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SCREENING OF BACTERIAL STRAINS FOR HEAVY METAL STRESS TOLERANCE, ANTIMICROBIAL POTENTIAL, AND OPTIMUM GROWTH AT A WIDE RANGE OF pH AND TEMPERATURES

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Abstract

The growing global population calls for sustainable and eco-friendly methods to enhance food production. One such solution is the use of plant growth-promoting rhizobacteria (PGPR), which can improve both plant and soil nutrient status, thereby boosting crop yields. In this study, PGPR were isolated from the Lower Dir region and assessed for their antibacterial activity against two phytopathogens, *Ralstonia solanacearum* and *Xanthomonas citri*, as well as their tolerance to heavy metals. The growth of these PGPR was evaluated across a wide range of pH levels and temperatures. Of the 59 isolates, 15 demonstrated antibacterial activity against *Ralstonia solanacearum*, and 13 showed resistance to *Xanthomonas citri*. The isolate DJ 15 exhibited the largest inhibition zones against both pathogens, measuring 33.33 mm for *Ralstonia solanacearum* and 18.16 mm for *Xanthomonas citri*. In terms of heavy metal tolerance, the highest growth rate (OD) was observed in the presence of lead (2.05), followed by nickel (1.61) and cadmium (1.38). Growth rates were also monitored at varying pH levels, with the highest recorded at pH 6 after three hours (0.22), and at pH 7 after 24 hours (1.38). Temperature variation also affected growth, with the highest growth rates at 50ºC (0.33) and 30ºC (1.39) after three and 24 hours, respectively. These findings highlight the potential of PGPR as effective biocontrol agents capable of tolerating heavy metals and combating plant pathogens, making them valuable for sustainable agricultural practices.

Key words: PGPR, Antimicrobial potential, Phytopathogens, Incubation, Phytoremediation.

Introduction

Agricultural stability and sustainability are critical for meeting the growing global demand for food, especially as the population continues to increase (Becker & Fanzo, 2023). Over the past few decades, industrialization and urbanization have had significant effects on the natural resources used in food production (Feng *et al*., 2023). As the global population is projected to reach 7.8 billion by mid-2020, a rise from 7 billion in just a few years, this has created substantial challenges for world food security (Gu *et al*., 2021; Islam, 2024). Moreover, in recent decades, approximately 44.2 million people have lived below the poverty line due to limited access to basic food (Rabbit *et al*., 2023). In regions like South Asia and Africa, about 75% of the population relies solely on agriculture for their livelihood (Khan *et al*., 2023). Over the last five decades, advancements in agricultural techniques have significantly increased food production, diversified crops, and enabled year-round food cultivation, leading to greater food availability and resilience to seasonal changes (Hemathilake & Gunathilake, 2022).

However, food production and food security are increasingly threatened by environmental stresses, including both biotic (such as pests, diseases, and weeds) and abiotic stresses (such as climate change, drought, and extreme weather). These stresses reduce crop yields and complicate the assurance of a reliable food supply. To address these challenges, it is crucial to use natural resources efficiently and adopt sustainable farming practices, such as soil conservation, water management, and environmentally friendly techniques. This approach will not only boost food production but also contribute to long-term food security for future generations (Subedi *et al*., 2023; Verma *et al*., 2024). Globally, climate change has a profound effect on agriculture, impacting food production directly by altering agro-ecological conditions and indirectly by disrupting growth and income circulation (Alexandridis *et al*., 2023). These environmental shifts have led to changes in plant diversity, elevated atmospheric CO_2 levels, increased temperatures, and altered precipitation patterns (Kabir *et al*., 2023).

One of the most serious issues exacerbated by climate change is heavy metal contamination in soil due to rapid industrialization, which accelerates the accumulation of hazardous metals like mercury, lead, cadmium, and chromium. These metals pollute both soil and water, negatively affecting microorganisms and reducing crop yields (Angon *et al*., 2023; Naz *et al*., 2022). The toxicity of these metals varies depending on their concentration, and their presence in the environment poses a significant threat to agriculture. Bioremediation, the process of using

microorganisms to remove or neutralize these toxic metals, is a promising solution to this problem. Specifically, plant growth-promoting rhizobacteria (PGPR) have demonstrated potential in mitigating metal toxicity. These microorganisms can immobilize, mobilize, or transform heavy metals into less harmful forms, thereby improving soil health and promoting plant growth (Wrobel *et al*., 2023; Zhou *et al*., 2023).

Microorganisms play a vital role in enhancing nutrient availability to plants, offering an eco-friendly strategy to boost crop production. Plant-microbial interactions occur when plants provide a habitat for microorganisms, and in turn, these microbes aid in various metabolic processes. In response to plant root secretions, microorganisms produce specific products that enhance plant growth, development, pathogen resistance, and tolerance to environmental stresses. Plant growth-promoting rhizobacteria (PGPR) are particularly beneficial in this regard, as they can grow within, on, or around plant tissues and promote growth through several mechanisms. PGPR improve plant growth by enhancing nutrient uptake, activating plant hormones, and protecting plants from pathogens (Mathur & Ulanova, 2023; Jain *et al*., 2024).

In addition to their growth-promoting properties, PGPR are increasingly recognized for their potential as bio-pesticides. These bacteria produce antimicrobial metabolites, such as antibiotics, enzymes that degrade fungal cell walls, and hydrogen cyanide, which help control plant pathogens. Though the exact mechanisms are still not fully understood, these antimicrobial properties make PGPR effective against various pests and diseases (Sharma *et al*., 2023). Furthermore, PGPR function as biofertilizers by solubilizing essential nutrients, including both macro- and micronutrients, or by producing growth regulators that support plant development. These actions improve plant health, enhance productivity, and make PGPR valuable tools in sustainable agriculture (Chaudhary *et al*., 2024).

