, Ampicillin, Amoxycillin, Kanamycin, Tetracycline, Gentamicin, Chlotramphenicol</td>
</tr>
<tr>
<td>K/G-1</td>
<td>Ampicillin, Amoxycillin</td>
<td>Amikacin, Gentamicin, Olofoxacin, Kanamycin, Methicillin, Tetracycline, Chloramphenicol, Cefalime, Cefalaxin</td>
</tr>
<tr>
<td>St/P-5</td>
<td>Ceftriaxone, Gentamicin, Chloramphenicol</td>
<td>Amoxycillin, Ceftriaxone, Chloramphenicol, Cefalaxin, Kanamycin, Methicillin</td>
</tr>
<tr>
<td>Pr/G-2</td>
<td>Ampicillin, Amikacin, Gentamicin, Chloramphenicol</td>
<td>Kanamycin, Methicillin, Tetracycline, Amoxycillin, Cefoxitin, Cefalime</td>
</tr>
</tbody>
</table>

High resistance pattern for Lead following Bacillus. The MIC values for K/G-1 and St/P-5 against Lead was found to be 1100 µg/ml.

All the bacterial strains were tested for antibiotic sensitivity. The predominant isolates that are tolerant to cadmium and lead were found to be multi-antibiotic resistant (Ps/G-1, Ps/P-4, Ba/P-5, K/G-1, St/P-5, Pr/G-2).

In the present study, it was observed that the isolates having high MIC values for a set of metals exhibit high resistance pattern towards a group of antibiotics (Table 2).

A correlation between the resistance to high level of Cu (II) and Pb (II) and antibiotic in the bacterial species found in drinking water has long being established (Colomiris et al., 1984). Vajieh et al. (2003) also studied that multiple metal resistance bacterial isolates exhibit high resistance towards a group of antibiotics. All the strains (Ps/G-1, Ps/P-4, Ba/P-5, St/P-5 and Pr/G-2) were resistant to Methicillin, Cefalime and Kanamycin. Chloramphenicol showed high sensitivity to Ps/G-1, Ps/P-4, St/P-5, Pr/G-2 but were resistant to Ba/P-5 and K/G-1. Amoxycillin was tolerated by almost all the strains except K/G-1. Multiple tolerances occur only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria.

Heavy metal resistant microorganisms play an important role in the bioremediation of heavy metal contaminated soils (Ray and Ray 2009; R.A.I. et al., 2007). Contaminated environments like those in the vicinity of industries or industrial dump grounds accumulate a heavy load of toxic metal ions, organic ions, organic wastes and antibiotics (Hao et al., 1999) At high concentrations, heavy metal ions react to form toxic compounds in cells (Nies, 1999). Both Cd and Zn are considered as one of the most toxic heavy metals and they can appear either in water or soil of any polluted site because of their high mobility, especially in agricultural fields. Thus greatly threatens human health via
food chains (Goris et al., 2001). The application of metal-resistant bacteria for bioremediation offers attractive perspectives (Mergaey et al., 2003; Kamnev et al., 2003) as reviewed the role of soil microorganisms in phyto remediation. Soil microorganisms interacted with plants in many different ways to reduce metal ion toxicity and enhance metal ion absorption by plants. The toxic levels of heavy metals affect structural and permeability properties of inner membranes and organelles, cause inhibition of enzymatic activities, nutrient imbalances, decreases in rates of photosynthesis and transpiration (Green et al., 2000), stimulate formation of free radicals and reactive oxygen species resulting in oxidative stress (Sandalio et al., 2000), suppress seed germination and seedling growth (Bori et al., 1995), reproductive development (Sethia et al., 1989), seed yield and seed quality (Bori and Sethia, 1995) and induce deleterious anatomical and ultra structural changes in crop plants (Liu and Kotke, 2004; Srivastava et al., 2003).

CONCLUSION

Heavy metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and possibly be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget et al., 2005). The concentration of a toxic metal that affects the growth and survival of different microorganisms varies greatly. It is clearly indicated that domestic waste and industrial waste are responsible for the development of bacterial resistance along with the risk of human health and environment. The long term effect of pollutants has led to emergence of multi metal and multi antibiotic resistant bacteria in the study areas. The use of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils. Consumption of food crops contaminated with heavy metals is a major food chain route for human exposure. Thus, from the present study it can be concluded that the application of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

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REFERENCES


Isolation and characterization of heavy metal resistant bacteria and its effect on shoot growth of *Oryza sativa* inoculated in industrial soil

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Abstract: A total of twenty heavy metal resistant bacteria were isolated from industrial effluents, sewages, garages and petrol pumps of Barak valley region of Assam, India, against copper, zinc, and lead. Decrease in total count and microbial population diversity with increasing metal concentrations were observed. The predominant isolates obtained were *Pseudomonas sp.*, *Klebsiella sp.* and *Acinetobacter sp.* with minimum inhibitory concentration for heavy metals as 66μg/ml (for copper), 18μg/ml (for lead) and 180μg/ml (for zinc). The present study demonstrated that the isolates having higher tolerance to heavy metals have high resistance pattern towards a group of antibiotics. Pot experimental studies suggest that the isolated bacterial communities live in association with rhizosphere and able to withstand high heavy metal concentrations in contaminated soil. Further, it has been observed that a significant increase in shoot length of *Oryza sativa* in contaminated soil when inoculated with heavy metal resistant bacterial strains.

