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HEAVY METAL-TOLERANT RHIZOBACTERIAL ISOLATION FROM CONTAMINATED SOIL SAMPLES OF PARICCHA, BUNDELKHAND

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ABSTRACT: Heavy metal contamination poses a severe risk to soil health and crop productivity, particularly in industrially impacted regions. In this study, agricultural soils surrounding the Pariccha Thermal Power Plant, Bundelkhand, Uttar Pradesh, were sampled to isolate cadmium (Cd)-tolerant rhizobacteria from three different sites. A total of 35 bacterial isolates were obtained, among these, three (VYS1, RGB2, and RGB4) demonstrated strong Cd tolerance up to 500 µg/mL. These isolates were further evaluated for plant growth-promoting rhizobacteria (PGPR) traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia generation. All three isolates exhibited positive activity, with RGB4 showing the highest phosphate solubilization and ammonia production, while RGB2 produced comparatively higher IAA levels. The findings highlight the dual functionality of indigenous rhizobacteria from contaminated soils—tolerance to cadmium stress and expression of PGPR traits—suggesting their potential as preliminary candidates for bioremediation and sustainable agriculture. Future work should focus on molecular identification and in-planta validation to confirm their bioinoculant potential.

INTRODUCTION: Heavy metal pollution of soils has become a pressing environmental concern worldwide, particularly in regions experiencing rapid industrialisation and energy generation. Among these pollutants, cadmium (Cd) is considered one of the most hazardous due to its non-biodegradable nature, high mobility in soils, and entry into food chains [1].

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Cd disrupts plant physiological processes, reduces crop yields, and poses severe risks to human health when ingested through contaminated food [2]. Thermal power plants are recognized as major contributors to heavy metal contamination, as fly ash deposits and industrial effluents introduce Cd and other metals into surrounding ecosystems.

This issue is especially critical in the Bundelkhand region of India, a semi-arid zone with fragile agroecosystems. Studies conducted near the Pariccha Thermal Power Plant in Jhansi have confirmed elevated levels of Cd, Pb, and Cr in soils and vegetables, highlighting the environmental hazards of industrial emissions [3, 4].

Plant growth-promoting rhizobacteria (PGPR) represent a diverse group of beneficial microbes that colonise plant roots and enhance growth through nutrient solubilization, phytohormone production, nitrogen fixation, and stress alleviation. Notably, certain PGPR strains exhibit tolerance to heavy metals, enabling them to survive in contaminated soils while simultaneously promoting plant growth [5, 6].

This dual functionality positions Cd-tolerant PGPR as valuable candidates for integrated bioremediation and sustainable agriculture strategies. By immobilising or bioaccumulating Cd, these microbes reduce its bioavailability and enhance plant resilience under stress conditions [7]. Previous research has demonstrated the potential of PGPR consortia in mitigating heavy metal toxicity. Cd-tolerant strains, including Bacillus and Pseudomonas, have been shown to improve plant biomass and nutrient uptake under Cd stress [8]. PGPR-assisted phytoremediation has also been identified as a promising approach to reduce Cd accumulation in edible plant parts, thereby improving food safety [9]. However, microbial diversity and tolerance mechanisms vary with soil type, contamination history, and climatic conditions, necessitating localised investigations.

Exploring indigenous Cd-tolerant PGPR from contaminated soils is therefore crucial for developing sustainable bioremediation strategies. These microbes not only detoxify polluted soils but also enhance crop productivity under stress. Traits such as phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia release directly contribute to plant growth and nutrition. When applied as microbial consortia, PGPR often act synergistically, producing superior plant responses compared to single inoculants [10]. Leguminous crops such as moong bean (*Vigna radiata*) are particularly suitable for evaluating PGPR efficacy, given their symbiotic associations with rhizobacteria and importance in sustainable cropping systems. Thus, the isolation and characterisation of Cd-tolerant PGPR from industrially affected soils are essential steps toward eco-friendly solutions that integrate microbial bioremediation with agricultural productivity, addressing the global challenge of heavy metal pollution.