PGPR also play an important role in helping plants overcome both biotic and abiotic stresses, which is crucial for sustainable farming practices. They help plants resist environmental stresses such as drought, temperature fluctuations, and soil salinity, as well as biotic stresses, including pathogen attacks. By acting as bio-pesticides, biofertilizers, and stress control agents, PGPR improve crop yields and reduce the environmental impact of traditional agricultural practices. Current research focuses on identifying bacterial strains capable of tolerating heavy metal stress and protecting plants from pathogens, further enhancing the potential of PGPR in agriculture (Karnwal *et al*., 2023; Anand *et al*., 2023). This work aims to investigate and screen bacterial strains with the potential to tolerate heavy metal stress, exhibit antimicrobial properties, and grow optimally under a wide range of pH and temperature conditions. The increasing environmental challenges, such as soil contamination with heavy metals and climate-related stresses, pose significant risks to agricultural productivity and food security. By identifying and characterizing PGPR strains that can thrive in these adverse conditions, this research seeks to contribute to the development of sustainable agricultural practices. These bacteria can potentially be used as bio-pesticides and biofertilizers to enhance crop resilience, improve nutrient availability, and mitigate the effects of heavy metal toxicity, ultimately promoting a healthier and more productive farming environment.

Material and Methods

Sample collection and isolation: The present study was conducted to determine the growth effects of bacterial isolates at a wide range of pH, temperature, and to assess their growth under heavy metal stress and antimicrobial activities. The bacterial isolates were collected from the Institute of Biotechnology and Genetic Engineering at the Biochemistry and Molecular Genetics Lab, the University of Agriculture, Peshawar, Pakistan. Previously, 55 rhizospheric bacterial strains were isolated through the serial dilution method, purified, and screened for phosphorus, potassium, and zinc solubilization (Siraj *et al*., 2022).

Medium preparation and culture initiation: Rhizospheric isolated bacteria were grown in Luria-Bertani (LB) suspension media (pH ±7.2) at 28ºC with 180 rpm for overnight incubation. The isolates were cultured on nutrient agar plates and stored at 4ºC for further processes.

Determination of antibacterial activity: The plant pathogenic strains were collected from the Plant Pathology Lab, the University of Agriculture, Peshawar, Pakistan and identified in the concerned lab. The pathogenic strains tested for antimicrobial activity were *Xanthomonas citri* and *Ralstonia solanacearum*.

Disc diffusion method (DDM): The antimicrobial activity of the isolated strains was checked using the disc diffusion method (Bovo *et al*., 2023; Ahmad *et al*., 2016). In this method, a complex of LB agar media and LB suspension media was used for the growth of microbes. Pathogenic strains were cultured in LB suspension media for 24 hours, and then 20 µl of the cultured pathogenic strains were spread on LB agar media plates in a laminar flow hood. Three discs (Whatman filter paper) of 10 mm diameter were placed on LB agar media plates with sterile forceps, and then isolated strains were added in varying concentrations per disc. Overnight, the inoculation plates were incubated at 37ºC. After 24 hours, the zone of inhibition surrounding each disc was measured in millimeters, indicating the antibacterial activity of the chosen strains.

Heavy metals influence on the growth of bacterial isolates: To find the ideal growth at various doses, isolated bacterial strains were tested against toxic heavy metals as lead, cadmium, and nickel. A stock solution was made for each element at a pH of 7.2 and sterilized for 20 minutes at 121ºC. From each stock solution, 10 ml was added to prepare two sets of tubes: one inoculated with bacteria and the other a control (without bacteria). Both sets of tubes were kept in a rotary incubator at 180 rpm, 28ºC. Optical density (OD600) was recorded after 3 and 24 hours of incubation (Oves *et al*., 2023; Roy *et al*., 2022).

Determination of optimum growth at a wide range of pH and temperature: To determine the optimum growth of the isolates, a wide range of pH and temperatures was applied. The pH was adjusted to 4, 5, 6, 7, 8, 9, and 10 using 0.5N HCl and 1N NaOH with a pH meter. Both sets of tubes were incubated in a shaking incubator at 180 rpm, 28ºC. The optical density (OD_{600}) was recorded at different

time intervals (3 and 24 hours). A similar procedure was used for different temperatures (10ºC, 20ºC, 30ºC, 40ºC, and 50ºC), and OD600 was recorded after 3 and 24 hours of incubation (Zargar *et al*., 2022).

Indole acetic acid (IAA) assay: The Indole-3-acetic acid (IAA) assay is a standard method for detecting IAA production by bacterial strains. In this procedure, bacterial cultures are grown overnight in LB media at 37°C with shaking. After centrifugation, the supernatant is mixed with Salkowski's reagent, which contains iron chloride and sulfuric acid. The mixture is incubated in the dark at 28°C for three hours. A pink color development indicates the presence of IAA. The concentration of IAA is determined by measuring the optical density at 535 nm using a spectrophotometer, referencing a standard curve prepared with known IAA concentrations (Rai & Golinska 2023).

Statistical analysis

In the study, data analysis was carried out using statistical software packages such as Graph Pad Prism and Statistix 8.1, commonly used tools for analyzing experimental data in scientific way. To ensure the reliability and reproducibility of the results, the experiments were performed in triplicates which helps in minimizing any errors that may arise due to experimental variability and ensures that the results are consistent (Jan *et al*., 2022).

