An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain *Bacillus sp*. isolated from municipal waste


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ABSTRACT

The objective of our present study was to isolate heavy metal tolerant and antibiotic resistant microorganisms, the site selected being the K.M.C.’s Waste Dumping Yard, Dhapa, Kolkata, West Bengal, India, based on the extent of pollutants being discharged and the land waterways being polluted by the effluents and waste being dumped by the household and numerous industrial units. The isolated strain showed amylase & protease positive, spore forming, Gram-positive bacilli and exhibited growth in wide range of substrates, temperature (30°C-40°C) & pH (6.0-11.0). Based on the 16S rDNA molecular technique, it was found that the isolate was *Bacillus sp.* and has a mega plasmid. Moreover it was found to grow in presence of a wide range of metals namely nickel, cadmium, chromium and cobalt in the order Cd$^{2+}$ > Cr$^{6+}$ > Ni$^{2+}$ > Co$^{2+}$. And also it was observed that the isolate was resistant to a wide range of antibiotics namely Kanamycin (30µg/disc), Ampicillin (25µg/disc) and Methicillin (5µg/disc). Plasmid curing result showed the loss of antibiotic and heavy metal resistance profile in the isolated strain and confirms a relationship between antibiotic and heavy metal resistance with plasmid. Heavy metal tolerance test showed maximum microbial tolerance to cadmium and minimum tolerance to cobalt. This heavy metal resistant organism could be a potential agent for bioremediation of heavy metals polluted environment.

Keywords: *Bacillus sp.*, heavy metals, MTC, antibiotic resistance, plasmid curing, 16S rDNA gene sequencing.

INTRODUCTION

Heavy metal contamination is widespread. Heavy metals are defined as a group of metals whose atomic density is greater than 5g/cm$^3$. In nature, there are about 50 heavy metals of special concern because of their toxicological effect to human beings and other living organisms. Many of them, like Zn, Cu, Co, Ni, Mn, and Fe have the nutritional characteristics known as essential “trace elements” are necessary for living organisms [1] because at a certain concentration levels,
these elements participate some enzyme activities. When in excess concentrations, the toxic effects of these dual functional ions are revealed. Heavy metals cannot be degraded or destroyed because they are stable and so persistent environmental contaminants [2]. The environmental pollution by heavy metals comes from anthropogenic sources such as smelters, mining, power stations and the application of pesticides containing metal, fertilizer and sewage sludge.

By affecting the growth, morphology and biochemical activities, heavy metals influence the microbial population and resulting in decreased biomass as well as diversity. Therefore microbes have developed mechanisms to tolerate the metals either by presence of heavy metals through efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration [3]. Most mechanism reported involves the efflux of metal ions outside the cell, and genes for tolerance mechanisms have been found on both chromosomes and plasmids. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions.

An increasing problem for the treatment of different infectious diseases is bacterial resistance to antibiotics and other antimicrobial agents. It is thought that a correlation exists between metal tolerance and antibiotic resistance in bacteria [4] because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria [5,6,7]. Thus we need to be more careful of the drastic use of antibiotics in medical practice, and also more aware of other antimicrobials, that we put into the environment, such as heavy metals. So in this case plasmid isolation and curing was carried out to establish the correlation among heavy metal and antibiotic resistance properties with plasmid.

The present study reports the characterization of one of the isolate from K.M.C.’s Waste Dumping Yard, Dhapa, Kolkata, West Bengal, India. The basic objective was to screen out potential microbes, to characterize them and to explore their properties in various applications.

MATERIALS AND METHODS

1.1 List of Chemicals
All chemicals used for experiment were analytical grade. Cobalt nitrate, cadmium chloride, nickel sulfate, potassium dichromate were bought from Merck Specialties Pvt. Ltd, Worli, Mumbai, India. Antibiotics disks and Muller-Hinton agar media were bought from Himedia, India.

1.2 Sampling
The area under study for this work was identified based on the need, diversity and extent of pollutants produced by various industries located on South Kolkata. The site was identified on K.M.C.’s Waste Dumping Yard, Dhapa, Kolkata, based on the extent of pollutants being discharged and the land waterways being polluted by the effluents and waste being dumped by the numerous industrial units. Soil samples were collected for bacterial isolation from the polluted site in sterilized plastic bags and transported to the laboratory of Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University. The container was maintained at a temperature of 4ºC to ensure minimal biological activity. Processing of the samples for the isolation of bacteria was carried out within 24 hrs of sample collection. A 10-fold dilutions of fresh soil (1 g) were made in phosphate buffered saline (PBS) and 0.1 ml from each of these dilutions were spread on nutrient agar plates and incubated at 37ºC for 72 hrs.
1.3 Isolation and Identification of Heavy Metal Resistant Bacteria