Keywords: Antibiotic, Barak Valley, Heavy Metal, Resistance, Rhizosphere, Tolerance

Introduction

Pollution of the biosphere with trace elements has increased dramatically since the industrial revolution. Primary sources are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, agrochemicals and sewage. In addition, natural mineral deposits containing particularly large quantities of heavy metals are also found in many regions. The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Iqwe et al., 2005). Metal toxicity may affect all forms of life including microorganisms, plants and animals, but the degree of toxicity varies for different organisms. Heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities (Gille et al., 1998). Traces of these heavy metals are necessary as co-factors of enzymatic reactions, but high levels of them may cause extreme toxicity to living organisms due to inhibition of metabolic reactions. The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation reduction.

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Naturally occurring bacteria that are capable of metal accumulation have been extensively studied but it is difficult to imagine that a single bacterium could be capable to remove all heavy metals from its polluted site (Claussen, 2000). It is important to study the indigenous microorganisms in heavy metal polluted sites. It may provide new insight into bacterial diversity under unfavorable conditions, new isolates and probably new genetic information on heavy metal resistance, which could be exploited in re-vegetation in future (Fabienne et al., 2003). Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal uptake by crops, and thus affect food quality and safety (Mucihar et al., 2006). Heavy metal accumulation in soils and plants is of increasing concern because of the potential human health risks.

The objectives of this study were to investigate heavy metal stress in bacteria isolated from Barak valley region of Assam, India and to determine whether there is a
relationship between heavy metal stress and antibiotic resistance.

**Materials and methods**

**Sample collection:**
The present study was conducted in the Barak valley region of Assam, India (Latitude: 24°8' N to 25°8' N, Longitude: 92°15' E to 93°15' E, Area: 6992 sq km). Soil samples were collected from industrial effluents, sewages, garages and nearby petrol pump in sterile sealed plastic bags and immediately brought to the laboratory. The samples were mixed with 50 ml distilled water and filtered.

**Isolation and identification of heavy metal resistant bacteria:**

Samples were streaked on Pseudomonas Isolation Agar (PIA), Klebsiella Isolation Agar (KIA) and Starch Agar and incubated at 37°C for 24 hrs for recovery of pure isolates. Pure cultures were obtained and their biochemical and morphological characters were studied. The predominant bacterial genera isolated were finally characterized and identified by standard identification methods (Holt et al., 1994; Cappuccino and Sherman, 2005).

**Determination of minimum Inhibitory concentration (MIC):**

All the twenty isolates were checked for metal tolerance. MIC was determined against respective heavy metals Cu (CuSO₄·5H₂O), Pb [(Cl₃COO)₂·Pb·3H₂O] and Zn (Zinc Metal Powder) by gradually increasing the concentration of the heavy metals on nutrient agar (NA) plates until the strains failed to give colonies on the plate. The initial concentration used was 50µg/ml and the broth concentration was gradually increased by 10 15µg/ml each time on NA plates. The growth of cultures on test concentration was transferred to the higher concentration by streaking on the plate. MIC was recorded when the isolates failed to grow on plates.

**Test for antibiotic resistance of bacterial isolates:**
The isolates were tested for antibiotic sensitivity according to Kirby-Bauer disc diffusion method (Bauer et al. 1966) to 12 antibiotics. The concentrations of the disc used were Amikacin (30 mcg), Amoxycillin (10 mcg), Ampicillin (25 mcg), Cefalexin (30 mcg), Cefazoline (5 mcg), Ceftriaxone (30 mcg), Chloramphenicol (10 mcg), Gentamicin (50 mcg), Kanamycin (5mcg), Methicillin (30mcg), Otoxacin (5mcg), Tetracycline (30mcg). The selected isolates were freshly inoculated on saline water or peptone water, their turbidity was checked by comparing with Mc. Farland solution and inoculated on Mueller-Hinton Agar. The selected antibiotics were placed on the plate and incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured to the nearest mm and the isolates were classified as resistant (R), intermediate (I) and susceptible (S) following the standard antibiotic disk sensitivity testing method.

**Pot experiment:**

Pot experimental studies were performed to determine the bioremediation potential of Heavy metal resistant bacteria (HMRB). Heavy metal contaminated soil collected from paddy field near industrial sites was put in five different pots. Pots were coded according to the inclusions used (Ps-1, Ps-2, Ba-6 and K-1) and compared them with a control set (C). The bacteria showing the highest MIC for heavy metals were selected and inoculated in nutrient broth for the formation of bio fertilizers (Table 1). The broth was kept in rotator shaker incubator at 37°C for 4-5 days. On the other hand seeds of Oryza sativa were soaked in petriplates containing sterile water for 24 hrs and sown on pots. The bacterial broth serving as bio fertilizers and distilled water was added to each pot every day. The shoot length was measured up to 15th day.

**Table 1:** Pots are marked according to the inclusions used; to each pot broth and distilled water were added on routine basis.

