

## Bioremediation of Cadmium Contaminated Effluent by *Sporosarcina luteola*: A Bacterium Isolated from Soil near Wazirpur Industrial Area, New Delhi, India

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Heavy metals pollution is emerging as a threat to ecological systems causing various problems to mankind, plants and animals. Aim of the present study was to isolate and identify cadmium tolerant bacteria from the soil of Wazirpur industrial area of New Delhi (India). The study involved physico-chemical characterization of the polluted soil which was found to contain high concentration of iron, manganese and cadmium at 352, 15.3 g/kg soil and 3.16 ppm, respectively. One bacterial strain was identified as *Sporosarcina luteola* on the basis of morphological, biochemical and phylogeny analysis. Strain *Sporosarcina luteola* was highly resistant to Cd up to 5mM (mM= millimolar) when cultured in solidified nutrient agar plates and 7.2 mM in nutrient broth. *Sporosarcina luteola* has also showed substantial growth in presence of Co, Pb, Fe and Mn upto 2.0, 2.0, 3.5 and 4.0 mM, respectively in liquid medium. Optimum growth of identified bacteria was shown at 37 °C, 7.0 pH and it tolerated up to 3 % sodium chloride (w/v). This is reported for the first time that *Sporosarcina luteola* (metal-tolerant bacteria) has potential of removal of cadmium from industrially contaminated soil.

**Keywords:** Cadmium, *Sporosarcina luteola*, Bioremediation, Metal resistant, Antibiotic resistance.

### INTRODUCTION

Due to uncontrolled industrial pollution and human induced sources, large amount of metals are being released which are ultimately accumulating into the soil. The discharge of effluents from the industrial units is severely damaging the health of human, animal and aquatic species enhancing the burden on ecosystem [1].

In India, most of the industries are discharging their effluents in to the rivers without following proper treatment protocols to cut down their cost [2]. Delhi has the highest number of small-scale industries in India. Rules for waste treatment are flexible for small-scale industries due to less amount of waste produced by each industry. Industries are taking advantage of loopholes in the law by releasing untreated effluents into the river Yamuna, which contributes a large volume of potable water for Delhi. This untreated wastewater has resulted in health and environmental risk to the people of Delhi and surrounding areas [3]. Inadequately treated or untreated waste water effusion on land might cause environmental problems related to water table, soil and crop productivity. Heavy metals present

in the industrial effluent are imposing life threatening lethal effects by crossing the critical limits [4]. Once in the soil, these metals and their compounds gets deposited at the place of contamination because of their fairly immobile nature. Some heavy metals at higher concentrations can travel and gets added into the crops, fruits and drinking water (bioaccumulation) and be potentially dangerous for human health.

Heavy metals are polluting the environment through processing of ore for metals extraction and electronic industries [5,6]. Surface and/or groundwater bodies near the industrial areas receive untreated effluent containing elevated levels of several toxic pollutants along with heavy metals posing risk of mutations and cancer in human [7]. In India, many dense urban areas with significant industrial waste generation have contaminated soil [8-10]. Higher heavy metals content have been found in soils near industrial areas of several industrial cities of India [11,12]. Soils of sewage irrigated area near Bhopal were found to contain higher amount of available Zn, Cu and Pb contents. Many studies [13-15] have revealed that the level of contamination is high near roads with the presence of Pb, Zn, Cu, Cd, etc. in toxic amounts.

Cadmium is a dangerous metal characterized by highly stable ions. Population living near roads and industries (fertilizer and metal ore processing) have high chances of heavy metal exposure. It is non-degradable in nature and thus, once released, it persists in the environment. Cadmium is known to bind with respiratory enzymes causing oxidative stress, which may be the etiology of several diseases including cancer. Intake of cadmium in form of fumes and particles can have frightening poisonous effects if exceeds critical limits. Although acute pulmonary effects and deaths are uncommon, sporadic cases have been reported. The health risks of cadmium exposure are exuberated by the relative inability of human beings to excrete cadmium. It is excreted and then reabsorbed by the kidney resulting in toxic levels. High concentrations of cadmium in kidney as risk factor for prostate cancer has also being investigated [16,17].

