Chromium Resistance and Plant-Growth Promotion by a Pseudomonas sp. Isolated from Tannery Effluent

Sania Sohail[1], Sowmya Kumar[2]
Department of Life Sciences, Mount Carmel College (Autonomous), Bengaluru, Karnataka, India

***Abstract*—Three bacterial colonies were isolated from tannery effluent collected from Negalkeni Village, Pallavaram, Chennai. All isolates were tested for their ability to grow in Cr(VI)-spiked nutrient media at increasing concentrations (50, 100, and 150 mg/L). Of the three, only one strain was able to grow in Cr(VI)-supplemented medium for 5 days. This strain was identified as a *Pseudomonas* species based on primary and secondary biochemical tests. Additionally plant-growth-promoting (PGP) activities, including phosphate solubilization, siderophore production, and HCN production, were also tested and found to be positive for this isolate.**

***Index Terms*—Cr (VI), phosphate solubilization, siderophore, HCN, IMViC**

I. INTRODUCTION

Chromium contamination has become a serious environmental issue, especially in regions located near industrial areas. Leather tanning industries employ chromium salts in a process called chrome tanning which results in the hexavalent chromium to be discharged in the effluents. (Sánchez et.al.2021) When this wastewater enters the environment without proper treatment, it contaminates soil and water and poses long-term risks. Among all the forms of chromium found in nature, hexavalent chromium [Cr(VI)] is the most harmful. (Tagliari) It dissolves easily in water, spreads quickly, and can enter living cells where it causes oxidative stress, DNA damage, and even cancer. Because of its toxicity and mobility, Cr(VI) is considered one of the most dangerous industrial pollutants. Mishra, S., & Bharagava, R. N. (2016)

Conventional methods used to treat chromium-rich wastewater—such as precipitation, ion exchange, or membrane-based systems—can be effective, but they are also costly, energy-demanding, and generate large amounts of secondary chemical sludge chemical waste.(Khulbe.et.al 2018)(Xie,2024). One promising approach is microbial detoxification, where naturally occurring bacteria in heavy metal expose fields or waterbodies convert toxic Cr(VI) into trivalent chromium [Cr(III)], a far less toxic and less mobile form.(Mishra et.al 2021)This biological conversion is especially attractive because it does not require harsh chemicals and can work well even at low chromium concentrations. Therefore several bioremediation techniques like phytoremediation and bioaccumulation have been carefully employed to remove chromium toxicity as well as remain an active area of research.

In recent years, attention has shifted toward bacteria that can do more than just detoxify heavy metals but work in conjugation with plants that can help immobilize heavy metals by phytoremediation. However some plants have shown the ability to tolerate heavy metals but it significantly affects their growth making it difficult for plants to survive. Silambarasan et al., 2019a)

Emerging evidence suggests that PGPB inoculation significantly enhances plant growth, photosynthetic activity, stress resilience, and overall phytoremediation capacity in heavy metal–contaminated soils. (Liu et al., 2019)PGP bacteria help counter these effects by producing molecules like indole-3-acetic acid (IAA), releasing siderophores to improve iron availability, solubilizing phosphate, producing ammonia, and synthesizing ACC deaminase that helps plants cope with stress. Ouledali et al., 2019 A bacterial strain that can both detoxify Cr(VI) and promote plant growth is particularly valuable for restoring polluted soils through environmentally friendly approaches such as phytoremediation.

Despite this potential, there is still limited information about bacteria that combine strong Cr(VI)

detoxification with robust PGP traits, especially those directly isolated from industrial discharge, where metal concentrations are extremely high. Identifying and studying these strains can lead to effective solutions for rehabilitating contaminated areas.

The present study focuses on the isolation and characterization of a Cr(VI)-detoxifying, plant growth-promoting bacterial strain obtained from industrial effluent. The study examines the strain's ability to tolerate and reduce Cr(VI), evaluates its biochemical and molecular characteristics, and explores its PGP properties. This work contributes to developing practical, sustainable approaches for managing chromium pollution and restoring degraded environments.

