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Isolation and characterization of Indole Acetic Acid (IAA) producing rhizospheric bacteria from Maize (*Zea mays*) plants

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Abstract

Auxins are plant hormones which regulate various physiological processes like root elongation, cell division and help in overall plant growth. Indole-3-acetic acid (IAA) is an important naturally occurring auxin which is normally endogenously produced by the plants but recent researches have demonstrated that plant growth-promoting rhizobacteria (PGPR) also synthesize auxins which affect plant development. The rhizospheric bacteria of *Zea mays* plant includes auxin producing bacteria which can have profound effect on root architecture, nutrient uptake and overall vigor of plant. In the present study, auxin producing ability of rhizospheric bacteria isolated from *Zea mays* was examined and their potential to promote the plant growth was studied. The results of the study indicated that out of ten auxin producing strains two had high auxin producing ability. The inoculation of these cultures on Wheat seeds exhibited positive influence on seed germination and seedling growth. Thus, these cultures have potential for being used as bioinoculants for improving growth of different plants and they offer sustainable alternative to chemical fertilizers.

Keywords

Auxin-producing bacteria, Indole-3-acetic acid (IAA), Maize, Plant growth promoting rhizobacteria (PGPR), Sustainable agriculture.

Introduction

Plant synthesizes different hormones endogenously which help in regulating physiological processes and growth of plants. Auxins are important plant hormones which are produced primarily at the tip of growing roots and shoots. They help in cell elongation, cell division, cell differentiation, embryonic development, root and stem tropisms, apical dominance and transition to flowering. Different types of naturally occurring auxins include Indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid, Phenylacetic acid, Indole-3-butyric acid and Indole-3-propionic acid. Recently, researchers highlighted that many plant hormones including auxins are also produced by microorganism particularly plant growth-promoting rhizobacteria (Arora *et al*., 2020). Plant growth-promoting rhizobacteria (PGPR) which colonize in rhizospheric soil of the plants and affect the plant growth as they help in increasing nutrient uptake, provide protection against pathogens and improve overall growth of plants (Glick, 2022). Auxin producing PGPR have been shown to stimulate growth of roots, increasing nutrient uptake and enhances overall vigour of plants (Kaur *et al*., 2021). Rhizospheric bacteria of Maize have been shown to produce IAA, promote lateral root formation which help in increasing root biomass and improves water and nutrient uptake (Sharma *et al*., 2020). Studies have also shown that IAA producing rhizospheric bacteria enhances Maize growth under normal as well as in environmental stressed conditions of drought, salinity and nutrient deficiency (Zhao *et al*., 2022; Mishra *et al*., 2022). These bacteria also contribute to plant resilience by mediating responses to abiotic stresses, thereby enhancing crop productivity in challenging environments (Zhang *et al*., 2021; Naji *et al*., 2025).

In addition to their role in promoting plant growth, auxin-producing rhizobacteria have been identified for their potential as biocontrol agents. They suppress soil-borne pathogens through the production of antimicrobial compounds and competition for root space (Khan *et al*., 2020). Furthermore, auxin-producing bacteria can improve plant health by inducing systemic resistance, further enhancing plant defence mechanisms (Sharma *et al*., 2021). The ability of these bacteria to act as biocontrol agents while promoting plant growth reduces the dependency on chemical fertilizers and pesticides, aligning with sustainable agricultural practices (Olanrewaju *et al*., 2021; Olanrewaju *et al*., 2020; Sujithra V. and Kanchana, 2020). This offers a promising alternative for sustainable crop production that minimizes the environmental impact of conventional agricultural practices.

Maize (*Zea mays*), one of the most widely cultivated and economically significant crops worldwide, benefits significantly from the action of auxin-producing rhizobacteria. These bacteria not only help enhance Maize growth but also contribute to improving soil health by increasing microbial diversity and promoting nutrient cycling (Khan *et al*., 2021). As Maize cultivation faces challenges from changing climate conditions, the application of auxin-producing bacteria as bioinoculants presents a potential solution for improving crop productivity while reducing the environmental footprint of conventional farming practices (Mishra *et al*., 2022; Raza *et al*., 2021).