Although many techniques can be employed for the treatment of soil, the ideal treatment should not be only suitable, but appropriate and applicable to the local conditions also. Presently there are a number of conventional processes being used for removal of heavy metals but these methods have low efficiency and high cost [18]. Scientists have focused their dedicated steps towards providing an alternative, more efficient and low cost method for treatment and removal of heavy metals. Biological methods have been thought to be adapted for elimination of heavy metals due to the enhanced effectiveness and low cost as it uses the metabolic potential of microbial species and plants used in the process [19]. In this experimental study, an effort was focused on isolation and characterization of metal tolerant bacteria from contaminated industrial soil.

## EXPERIMENTAL

**Sample collection and chemical analysis:** Soil from Wazirpur Industrial Area of New Delhi (Longitude 77°09'51" East and Latitude 28°41'59.28" North) was used for the study. Sample was taken from 6-10 inches below the surface in polyethylene bags and brought to the laboratory. For establishing pH and electrical conductivity, 5 g soil was mixed with 25 mL distilled water and incubated for 1 h in a rotatory shaker at 170 rpm. It was then filtered with Whatman 1 paper. For heavy metals extraction, 10 g soil was mixed with 20 mL of diethylenetriaminepentaacetic acid (DTPA) [20] and mechanically shaken for 2 h. It was then filtered with Whatman No.42 and 0.45 µ filter and analyzed by atomic absorption spectrometry (AAS) at National Testing Laboratory, New Delhi, India.

**Bacterial isolation by spread plate method:** Thoroughly mixed soil was enriched for manganese, cadmium, lead, cobalt, nickel, iron, copper and zinc resistant clones by serial dilution method. A 10-fold dilution of stock (1 g fresh soil in 1 mL distilled water) was prepared by using distilled water and with the help of micropipette 100 µL inoculum was applied on nutrient agar plates containing each metal with a sterilized glass spreader. Plates were observed for the development of bacterial colonies during an incubation period of 5 days at 37 °C. After completion of incubation period a number of strain were selected on the basis of morphological parameters. Maximum resistance was determined by gradually increasing metal concentration in the media until the bacterial strains were unable to develop colonies [21]. The initial metal concentration used was 0.1 mM. The

solution of cadmium chloride, cobaltous chloride, lead acetate, manganous sulphate, ferrous sulphate, copper sulphate, zinc sulphate and nickel sulphate amended with nutrient agar and sterilized by autoclaving. Minimum inhibitory concentration (MIC) was determined. Potential bacterial isolates were mixed in 10 % glycerol and preserved at -20 °C in cryovials tubes.

**Growth of bacterial strain on nutrient broth:** Exponential phase cells of isolated strain (24 h old) were inoculated into nutrient broth medium supplemented with different degrees of heavy metal concentrations (in mM) *i.e.*, Cd 1.0-7.2; Pb 1.0-2.0; Co 1.0-2.0; Fe 1.0-3.5; Mn 1.0-4.0; Ni, Cu and Zn - 1 mM each. The cultures were incubated for 4 days at 30 °C in shaking incubator (REMI, India) and agitated at 150 rpm. Growth rate of the strain was determined by taking absorbance at wavelength of 600 nm (OD600) using Spectronic 200 Spectrophotometer (Thermo Scientific, India). Experiment was carried out in triplicate [22].

**Morphological, physiological and biochemical characteristics:** For analysis of morphological characteristics, bacterial gram staining technique was used to stain the cells and observed with the help of microscope (Table-1). The ideal pH, temperature and salt tolerance were optimized [23]. The isolate was grown in nutrient broth medium maintained at different pH values and incubated at various temperature ranging from 10-50 °C. Salt tolerance was also checked at increasing NaCl concentrations ranging from 1 to 3 %. The optical density of the growing cultures at log phase conditions was observed at 600 nm to determine the growth. Hi 25TM rapid biochemical identification kit was used for the assessment of different carbon source utilization. Various physiological characteristics were determined by following the instructions given in Bergey's manual [24]. Disc Diffusion method was used for the detailed examination of antibiotic profile of bacterial isolate.

**16S rDNA gene amplification:** Genomic DNA was amplified as per standard PCR protocols and universal primers [(27F 5'-AGAGTTTGATCATGGCTCAG-3'); (1492R 5'-TACGGCTA CCTTGTACGACTT-3')] used in our previous study [21].