Heavy metals incorporated media were used for the selective isolation of heavy metals resistant bacteria. Basal media nutrient agar (NA) incorporated with salts of heavy metals like Cd$^{2+}$, Cr$^{6+}$, Ni$^{2+}$, Co$^{2+}$ were prepared separately. The concentration of each heavy metal was maintained as follows:

- CdCl$_2$: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5mg/ml
- K$_2$Cr$_2$O$_7$: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5mg/ml
- NiSO$_4$: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5mg/ml
- Co(NO$_3$)$_2$: 0.1, 0.2, 0.3, 0.4, 0.5mg/ml

After the incubation period (24-48 hrs.) the plates were observed for any kind of growth on the media. The isolated and distinct colonies on these selective media were sub cultured repeatedly on the same media for purification. The pure culture was identified on the basis of their morphology and biochemical characters [8].

1.4 Study of Colonial Morphology

Isolated colonies of purified bacterial strain grown on solidified agar plates were observed and data was recorded regarding the form (circular, filamentous and irregular); elevation (flat, convex, and umbonate); margin (entire, undulate, erose and filamentous) ; and optical feature (opaque, translucent, and transparent) of the colonies [9].

1.5 Study of Motility Character

The test can be used to check for the ability of bacteria to migrate away from a line of inoculation to physical features like flagella. Craigie’s method is used to perform this test. The semi solid nutrient agar medium (contains 0.2%-0.5% of agar) test tube is inoculated with the test organism into the central glass tube and incubated at the relevant temperature for 18-24 hrs.

1.6 Study of Cellular Morphological Characteristics

For determining the shape and gram character, bacterial film was stained by Gram’s Method. The slide was examined under the microscope (oil immersion, 100 X). Other staining procedures were conducted for detection of endospore, capsule as per standard protocol.

1.7 Biochemical Characterization

Biochemical characterization were studied to detect the presence of enzymes namely gelatinase, oxidase, catalase, urase, nitrate reductase, casein hydrolase and amylase. Other tests also were performed included indole test, methyl red and Voges Proskauer test, citrate utilization test, H$_2$S production test.

1.8 Physiological Characterization

To select the heavy metal tolerance strains, it is necessary to standardize the cultural and physiological conditions of the selected organisms. Among the physico-chemical conditions, temperature, pH and salt (NaCl) concentration are of great importance on the bacterial growth.

1.8.1 pH Profile

pH is a limiting factor, which governs bacterial growth. To determine the pH optima, nutrient broth medium meant for growth of the isolates was adjusted to different pH ranging from 6.0-11.0 and was seeded with 0.1ml inoculum. Post overnight growth at 37°C under shaking condition, growth was measured in terms of OD at 600 nm using colorimeter.
1.8.2 Temperature Profile
For determination of optimum temperature, 0.1ml inoculation was provided into nutrient broth medium and overnight incubation was done at different temperatures like 30, 37, 40 and 50°C. The growth was measured in terms of OD at 600 nm through colorimeter.

1.8.3 Salt (NaCl) Concentration Profile
To study the optimum salt concentration on the bacterial growth, different concentration of NaCl ranging from 0.5% to 10% were used in nutrient broth. The growth was measured in terms of OD at 600 nm using colorimeter.

1.9 Molecular Characterization
The molecular characterization was done on the basis of 16S rDNA sequence analysis. This analysis was performed by Xcelris Labs Ltd. (Sydney House, Ahmedabad, India). DNA was isolated and evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1398 bp rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the ndatabase of NCBI genbank. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4. The sequence obtained was submitted to NCBI GenBank.

1.10 Maximum Tolerance of Heavy Metals
The metals used in the study and detailed procedure to determine the tolerance property, in terms of Maximum Tolerable Concentration (MTC) [10]. The Maximum Tolerable Concentration (MTC) of heavy metal was designated as the highest concentration of heavy metal that allows growth after 2 days i.e., 48 hrs [11].

Maximum Tolerable concentrations of the strain against CdCl$_2$, K$_2$Cr$_2$O$_7$, NiSO$_4$ and Co(NO$_3$)$_2$ have shown that isolated strain was capable of growing at high concentration of heavy metals in the media. MTCs of metal salts are given in Table 4.