<table>
<thead>
<tr>
<th>Pot Labelling (codes)</th>
<th>Bacterial strains inoculated</th>
<th>Broth added</th>
<th>Distilled water added</th>
<th>Total volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps-1</td>
<td>Pseudomonas sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Ps-2</td>
<td>Pseudomonas sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Ba-6</td>
<td>Bacillus sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>K-1</td>
<td>Klebsiella sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

**Results**

**Isolation and identification of heavy metal resistant bacteria:**

Twenty heavy metal resistant bacteria isolated from sewage of industrial effluents, garages and petrol pumps of Cachar district of Assam, India, against copper, zinc, and lead. After performing the biochemical tests...
(Table 2), it was deciphered that the isolated bacterial strains belong to genus *Pseudomonas* sp., *Bacillus* sp. and *Klebsiella* sp.

**Table 2**: Biochemical tests of all the isolated strains

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Gram</th>
<th>Indole</th>
<th>MR</th>
<th>VP</th>
<th>Glucose</th>
<th>Nitrile Reduction</th>
<th>Starch Hydrolysis</th>
<th>Urease Test</th>
<th>Lysine Test</th>
<th>Catalase Test</th>
<th>Gelatin Hydrolysis</th>
<th>Oxidase</th>
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<tbody>
<tr>
<td>Ps-1</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ps-2</td>
<td>Rod</td>
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<td>Ps-3</td>
<td>Rod</td>
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<td>Rod+</td>
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<tr>
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<td>Rod+</td>
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<td>Rod+</td>
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<td>Ba-6</td>
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<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ki-5</td>
<td>Rod-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ki-6</td>
<td>Rod-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Minimum inhibitory concentrations for heavy metals:**

Among all the twenty isolated bacteria, eight isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 30µg/ml to 1800µg/ml (Table 3). *Pseudomonas* sp. exhibited high resistance with MIC for heavy metals as 60µg/ml (for copper), 170µg/ml (for lead) and 1000µg/ml (for zinc). *Klebsiella* sp. showed 50µg/ml (for copper), 180µg/ml (for lead) and 1500µg/ml (for zinc) whereas *Bacillus* sp. showed the values similar to *Klebsiella* sp. except lead (150µg/ml). Some isolates also exhibit multiple tolerance to heavy metals (Ps-1, Ps-4, Ba-6 and K-1). Heavy metal tolerance test indicated highest tolerance to zinc by Ps-1 and Ps-4 (1800µg/ml), copper by Ps-4 (60µg/ml) and lead by Ki-1 (100µg/ml).
Table 3: Tolerance of bacterial isolates to heavy metals (Cu, Pb and Zn)

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Strain No.</th>
<th>Minimum Inhibitory Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Copper</td>
</tr>
<tr>
<td>Ps-1</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Ps-2</td>
<td></td>
<td>60 μg/ml</td>
</tr>
<tr>
<td>Ps-3</td>
<td></td>
<td>40 μg/ml</td>
</tr>
<tr>
<td>Ps-4</td>
<td></td>
<td>60 μg/ml</td>
</tr>
<tr>
<td>Ps-5</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Ps-6</td>
<td></td>
<td>45 μg/ml</td>
</tr>
<tr>
<td>Ps-7</td>
<td></td>
<td>40 μg/ml</td>
</tr>
<tr>
<td>Ps-8</td>
<td></td>
<td>35 μg/ml</td>
</tr>
<tr>
<td>Ba-1</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Ba-2</td>
<td></td>
<td>30 μg/ml</td>
</tr>
<tr>
<td>Ba-3</td>
<td></td>
<td>35 μg/ml</td>
</tr>
<tr>
<td>Ba-4</td>
<td></td>
<td>45 μg/ml</td>
</tr>
<tr>
<td>Ba-5</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Ba-6</td>
<td></td>
<td>45 μg/ml</td>
</tr>
<tr>
<td>Kl-1</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Kl-2</td>
<td></td>
<td>35 μg/ml</td>
</tr>
<tr>
<td>Kl-3</td>
<td></td>
<td>45 μg/ml</td>
</tr>
<tr>
<td>Kl-4</td>
<td></td>
<td>40 μg/ml</td>
</tr>
<tr>
<td>Kl-5</td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>Kl-6</td>
<td></td>
<td>40 μg/ml</td>
</tr>
</tbody>
</table>

Antibiotic sensitivity and resistance pattern of heavy metal resistant isolates:

All the predominant isolates having high MIC values for a set of metals exhibited high resistance pattern towards a group of antibiotics. It was observed that most of the metal tolerant strains (Ps-1, Ps-4, Ba-6 and Kl-1) were resistant to amoxylicillin, ampicillin, cefalexin, cefixime, kanamycin, methicillin and tetracycline (Table-4). The present study showed some resemblance with the long back work of Calomiris et al., (1984) who found a correlation between the resistance to high level of Cu(I), Pb(I), Zn(I) and antibiotic in the bacterial species found in drinking water.