METHODOLOGY:

Sample Collection and Soil Characterisation: Soil samples were collected from agricultural fields surrounding the Pariccha Thermal Power Plant, Bundelkhand, Uttar Pradesh, India. Three distinct sites were selected within a 3 km radius of the plant, each representing agricultural land impacted by fly ash deposition and effluent runoff. Sampling was performed at a depth of 10–15 cm using sterile augers. At each site, three subsamples were pooled to form a composite sample (~500 g), homogenised, air-dried, and sieved through a 2 mm mesh before analysis [11]. The physicochemical parameters pH, Electrical Conductivity (EC), Organic Carbon, Soil texture, Moisture content, Heavy metal concentration were also determined by following the standard protocols.

Isolation of Cd-tolerant Rhizobacteria: Rhizobacteria were isolated using the serial dilution and spread plate method. One gram of soil was suspended in 9 mL of sterile distilled water and serially diluted up to 10^{-6}. Aliquots (0.1 mL) of appropriate dilutions were plated on nutrient agar (NA) and tryptic soy agar (TSA) supplemented with cadmium chloride ($CdCl_2$, 100 µg/mL) to selectively enrich Cd-tolerant strains [11]. Plates were incubated at 28 ± 2 °C for 48–72 h. Distinct colonies were selected based on morphological characteristics (size, shape, color, margin, elevation) and purified by repeated streaking on fresh agar plates [12].

Cadmium Tolerance Screening of Rhizobacterial Isolates: Tolerance was assessed on nutrient agar plates containing $CdCl_2$ concentrations ranging from 100–500 µg/mL in 100 µg/mL increments. Inoculum density was standardized to OD600 = 0.1. Growth was defined as visible colony formation after 72 h incubation, confirmed in triplicate assays. Scoring criteria: **++** = strong growth (>75% streak coverage), **+** = moderate growth (25–75% coverage), **–** = no growth (<25% coverage).

Screening of Plant Growth-promoting Rhizobacteria (PGPR) Activities: Cd-tolerant isolates were further evaluated for PGPR traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia generation, using conventional microbiological assays.

Phosphate Solubilization: Isolates were inoculated on Pikovskaya's agar medium containing insoluble tricalcium phosphate. Plates were incubated at 28 ± 2 °C for 7 days. Clear halo zones around colonies indicated phosphate solubilization [13, 11].

Indole-3-acetic Acid (IAA) Production: IAA production was quantified using the colorimetric method. Bacterial cultures were incubated at 28 ± 2 °C in nutrient broth supplemented with 0.1% L-tryptophan for 72 h under shaking conditions. Cultures were centrifuged, and 1 mL of supernatant was mixed with 2 mL of Salkowski reagent (containing 1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% perchloric acid). The development of a pink color indicated IAA production [14, 12].

Ammonia Production: Ammonia production was assessed by inoculating isolates in peptone water broth and incubating at 28 ± 2 °C. After incubation, 1 mL of culture supernatant was treated with Nessler's reagent. The appearance of a yellow-brown coloration indicated ammonia production, which was quantified spectrophotometrically at 450 nm [14, 16].

RESULTS:

Soil Properties and Cadmium Concentrations: Soils were slightly acidic (pH 6.2–6.8), with moderate EC (0.9–1.4 dS/m), low organic carbon (0.35–0.52%), sandy loam to loam texture, and moisture content of 8–12%. Cadmium concentrations were evaluated site wise and found as follows:

- Site 1 (VYS group): 4.8 ± 0.3 mg/kg
- Site 2 (TPP group): 3.9 ± 0.2 mg/kg
- Site 3 (RGB group): 5.2 ± 0.4 mg/kg

TABLE 1: SOIL CHARACTERISTICS AND MICROBIAL ISOLATION SUMMARY AT SAMPLING SITES NEAR PARICCHA THERMAL POWER PLANT

Site Code	Location Descriptor	pH	EC (dS/m)	OC (%)	Texture	Moisture (%)	Cd (mg/kg)	Isolates Obtained	Cd-tolerant Isolates
VYS (Site 1)	Adjacent to fly ash deposition zone	6.2	1.4	0.52	Sandy loam	12	4.8 ± 0.3	12	3
TPP (Site 2)	Near effluent channel	6.8	1.1	0.35	Loam	9	3.9 ± 0.2	9	2
RGB (Site 3)	Agricultural field downstream	6.7	0.9	0.41	Sandy loam	8	5.2 ± 0.4	14	7