Results

Antimicrobial assay: In the previous study, 59 rhizospheric bacteria were isolated from the soil. Two different phytopathogens, i.e., *Ralstonia solanacearum* and *Xanthomonas citri,* were checked for their antimicrobial potentials. A total of 15 isolates showed resistance against *Ralstonia solanacearum*. Maximum zones of inhibition were shown in isolates DJ15 (33.33 mm), followed by DJ24 and DJ07 (30.66 mm, 22.66 mm). Similarly, a minimum zone of inhibition was shown in DJ26 (7.5 mm), followed by DJ09 and DJ04 (9.66 mm, 10.33 mm) as compared to the control (34 mm) (Fig. 1). Out of the total isolates, 13 isolates showed resistance against *Xanthomonas citri,* while the remaining ones showed no response as such. Out of 13 isolates, the maximum zone of inhibition was shown in DJ59 (18.16 mm), followed by DJ15 and DJ17 (16.46 mm, 15.33 mm), etc. Isolates DJ27 (8 mm), followed by DJ32 and DJ31 (10.4 mm, 12.36 mm), and showed a minimum zone of inhibition with prescribed value as compared to control (33 mm) (Fig. 2).

Determination of optimum growth of isolated bacteria under metal stress: In order to check the resistance against heavy metal stresses isolated rhizospheric bacteria, three different toxic heavy metals, i.e., $PbNO_3$, $CdNO_3$, and Ni $(NO_3)_2$, were added in broth media as described in materials and methods. A total of 15 bacterial isolates were chosen to check their resistance against lead (Pb), cadmium (Cd), and nickel (Ni), respectively.

Lead nitrate ($PbNO_3$) stress and growth rate measurement after an interval of 3 and 24 hours of incubation: A total of 15 isolates were tested against lead nitrate stress, and their growth rate (OD_{600}) was recorded using spectrophotometry after incubation at different intervals of time (3-24 hours). After 3 hours of incubation, the highest growth rate (OD) was recorded by DJ23 (1.07), followed by DJ8 and DJ22 (0.95, 0.92). The lowest growth rates were recorded by DJ21 (0.12), followed by DJ12 and DJ67 (0.51, 0.55), as shown in (Fig. 3). After 24 hours of incubation, the highest growth rate (OD) was recorded by DJ3 (2.05), followed by DJ23 and DJ7 (1.75, 1.74). Similarly, the lowest growth rates were recorded by DJ1 (1.09), followed by DJ12 and DJ24 (1.19, 1.46) (Fig. 4).

Cadmium nitrate ($CdNO_3$) stress and growth rate measurements after 3 and 24 hours of incubation: A total of 15 isolates were tested against cadmium nitrate stress, and their growth rate (OD_{600}) was recorded using spectrophotometry after different time intervals of incubation. Among these isolates, the highest growth rate (OD) was recorded by DJ31 (0.40), followed by DJ3 (0.38) and DJ2 (0.29) after 3 hours of incubation. While the lowest growth rate was recorded by DJ67 (0.16), followed by DJ8 (0.17) and DJ21 (0.17), as shown in (Fig. 5). Similarly, after 24 hours of incubation, the highest growth rate (OD) was recorded by DJ31 (1.38), followed by DJ10 (1.38) and DJ2 (1.13). Among these isolates, the lowest growth rate was recorded by DJ12 (0.21), followed by DJ67 (0.34) and DJ22 (0.50), as shown in (Fig. 6).

Nickel nitrate ($NiNO_3$) stress and growth rate measurements after 3 and 24 hours of incubation: Similarly, in the case of $NiNO_3$ stress, a total of 15 isolates were tested against nickel nitrate stress, and their growth (OD_{600}) was recorded using spectrophotometry after 3 and 24 hours of incubation. Among these isolates, the highest growth rate was recorded in isolates DJ12 (0.98), followed by DJ21 and DJ24 (0.80, 0.70). While the lowest growth rate was recorded for DJ29 (0.23), followed by DJ3 and DJ4 (0.36 and 0.49) after 3 hours of inoculation (Fig. 7).

After 24 hours of incubation, the highest growth rate (OD) was recorded for DJ22 (1.61), followed by DJ8 (1.49) and DJ29 (1.44). Among these isolates, the lowest growth rate was recorded by DJ4 (0.84), followed by DJ24 (1.05) and DJ67 (1.06), as shown in Fig. 8).

Determination of optimum growth rate of bacterial isolates at a wide range of pH: To find out the optimum growth rate of isolated bacteria at a wide range of pH (4-10). A total of 30 isolated bacteria were checked and showed significant results at a wide range of pH adjusted at two different time intervals, i.e., after 3 and 24 hours of incubation. The growth rate (OD_{600}nm) was measured at 600 nm.

Growth rate (OD) recorded after 3 and 24 hours of incubation at a wide range of pH: After 3 hours of incubation at pH 4, the highest growth rate (OD) was recorded by DJ1 (0.34), followed by DJ73 and DJ27 (0.23, 0.29), while the lowest growth rate was recorded by DJ7 (0.11), followed by DJ57 and DJ11 (0.10, 0.11), as shown in (Figs. 9 and 10). Similarly, at pH 5 the highest growth rate was recorded by DJ51 (0.19), followed by DJ22 and DJ21 (0.17, 0.18) respectively, while the lowest

growth rate (OD) was recorded by DJ11 (0.09), followed by DJ4 and DJ24 (0.09, 0.09). Growth rates recorded after 3 hours of inoculation at pH 6, 7, 8, 9, and 10 were presented in (Figs. 9 and 10). After 24 hours of incubation at pH 4, the highest growth rate (OD) was recorded in strain DJ7 (0.28), followed by DJ51 and DJ13 (0.24, 0.25), while the lowest growth rate was recorded in DJ8 (0.10), followed by DJ21 and DJ56 (0.11, 0.10), as shown in (Figs. 11 and 12). Similarly, at pH 5 the highest growth rate was recorded by DJ5 (1.47), followed by DJ24 and DJ21 (1.41, 1.43), respectively. While the lowest growth rate was recorded by DJ25 (0.13), followed by DJ11 and DJ8 (0.15, 0.13) (Figs. 11 and 12).