Effect of HMRB on the Shoot growth of Oryza sativa inoculated in industrial soil:

The effects of HMRB on shoot elongation of Oryza sativa in industrial soil, collected from paddy field nearby paper industry is shown in Figure 1. No growth was observed for the first two days, but after 3rd day, the shoots began to develop in some pots. After 15 days of inoculation, it was observed that the pot marked as Ps-1 had a remarkable shoot growth of 34±2.0 cm when compared with control pot with shoot length of 23±1 cm. The lower shoot growth by Ps-4 and Ba-6 reveals the incompetence of the isolates as bio fertilizer.

Table 4: Antibiotic pattern of some selected bacterial isolates.

<table>
<thead>
<tr>
<th>Antibiotic disc</th>
<th>Diameter of inhibition zone (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pe-1</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>13 (I)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>NI</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>7 (R)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>NI</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12 (I)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5 (I)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>11 (S)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>NI</td>
</tr>
<tr>
<td>Methicillin</td>
<td>NI</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2 (b)</td>
</tr>
</tbody>
</table>

NI= no inhibition zone; Diameter of disc=6mm.
Letters in parenthesis indicate sensitivity: R = Resistance; I = Intermediate; S = Susceptible.

Figure 1: Shoot length (in cm) of Oryza sativa seedlings in heavy metal contaminated industrial soil, inoculated with HMRB and control.
Discussion

A decrease in growth (cfu/g) of bacterial colonies was observed on increasing the heavy metal concentration on culture plates at any given time interval compared to the control without metal amendment. The lower values of microbial load at higher metal concentrations showed correlation with the study of Anyanwu et al., (2011). Contaminated environments like those in the vicinity of industries or industrial dump grounds accumulate a heavy load of toxic metal ions, organic ions, organic wastes and antibiotics. The present study suggests that the microorganisms resistant to antibiotics and tolerant to metals appear to be the result of exposure to metal contaminated environment, which is fairly consistent with the findings of Ramteke (1997). This fact was also established by other researchers that multiple metal resistant bacterial isolates exhibits high resistance towards a group of antibiotics (Vajjhe et al., 2003). Based on the MIC values and antibiogram pattern of the isolated strains and as studied by Bruins et al., (2003), Pseudomonas sp. shows resistance to a variety of toxic substances, heavy metals and antibiotics, which have generated a high degree of interest in the area of environmental bioremediation. Pot experimental studies demonstrated that the isolate Ps-1 (Pseudomonas sp.) live in association with rhizospheric soil are able to withstand high heavy metal concentrations in contaminated soil. Although a number of studies have demonstrated the importance of bacterial inoculation for plant growth and heavy metal accumulation in heavy metal polluted environments (Abou Shanab et al., 2003; Idris et al., 2004; Khan, 2005; Sheng and Xia, 2006). It is evident from the present study that the application of HMRE specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

Conclusion

Heavy metal contamination of soil by industrial effluents, sewages, garage wastes and petrol pumps can induce serious problems to soil, cropping and vegetation which subsequently hamper human health. The long term effect of pollutants has led to emergence of multi-metal and multi-antibiotic tolerant bacteria in the study area. Pot experimental studies demonstrated a significant increase in shoot length of Oryza sativa in contaminated soil when inoculated with heavy metal resistant bacterial strains. Further research will expand the knowledge of the microbial genetics, their application and bio-absorption of heavy metals from contaminated sites. In addition, we need to understand the mechanisms involved in immobilization and transfer of metals into the bacterial cell in order to develop future strategies.

Acknowledgements

The authors wish to extend their grateful thanks to Department of Biotechnology, Govt. of India, New Delhi for the establishment of Institutional Level Biotech Hub and Bioinformatics Centre in Gurucharan College, Silchar, India. The authors are also thankful to Department of Microbiology, Assam University, Silchar, India for providing laboratory facility to carry out initial research work.

References


Source of support: Nil
Conflict of interest: None Declared
Isolation of Heavy Metal Resistant Bacteria for Sustainable Crop Production

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Abstract
Thirty five heavy metal resistant bacteria were isolated from contaminated crop field of Northern Assam, India, against copper, zinc, cadmium and lead. The predominant isolates were identified as Pseudomonas sp., Klebsiella sp., Staphylococcus sp., Bacillus sp. and Pseudomonas sp. Some isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals as 60 µg ml⁻¹ (for copper), 130 µg ml⁻¹ (for lead) 130 µg ml⁻¹ (for cadmium) and 1300 µg ml⁻¹ (for zinc). They also showed multiple heavy metal tolerance and were multi-antibiotic resistant. There was decrease in total count and microbial population diversity with increasing metal concentrations. The present study showed a correlation between heavy metal resistance and antibiotic tolerance among bacterial isolates. The effect of heavy metal resistant strains on Oryza sativa inoculated in contaminated soil showed a remarkable increase in the shoot length when compared with control pots.