1.11 Study of Antibiotic Resistance
To detect the antibiotic susceptibility on different antibiotics, the bacterial strain was cultured on Muller-Hinton agar plates. Using the methods of [12,13,14] the isolated microbes were assayed for their sensitivities to the antibiotics. For this test, eleven antibiotics (from Himedia) were chosen. These antibiotics were Chloramphenicol (10µg/disc), Streptomycin (25µg/disc), Tetracycline (10µg/disc), Norfloxacin (10µg/disc), Rifampicin (15µg/disc), Kanamycin (30mcg/disc), Neomycin (30mcg/disc), Ampicillin (25mcg/disc), Nalidixic acid (30µg/disc), Methicillin (5µg/disc), Cotrimoxazole (35µg/disc). The bacterial cultured solution was spread (after 16 hrs growing condition) on Muller-Hinton agar plates and antibiotic discs were placed. All the plates were incubated at 37°C for 24 hrs. On the basis of zone diameter, the isolated strain was classified as resistant or sensitive [15]. Control plates were incubated without antibiotic discs. All the experiments were carried out in triplicate.
1.12 Isolation and Curing of Plasmid DNA
Plasmid was isolated using standard method [16]. The isolated plasmid was characterized by agarose gel electrophoresis according to the standard procedure [17]. The plasmid curing was carried out according to standard protocol [18]. The isolated strain was subjected to plasmid curing by chemical agent ethidium bromide (EtBr). To detect the loss of antibiotic resistance, the EtBr treated culture was transferred to the LB plates amended with the respective antibiotics with proper concentrations Kanamycin (30µg/disc), Ampicillin (25µg/disc), Methicillin (5µg/disc). Then the same culture of the strain which already had lost antibiotic resistance was subjected to metal tolerance (Cadmium 1.0mg/ml, Cobalt 0.4 mg/ml, Nickel 0.5 mg/ml, chromium 0.75 mg/ml).

RESULTS

2.1 Isolation of Heavy Metal Tolerant Bacteria
The heavy metal tolerance property of the isolated strain was confirmed by growing it on heavy metal containing media. One of them was selected for our study and the isolated heavy metal tolerant strain was designated as Strain P.

2.2 Identification of Isolated Bacterial Strain
2.2.1 Colonial Morphology
Strain P possessed large colony. The colonial morphology of the strain was circular, elevation was convex, colonial margins were entire type. A detailed result for colonial morphology has been given in Table 1

<table>
<thead>
<tr>
<th>Shape</th>
<th>Circular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Large</td>
</tr>
<tr>
<td>Color</td>
<td>White</td>
</tr>
<tr>
<td>Margin</td>
<td>Entire</td>
</tr>
<tr>
<td>Elevation</td>
<td>Convex</td>
</tr>
<tr>
<td>Surface</td>
<td>Smooth &amp; Shiny</td>
</tr>
<tr>
<td>Opacity</td>
<td>Opaque</td>
</tr>
</tbody>
</table>

2.2.2 Cellular Morphology
Cellular morphology such as arrangement, shape and Gram reaction were observed during Gram staining of isolated strain. Cellular shape of the strain was found as rod, whereas cellular arrangement was found in chain and scattered form, and the isolated strain was Gram positive. Details for cellular morphology and Gram reaction are given in Table 2.

<table>
<thead>
<tr>
<th>Gram straining</th>
<th>Gram +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Rod</td>
</tr>
<tr>
<td>Arrangement</td>
<td>chain and scattered</td>
</tr>
<tr>
<td>Spores</td>
<td>Present</td>
</tr>
<tr>
<td>Capsule</td>
<td>Present</td>
</tr>
<tr>
<td>Cell motility</td>
<td>Motile</td>
</tr>
</tbody>
</table>

2.2.3 Biochemical Characteristics
The results of biochemical Characterization experiments are shown in Table 3.
Table 3. Biochemical Characterizations of Isolated Strain P.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Starch hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>Protein hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>03</td>
<td>Citrate utilization</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td>Urea hydrolysis</td>
<td>–</td>
</tr>
<tr>
<td>05</td>
<td>Indole production</td>
<td>–</td>
</tr>
<tr>
<td>06</td>
<td>Methyl red test</td>
<td>+</td>
</tr>
<tr>
<td>07</td>
<td>Voges Proskauer(VP) test</td>
<td>–</td>
</tr>
<tr>
<td>08</td>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>09</td>
<td>Gelatin liquefication</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Catalase</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>Oxidase</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Triple Sugar Iron Test</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Sulfide Indole Motility Test</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Sugar fermentation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>Acid + Gas</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>Acid + Gas</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>Acid + Gas</td>
</tr>
<tr>
<td></td>
<td>Mannitol</td>
<td>Acid + Gas</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>Acid + Gas</td>
</tr>
</tbody>
</table>

2.2.4 Physiological Characterization

The physiological properties of the isolated strain P was concluded in terms of pH and temperature. The isolate strain P was found to grow within a pH range of 6.0-11.0 with optimum growth at pH 7.0 (Fig. 1). The temperature range found suitable for growth of isolated strain was between 30-40°C, optimum being at 37°C (Fig.2). These physiological conditions are reported as the optimum for most of the functions in a living organism.