Determination of optimum growth rate of bacterial isolates at different temperatures: To find out the optimum growth rate of isolated bacteria at different temperatures, 20ºC, 30ºC, 40ºC, and 50ºC, respectively. A total of 30 isolated bacteria were checked and showed significant results after 3 and 6 hours of incubation. The growth rate (OD_{600}nm) was measured at 600nm.

Growth rate (OD) recorded at variable temperature after 3 and 24 hours of incubation: After 3 hours of incubation at 10ºC temperature, the highest growth rate (OD) was recorded by DJ33 (0.46), followed by DJ4 and DJ8 (0.39, 0.43). While the lowest growth rate was recorded by DJ1 (0.09), followed by DJ23 and DJ65 (0.12, 0.11), as shown in figures 13 & 14. Similarly, at 20ºC the growth rate was recorded as DJ33> DJ27> DJ56 (0.42, 0.39, 0.38), while the lowest growth rate was recorded by DJ57< DJ28 (0.19, 0.22). Other temperatures, like 30ºC, 40ºC and 50ºC, also affect the growth rate of different isolates with variable numbers of values shown in Figures 13 and 14. After 24 hours of incubation at 10ºC temperature, the highest growth rate (OD) was recorded by DJ30 (1.42), followed by DJ27 (1.30) and DJ33 (1.27). While the lowest growth rate was recorded by DJ1 (0.21), followed by DJ5 (0.26) and DJ67 (0.32), as shown in (Figs. 15 & 16). Similarly, at 20ºC temperature, the highest growth rate was recorded by DJ27 (1.66), followed by DJ55 (1.58) and DJ12 (1.54), respectively. While the lowest growth rate was recorded by DJ7 (1.11), followed by DJ56 (1.19) and DJ67 (1.25). Growth rates affected by various ranges of temperature, like 30, 40, 50, etc., are also given in Figures15 and 16, respectively.

Capacity of IAA production by bacterial isolates: To determine the production of indole acetic acid concentration, the isolated strains were inoculated in the LB media. After 2 hours of incubation, growth rate (OD) was recorded through spectrophotometry and converted into known concentration. The maximum IAA was produced by DJ34, followed by DJ7 and DJ16, respectively. The minimum IAA was produced by DJ30, followed by DJ9 and DJ35, respectively, as shown in (Fig. 17).

Discussion

Agriculture plays a vital role in the global food production system, which is essential to meet the increasing food demand. With the world's population projected to reach around 9 billion by the middle of this century, there is a growing need to enhance food production to cater to this demand. However, several challenges have arisen over the last few decades, primarily driven by urbanization and industrialization. These processes have resulted in the overexploitation of natural resources, leading to a depletion of fertile soil, water resources, and biodiversity, which in turn contributes to food insecurity. In light of this, there is an urgent need to explore and implement innovative agricultural practices that can sustainably increase food production without further damaging the environment. One promising solution is the utilization of novel agricultural approaches, such as the use of beneficial microorganisms, to maintain agricultural sustainability while boosting food production.

In this study, 15 isolated rhizospheric bacteria were screened for their antimicrobial properties against plant pathogens, specifically *Ralstonia solanacearum* and *Xanthomonas citri*, which are known to cause significant diseases in crops. The aim was to identify rhizobacteria that can be used as biocontrol agents to protect plants from these pathogens, reducing the need for harmful chemical pesticides. *Ralstonia solanacearum* is a soil-borne bacterium responsible for bacterial wilt disease in many important crops, while *Xanthomonas citri* is the causative agent of citrus canker, a major problem for the citrus industry. The results showed that several bacterial isolates exhibited varying degrees of antimicrobial activity against these pathogens.

For *Ralstonia solanacearum*, three isolates, namely DJ15, DJ24, and DJ07, demonstrated strong inhibition, showing the potential of these bacteria to act as biocontrol agents against this pathogen. On the other hand, isolates DJ26, DJ09, and DJ04 showed relatively weaker inhibition, suggesting that their antimicrobial potential may not be as effective against *Ralstonia solanacearum*. For *Xanthomonas citri*, a larger proportion of isolates, specifically 13, displayed significant antimicrobial activity. Notably, isolates DJ59, DJ15, DJ17, and DJ63 showed the highest levels of inhibition, further supporting the idea that rhizospheric bacteria can be harnessed to fight plant diseases. In contrast, isolates DJ27, DJ32, and DJ31 exhibited weaker antimicrobial effects, which highlights the variability in antimicrobial activity among different bacterial isolates. These findings are consistent with previous studies (Silva *et al*., 2024; Ke *et al*., 2023), which also reported varying degrees of antimicrobial efficacy from different bacterial strains.

In addition to their antimicrobial properties, the ability of plant growth-promoting rhizobacteria (PGPR) to tolerate heavy metal stress was also investigated in this study. Heavy metals, such as lead (Pb), cadmium (Cd), and nickel (Ni), are increasingly contaminating agricultural soils due to industrial activities and can be toxic to plants and microorganisms. However, some bacteria have evolved mechanisms to resist heavy metal toxicity and can potentially be used in bioremediation strategies to clean up polluted soils. The study revealed that several bacterial isolates exhibited significant resistance to the three heavy metals tested: $PbNO_3$, $CdNO_3$, and Ni $(NO_3)_2$ (Zulfiqar *et al*., 2022).