Sequencing and phylogeny for sequencing the PCR products (1.5 Kb) were taken, purified using PCR-DNA and Gel Band purification kit and sent to Biolinkk, India for sequencing. Phylogenetic tree was constructed using MEGA6.06. Sequences has been deposited in Gen Bank with accession no. KX870873.

## RESULTS AND DISCUSSION

The pH and electrical conductivity of the soil were found to be 6.90 and 516 µS, respectively. Soil sample was found to contain significant concentration of Mn (352 g/kg) and Fe (15.3 g/kg). The concentration of various heavy metals is shown in Table-2.

The bacterial colonies of *Sporosarcina luteola* (EP3) were circular in shape and creamish in colour. They were able to grow well on solid medium supplemented with cadmium concentration as high as 5 mM after an incubation period of 48 h. Bacterial cultures have shown multiple heavy metal tolerance property when cultured on nutrient agar medium supplemented with Mn (3 mM), Co (1 mM), Pb (1 mM) and Fe (1 mM). However, no growth was observed on plates supplemented with nickel, copper and zinc. Growth of *Sporosarcina luteola* (EP3)

TABLE-1  
BIOCHEMICAL, MORPHOLOGICAL, PHYSIOLOGICAL CHARACTERISTICS AND  
EFFECT OF ANTIBIOTICS ON THE ISOLATED SOIL BACTERIA EP3

Morphological tests		Antibiotic	Diameter of zone of inhibition (mm)
Colony configuration	Circular	Chloramphenicol	35 (S)
Cell shape	Rod	Neomycin	21 (S)
Colony elevation	Flat	Ciprofloxacin	40 (S)
Colony margin	Entire	Amoxicillin	40 (S)
Colony surface	Smooth	Ampicillin	24 (S)
Colony colour	Creamish	Kanamycin	0 (R)
Opacity	Opaque	Chlorotetracycline	27 (S)
Gram's reaction	-ve	Ceftazimide	40 (S)
Biochemical tests		Physiological tests	
ONPG	+	Growth at 20 °C	+
Lysine utilization	-	Growth at 30 °C	++
Ornithin utilization	-	Growth at 37 °C	+++
Urease detection	-	Growth at 40 °C	+
Phenylalanine deamination	-	Growth at 45 °C	-
Nitrate reduction	-	NaCl 1 %	+++
H <sub>2</sub> S production	-	NaCl 2 %	++
Citrate utilization	+	NaCl 3 %	+
Voges proskauer's	-	NaCl 4 %	-
Methyl red	-	pH 5	-
Indole test	-	pH 6	++
Malonate utilization	+	pH 7	+++
Esculin hydrolysis	-	pH 8	++
Arabinose, xylose, Adonitol	-	pH 9	+
Rhamnose	-	pH 10	-
Cellobiose, melibiose, saccharose, raffinose, trehalose	-		
Glucose, lactose	+		
Oxidase test	+		

+ = scant growth/positive; ++ = moderate growth; +++ = abundant growth; -- = no growth/negative; R = Resistant; S = Susceptible

TABLE-2  
PHYSICO-CHEMICAL CHARACTERIZATION OF SOIL SAMPLE

Metal ions	Conc. (ppm)	Metal ions	Conc. (ppm)
Manganese	15.3	Copper	1.95
Lead	0.028	Zinc	0.22
Iron	352	Cobalt	0.11
Nickel	0.28	Cadmium	3.16

was studied in nutrient broth incorporated with cadmium (7.2 mM), Mn (4 mM), Pb (2 mM), Fe (3.5 mM) and Co (2 mM) respectively. The relative order of multiple heavy metals resistance shown by bacterial strain EP3 was found to be Cd > Mn > Fe > Co = Pb 9 as evident from Fig. 1.

*Sporosarcina luteola* (EP3) is a rod shaped Gram negative bacterium. The strain have shown growth between 20-40 °C with optimal temperature of 37 °C. The pH profile study revealed that bacterial strain EP3 can survive under pH 6.0-9.0, with an optimal growth rate at pH 6.0-7.0. In acidic environment (pH 5), EP3 growth rate decreased rapidly by showing no visible growth. *Sporosarcina luteola* (EP3) survived when exposed to alkali environment (pH 8-9) but the rate of growth was decreased with increasing pH values. *Sporosarcina luteola* (EP3) have tolerated NaCl concentration upto 3 % with an optimal growth at 1 %. Bacterial strain was also studied for susceptibility against various antimicrobial agents. *Sporosarcina luteola* (EP3) was also found to be susceptible against antibiotics used except to kanamycin.

