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Isolation and characterization of cadmium resistant bacteria from industrial areas in Patna and their potential for plant growth promoting characters

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Abstract: *Heavy metals are present in the soil, geologic and anthropogenic activities that increase the concentration of these elements to amounts that are harmful to both plants and animals. The use of metal- resistant plant growth promoting Rhizobacteria (PGPR) constitutes an important tool for the tolerance of the plants to heavy metals which helps in promoting root and shoots growth. In this study, bacterial communities from two industrial sites were characterized in order to isolate, and identify cadmium resistant bacteria. Four cadmium-resistant bacteria were isolated from two soil samples from heavy metal-contaminated sites, and were evaluated for their plant growth promoting characters. Bacterial strains showing positive results of PGPR characteristics may be utilized to augment plant growth and suppress plant diseases. The study provides an insight in the development of phytoremediation in cadmium contaminated soil.*

Keywords: *Cadmium, Bacteria, Isolation, MIC, PGPR.*

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Introduction:

Heavy metal pollution is a serious global environment problem due to the rapid development of industries as it adversely affects plant growth. Cadmium is considered to be one of the most harmful metal pollutants (Huang et al., 2019). The toxic effect of Cd pollution and its physical disturbance can influence plant survival and reproductive success (Deng et al., 2007). However, heavy metals affect the growth, morphology and metabolism of soil microbes through functional disturbance, protein denaturation or the destruction of integrity of cell membrane (Leita et al., 1995). Extensive data suggest that Cadmium is the toxic heavy metal and it is included in the black list of several international agreements established to regulate the input of cadmium into the environment (Brussels, 2006). Cadmium is toxic even at low concentration of 0.001-0.1mg L-1 and can be accumulated in the human body through the food chain through plants and diet, developing several chronic diseases (Gench et al., 2020) and the effects of cadmium toxicity.

Among microbes, bacteria, yeast and protozoans are generally the first category considered to be present

in the environment (Mahvi, 2004). Cadmium resistant bacteria have been isolated in numerous study explaining the mechanism of Cd uptake in bacteria. Moreover, different cadmium resistant bacterial species are used for the elimination of Cd, the isolation of potential Cd-resistant bacteria and characterization of Cd-removal properties are still required in order to eliminate the Cd contaminant from the environment.

Inoculation of plants with heavy metal-resistant plant growth promoting rhizobacteria (PGPR) has been found as an interesting option to improve plant performance under stress conditions. PGPR plays an important role in developing plant growth and reducing the toxicity of various heavy metals to plants. Moreover, microorganisms have large specific surface areas and high metabolic activity, which makes them particularly susceptible to the presence of heavy metals in the soils. A number of soil microorganisms have been reported effective in the remediation of heavy metals. For example, (Suksabye et al., 2016) reported that the addition of Pseudomonas aeruginosa or Bacillus subtilis to the soil could decrease the quantity of Cd in rice grains obtained from Cd contaminated soil as a result of cadmium remediation property.

The study focuses on to isolation and enumeration of cadmium resistant bacteria found in soil microflora as it is an important habitat for a diverse group of microorganisms, and to characterize the bacteria based on morphological and cultural characteristic. Minimum inhibitory concentration for each against the isolates and promoting their PGPR characteristics.

Materials and Methods:

Study area and sample collection: Two soil samples were taken from the Industrial areas in Patna i.e., Ganges area (Between 22° 30' and 31° 30' N and 73° 30' and 89° 0' E), and Bihta (Between 25° 31' and 10º 11' N and 84° 48' and 54° 05' E) area where the soils were contaminated with heavy metals like Cadmium. The collected two samples were labeled sterilized bottles and then moved to the lab and kept at 4°C during experiment. Nutrient Agar media and stock solution of 1M CdCl2 was prepared for 4 different concentrations of cadmium chloride i.e., control (0mM), 0.1mM, 0.2mM, 0.4mM and pouring was done.

Isolation and Enumeration of cadmium-resistant bacteria from different samples: Bacteria were isolated based on the enrichment culture technique in which dilution of sample upto 10-4 were prepared on the nutrient agar media and incubated at 37^{O}C for 24 hours. The growth was observed and enumeration was done in cfu/ml. All the colonies were distinctly characterized and single colony from different concentration was re-streaked on CdCl2 supplemented nutrient agar plate and again incubated at 37^{O}C for 24 hours. Growth was observed and the process was repeated until pure culture was obtained.

Characterization of Cd-Resistant Bacteria: Among the selected bacterial isolates, morphological characteristics were done through Gram's stain using Bergey's Manual of Determinative Bacteriology (Holt, 1994). Morphological characteristics i.e., colony colour, texture, elevation, margin, Gram's stain and shape under the microscope was observed.