1. Introduction
The quality of life on earth is inextricably linked to overall quality in the environment. The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Iwas et al., 2005). The use of domestic and industrial effluents, which may contain high concentrations of heavy metals on agricultural lands, is a common practice in some parts of the world. These toxic metals, when concentrated on plant tissues can have damaging effects on the plants themselves and may also pose health hazards to man and animals (Kumar et al., 2010). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. It is important to study the indigenous microorganisms in heavy metal polluted sites. Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal uptake by crops, and thus affect food quality and safety (Mutschke et al., 2006). Microorganisms are generally the first to be affected by the discharges of heavy metals into the environment. Microbial ecosystem can drastically alter the fate of the metal entering into aquatic or soil environments (Brown, 1996). Yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure (Medlik et al., 1996). In order to survive in heavy metal polluted environments, many microorganisms have developed resistance to toxic metal ions. These mechanisms include: metal exclusion by permeability barriers, active transport of the metal away from the cell organism, intracellular sequestration of the metal by protein binding, extracellular sequestration, enzymatic detoxification of the metal to a less toxic form and reduction in metal sensitivity of cellular targets (Bruins et al., 2000). The detoxification mechanisms may be directed against one metal or a group of chemically related metals. Furthermore, the detoxification mechanisms may vary depending on the type of microorganism. This transformation of the contaminant is an incidental reaction catalyzed by enzymes present in the cell's metabolic system (Prasenjit and Sunatthi, 2005). The objectives of this study were to investigate heavy metal stress on bacteria, isolated from contaminated sites of Cachar district of Assam, India and to evaluate the bioremediation potential of the strains for better crop improvement.

2. Materials and Methods
2.1 Isolation and identification of bacteria
Soil sample were collected from industrial effluents, scwages, garages and petrol pumps of Cachar district of Assam, India in sterilized polyethylene bags and were immediately brought to the laboratory. Samples were then streaked on selective media with the help of calibrated loop and incubated at 37°C for 24 hrs for recovery of potent isolates. Morphological characteristics of recovered isolates viz., Colony morphology (colour, shape, margin, elevation and surface) and cell morphology (shape, arrangement, gram reaction) were studied. Various biochemical tests were also performed for identification of isolates (Holt et al., 1994; Cappuccino and Sherman, 2005).

3.2. Screening and determination of minimum inhibitory concentration (MIC) of HMRR
Bacterial isolates were screened by growing them on heavy metal incorporated nutrient agar media and MIC of heavy metal resistant bacteria (HMRRB) were determined by gradual increasing the concentration of heavy metals in the media. Heavy metals used were Cd²⁺ (CdCl₂), Cu²⁺ (CuSO₄·5H₂O), Pb²⁺ [Pb(NO₃)₂], and Zn²⁺ (Zinc metal powder), with starting concentration of 50 µg ml⁻¹. The concentration of heavy metals on NA plates were increased each time until the strain failed to grow on the plates. The culture growing on last concentration was transferred to the higher concentration by streaking on the plates. MIC was noted when the isolates failed to grow on plates.

3.3. Antibiogram pattern of recovered isolates
All recovered isolates were characterised for their resistant pattern by Kirby Bauer disc diffusion method and Double disc diffusion method. The commonly used antibiotics were Ampicillin, Amikacin, Amoxycillin, Chloramphenicol, Cefixime, Gentamicin, Kanamycin, Cefalexin, Methicillin, Ofloxacin, Tetracycline and Cefixime. The recovered isolates were inoculated in Muller Hinton Broth and antibiotic discs were placed equidistantly on the surface of the Muller Hinton Agar (MHA) plates. After incubation at 37°C for 24 hrs, the zone of inhibition was measured in mm and the results were interpreted using standard chart that relates the zone diameter to degree of microbial resistance (Bauer et al., 1966).

3.4. Pot experiment
Pot experiments were performed to determine the bioremediation potential of HMRRB isolates. Heavy metal contaminated soil collected from paddy field nearby industrial sites and garages are put in 2 different sets, each set containing 6 pots. Pots were watered according to the inoculums added (IR, SI, PR, KI and AU) and compared them with a control (C) set (Table 1). The bacteria showing the highest MIC was taken and inoculated in nutrient broth, for the formation of biofertilizers. The broth was kept in rotator shaker incubator at 37°C for 4-5 days. Seeds of Oryza sativa were soaked in petriplates containing sterile water for 24hrs and sown on all 12 pots. The bacterial broth serving as biofertilizers and distilled water was added to each pot every day. The shoot length was measured at 5 days interval up to 15th day.

3.5. Results and Discussion
The turbidity of microbial enrichment broth was taken as a primary indicator for microbial growth. Enriched culture was streaked with the help of calibrated loop on Nutrient agar and further on different selective media such as Phenylalanine Agar (PAA), Maltose Salt Agar (MSA), Starch Agar, Macconkey Agar and Pseudomonas Isolation Agar (PIA) for isolation of different bacteria from different soil samples. A total of 62 isolates were recovered from 10 soil samples.

3.1. Morphological and biochemical characterization of recovered isolates
62 types of bacteria were recovered from samples and identified by their colony characterization and gram staining and biochemical testing (Holt et al., 1994; Smith, 1986) and were identified as Proteus sp., Klebsiella sp., Staphylococcus sp., Bacillus sp. and Pseudomonas sp. The highest prevalence was observed by Pseudomonas sp. (19%) and Klebsiella sp. (17%), about 23% of the total isolates remains unidentified.