16S rDNA amplification showed the fragment of 1.5 Kb/1500 bp and concentration of 49.0 ng/ul (P-1.7). Sequence

comparison with present database revealed the highest sequence similarity (99 %) of EP3 with *Sporosarcina luteola* (NBRC 105378, accession number NR: 114283.1). ClustalX Phylogeny analysis have also indicated that EP3 belongs to *Sporosarcina luteola*. The neighbour joining phylogenetic tree was generated using MEGA6.06 software as shown in Fig. 2.

The soil in and around Wazirpur industrial area is polluted by various heavy metals. Strain EP3 isolated from this contaminated site was found to be metal tolerant to various heavy metals. The concentration of heavy metals (Mn 4 mM; Cd 7.2 mM; Pb 2 mM; Fe 3.5 mM and Co 2 mM) tolerated by isolated bacterial strain was significantly higher when compared to the minimal inhibitory concentration (Cd<sup>2+</sup> 0.5 mM and Co<sup>2+</sup> 1 mM) of *E. coli* based model studied by Nies [25]. In this study, bacterial strain EP3 demonstrated resistance against cadmium upto 7.2 mM and was identified as *Sporosarcina luteola*. Growth rate of the soil isolate in the presence of Cd, Pb, Mn, Co and Fe was consistently slower in comparison with the control; results concurrent with studies reported earlier [21,22,26,27].

Two strains *Chryseobacterium indoltheticum* and *Acinetobacter tjernbergiae* were isolated having tolerance limit of 200 mg/L to Cd<sup>2+</sup> [28]. Strains of *Pseudomonas aeruginosa* and *Enterobacter cloacae* were reported to have tolerance limit of 400 and 300 mg/L to Cd<sup>2+</sup>, respectively [29]. In the present study, *S. luteola* was grown in presence of Mn, Co, Fe, Pb at 4 mM, 2 mM, 3.5 mM and 2 mM, respectively. Results revealed multiple heavy metal resistance capability of *Sporosarcina luteola* (EP3). It is quite significant as no other studies have reported such potential of heavy metal-resistant bacteria.