The objective of this study was to isolate and characterize auxin-producing rhizospheric bacteria from Maize plants, with a focus on evaluating their potential to synthesize IAA and their impact on plant growth. By understanding the functional diversity of these bacteria and their mechanisms of action, we hope to identify effective candidates for use as bioinoculants that promote sustainable agricultural practices (Sharma *et al*., 2020; Riaz *et al*., 2021; Ali *et al*., 2021; Khan *et al*., 2021). The results from this research will contribute to the development of eco-friendly agricultural strategies that enhance crop yields and improve soil health, ultimately contributing to the sustainability of Maize cultivation.

Materials and Methods

1. Collection of soil samples

Soil samples were collected from the rhizosphere of Maize plants in Sabakheda village, Mandsaur District, Madhya Pradesh, India, located approximately 180 km from Ujjain. Using a sterile spatula, rhizospheric soil adhering to the root surface was carefully collected into sterile polythene bags. The samples were transported to the laboratory and stored at room temperature. Isolation of rhizospheric bacteria was initiated within 2-3 days of sampling to ensure microbial viability (Randevi *et al*., 2024).

2. Isolation and identification of rhizospheric bacteria

Rhizospheric bacteria were isolated from soil samples using the serial dilution method. One gram of soil was suspended in 100 ml of sterile distilled water, followed by serial dilution from 10^{-1} to 10^{-6}. A 0.1 ml aliquot of the 10^{-6} dilution was spread onto sterile nutrient agar plates and incubated at 37°C for 24 hours. Morphologically distinct colonies were picked and transferred onto Nutrient agar slants. The streak plate method was used to obtain pure cultures of rhizospheric bacteria. Single colonies were further purified and stored on fresh Nutrient agar slants at 4°C. Morphological characterization of the pure isolates was performed using a light microscope at 40X magnification (Sherpa *et al*., 2021; Chowhan *et al*., 2022).

3. Indole Acetic Acid (IAA) producing assay of rhizospheric bacteria

The production of indole-3-acetic acid (IAA) by rhizospheric bacteria was determined following a method described by Oo *et al.*, (2020). Fresh 24-hour rhizobacterial cultures were inoculated into test tubes containing Nutrient broth supplemented with 0.1% tryptophan. The inoculated tubes were incubated at 37°C for 48 hours. After incubation, 5 ml of Salkowski reagent was added to each tube, and the mixture was kept in the dark at room temperature for 30 minutes. An uninoculated medium served as the control. The development of a pink color indicated IAA production, whereas no color change showed a negative result. The absorbance of the samples was measured at 530 nm using a spectrophotometer (Oo *et al.*, 2020).

A standard curve was generated for the quantification of IAA (Indole-3-acetic acid) in solution, using an uninoculated medium with a reagent as a control. The concentration of IAA in the culture was expressed in mg/ml. Standard solutions were prepared in nutrient broth medium at varying concentrations of 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10,15, 20, 25 and 30 mg/ml. To prepare the stock solution, 100 mg of IAA was added to a 10 ml beaker containing acetone, and the mixture was stirred using a glass rod until the IAA was completely dissolved. This resulted in a 10 mg/ml stock solution, which was then distributed into a series for the dilution series.

The solution was incubated at room temperature for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at 530 nm (OD530). The color change across the gradient of concentrations was observed within a few minutes. Absorbance data was recorded and the IAA concentrations in bacterial cultures were calculated based on the standard curve created using known IAA concentrations (Oo *et al*., 2020).

4. Effect of auxin producing rhizospheric bacteria on seed germination and seedling growth

Wheat seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, followed by thorough rinsing with sterile water. Sterilized seeds were soaked in bacterial suspensions (10^{-6} CFU/ml) for 30 minutes and then placed in sterile Petri plates containing moistened filter paper. The seeds were allowed to germinate at room temperature and growth parameters like root and shoot growth were recorded after 5 days (Oo *et al.*, 2020). The vigor index was calculated using the mathematical expression described below (Mahadevamurthy *et al*. 2016).