3.2. MIC of the bacterial isolates against some selected heavy metals
All the bacterial isolates were screened for heavy metal tolerance by growing them on heavy metal incorporated media. Out of which, 35 isolates showed heavy metal tolerance against Cu, Cd, Pb and Zn with MIC ranging from 50 to 1800 µg ml⁻¹ for different heavy metals (Table 2). This confirms the emergence of highly heavy metal resistant bacteria in the polluted sites. A better opportunity for bioremediation of heavy metals by bacteria was observed when compared with the work done by Rajbenabi, 2008, where MIC for heavy metals ranges from 150 µg ml⁻¹ to 500 µg ml⁻¹. Heavy metal tolerance test indicated highest tolerance to Zinc by Ps-1 (1800 µg ml⁻¹), Copper by Kl-1 and Ps-1 (60 µg ml⁻¹), Lead by Pr-2 (180 µg ml⁻¹) and Cadmium by Ps-3 (130 µg ml⁻¹). It was observed that, none of the Proteus sp., Bacillus sp. and Staphylococcus sp. can tolerate the copper stress even at 50 µg ml⁻¹. The present study also fails to recover cadmium resistant Bacillus sp. and Klebsiella sp.

3.3. Antibiotic tolerance and sensitivity
Most of the isolates in the present study showed multiple antibiotic resistance to allow three heavy metals. 6 strains of Pseudomonas sp. and 5 strains of Staphylococcus sp. showed high resistance towards a group of antibiotics and were multi-metal resistant. The present study showed some resemblance with the long back work of Calomiris et al., (1984), Rashid et al., (2009), who found a correlation between the resistance...
Table 1: Pots are marked according to the inoculums used to each pots broth and distilled water were added on routine basis

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Control</td>
<td>No</td>
<td>50 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Ps</td>
<td>Pseudomonas sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>St</td>
<td>Staphylococcus sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Pr</td>
<td>Proteus sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Kl</td>
<td>Klebsiella sp.</td>
<td>5 ml</td>
<td>45 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>All</td>
<td>Mixture of Ps, Pr, Kl (5+5+5=15)</td>
<td>30 ml</td>
<td>50 ml</td>
<td></td>
</tr>
</tbody>
</table>

A-Poti Labelling (codes); B-Bacterial strains inoculated; C-Broth added; D=Distilled water added; E=Total volume.

Table 2: Minimum inhibitory concentration of potent isolates on respective heavy metals

<table>
<thead>
<tr>
<th>A</th>
<th>Strain</th>
<th>Cu (µg ml⁻¹)</th>
<th>Cd (µg ml⁻¹)</th>
<th>Pb (µg ml⁻¹)</th>
<th>Zn (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Pr.2</td>
<td>50</td>
<td>180</td>
<td>1500</td>
<td>1430</td>
</tr>
<tr>
<td>C</td>
<td>He.4</td>
<td>NG</td>
<td>150</td>
<td>1430</td>
<td>1500</td>
</tr>
<tr>
<td>D</td>
<td>Ps.3</td>
<td>50</td>
<td>120</td>
<td>130</td>
<td>1800</td>
</tr>
<tr>
<td>E</td>
<td>Kl.1</td>
<td>60</td>
<td>NG</td>
<td>150</td>
<td>900</td>
</tr>
<tr>
<td>F</td>
<td>St.5</td>
<td>NG</td>
<td>110</td>
<td>150</td>
<td>1500</td>
</tr>
</tbody>
</table>

A=Bacterial isolates; B=Proteus sp.; C=Klebsiella sp.; D=Pseudomonas aeruginosa; E=Klebsiella pneumonia; F=Proteus mirabilis and Staphylococcus sp. resistant to heavy metals and antibiotics.

3.4. Effect of HMRR on the shoot growth of *Oryza sativa* inoculated in industrial soil

The effects of HMRR on shoot elongation of *Oryza sativa* in industrial soil, collected from pulp field nearby paper industry is shown in Figure 1. No growth was observed for the first two days, but after 3rd day the shoots began to develop in some pots. On 5th day, growth pattern was observed except the pots marked as Kl, Pr and C. It was found that the pots marked as Ps had a remarkable shoot growth of 34 cm when compared with control pot with shoot length of 23 cm.

3.5. Effect of HMRR on the shoot growth of *Oryza sativa* inoculated in garbage soil

The efficacy of potent isolates was also tested in heavy metal contaminated garbage soil (Figure 2). Significant shoot growth was observed in all the pots including control after 3rd day of inoculation. Overall, the control pot without any bacterial inoculum added, showed a negligible difference inferring the incompetence of HMRR as biofertilizer in garbage soil. On 15th day, the highest growth of *Oryza sativa* was observed in pots marked as Pr (25 cm) and Kl (24.5 cm) whereas the control pot grows upto 23 cm.