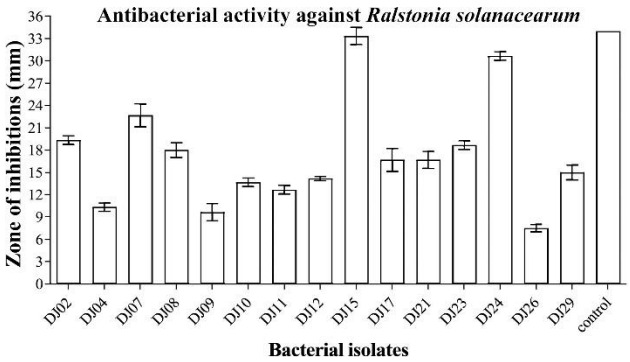

Fig. 1. Antibacterial activity shown by bacterial isolates against *Ralstonia solanacearum*. The highest zone of inhibition was recorded in DJ15, followed by DJ24.

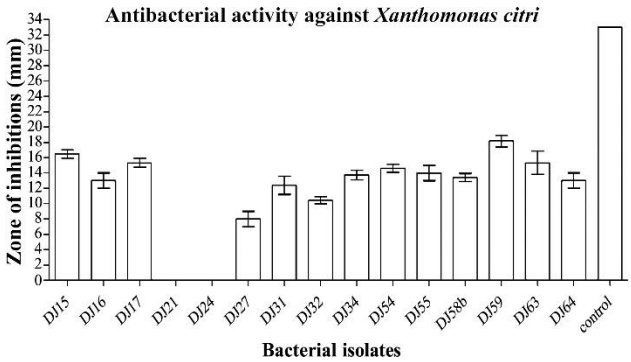

Fig. 2. Antibacterial activities shown by bacterial isolates against *Xanthomonas citri*. The highest zone of inhibition was shown by DJ59, followed by DJ15 and DJ17.

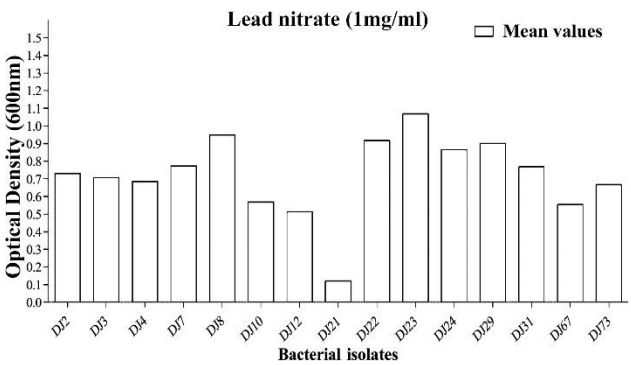

Fig. 3. Growth rate (OD) of bacterial isolates against $PbNO_3$ stress after 3 hours of incubation was recorded. The highest growth rate was recorded against lead nitrate stress by DJ23 (1.07) and DJ8 (0.95).

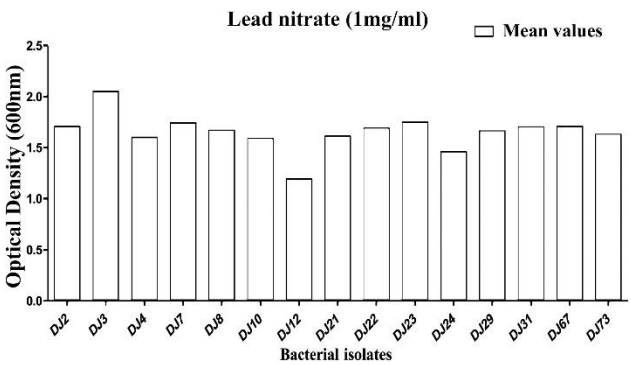

Fig. 4. Growth rate (OD) of bacterial isolates against $PbNO_3$ stress after 24 hours of incubation was recorded. The highest growth was recorded against lead nitrate stress by DJ3 (2.05) and DJ23 (1.75).

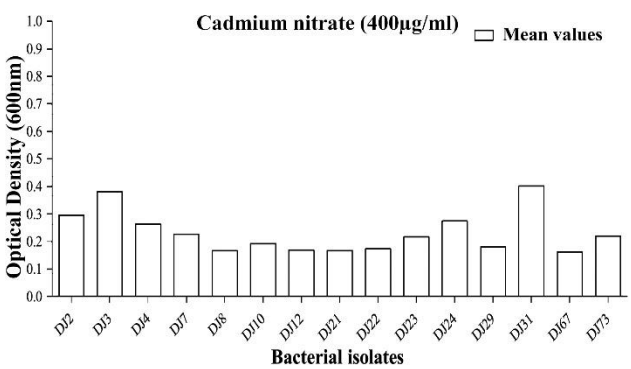

Fig. 5. Growth rate (OD) of bacterial isolates against $CdNO_3$ stress after 3 hours of incubation was recorded. The highest growth was recorded against cadmium nitrate stress by DJ31 (0.40) and DJ3 (0.38).

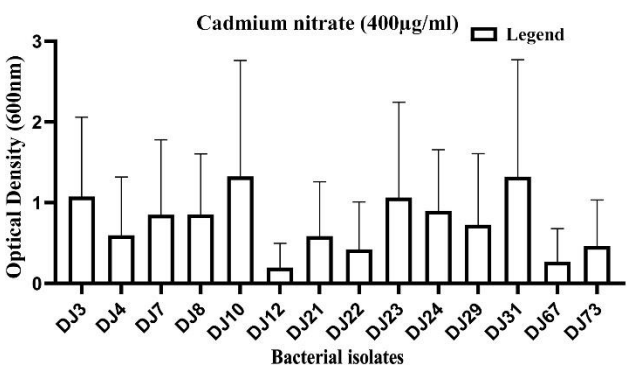

Fig. 6. Growth rate (OD) of bacterial isolates against $CdNO_3$ stress after 24 hours of incubation was recorded. The highest growth was recorded against cadmium nitrate stress by DJ31 (1.38) and DJ10 (1.38).

