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Review Article

Isolation and Characterization of Bacterial Diversity from Wheat Rhizosphere

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Abstract

Rhizosphere is considered to be an important spot where microbes have an interaction with plant roots. The soil sample from wheat rhizosphere is taken for microbial isolation and isolated microbes were studied on basis of their morphology and biochemistry. All of the isolated strains were examined morphologically and showed variable parameters viz. colony colour, colony shape etc. the bacterial strains showed varied morphology as circular, Punctiform, undulate and irregular colonies. The bacterial stains A, B, C, D, E & F showed positive Citrate test and Catalase test was positive for the bacterial stains A, D, E, I, L and N. Some strains B, C, M & J showed plant growth promoting activity too.

Key words: *Rhizosphere, Micro-organisms, PGPRs.*

Introduction

The rhizosphere is considered to be a hotspot of bacterial diversity and a rich source of various microorganisms which favors the plant growth and survival. The rhizosphere micro-environment is that biologically active area of soil where microorganisms interact with plant roots and it is of great importance for plant health, as well as for nutrient cycling (Fang et al., 2022). The microorganisms living in plant rhizosphere also play a significant role in affecting the plant growth and productivity. Plant-growth-promoting rhizobacteria (PGPRs) are rhizosphere bacteria that can enhance plant growth through a wide variety of mechanisms, like phosphate solubilization, siderophore production, production of enzymes to mineralize N, S orP from organic compounds to provide plants corresponding inorganic ions, nitrogen fixation (Vessey, 2003; Fischer *et al*., 2007; Cummings, 2009; Meena *et al*., 2020; Fausi *et al*., 2021; Bhat *et al*., 2023). Wheat (*Triticum aestivum*) is one of the world's most important cereal crops because wheat is used for manufacturing of a majority of food products such as bread, cakes, noodles, or cookies (Wolinska *et al*., 2020). The vide usage of wheat in food items leads to its selection in present study.

A number of bacterial species associated with the plant rhizosphere have been isolated and were found belonging to the genera *Azospirullum, Alcaligenes, Azotobacter, Acinetobacter, Bacillus, Enterobacter, Flavobacterium, Pseudomonas, Rhizobium and Serratia* are able to exert a beneficial effect on the plant growth (Rawat *et al*., 2011; Wolinska *et al*., 2020).

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It is generally assumed that PGPR trigger an increase in root surface area which results in an increased mineral uptake and in turn, enhances the shoot biomass accumulation. PGPR improves plant growth directly or indirectly like by suppression of plant disease, improvement of nutrient acquisition or phytohormone production. PGPR also increase plant height, grain weight and grain yield. Therefore, application of PGPR with low fertilizer rates could be a viable supplementary strategy for maximum benefits in terms of cost of production and sustaining productivity. Hence, keeping in mind all the above aspects, the present study was conducted to isolate and identify the microbial species in rhizosphere of wheat plant on morphological and biochemical basis.

Materials and Methods

Sample Collection

Soil and root samples of the wheat were collected aseptically in sterile bags from the fields at Yamuna Nagar.

Isolation of bacterial colony

The solidified nutrient agar (NA) plates were taken and the soil serial dilutions from all the test tubes were poured into the plates and the plates were then kept in incubators at 37^0 C for 24 hrs. The plates were then observed for microbial growth and were sealed with paraffin and kept at 4^0 C for further studies and tests. The colonies so obtained were numbered alphabetically

for documentation. The slants were also prepared and preserved for each plate for further experiments.

Morphological Characterization

Morphological characteristics viz., colony morphology (color, shape, elevation and growth) and cell morphology (shape, color and gram staining) of the recovered isolates were studied.

Biochemical Characterization

The various biochemical characteristics viz., Catalase test, Citrate test, Gram staining, plant growth promoting effect of rhizobacteria on seed germination were carried out.

1. Catalase test for H_2O_2 production:

Most aerobes and anaerobes have the ability to show catalase activity. Actually these organisms use O_2 to produce H_2O_2 which is toxic to their enzyme system. Hence, these organisms produce enzyme catalase which converts H_2O_2 to H_2O & O_2. Bacterial colony was picked from the plate and transferred on a microscopic glass slide in a drop of water. A few drops of 3% H_2O_2 over the culture and observed for appearance of bubbles in 20 seconds indicating positive catalase activity

2. *Citrate Test*

Citric acid is an intermediate of metabolic product of Krebs's cycle which oxidizes pyruvate to CO_2. The bacteria must have the ability to transport it across the membrane. This test is based on the ability of an organism to utilize citrate as it is the only source of carbon and ammonia as nitrogen. Simmon's citrate agar medium was prepared and used for streaked with the cultured strains. After incubation at 37^0C for 48 hrs the plates were examined for the change in medium green colour to blue due to change of pH as it becomes alkaline by citrate utilization.