Vigor index = Seed germination (%) x [Mean Root Length + Mean Shoot Length]

5. Statistical analysis

Statistical analysis was conducted to assess the significance of the results obtained from the auxin production assay and seedling growth experiments. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The mean value at $p \leq 0.01$ was considered significant.

Results

1. Isolation and characterization of rhizobacterial cultures:

A total of ten rhizobacterial isolates were obtained from the rhizospheric soil samples collected from Maize crops in Sabakheda village, Mandsaur district. These isolates showed distinct morphological features, such as variations in colony size, shape, color, margin and elevation. Colony characteristics of rhizobacterial isolates are summarized in Table 1. It was seen that isolates B161, B207, B200 and B212 had white pale colonies, isolates B162, B163 and B255 had lemon yellow colonies, isolate B213 had mustard yellow colonies and B160 had peach coloured colonies on the nutrient agar medium. The isolates B161 and B162 are in shape of long rods, B207, B160, B255, B213 and B163 have cocci shape, B259, B200 and B212 have rod shape. B161, B255 and B259 isolates have entire margin and convex elevation, isolate B207 has undulate margin and irregular elevation, isolate B160 has serrate margin and drop like elevation, isolate B162 has serrate margin and convex elevation, B163 has entire margin and flat elevation, B200 and B213 have serrate margin and flat elevation while B212 has undulate margin and flat elevation. During microscopic examination it was observed that seven cultures (B207, B160, B162, B255, B163, B212 and B213) were gram positive and three were gram negative (B161, B259 and B200).

Table 1. Morphological characteristics of isolated rhizospheric bacteria

S. No.	Culture number	Colour of colony	Margin of colony	Elevation of colony	Gram reaction	Shape of cells
1.	B161	White pale	Entire	Convex	Gram negative	Long rods
2.	B207	White pale	Undulate	Irregular	Gram positive	Cocci
3.	B160	Peach	Serrate	Drop like	Gram positive	Cocci
4.	B162	Lemon yellow	Serrate	Convex	Gram positive	Long rods
5.	B259	Yellow	Entire	Convex	Gram negative	Rod
6.	B255	Light yellow	Entire	Convex	Gram positive	Cocci
7.	B163	Yellow	Entire	Flat	Gram positive	Cocci
8.	B200	White	Serrate	Flat	Gram negative	Rod
9.	B212	White	Undulate	Flat	Gram positive	Short rods
10.	B213	Mustard yellow	Serrate	Flat	Gram positive	Round

2. IAA production by rhizospheric bacteria

The results of the IAA assay showed that the majority of the bacterial isolates exhibited positive IAA production with varying levels of efficiency as shown in the Table 2. The control sample showed no detectable IAA production, confirming their inability to synthesize auxin under the given conditions.

Among the tested bacterial cultures, B200 (7.42 mg/ml) and B207 (7.01 mg/ml) demonstrated the highest IAA production. These strains are promising candidates for plant growth-promoting applications due to their significant auxin synthesis, which is known to enhance root elongation and overall plant development.

Table 2. IAA production by the isolated rhizobacteria

S. No.	Culture number	IAA test	IAA Concentrations (mg/ml)
1.	Control	Negative	0
2.	B212	Positive	3.697
3.	B213	Positive	4.150
4.	B161	Positive	4.725
5.	B255	Positive	4.777
6.	B163	Positive	5.931
7.	B160	Positive	6.539
8.	B162	Positive	6.629
9.	B207	Positive	7.012
10.	B259	Positive	6.742
11.	B200	Positive	7.425

Moderate IAA production was observed in isolates B259 (6.74 mg/ml), B160 (6.53 mg/ml), B162 (6.62 mg/ml) and B163 (5.93 mg/ml), suggesting their potential for contributing to plant growth enhancement. Other isolates such as B161 (4.72 mg/ml), B255 (4.77 mg/ml), B213 (4.15 mg/ml) and B212 (3.69 mg/ml) also displayed positive IAA production but at comparatively lower levels. The control did not show any detectable IAA production.