II. MATERIALS AND METHOD

2.1 Collection of samples

Leather industry effluents were collected from the surrounding areas of Negalkeni village, Pallavaram, Chennai and stored in refrigerator for avoiding further contamination in the wastewater.

2.2 Isolation of Cr resistant bacteria

For isolating chromium-resistant bacterial strains, 1 g of sample was subjected to serial dilution and spread onto PYE (Peptone–Yeast Extract) agar plates amended with 100 µg/mL Cr(VI) in the form of potassium dichromate ($K_2Cr_2O_7$). Plates were incubated at 30°C for 24 hours, after which bacterial growth was examined. Individual colonies showing growth were transferred using a sterile inoculation loop and re-streaked onto fresh PYE agar plates containing the same concentration of Cr(VI) (100 µg/mL) to obtain purified isolates.(Das and Mishra 2010)

The chromium tolerance of the isolates was evaluated by growing them in PYE medium amended with increasing concentrations of Cr(VI) (50, 100, 150 and 250 mg/L). Isolates that showed noticeable growth at 100 mg/L Cr(VI) after 48 hours of incubation at 30°C were classified as chromium-resistant. Only one isolate was able to grow under these conditions, and this strain was chosen for subsequent analyses.

2.3 Microbiological assay

A series of biochemical assays were performed on the bacterial isolate, including oxidase, Voges–Proskauer (V-P), catalase, citrate utilization, indole production, triple sugar iron (TSI) reaction, and the methyl red test. All tests were carried out following established protocols and standard biochemical identification guidelines.

PGP trait of the isolate under Cr stress

2.4 Phosphate solubilisation

Pikovskaya agar plates were used with certain modifications for qualitative screening of phosphate solubilizing activity of bacterial isolates. The reaction was considered positive when a clear halo surrounding the spot inoculated bacterial colonies were observed after 7 days of incubation at 30°C (Nautiyal 1999).

2.5 Siderophore production

Chrome azurol sulfonate (CAS) agar solid medium was used to screen siderophore production by bacterial isolates. 60.5 mg CAS was dissolved in 50 ml water and mixed with 10 ml iron (III) solution (ImM $FeCl3.6H_2O$, 10mM HCI) to prepare 1 litre of blue agar. This solution was slowly added to 72.9 mg HDTMA dissolved in 40 ml water and the resultant dark blue liquid was autoclaved. Each plate was poured with 30 ml of blue agar (Gopal et al 2009) The reaction was considered positive when an orange halo surrounding the bacterial colony appeared due to removal of iron from CAS by the siderophore (Bruins et al 2000).

2.6 HCN Production

HCN production by the bacterial isolate was tested qualitatively following the method of Bakker and Schippers (1987). The bacterial isolate was streaked on King B medium amended with glycine at 4.4 g/l. In the upper lid of the petri plate, sterile filter paper saturated with picric acid solution (2.5 g picric acid, 12.5 g of $Na_2C\ O_3$. 1000 ml of distilled water) was placed. The dishes were sealed with parafilm and incubated at 28°C for 48 hours. A change of colour of the filter paper from yellow to light brown, brown or reddish brown was recorded as weak (+), moderate (++) or strong (+++) reaction respectively.

III. RESULTS

3.1 Screening of microorganisms for chromium resistance

The chromium tolerance of the isolates was assessed on nutrient agar plates amended with increasing concentrations of Cr(VI) (20–200 mg/L). Among the recovered colonies, three demonstrated the ability to grow at 100 mg/L Cr(VI). Of these, one isolate exhibited robust growth even at 200 mg/L Cr(VI). This

high-tolerance strain was selected for subsequent experiments.

3.2 Morphological and biochemical profile

The isolate produced circular, moist colonies with a distinct blue–green pigmentation and an opaque appearance on nutrient agar. Gram staining revealed Gram-negative rods that were non-spore forming. Motility tests were positive, indicating that the organism is motile under the conditions tested.