Isolation of Cd-tolerant Rhizobacterial Strain: Out of the three agricultural soil samples taken in the vicinity of the Pariccha Thermal Power Plant, Bundelkhand, 35 bacterial isolates were obtained using serial dilution and plating methods. Sample 1 yielded 12 isolates, Sample 2 yielded 9 isolates, and Sample 3 yielded 14 isolates. A series of streaking was used to purify all the isolates and the isolates were kept in nutrient agar slants awaiting cadmium tolerance screening and plant growth-promoting activity activities.

Cadmium Tolerance Screening of Rhizobacterial Strains: Cadmium tolerance of the rhizobacterial isolates was tested on nutrient agar plates supplemented with $CdCl_2$ concentrations ranging from 100 to 500 µg/mL. Growth responses were recorded after 72 hours of incubation. All isolates showed strong growth at 100 µg/mL, while tolerance decreased with increasing concentrations. Among the VYS group (Sample 1), isolates VYS-1 tolerated cadmium up to 500 µg/mL, while VYS-4 grew up to 300 µg/mL and VYS-7andVYS-9only up to 100 µg/mL. In the TPP group (Sample 2), isolate TPP-3 tolerated cadmium up to 300 µg/mL, whereas TPP-8 and TPP-9 grew only at 100 µg/mL. In the RGB group (Sample 3), isolates RGB-2 and RGB-4 tolerated cadmium up to 500 µg/mL, RGB-1 up to 400 µg/mL, while RGB-6 and RGB-7 grew only at 100 µg/mL. The isolates VYS-1, RGB-2, and RGB-4 demonstrated tolerance up to 500 µg/mL Cd, making them the most resistant strains among the tested collection.

TABLE 2: GROWTH RESPONSE OF SELECTED ISOLATES AT DIFFERENT CD CONCENTRATIONS

Isolates	Cadmium concentration (µg/mL)				
	100	200	300	400	500
VYS-1	++	++	+	+	+

VYS-4	++	++	+	-	-
VYS-7	++	-	-	-	-
VYS-9	++	-	-	-	-
TPP-3	++	+	+	-	-
TPP-8	++	-	-	-	-
TPP-9	++	-	-	-	-
RGB-1	++	+	+	+	-
RGB-2	++	+	+	+	+
RGB-4	++	++	+	+	+
RGB-6	++	-	-	-	-
RGB-7	++	-	-	-	-

Legend: ++ = strong growth; + = moderate growth; – = no growth

PGPR Activity Screening: He three cadmium-tolerant isolates **(**VYS-1, RGB-2, and RGB-4**)** were evaluated for their plant growth-promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia generation. All three isolates demonstrated positive activity in each assay. On Pikovskaya's agar, the isolates produced distinct halo zones around colonies, confirming phosphate solubilization. The solubilization index was highest for RGB-4, followed by RGB-2 and VYS-1. In the IAA production assay, all isolates developed a pink coloration with Salkowski's reagent, indicating IAA synthesis, with RGB-2 showing comparatively higher absorbance values. In the ammonia production assay, all three isolates produced yellow to brown color with Nessler's reagent, confirming ammonia generation, with VYS-1 showing moderate activity and RGB-4 the strongest response.