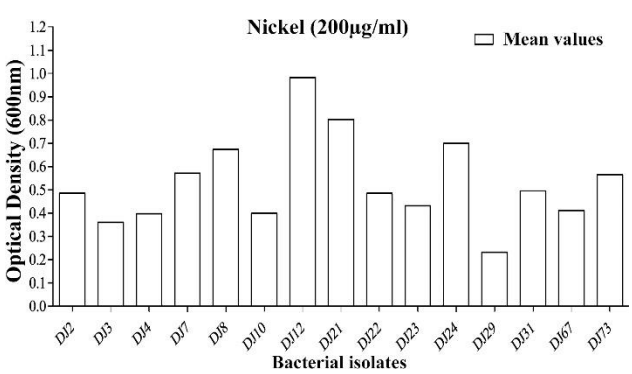

Fig. 7. Growth rate (OD) of bacterial isolates against $NiNO_3$ stress after 3 hours of incubation was recorded. The highest growth rate was recorded against nickel nitrate stress by DJ12 (0.98) and DJ21 (0.80).

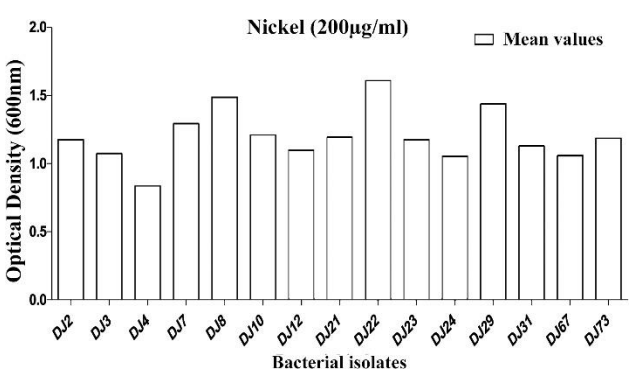

Fig. 8. Growth rate (OD) of bacterial isolates at $NiNO_3$ stress after 24 hours of incubation was recorded. The highest growth rate was recorded against nickel nitrate stress by DJ22 (1.61) and DJ8 (1.58).

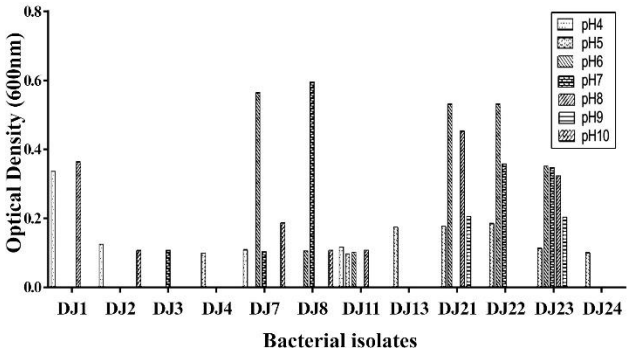

Fig. 9. Growth rate (OD) of bacterial isolates at a wide range of pH after 3 hours of incubation was recorded. DJ8, DJ7, and DJ21 showed the highest growth rate at different pH levels.

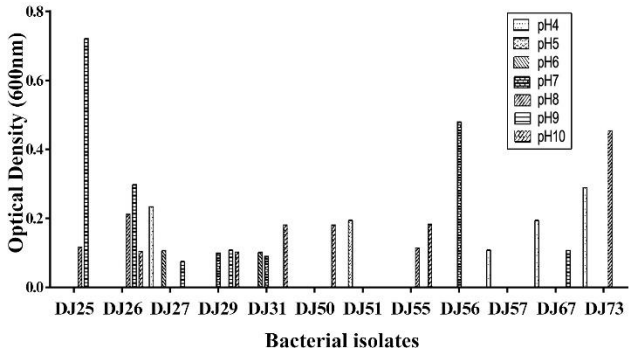

Fig. 10. Growth rate (OD) of bacterial isolates at a wide range of pH after 3 hours of incubation was recorded. DJ25, DJ56, and DJ73 showed the highest growth rate at different pH levels.

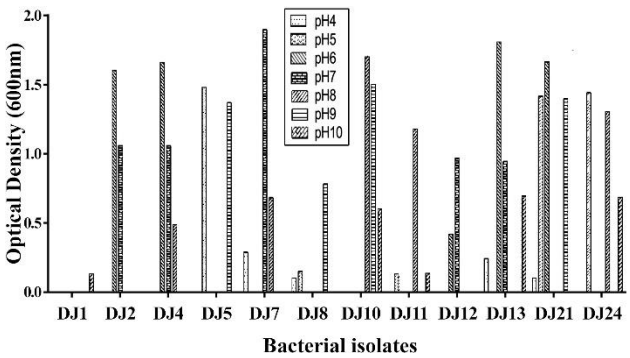

Fig. 11. The growth rate (OD) of bacterial isolates at a wide range of pH after 24 hours of incubation was recorded. DJ7, DJ13, and DJ10 showed the highest growth rate at different pH levels.

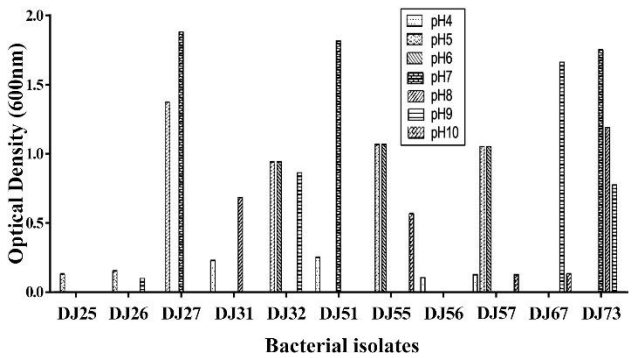

Fig. 12. The growth rate (OD) of bacterial isolates at a wide range of pH after 24 hours of incubation was recorded. DJ27, DJ51, and DJ73 showed the highest growth rate at pH 7.