Determination of minimum inhibitory concentration: The MIC of Cd2+ was determined to evaluate the growth in a liquid medium of isolated Cd-resistant bacteria. MIC of Cd resistant isolates were determined using absorbance method. Single colonies were inoculated into nutrient broth containing different concentrations of CdCl2 (0mM, 0.5mM, 1mM, 2mM) and shaken overnight at 37°C at 160 RPM. Growth is assessed after incubation and the MIC value is calculated.

Determination of PGPR characteristics: It was determined by the Indole-3-acetic acid (IAA) method. For evaluation and quantification of Indole-3-acetic acid production by Cd-resistant isolates, sterilized test tubes containing 5 ml of tryptone broth were taken and inoculated aseptically by taking the growth from 18 to 24 hrs culture. After that it was shaken for 24-48 hrs at 37 °C. Addition of 0.5 ml of Kovac's reagent to the growth culture and the indole ring's presence or absence was observed.

Effect on PGPR on plant growth: Soil treatment was done with selected isolates to observe the PGPR characteristic in plant growth. Once isolated, they can be screened for ability to enhance germination of chickpea. Inoculation of isolates on chickpea *(Cicer arietinum)* plant and it's growth was observed for few days.

Results:

Isolation and Enumeration of cadmium-resistant bacteria from samples: The CFU/ml for the

soil sample taken from the Ganges area in presence of cadmium was (3.8×10^2 cfu/ml) while that of the soil taken from the Bihta area showed CFU/ml of 0.6×10^2. (Table 1).

Table 1. Table showing cfu/ml of different samples at different cadmium concentrations.

	Cadmium concentration			
Samples	***0mM***	***0.1mM***	***0.2mM***	***0.4mM***
Ganges	10.4×10^2	1.7×10^2	2.1×10^2	3.8×10^2
Bihta	8.1×10^2	1.1×10^2	0.6×10^2	0.1×10^2

Cultural and morphological characteristics of selected bacterial colonies: A total of four isolates of different morphologies were isolated from two Cd-contaminated soil samples using nutrient agar plate containing 0mM, 0.1mM and 0.2mM and 0.4mM Cd2+ which was designated as isolate I, isolate II, isolate III and isolate IV. Among them, isolate 3 was fuzzy white, rough texture having *Bacillus* shape and Gram positive (Table 2). According to the Manual of Determinative Bacteriology, comparing isolate 3 with other bacteria, it showed resemblance similar to *Bacillus* species.

Table 2. Culture and morphological characteristics of different isolates.

Isolates	***Colony colour***	***Texture***	***Elevation***	***Margin***	***Gram's staining***	***Shape***
I	White	Creamy	Flat	Regular	+ve	*Coccus*
II	Off-White	Smooth	Flat	Entire	ve	*Coccus*
III	Fuzzy White	Rough	Opaque	Irregular	+ve	*Bacillus*
IV	White	Creamy	Flat	Regular	+ve	*Streptococcus*

Evaluation of Cadmium Resistance (MIC test): The MIC values of three isolates were determined. Among them, the MIC value of isolate 1, 2, 3 was 0.2mM, 0.8mM and 2mM respectively. It was determined by calculating the optical density of the isolates using a spectrophotometer and a graph was drawn based on it's observation. (Figure 1).

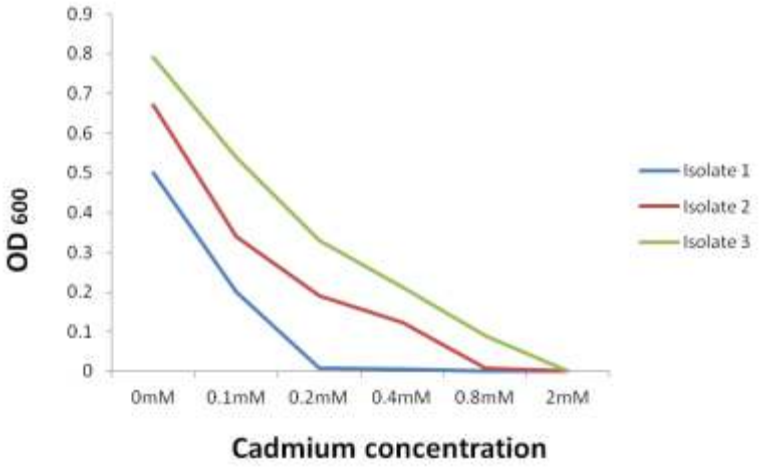

Fig. 1. Graph representing MIC of different concentrations

Determination of PGPR characteristics: The plant growth promoting characteristics were observed with the four selected bacterial isolates (Table 3). In the presence of tryptophan, all the cadmium resistant bacteria showed positive results as red indole ring was observed (Figure 2).