The effect of NaCl concentration on growth of the isolated P was also conducted. The optimum growth for isolated strain was found at 0.5% NaCl concentration (Fig.3).
2.3 Molecular Characterization
N-BLAST search of the 16S rDNA sequence of isolated strain P was reported by Xcelris Labs Ltd. (Sydney House, Ahmedabad, India) and reveals that the isolate bears maximum similarity (99%) and identified as Bacillus sp. TDSAS2-2CS-2010. The sequence obtained was submitted to GenBank and the GenBank accession no. is HM752770.

2.4 Determination of the Effect of Heavy Metals on Bacterial Growth
The Gram positive Bacillus sp. exhibited different growth patterns in presence of different heavy metals. The growth curves for Strain in presence of different metal concentrations are shown in Fig 4.

The isolated strain showed different levels of tolerance to the metals under investigation and MTC were used to determine metal tolerance of bacteria isolated from soil (Table 4). It was found that among four experimental heavy metals, the strain showed high resistance to cadmium showing the growth of microorganisms up to 1.0mg/ml (i.e.1000 µg/ml) and less resistance to cobalt showing growth of microorganism up to 0.4mg/ml (i.e.400 µg/ml) and the pattern of metal tolerance were in the order Cd²⁺ > Cr⁶⁺ > Ni²⁺ > Co²⁺.

The microbial growth decreased with the increase in concentration of heavy metals indicating toxic effect of the heavy metals on the growth of microorganisms [19].

<table>
<thead>
<tr>
<th>Isolated Strain</th>
<th>Cd²⁺</th>
<th>Cr⁶⁺</th>
<th>Ni²⁺</th>
<th>Co²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.</td>
<td>1.0mg/ml</td>
<td>0.75mg/ml</td>
<td>0.5mg/ml</td>
<td>0.4mg/ml</td>
</tr>
</tbody>
</table>
Fig. 4 Growth curves of Strain *Bacillus* sp. in presence of different concentrations of different heavy metals. The X axis depicts time in hours where as Y axis represents bacterial growth presented in terms of OD at 600 nm

*a.* Growth curve of Strain *Bacillus* sp. in presence of Cd$^{2+}$ (CdCl$_2$).

*b.* Growth curve of Strain *Bacillus* sp. in presence of Cr$^{6+}$ (K$_2$Cr$_2$O$_7$).

*c.* Growth curve of Strain *Bacillus* sp. in presence of Ni$^{2+}$ (NiSO$_4$).

*d.* Growth curve of Strain *Bacillus* sp. in presence of Co$^{2+}$ (Co(NO$_3$)$_2$).

2.5 Antibiotic Susceptibility Test

In the present study, *Bacillus* sp. exhibited highest resistance to Kanamycin (30µg/disc) followed by Ampicillin (25µg/disc) and Methicillin (5µg/disc). Results are given in Table 5. This result indicates that the strain *Bacillus* sp. exhibits resistance to wide spectrum of antibiotics, i.e.; multiple drug resistance patterns.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>Bacillus</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol (10µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Streptomycin (25µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Tetracycline (10µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Norfloxacin (10µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Rifampicin (13µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Kanamycin (30µg/disc)</td>
<td>Resistance</td>
</tr>
<tr>
<td>Neomycin (30µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ampicillin (25µg/disc)</td>
<td>Resistance</td>
</tr>
<tr>
<td>Nalidixic acid (30µg/disc)</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Methicillin (5µg/disc)</td>
<td>Resistance</td>
</tr>
<tr>
<td>Cotrimoxazole (35µg/disc)</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>
2.6 Plasmid Profile and Curing

The plasmid DNA was successfully isolated from the strain *Bacillus sp.* Plasmid profile of the isolated strain exhibits a single band indicating the presence of a mega plasmid (Fig. 5).