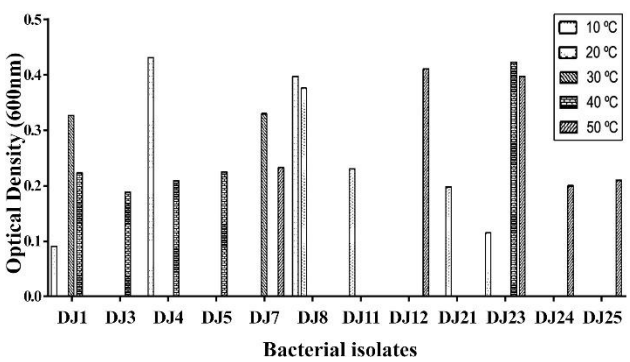

Fig. 13. Growth rate (OD) of bacterial isolates at different temperatures after 3 hours of incubation was recorded. The highest growth rate was recorded by DJ4 at 10ºC, DJ23 at 40ºC, and DJ12 at 50ºC, respectively.

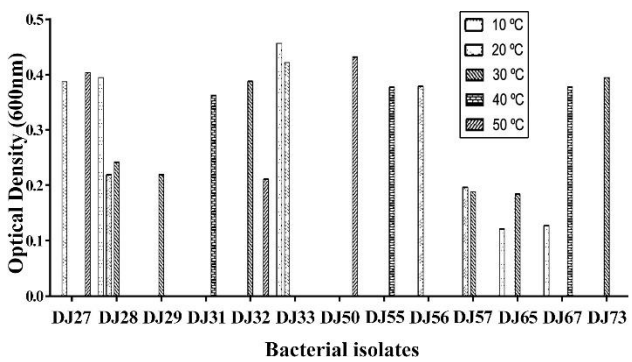

Fig. 14. Growth rate (OD) of bacterial isolates at different temperatures after 3 hours of incubation was recorded. The maximum growth rate was recorded by DJ33 at 10, 20ºC, DJ50 at 50ºC, and DJ28 at 10, 50ºC, respectively.

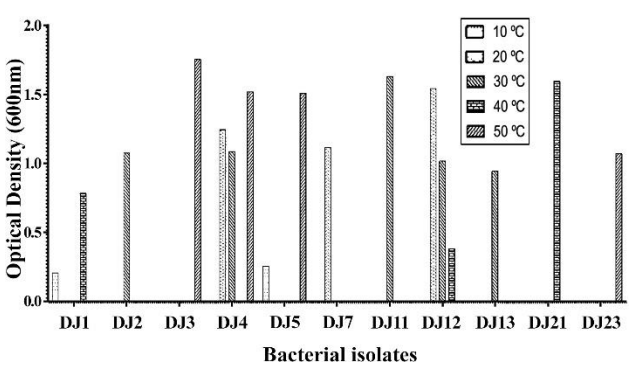

Fig. 15. The growth rate (OD) of bacterial isolates at different temperatures after 24 hours of incubation was recorded. The highest growth rate was measured by DJ3 at 50ºC, DJ11 at 30ºC, and DJ21 at 40ºC, respectively.

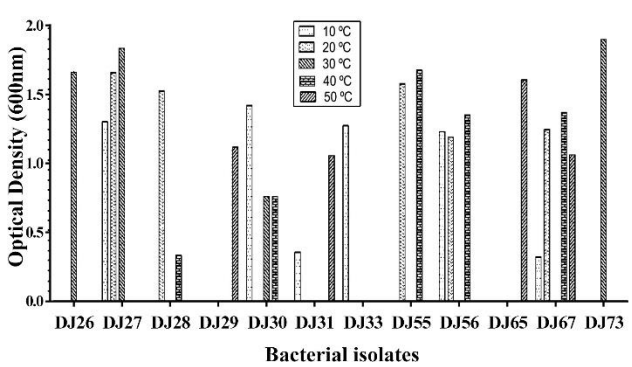

Fig. 16. Growth rate (OD) of bacterial isolates at different temperatures after 24 hours of incubation was recorded. The maximum growth rate was recorded by DJ73 at 30ºC, DJ27 at 30ºC, and DJ26 at 30ºC, respectively.

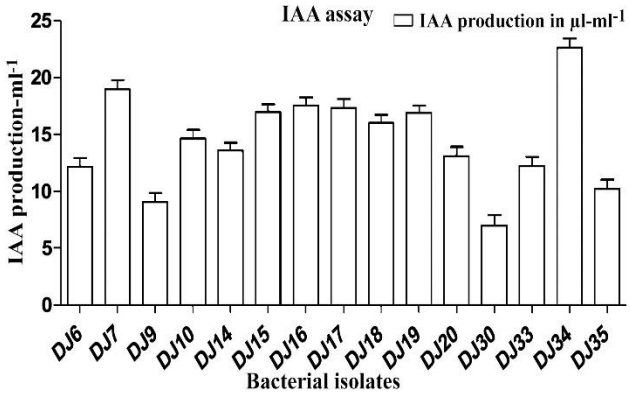

Fig. 17. Indole acetic acid productions by bacterial isolates. The maximum IAA was produced by DJ34, DJ7, and DJ16, respectively.