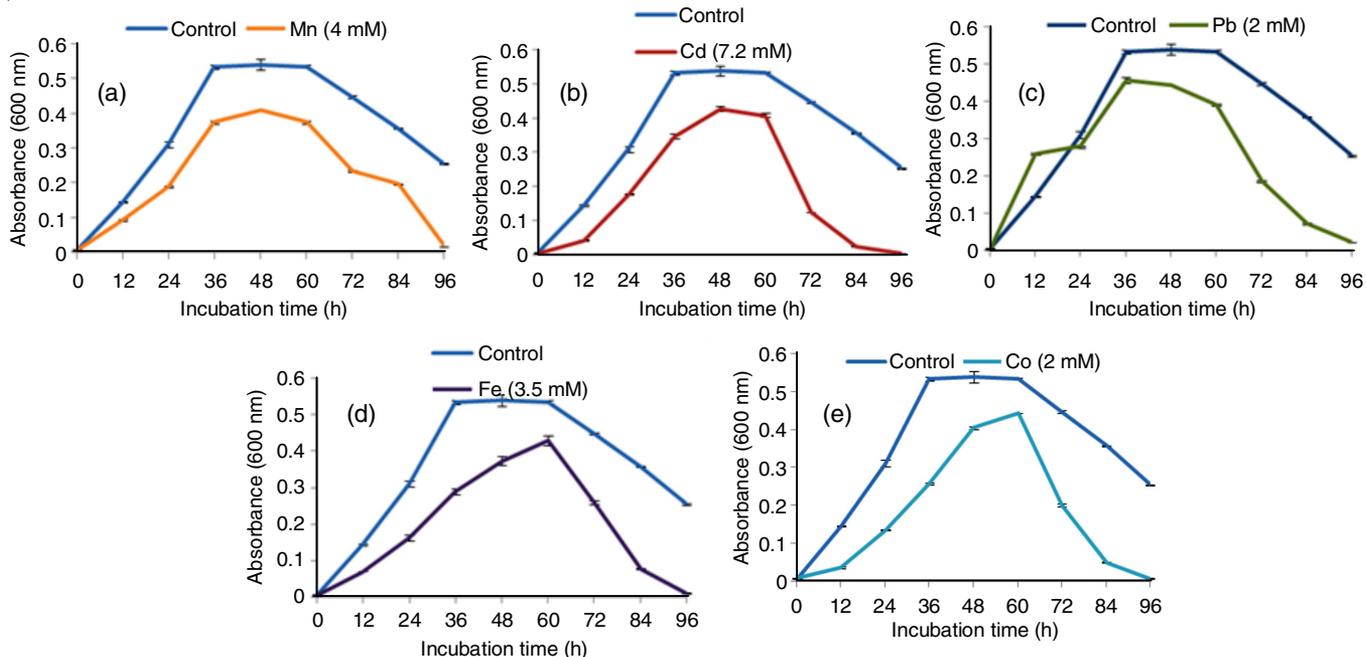


Fig. 1. Mean ( $\pm$  SE) of absorbance carried out to study the effect of different heavy metals (Mn: 4 mM; Cd: 7.2 mM; Pb: 2 mM; Fe: 3.5 mM and Co: 2 mM) on growth kinetics of *Sporosarcina luteola* (EP3) against control. Where, (n = 3), control - EP3 grown in nutrient broth without any metal

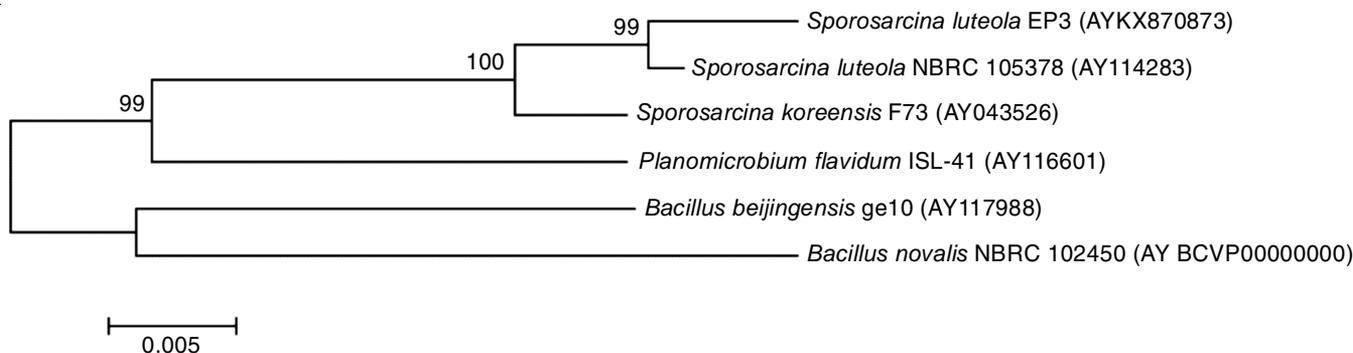


Fig. 2. Phylogenetic tree made using neighbour joining method. Bacterial isolate identified as *Sporosarcina luteola*

*Sporosarcina luteola* (EP3) exhibited growth at 20–40 °C, pH (6–9) and salt concentration as high as 3 %. These physiological characteristics could be useful in treatment of wide range of effluents and also found to be resistant to kanamycin. This property of bacteria could be useful for cloning and recombinant studies [30].

## Conclusion

This study concluded that native bacteria *Sporosarcina luteola* (EP3) present in soil isolated from nearby areas around industries have the property of cadmium resistance and ability to remediate cadmium from their environment ensuring more efficient and low cost clean-up process. Furthermore, it is potentially a cost effective method as this bacteria is abundantly present in the environment. Therefore, it can be used as potent bacteria in removing cadmium from aqueous solutions. Further studies need to be conducted with samples from different industrial areas and culture grown under varied conditions to replicate results of this study, to standardize procedures for isolation of bacteria and their role in bioremediation.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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