TABLE 3: PGPR ACTIVITIES OF CADMIUM-TOLERANT ISOLATES

Isolate	Phosphate Solubilization	IAA Production	Ammonia Production
VYS-1	+ (moderate halo)	+ (positive)	+ (moderate)
RGB-2	+ (clear halo)	++ (strong)	+ (positive)
RGB-4	++ (large halo)	+ (positive)	++ (strong)

Legend: ++ = strong activity; + = positive activity

DISCUSSION: The activity screening of three cadmium-tolerant isolates (VYS 1, RGB 2, and RGB 4) demonstrated their ability to promote plant growth through phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia generation. All isolates tested positive in these assays. Distinct halo zones formed around colonies on Pikovskaya's agar confirmed phosphate solubilization, with RGB 4 exhibiting the highest solubilization index, followed by RGB 2 and VYS 1. In the IAA assay, all isolates produced a pink coloration with Salkowski reagent, indicating IAA synthesis, with RGB 2 showing relatively higher absorbance. Ammonia production was confirmed by yellow-brown coloration with Nessler's reagent, with RGB 4 displaying the highest activity and VYS 1 moderate activity.

Isolation and characterization of cadmium-tolerant rhizobacteria from agricultural soils near the Pariccha Thermal Power Plant revealed acidic soil conditions (pH 6.2–6.8), which are known to increase cadmium bioavailability and solubility [17]. Similar acidic conditions in Indian agricultural soils have been associated with enhanced heavy metal mobility and ecological risk [18]. Acidic soils impose stress on microbial communities but also provide niches for metal-tolerant strains. A total of 35 isolates were obtained, with VYS 1, RGB 2, and RGB 4 tolerating up to 500 µg/mL Cd, whereas isolates from the TPP group survived only up to 300 µg/mL. This variation aligns with previous findings that isolates from highly contaminated soils exhibit greater resistance than those from moderately affected soils [19]. Comparable studies have reported tolerance levels of 400–600 µg/mL in rhizobacteria, placing the present isolates at the higher end of global cadmium resistance [20].

The PGPR activities of these isolates further enhance their potential application. RGB 4 exhibited the strongest phosphate solubilization, consistent with earlier reports highlighting the importance of phosphate-solubilising bacteria in mobilising insoluble phosphorus to improve nutrient uptake [12].

The ability of RGB 4 to maintain high activity under cadmium stress, in contrast to reports of reduced solubilization under heavy metal conditions [22], underscores its robustness. All isolates produced IAA, with RGB 2 showing the highest activity. IAA production by PGPR is a well-established mechanism for promoting root elongation and nutrient uptake [23]. Although heavy metal stress has been shown to reduce IAA production in bacteria [24], the present isolates retained activity, indicating resilience.

Ammonia production was observed in all isolates, with RGB 4 being the most active. Ammonia release enhances nitrogen availability in the rhizosphere, supporting plant–microbe associations [24]. The uniform ammonia production observed here contrasts with previous studies where variability among cadmium-tolerant strains was reported [22], suggesting that these isolates are particularly effective in balancing stress tolerance with nutrient-enhancing traits.

Overall, the comparative analysis of VYS 1, RGB 2, and RGB 4 demonstrates their dual functionality: high cadmium tolerance (up to 500 µg/mL) and strong PGPR activity. This bifunctional nature qualifies them as promising bioinoculants for sustainable agriculture and bioremediation. Their performance is comparable to, and in some cases exceeds, previously reported isolates, such as those enhancing soybean growth under cadmium stress [20]. These findings highlight the potential of indigenous PGPR strains to improve crop productivity in polluted soils of Bundelkhand, integrating microbial biotechnology with eco-friendly remediation strategies.

CONCLUSION: This study isolated and characterized cadmium-tolerant rhizobacteria from soils near the Pariccha Thermal Power Plant, Bundelkhand. Three isolates (VYS1, RGB2, RGB4) demonstrated tolerance up to 500 µg/mL Cd and expressed key PGPR traits, including phosphate solubilization, IAA production, and ammonia generation. These results suggest that indigenous microbes in contaminated soils can simultaneously withstand heavy metal stress and support plant growth-promoting functions. Given the qualitative nature of PGPR assays and the absence of molecular identification or in-planta validation, the present work should be considered preliminary. Future studies must focus on 16S rRNA sequencing, quantitative PGPR measurements, and greenhouse/field trials to confirm bioinoculant potential. Despite these limitations, the study provides site-specific evidence of microbial adaptation to cadmium contamination and establishes a foundation for further bioremediation research in Bundelkhand.

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CONFLICT OF INTEREST: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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