When exposed to $PbNO_3$ at a concentration of 1 mg/ml, isolate DJ23 showed the highest resistance after 3 hours of incubation, followed by DJ8, DJ22, and DJ29. In contrast, isolates such as DJ21, DJ12, DJ67, and DJ10 demonstrated weaker resistance, indicating that some bacterial strains are more capable of surviving in environments contaminated with lead. After 24 hours, DJ3 exhibited the highest resistance, which was followed by DJ23, DJ7, and DJ67. The results highlight the diversity in the heavy metal tolerance of PGPR strains, which can be important for their potential application in polluted environments. This is consistent with previous research (Gupta *et al*., 2024; Mondal *et al*., 2023 and Chandwani *et al*., 2023), which demonstrated that certain PGPR isolates can thrive in heavy metal-contaminated environments and may assist in reducing soil contamination levels.

Similarly, the ability of PGPR to resist cadmium nitrate ($CdNO_3$) was tested, with DJ31 showing the highest resistance at a concentration of 400 µg/ml after 3 hours of incubation. Other isolates, such as DJ3, DJ2, and DJ24, also showed considerable tolerance, while strains like DJ67, DJ8, DJ21, and DJ12 exhibited lower resistance. After 24 hours of incubation, DJ31 remained the most resistant, followed by DJ10, DJ2, and DJ23, suggesting that this strain could be a promising candidate for bioremediation applications in cadmium-contaminated soils. Previous studies (Zulfiqar *et al*., 2023; Renu *et al*., 2022) have also shown that some bacterial strains are capable of degrading or sequestering cadmium, reducing its toxicity to plants and other organisms.

In the case of nickel nitrate ($Ni(NO_3)_2$), the highest growth rate was observed for isolate DJ12 at a concentration of 200 µg/ml after 3 hours of incubation, followed by DJ21, DJ24, and DJ8. In contrast, isolates like DJ29, DJ3, DJ4, and DJ10 showed lower growth rates, indicating varying degrees of nickel tolerance among the bacterial strains. After 24 hours, DJ22 exhibited the highest resistance, followed by DJ8, DJ29, and DJ7, while DJ4, DJ24, DJ67, and DJ3 demonstrated weaker resistance. These results are similar to those of Rasouli *et al*., (2023), who observed varying heavy metal resistance levels among different PGPR strains.

In addition to their tolerance to heavy metals, the ability of these bacterial isolates to grow under different environmental conditions, such as varying pH levels and temperatures, was also assessed. The growth of bacteria is influenced by factors such as soil pH and temperature and identifying strains that can thrive under extreme conditions could enhance their application in diverse agricultural environments (Wani *et al*., 2023). The pH tolerance of 30 bacterial isolates was tested, revealing that DJ25 exhibited the highest growth rate at pH 9, followed by DJ7 at pH 7 and DJ7 at pH 6 after 3 hours of incubation. On the other hand, DJ27 showed the lowest growth rate at pH 9, followed by DJ31 at pH 7 and DJ11 at pH 5. After 24 hours, DJ73 showed the highest growth rate at pH 8, followed by DJ7 at pH 7 and DJ27 at pH 7. These findings suggest that some bacterial strains can thrive under alkaline conditions, which could be useful in soils with higher pH levels (Aqel *et al*., 2024). Temperature also plays a crucial role in the growth of PGPR. After 3 hours of incubation, the highest growth rate was recorded by DJ33 at 10°C, followed by DJ4 at 10°C and DJ24 at 50°C, indicating that these strains could tolerate a wide range of temperatures. The minimum growth rate was noted by DJ1 at 10°C, followed by DJ23 and DJ65 at 10°C, highlighting that some bacterial strains are more sensitive to temperature variations (Kumar *et al*., 2023; Aqel *et al*., 2023).

Finally, the biochemical characterization of the bacterial isolates revealed the production of indole acetic acid (IAA), a plant growth hormone known for its role in stimulating root growth and improving nutrient uptake. The IAA production by potassium-solubilizing bacteria (KSB) isolated from rice paddy soil, as reported by Sharma *et al*., (2020), suggests that these bacteria could not only help plants tolerate stress but also promote plant growth by enhancing nutrient availability (Sharma *et al*., 2024; Saheewala *et al*., 2023).

Conclusion

In conclusion, this study underscores the promising potential of rhizospheric bacteria as effective biocontrol agents against plant pathogens and as valuable tools for enhancing plant growth in heavy metal-contaminated environments. The diverse antimicrobial activities, heavy metal resistance, and environmental adaptability exhibited by the bacterial isolates highlight their significance for agricultural applications. Among the 59 strains screened, 15 demonstrated strong antimicrobial activity against *Ralstonia solanacearum*, and 13 effectively inhibited *Xanthomonas citri*, emphasizing their potential as natural alternatives to chemical pesticides. Additionally, the isolates displayed remarkable resistance to heavy metals, particularly lead nitrate, followed by nickel and cadmium, showcasing their ability to thrive in polluted soils. The ability of these PGPR to grow optimally across various pH levels and temperature ranges further underscores their versatility in diverse environmental conditions. Overall, these findings point to the potential for developing sustainable agricultural practices that enhance crop productivity while mitigating the harmful effects of environmental contaminants, ultimately contributing to more eco-friendly and resilient agricultural systems. Further research into the characterization and application of these PGPR could pave the way for innovations in sustainable farming, offering a viable solution to the growing challenges of food security and environmental sustainability.

Recommendation

The findings of this study emphasize the importance of PGPR as a valuable resource for advancing sustainable agriculture. By further exploring their mechanisms, environmental adaptability, and potential for multi-stress tolerance, we can better harness their benefits in promoting crop productivity, enhancing soil health, and mitigating the impact of heavy metal contamination. Further research in these areas will contribute significantly to developing eco-friendly, efficient, and resilient agricultural systems for the future.

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