Table 3. Indole test determination

Isolates	*Inference*	*Result*
I	Red ring	Positive
II	Red ring	Positive
III	Red ring	Positive
IV	Red ring	Positive

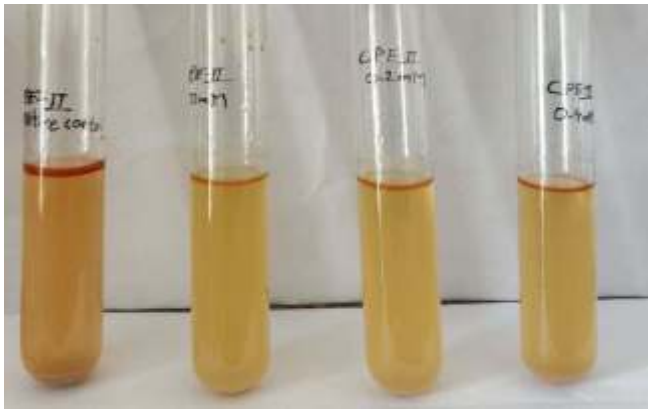

Fig. 2. Indole Test Results

Effect of PGPR on plant growth: Isolate II, III and IV significantly increased the growth of chick pea *(Cicer arietinum)* as compared to isolate I where no inoculation of PGPR was done. Treatment with all the isolates i.e., without PGPR (isolate I), isolate II isolate III and isolate IV produced maximal root and shoot length of 22.5 cm, 23.1 cm, 22.9 cm, 22.4 cm respectively after 32 days (Figure 3). Collectively these results proposed that the isolates possess the traits associated with plant growth promotion.

Fig. 3. Soil treatment with and without PGPR

Discussion:

Microorganisms have evolved the mechanisms to cope with a variety of harmful metals for their survival in the environment accumulated with such metals. Many studies use soil microflora to evaluate the ecological status of heavy metal contaminated soils (Stefanowicz et al., 2010; Gomez-Sagasti et al., 2012; Burges et al., 2015). Presence of heavy metal can influence the diversity and also change the community structure and function (Luo et al., 2019; Duan et al., 2020). In this study, we found that there was a resistant effect of cadmium concentration on bacterial diversity which showed significant outcome for PGPR properties.

Present study was carried out to investigate the isolation, characterization and application of cadmium resistant bacteria, a heavy-metal resistant bacteria whose resistance for Cd may result in better growth of plants under the stress of the heavy metal. In this study, two different soil samples were collected from the Industrial areas in Patna and the sample collected from Ganges area was found to be the one that showed highest bacterial diversity which is quite similar to the work of (Munoz et al., 2006) and minimum Cd- resistant bacterial colonies were isolated from the Bihta area. As the isolated cadmium resistant bacteria showed the PGPR characteristics, it is similar to the work done by (Yan et al., 2020) but dissimilar to the work done by (Ansari et al., 2007) as there the cadmium was used as a phosphate fertilizer which proved to be harmful to the soil texture, aeration and its porosity. Moreover, the result of isolating cadmium resistant bacteria showed that the Gram-positive *coccus* mostly inhabited in metal contaminated rhizosphere soil. Rhizosphere bacteria of

heavy metal tolerant plants have important roles in plant growth enhancement and remediation of heavy metal contaminated soils.

The result indicated that the *cocci* species isolates may have a great possibilities in the application of metal resistant bacteria for bioremediation which offers appealing approach to the role of soil microorganisms in phytoremediation. The optical density was decreased with the increase of Cd concentration which indicated the toxic effect of Cd on the growth of bacteria. At each concentration of CdCl2 (0mM, 0.5mM, 1mM, 2mM) the red indole ring was formed which proved that the indole test was positive which inferred the presence of PGPR characteristics. Results from our study indicate that PGPR isolates applied as soil treatment significantly increased plant growth and development. (Fatima et al., 2009) also demonstrate that germination rate, root and shoot growth of plant were increased by IAA and PGPR. The application of cadmium-resistant bacteria is a promising approach as it not only detoxifies or bioremediates the soil but also improves the plant growth by producing plant growth hormones such as Indole-3-acetic acid (IAA), gibberellins etc. (Wang et al., 2016).

From this study, it can also be inferred that these populations of bacteria actually detoxified cadmium through some sort of sequestration or precipitation mechanism. The use of these microorganisms is also a boon as it acts as a bio sorbent of heavy metals from contaminated soil. The isolation of microorganisms that are both metal-resistant and efficient in producing plant growth-promoting compounds could prove applicable as inocula for processes of re-vegetation and phyto-remediation. Therefore our study provides a baseline for bacterial communities in the cadmium polluted soils.

Conclusion:

During present study cadmium resistant bacteria was isolated and characterized. It was noticed that bacteria with resistance to cadmium can influence better growth to the plants. The bacteria can also help in remediation of contaminated sites and reduce cadmium phytotoxicity. This study shows the ability of cadmium-resistant bacteria to produce plant growth promoting hormones in the rhizosphere and other PGP activities as it not only detoxifies or bioremediates of the soil but also improves the plant growth by producing plant growth hormones such as Indole-3-acetic acid (IAA), gibberellins etc (Wang et al., 2016). Additionally, more studies are needed to elucidate the molecular mechanisms behind cadmium resistance in these genera.

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