3. Effect of high auxin producing rhizospheric bacteria on seed germination and seedling growth

The effect of selected high auxin producing plant growth-promoting rhizobacteria (PGPR), namely B207 and B200 on seedling growth of wheat seeds was evaluated in Petri plates. Several growth parameters were analyzed, including germination percentage, root length, shoot length, dry weight and vigour index. The results show that the germination percentage of control seeds and seeds treated with B207 was 90%, while seeds treated with B200 exhibited 100% germination, indicating a positive influence on seed germination. The p-value calculated was $p \leq 0.0001$ indicating highly significant differences between the culture isolates.

Regarding root growth, the control seeds had a mean root length of 8.28 ± 0.92 cm. Seeds treated with B207 and B200 showed improved root growth, with mean root lengths of 8.54 ± 1.11 cm and 11.24 ± 1.15 cm respectively. The treatment with B200 demonstrated the most significant improvement in root growth, significantly outperforming the control and other isolate.

With respect to shoot growth, the mean shoot length of control seeds was 6.82 ± 1.25 cm, while those treated with B207 and B200 had mean shoot lengths of 8.36 ± 1.19 cm and 8.64 ± 0.54 cm respectively. Again, B200 showed the highest shoot growth, indicating its beneficial effect on seedling development.

The mean dry weight of the control root was 0.188 ± 0.001 gm, while those treated with B207 and B200 had significantly higher root dry weights of 0.291 ± 0.001 gm and 0.816 ± 0.001 gm respectively. The highest root dry weight was observed in B200, suggesting superior root development. Similarly, the control seeds had a mean shoot dry weight of 0.052 ± 0.001 gm, while the mean shoot dry weights for seeds treated with B207 and B200 were 0.067 ± 0.001 gm and 0.400 ± 0.01 gm respectively. Again, B200 significantly enhanced shoot dry weight, demonstrating its role in improving overall plant health.

Table 3. Effect of high auxin producing rhizobacteria on seed germination and seedling growth

S. No.	Culture isolate	Percentage germination	Root growth		Shoot growth		Seed vigour
			Root length (cm)	Dry weight (gm/ 5 seeds)	Shoot length (cm)	Dry weight (gm/ 5 seeds)	
1.	Control	90	8.28± 0.92	0.188± 0.001	6.82 ±1.25	0.052± 0.001	1359±111.51
2.	B207	90	8.54 ±1.11	0.291± 0.001	8.36 ±1.19	0.067± 0.001	1521±156.79
3.	B200	100	11.24±1.15	0.816± 0.001	8.64 ±0.54	0.400± 0.01	1988±155.52

The vigour index, which combines germination percentage, mean root length and mean shoot length, was also positively affected by the bacterial treatments. The vigour index of control seeds was 1359 ± 111.51, while seeds treated with B200 exhibited the highest vigour index at 1988 ± 155.52, followed by B207 at 1521 ± 156.79. These results suggest that B200 was the most effective treatment, significantly enhancing seedling growth and vigor compared to the control and other isolate.

Overall, these findings demonstrate that PGPR, particularly B200, has a positive impact on seed germination and seedling growth of wheat, suggesting its potential for improving crop establishment and early growth stages.

Discussion

The results of this study highlight the significant role of auxin-producing rhizospheric bacteria in enhancing the growth and development of Maize plants. The isolated bacterial strains demonstrated substantial auxin production, particularly indole-3-acetic acid (IAA), which has been previously shown to positively influence root and shoot growth. The increase in root length, biomass and overall plant vigor in wheat seedlings treated with these bacteria can be attributed to the IAA-mediated modulation of root architecture. These findings align with earlier studies that have reported the beneficial effects of IAA-producing bacteria on plant growth by stimulating lateral root formation, which in turn improves nutrient and water absorption (Arora *et al*., 2020; Mishra *et al*., 2022).