3. *To check plant growth promoting effect of rhizobacteria on seed germination*

Moong beans were incubated in NA medium followed by their transferring when germinated onto MS medium and were incubated for 24 hours. After 24 hrs it was observed that some seeds show germination in plates while others did not. It was concluded that the microorganisms promote the growth of moong beans in which they were seen germinated.

4. *Gram staining of isolated bacteria*

Gram staining is a bacteriological laboratory technique used to differentiate bacteria by chemical & physical properties of their cell walls by detecting peptidoglycan which is present in cell wall of gram positive bacteria. Gram positive bacteria retain the crystal violet dye and thus are stained violet while the gram negative bacteria do not. After washing a counter stain is added i.e. safranin that will stain these gram negative bacteria a pink color.

Results and Discussion

A. Morphological characterization

The isolated microbial strains were examined morphologically and were found to have different shapes like irregular, circular, undulate etc. colour of isolated colonies varied from off-white, white and pale yellow. Some colonies were flattened and some showed elevation and different growth patterns like slow, moderate and fast growers were observed among isolated samples. The morphological study of each isolated bacterial strains is shown in Table 1.

Table 1. Morphological characteristics of isolated bacteria:

Strain No. & soil dilution conc.	Shape	Color	Elevation (Yes/No)	Growth (Fast/moderate/ slow)
A (10^{-5})	Irregular	Pale Yellow	No (Flat)	Moderate
B (10^{-4})	Punctiform	White	No	Slow
C (10^{-7})	Irregular	Off White	Yes	Fast
D (10^{-7})	Undulate	Off White	Yes	Moderate
E (10^{-7})	Rhizoid	Off White	Yes	Fast
F (10^{-9})	Rhizoid	Off White	Yes	Fast
G (10^{-8})	Circular	Off White	No	Slow
H (10^{-10})	Irregular	White	No	Fast
I (10^{-10})	Irregular	White	No	Fast
J (10^{-6})	Curled	Pale Yellow	No	Slow
K (10^{-6})	Irregular	White	Yes	Moderate
L (10^{-6})	Undulate	White	No	Slow
M (10^{-6})	Punctiform	White	No	Fast
N (10^{-4})	Circular	White	No (Flat)	Moderate
O (10^{-8})	Punctiform	Off White	No	Fast

B. Biochemical tests

1) Catalase test for H_2O_2 production:

Oxygen bubbles were released bacterial stains A, D, E, I, L and N whereas all other strains did not show any bubbles (Figure 1). This shows that the organisms A, D, E, I, L and O are catalase producer and all others e.g. B, C, G, H etc are not catalase producer.

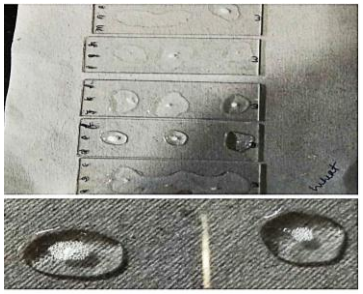

Figure 1 Bubble production showing the catalytic activity

2. Citrate Test

The growth of the organisms at the streaked regions in the plates containing Simmons citrate medium and color change from green to blue indicates citrate utilization. The color change from green to blue was observed in bacterial stains A , B ,C ,D ,E & F to a greater extent which shows citrate utilization in them while I & J shows a little change while others didn't show any change and hence didn't possess any citrate utilization (Figure 2 a & b).