Although a number of studies have demonstrated the importance of bacterial inoculation for plant growth and heavy metal accumulation in heavy metal polluted environments (Abou-Shanab et al., 2001; Ides et al., 2004; Khan, 2005; Shere and Xia, 2006). It is evident from the present study that the application of HMRR specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

4. Conclusion

Based on the present study, it could be concluded that it is possible to develop new bioremediation strategies with the inoculation of HMRR with co cropping or intercropping systems in order to enhance biological-extraction of metals.
from contaminated soils. A better understanding of the soil physico-chemical properties and plant-bacteria interaction is needed to optimize bioremediation potential followed by proper field investigations. The long term effect of pollutants has led to emergence of multi-metal and multi-antibiotic resistant bacteria in the study areas. Pot experiment study demonstrated that isolated strains of *Pseudomonas* sp., *Proteus* sp. and *Klebsiella* sp. could increase the growth of *Oryza sativa* in contaminated field, dedicating sites which are set aside for long term research purpose. Further research is still required to expand the knowledge of the microbial genetics to increase capabilities to degrade heavy metals from contaminated crop fields. In addition, we need to understand the mechanisms involved in mobilization and transfer of metals in order to develop future strategies and optimize the bioextraction process.

5. Acknowledgements

The authors wish to extend their grateful thanks to Department of Biotechnology, Govt of India, New Delhi for the establishment of Bioinformatics Centre and Institutional Level Biotech Hub in Gauhati University, Silchar, India. The authors are also thankful to Himachal Institute of Life Sciences, Patna Sahib, (H.P), India for providing laboratory facility to carry out initial research work.

6. References


Rajhanshi, J. 2008. Study on Heavy Metal Resistant Bacteria in Gobehwori Sewage Treatment Plant. Our Nature 6, 52-57


Copper tolerant rhizobacteria for sustainable cultivation of rice

Soumitra Nath1,2, Rubina Dahiya3, Indira Sharma4 and Praveen Pandey5

Soumitra Nath1,2, Rubina Dahiya3, Indira Sharma4 and Praveen Pandey5

In copper contaminated crop field, the natural role of Copper-tolerant rhizobacteria such as Bacillus sp., Pseudomonas sp., Staphylococcus sp., Streptococcus sp. and Monasella sp. in maintaining soil fertility is of much importance. Besides their role in metal detoxification, rhizobacteria also promote the growth of plants by other mechanisms such as production of growth promoting substances and siderophores. In the present study, copper (Cu) tolerant rhizobacteria were isolated from cultivated crop field of Barak Valley Region of Assam. For selective isolation of Cu tolerant rhizobacteria, nutrient agar was incorporated with cupric sulphate (CuSO4.5H2O) and the concentration of CuSO4.5H2O was maintained at 0.2 mM to 1.0 mM. An overall change in the microbial communities was observed in comparison with the control. The soil physico-chemical properties of the study soils were also performed. The pH range was found to be 4.7 - 5.3 which indicates strongly acidic nature of the soil. On the other hand, a set of pot experiment was performed to determine the deleterious effect of copper on rice field agro-eco system. Most of the rhizobacterial isolates were found to be gram negative. The minimum inhibitory concentration (MIC) and antibiotic sensitivity test of potential gram negative rhizobacteria was determined. Thus, the application of heavy metal tolerant rhizobacteria in contaminated crop field may be a suitable alternative for sustainable cultivation of rice.

Keywords: Copper, rhizobacteria, siderophore, pollutants, tolerant, detoxification

Biography

Mr. Soumitra Nath is a Research Scholar from Bioinformatics Centre, Gurusahani College Silchar under DBT funded project and registered for his Ph.D in Department of Microbiology, Assam University, Silchar (AUS). He has completed his Master degree from Department of Biotechnology, AUS and PG Diploma in Bioinformatics from Department of Life Science, AUS. He has also been appointed as AIOHOA, Lecturer in Department of Biotechnology and Bioinformatics Centre of Gurushahani College, Silchar.

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Determination of heavy metal stress on bacterial diversity
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\textsuperscript{b}Department of Microbiology, Assam University, India

In the present study total thirty heavy metal resistant bacteria were isolated from sewage of industrial effluents, garages and
piet pump of Cachar district of Assam, India, against copper, zinc, and lead. Samples were streaked on selective media and
incubated at 37\textdegree C for 24 hres for recovery of potent isolates. The isolated and distinct colonies were sub-cultured and
obtain in the form of pure culture and identified on the basis of their morphology and biochemical characters. There were
decreases in total count and microbial population diversity with increasing metal concentrations and the predominant isolates obtained
were Pseudomonas sp., Klebsiella sp. and Bacillus sp. Some isolates exhibited high resistance to heavy metals with minimum
inhibitory concentration (MIC) for heavy metals as 90 mg/ml (for copper), 180 mg/ml (for lead) and 1500 mg/ml (for zinc). They
also showed multiple heavy metal tolerance and were multi antibiotic resistant.

Biography
Saumitra Nath is a Research Scholar from Bioinformatics Centre, Guwahati College, under DBT funded project and registered for his Ph.D in
Department of Microbiology, Assam University, Silchar (ASS). He has completed his Master degree from Department of Biotechnology, ASS and
PG Diploma in Bioinformatics from Department of Life Sciences, ASS. He has also been appointed as Lecturer in Department of Biotechnology and
Bioinformatics Centre of Guwahati College, Silchar. His research aim is to find an efficient soil bacteria for better crop production in heavy metal
contaminated crop field.

nath.saumitra1@gmail.com
Pseudomonas aeruginosa strain SN1 16S ribosomal RNA gene, partial sequence

GenBank: KF031122.1

SOURCE
Pseudomonas aeruginosa

ORGANISM
Pseudomonas aeruginosa

KEYWORDS
bacteria; proteobacteria; gamma-proteobacteria; pseudomonadin; Pseudomonadaceae; Pseudomonas.