Auxin production by rhizobacteria is a well-documented mechanism of plant growth promotion. It is well established that IAA can influence various physiological processes, such as root elongation, differentiation and lateral root development, which are essential for improved plant nutrient uptake (Kaur *et al*., 2021; Zhao *et al*., 2022; Ahmed and Hasnain, 2010). The results observed in this study are consistent with these findings, as treated wheat plants showed improved root development compared to the control. Moreover, the enhanced shoot growth, as evidenced by increased shoot length and fresh and dry weights suggests that the auxin-producing bacteria also play a crucial role in stimulating above-ground growth, potentially by modulating hormonal balance and improving nutrient transport within the plant (Glick, 2022; Sharma *et al*., 2020).

In addition to growth promotion, the presence of auxin-producing bacteria in the Maize rhizosphere can improve plant resilience to environmental stresses. Several studies have reported that bacteria producing IAA and other phytohormones can mediate stress tolerance in plants, including resistance to drought, salinity and nutrient deficiencies (Zhang *et al*., 2021; Riaz *et al*., 2021; Naji *et al*., 2025). While this study focused primarily on the growth-promoting effects of auxin, future research should examine the role of these bacteria in enhancing Maize resilience to abiotic stresses, which is critical in the face of global climate challenges.

The potential of auxin-producing bacteria to act as biocontrol agents further underscores their importance in sustainable agricultural practices. Previous studies have shown that these bacteria not only promote plant growth but also suppress soil-borne pathogens through competition and the production of antimicrobial compounds (Khan *et al*., 2021). By reducing the reliance on chemical fertilizers and pesticides, these beneficial bacteria offer an eco-friendly alternative for sustainable crop management (Olanrewaju *et al*., 2021). Furthermore, the use of auxin-producing bacteria as bioinoculants can improve soil health by enhancing microbial diversity, thus contributing to long-term soil fertility and ecosystem sustainability (Raza *et al*., 2021).

In terms of practical applications, the isolation and characterization of auxin-producing bacteria from the Maize rhizosphere provide valuable insights into selecting effective candidates for bioinoculants. The high levels of IAA production observed in our study suggest that these strains can be harnessed for agricultural use, particularly in Maize cultivation. However, further investigations are needed to assess their efficacy in field trials, their interaction with different environmental factors and their compatibility with other agricultural practices (Ali *et al*., 2021). Additionally, exploring the mechanisms behind IAA synthesis and the genetic regulation of auxin production could lead to the development of more efficient and targeted bacterial strains for agricultural applications.

Overall, this study contributes to the growing body of knowledge on the role of auxin-producing rhizobacteria in plant growth promotion. The findings emphasize the potential of these bacteria as natural bioinoculants for improving Maize productivity and promoting sustainable agricultural practices, ultimately contributing to food security and environmental sustainability.

Conclusion

In this study, auxin producing rhizospheric bacteria from roots of Maize plants were isolated and characterized and their role in promoting seed germination and seedling growth has been demonstrated. The isolated strains exhibited significant IAA production, which positively impacted root and shoot development, leading to improved overall plant health and vigor. These findings highlight the potential of plant growth-promoting rhizobacteria (PGPR) as natural bioinoculants, offering a sustainable approach to enhance crop productivity while reducing dependence on chemical fertilizers and pesticides. The results emphasize the eco-friendly advantages of utilizing IAA-producing PGPR as an alternative to traditional agricultural practices. Further investigation into the mechanisms of IAA synthesis and its interaction with other plant growth-promoting traits could optimize the use of these bacteria in agriculture. This research contributes to the growing understanding of rhizospheric microbiota's role in boosting plant health and soil sustainability, opening the new way for the development of innovative, environmentally responsible farming practices.

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