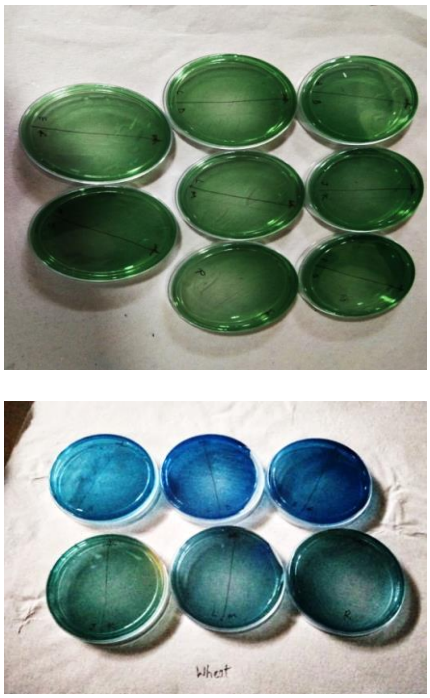

Figure 2 (a) shows the plates after streaking and before incubation while, (b) shows the plates after incubation and change in colour is noticed

3. Effect of PGPR on plant germination

After 24 hrs it was observed that strains B, C, M & J showed fully germinated seeds while a little germination in F and no germination was observed in N (Figure 3). The better growth pattern observed in above mentioned strains can be attributed to the PGPRs.

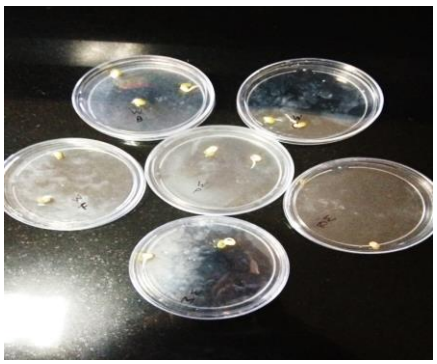

Figure 3 Germination in seeds due to presence of PGPR

4. Gram Staining

The following result was obtained after observing under microscope (Figure 4). The isolated strains of wheat shows two color variations, few were blue-violet (strain A & G) Gram's positive and others were pink (B, C, D, E, F, H & I) Gram's negative strains.

Figure 4 shows the gram stained slides of bacteria's isolated

Conclusion

From above all performed tests and analysis it is concluded that various bacterial strains are present in the wheat rhizosphere that affects the growth of the plant and show various characteristics properties that are depicted by the morphological and the biochemical analysis. The bacterial strains according to the carried study could be: *Acetobacter* (show catalase and oxidase activity), *Bacillus* (based on their cell morphology and gram reaction), *Strenotrophomonas* (based on cell motility and morphology), *Flavobacterium* (gram negative), *Azospirullum* and *Enterobacter* etc. The study concluded the presence of PGPR affect the plant growth and helps it in survival and increased growth rate and productivity. Further experiments and tests are needed to be carried out in view to characterize the concerned species.

References

[1] Bhat, M.A., Mishra, A.K., Jan,S., Kamal, M.A., Rahman, S., Shah,A. A. and Jan, A.T. (2023). Plant Growth Promoting Rhizobacteria in Plant Health: A Perspective Study of the Underground Interaction. Plants (Basel). 2023; 12(3): 629.

[2] Cummings, S.P. (2009). The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops: potential and problems. Environ. Biotechnol., 5 (2): 43-50.

[3] Fang, X.; Wang, H.; Zhao, L.; Wang, M.; Sun, M. (2022). Diversity and structure of the rhizosphere microbial communities of wild and cultivated ginseng. BMC Microbiol. 22, 2.

[4] Fasusi, O. A., Amoo, A. E. and Babalola O. O. (2021). Characterization of plant growth-promoting rhizobacterial isolates associated with food plants in South Africa. Antonie Van Leeuwenhoek. 114(10): 1683–1708.

[5] Fischer, S.E., Fischer, S.I., Magris, S., Mori. G.B. (2007). Isolation and characterization of bacteria from the rhizosphere of wheat. World J. Microbiol. Biotechnol., 23: 895-903.

[6] Meena, M.; Swapnil, P.; Divyanshu, K.; Kumar, S.; Harish; Tripathi, Y.N.; Zehra, A.; Marwal, A.; Upadhyay, R.S. (2020). PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. J. Basic Microbiol., 60, 828–861.

[7] Rawat, S., Izhari, A. and Khan, A. (2011). Bacterial Diversity in Wheat Rhizosphere and their Characterization. Advances in Applied Science Research, 2 (2): 351-356.

[8] Vessey, J.K. (2003) Plant Growth Promoting Rhizobacteria as Biofertilizers. Plant and Soil, 255, 571-586.

[9] Wolińska, A., Kuźniar, A. and Gałązka, A. (2020). Biodiversity in the Rhizosphere of Selected Winter Wheat (*Triticum aestivum* L.) Cultivars—Genetic and Catabolic Fingerprinting. Agronomy, 10(7), 953