REFERENCE

AUTHORS
Reddy, S., Sharma, I. L., Deb, B. and Pande, P.

JOURNAL
Direct Submission

COMMENT
#Assembly-Data-START#

PRAYER
Locaton/Quality:

DNA

1..1407

/article/Qualifies

Growth of Pseudomonas aeruginosa

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/strand=+1

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/country="India; Assam"

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//
Pseudomonas aeruginosa strain SN3 16S ribosomal RNA gene, partial sequence

GenBank: KF031123.1

**LOCUS** KF031123

**ID:** KF031123

**DEFINITION** Pseudomonas aeruginosa strain SN3 16S ribosomal RNA gene, partial sequence.

**ACCESSION** KF031123

**VERSION** KF031123.1

**SOURCE** Pseudomonas aeruginosa

**ORGANISM** Pseudomonas aeruginosa

**References**

Nath,S., Deb,B., Sharma, I. and Fendey,P.

**JOURNAL** Submitted (19-MAY-2013) Bioinformatics Centre, Guruvaran College, College Road, Silchar, Assam 788004, India

**COMMENT** 

**FRACTIONS**

**SOURCES**

**QUALIFIER**

**/ORGANISM** Pseudomonas aeruginosa

**/mol_type** Generic DNA

**/strain** SN3

**/db qualifiers**

**/country** India

**/annotation**

**Source**

**/product** 16S ribosomal RNA

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www.ncbi.nlm.nih.gov/nuccore/KB031123

1/1
Pseudomonas aeruginosa strain SN4 16S ribosomal RNA gene, partial sequence

GenBank: KF447770.1

ORIGIN

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Pseudomonas aeruginosa strain SN5 16S ribosomal RNA gene, partial sequence

GenBank: KF447771.1

### Sequnce:

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DEFINITION  Pseudomonas aeruginosa strain SN5 16S ribosomal RNA gene, partial sequence.
ACCESSION   KF447771
VERSION     KF447771.1
ID  G1532214543
SOURCE      Pseudomonas aeruginosa
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REFERENCE    1 (Isaaq T n 1969)
AUTHORS      Isaaq T
TITLE        Isolation and characterisation of heavy metal resistant and
            antibiotic resistant Pseudomonas aeruginosa from contaminated crop
            field of Aman, India
JOURNAL      Unpublished
REFERENCE    2 (Isaaq T to 1969)
AUTHORS      Isaaq T
PPENDIX      Direct Submission
JOURNAL      Submitted (19-Jul-2013)
ABSOLUTE CENTRE, Wroclaw University College,
            Gielchow College Road, Gielchow, Aman 78004, India
COMMENT      Assembly-Data-START##
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              #Assembly-Data-END##
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            /gd_ek="taxon1"
            /country="India; Aman; Bank valley region"
            /note="Heavy metal tolerant strain"
            /mol=1388
            /product="16S ribosomal RNA"
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            479 cgcggcc gattgaggg ctcgctcgct ctcgctcgct cggcaggtc cggcaggtc
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            1311 aagcgccg gatgaggaa gttctttcct gttcttcg gaggcgggt gcgggcgctg
```


1/1
TRAININGS ATTENDED

1. Attended a 3 days training programme on “Bioinformatics: Current Approaches and Applications” organized by Guahati University, Guwahati on 21st – 23rd Mar, 2011.

2. Attended a 3 days training programme “Techniques in Molecular Biology & Their Applications” organized by State Biotech Hub College of Veterinary Science, Gauhati, Assam on 21st to 23rd December, 2011.

3. Attended 42 days training programme on “Role of Heavy Metal tolerant rhizobacteria in sustainable cultivation of rice” in Himachal Institute of Life Science, Himachal Pradesh from 21st April to 2nd June, 2012.

4. Attended 5 days Clinical Investigator Development Program on “Genetics and Epidemiology- Study Design and Statistical Methods” in National Institute of Biomedical Genomics, Kalyani, West Bengal from 1st April to 5th April, 2013.

WORKSHOPS ATTENDED

1. Attended 2 days National Workshop on “Application of Bioinformatics in Agricultural Research and Biofertilizer Technology” organized by Gurucharan College, Silchar, Assam on 14th and 15th May, 2011.

2. Attended 2 day Workshop on “Recent Advances in Computational Biology and structural based drug Designing” conducted by Biotechnology-2012 conference (OMICS group) at Hyderabad International Convention Centre, India on 13th -15th Sept, 2012.

CONFERENCES ATTENDED

1. Poster presentation in “3rd World Congress on Biotechnology” held at Hyderabad International Convention Centre, India during September 13th - 15th, 2012.


3. Poster Presentation in “100th Indian Science Congress” held at Calcutta University, Kolkata during 3rd – 7th January, 2013.