Biochemical profiling showed that the strain was oxidase positive, catalase positive, citrate positive, and indole positive, with acid, gas, and H_2S production observed in TSI medium. In contrast, both the methyl red and Voges–Proskauer tests were negative. Collectively, the colony morphology, pigment production, and oxidative biochemical traits strongly indicate that the isolate is most likely a member of the genus *Pseudomonas*.

Test	Result
Colony morphology	Circular; moist; opaque
Pigmentation	Blue–green
Gram reaction and cell shape	Gram-negative rods
Spore formation	Absent
Motility	Positive
Oxidase	Positive
Catalase	Positive
Methyl red (MR)	Negative
Voges–Proskauer (VP)	Negative
Citrate utilization	Positive
Indole production	Positive
Triple Sugar Iron (TSI): Production	Positive
TSI: H_2S production	Positive
TSI: Gas production	Positive

Table 1. Summary of morphological and biochemical characteristics of the isolate

3.3 Phosphate solubilisation

Phosphate solubilization ability was assessed to determine the plant-growth-promoting potential of the chromium-resistant isolate. When grown on modified Pikovskaya's agar, the strain produced a distinct clear halo zone around the colonies after 7 days of incubation at 30 °C, indicating positive phosphate-solubilizing activity.

The ability of the isolate to solubilize phosphate is particularly important in metal-contaminated soils, where elevated Cr(VI) levels often interfere with nutrient uptake, especially phosphorus. Such nutrient limitation commonly restricts plant growth in polluted environments.(Gupta &Kumar)

3.4 Siderophore production

The ability of the isolate to secrete siderophores was examined on Chrome Azurol Sulfonate (CAS) medium. The development of a clear orange zone around the colony indicated that the strain actively released iron-chelating compounds capable of removing Fe^{3+} from the CAS dye complex. This confirmed a positive siderophore response.

Siderophore secretion is an important plant-growth-promoting attribute because, although iron is abundant in soils, it usually occurs in forms that are poorly soluble and difficult for plants to absorb. (Roskova et al., 2022) Microbial siderophores convert this unavailable iron into bioaccessible forms, thereby improving plant nutrition and supporting growth, especially under metal-stressed conditions where nutrient availability is further reduced.

3.5 HCN Production

Hydrogen cyanide (HCN) production was evaluated to assess the biocontrol potential of the chromium-resistant isolate. The strain tested positive for HCN synthesis, indicating its ability to release this volatile antimicrobial compound. HCN is widely recognized as one of the key metabolites produced by plant-associated *Pseudomonas* spp., where it contributes to the suppression of soil-borne pathogens, particularly those responsible for root-rot diseases. By inhibiting the growth of competing or harmful microorganisms in the rhizosphere, HCN-producing PGPR enhance plant health and provide a protective advantage to the host plant.(Deshwal 2013)

Table 2. Plant-growth-promoting traits of the chromium-resistant *Pseudomonas* sp. isolate

PGP Trait	Method Used	Observation	Result
Phosphate solubilization	Modified Pikovskaya's agar (7 days, 30 °C)	Clear halo zone around colonies	Positive
Siderophore production	CAS agar assay	Orange zone around colony (Fe^{3+} chelation)	Positive
HCN production	Bakker & Schippers method (1987)	Color change indicating HCN release	Positive

IV. CONCLUSION

In this study, we isolated a chromium-tolerant *Pseudomonas* strain from industrial effluent. The isolate grew well in the presence of Cr(VI) and showed several plant-growth-promoting traits. It could solubilize phosphate and produced siderophores and HCN, all of which can support plant growth in soils affected by heavy metals.

This work was carried out under controlled laboratory conditions. The next phase should involve testing the strain in soil, pot trials, and field systems, where many external factors influence bacterial survival and activity. Using this isolate as part of a microbial consortium may also improve its stability and overall performance. Understanding how such bacteria help plants take up or avoid chromium will further strengthen the use of PGPB-assisted phytoremediation in different environments. Field trials and multi-strain approaches will help confirm how effective this *Pseudomonas* isolate is for chromium cleanup and plant growth support.

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