![Fig. 5 Plasmid profile of the isolated strain P subjected to electrophoresis on 1% agarose gel.](image)

Plasmid curing is carried out to confirm whether the genes for resistance are encoded by genomic DNA or plasmid DNA. Here, attempt was made to cure antibiotic resistant marker from the strain using chemical agent, Ethidium bromide. After plasmid curing, it was observed that the strain was sensitive to antibiotics but previously that was resistant to those antibiotics. The growth of the control culture with EtBr was reduced due to may be the effect of EtBr with respect to without EtBr culture. After the plasmid curing, there was loss of resistance to the heavy metals, but previously the resistance properties of the *Bacillus sp.* was found (Fig. 4). The above result indicates that the genes for antibiotic and heavy metal resistance of the isolated strain may be reside on plasmid DNA.

**DISCUSSION**

This investigation highlights the presence of metal ions in the soil sample studied and shows the prevalent occurrence of metal tolerant microbial population in the K.M.C.’s Waste Dumping Yard, Dhapa, Kolkata.

Initially, several bacterial strains were purified out of which few showed consistent behaviour and were revived after being refrigerated for few weeks. Finally, one of the isolated strains was selected for characterizations which were consistent for its tolerance behavior; the isolated strain was designated as **Strain P**.

Since salt (NaCl) concentration, temperature and pH have roles in enzymatic function as well as overall metabolic efficiency, these factors do have an effect on survivability. The salt (NaCl) concentration, temperature and pH profile of the strain indicate that the strain has the ability to survive in an adverse condition.

The strain has an ability to grow up over wide range of substrates such as glucose, sucrose, galactose, maltose, mannitol, lactose. This indicates towards the aerobic metabolic pathway as these substrates could be easily assimilated and directly enters Tri Carboxylic Acid (TCA) Cycle. In a stressed environment tolerance mechanisms develop in the organism to survive [20], and they also play an important role in the cycling of toxic metals in the biosphere. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena.
among heavy metal resistant bacteria. In this piece of work we observed that the *Bacillus sp.* has multiple heavy metal tolerance property and resistance to $\text{Cd}^{2+}$, $\text{Cr}^{6+}$, $\text{Ni}^{2+}$ and $\text{Co}^{2+}$. Chromium appeared as more toxic than cadmium. The combined resistance to heavy metals was also reported by [21]. These reports support that the metal resistances of the bacteria were interrelated to each other. The resistance mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance factor from one cell to another [22,23]. Even after plasmid curing, the *Bacillus sp.* became sensitive to all the heavy metals tested suggesting plasmid mediated resistance. Multiple metal resistances has been found in other bacteria and the resistance mechanisms and genes involved are typically not common [24]. This heavy metal resistant organism could be a potential agent for bioremediation of heavy metals polluted environment. Because heavy metals cannot be degraded or destroyed, the introduction of heavy metals in various forms, in the environment can produce considerable modifications of the microbial communities and their activities for their survivability [25]. It was also observed in our study.

The strain exhibits resistance to a group of antibiotics namely Kanamycin, Ampicillin and Methicillin and this is supported by the earlier studies by [26,27,28]. The presence of resistance gene in mega plasmid was confirmed by the result of plasmid curing. Thus, above result clearly confirmed the association between the antibiotic and heavy metal resistance with plasmid. The same result was reported by many investigators [29,30]. These results reflect the earlier reports [31] that the combined expressions of heavy metal tolerance and antibiotic resistance may not be a chance phenomenon rather these are the results of selection by heavy metals present in an environment [32,33]. In the stressed environmental condition, the bacterial strains may carry plasmid not only in terms of the frequency but also in size [34]. Tanaka *et al* [35] have reported the presence of large plasmid in *Bacillus sp.* Similarly in the current study a mega plasmid was observed in the isolated strain *Bacillus sp.* (Fig. 5).

**CONCLUSION**

Bacterial resistance to both antibiotics and heavy metals can readily be isolated from the natural environment, with greater abundance noted for polluted waste sites such as K.M.C.’s Waste Dumping Yard, Dhapa, Kolkata.

The heavy metal tolerant soil bacteria are a potential indicator of toxicity of heavy metals to other forms of life [36]. In this study it is proved that these high cadmium and chromium tolerant bacteria confirmed the contamination by these metals in the study locations of Kolkata.

The future prospect lies in the application of this microorganism for purposes like heavy metal remediation and potential use in extracting rare metals from dilute solution or removing toxic metals from industrial effluents [37]. The present studies inform us that the isolated *Bacillus sp.* has the properties to resist a wide range of heavy metals and antibiotics; it may be harmful to human being as well as to the animals. The present investigation has widened the scope for research and development of metal tolerance and antibiotic resistance from bacterial origin.

**Acknowledgements**

The authors are pleased to acknowledge the Xcelris Labs Ltd. (Sydney House, Ahmedabad, India) for identification of microbial culture using 16S rDNA based molecular technique.
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