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ORIGINAL RESEARCH

Plant Growth Promoting Effects of Native Microbial Isolates From *Zea Mays L.*

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Abstract

Maize (Zea mays L.) alongside rice and wheat, stands as one of the three most crucial agronomic crops globally in terms of productivity. In Tamil Nadu, districts like Coimbatore, Salem, Erode, and Virudhunagar constitute 77 percent of the total area of maize cultivation. Pests and diseases have developed as a result of constant cropping and a congenial environment. Inoculating crops with plant growth-promoting rhizobacteria (PGPR) presents an attractive option for widespread adoption, as it could substantially reduce reliance on chemical pesticides and fertilizers. This reduction would mitigate environmental contamination and alleviate associated negative impacts on both ecosystem and human health. The objective of the study is to isolate microorganisms from maize fields and to determine their plant growth-promoting traits. Samples of soil and rooted maize plants were obtained from several locations in and around Coimbatore, and standard microbiological techniques were appiled during processing. The biochemical assays were performed on fifty-four strains that had been isolated. Utiiizing various isolated PGPR attributes such as ammonia, indole acetic acid (IAA), and siderophores, alongside employing pectinase, cellulase, protease assay, and dual culture technique, to combat the fungal pathogen, Fusarium moniliforme. The majority of the isolates exhibited at least one positive attribute, indicating their potential as PGPR. Out of 54 isolates, 50 demonstrated ammonia production ability, 38 isolated can produce indole acetic acid, 14 tested positive for siderophore, 11 exhibited cellulase production, 8 isolates are pectinase producers, and 26 showed antagonistic behavior against Fusarium moniliforme, which is a maize pathogen. These isolates' ability to promote plant development and exert biocontrol activity suggests that they could be a valuable bloinoculant for sustainable agriculture.

Keywords

Maize, PGPR, IAA, Fusarium moniliforme, siderophores

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1. Introduction:

Maize (*Zea mays L.*) alongside rice and wheat, stands as one of the three most crucial agronomic crops globally in terms of productivity. Due to the rising demand for food and animal feed, it is a significant crop in both semi-arid and temperate climate regions. It is well recognized that the extensive use of agrochemicals to increase crop cultivation has detrimental impacts on arable soils *(Kozdro´j et al. 2004)*. Soil microbial communities are pivotal and frequently distinctive contributors to ecosystem functions, constituting some of the most intricate, diverse, and crucial assemblies within the biosphere *(Zhou et al. 2003)*.

For several decades, research has been conducting into the potential role of soil microorganisms in enhancing plant fertility and reducing dependence on fertilizers. Beneficial free-living soil bacteria associated with roots and other plant tissues, employ diverse mechanisms to augment nutrient supply to crops *(Kloepper et al. 1989; Saharan and Nehra, 2011)*. They can either promote plant growth in direct manner and also in indirect manner. The direct mechanism involves fixation of nitrogen, solubilization of phosphorus, synthesis of siderophore and phytohormone synthesis. In contrast, indirect promotion occurs when rhizobacteria reduce or eradicate the determental effects of one or more pathogenic microbes *(Penrose and Glick, 2003)*. This is achieved through the synthesis of anatagonistic chemicals, enhancement of plant defense, utimately leading to stimulate plant growth. Numerous bacteria, such as *Klebsiella, Bacillus, Azospirillum, Herbaspirillum, Burkholderia and Pseudomonas spp.*, have been discovered to be linked with cereals like maize and other crops. It has been demonstrated that introducing these bacteria into maize increases crop yield *(Wu et al. 2005; Mehnaz et al. 2010)*.

In the current study, rhizosphere bacteria were isolated from several areas in and around Coimbatore that grow maize, and they were then identified and characterized based on their functional characteristics related to biocontrol and plant development promotion.

2. Materials and Methods:

2.1. Bacterial strain isolation and sampling:

Ten different soil samples and plants with roots intact were collected from different maize growing regions in around Coimbatore. These regions are prominent maize growing regions in the district. Soils that adhered loosely were shaken and separated from the roots and removed of, while intact root systems were gathered. After that, root sections with adhering rhizosphere soil were placed in 100 mL of sterilized water and agitated for 10 to 15 minutes. All sample suspensions performed three replications of serial dilution up to 10-7. For the initial screening of the isolates, 100 µl of each dilution, ranging from 10-3 to 10-7, was spread plated onto nutrient agar plate and incubated for 24 to 48 hours at 37ºC . On the basis of colony morphology, 54 strains were selected and stored at -20 ºC in nutrient broth containing 60% glycerol. Furthermore, the in vitro plant growth promoting and biocontrol capabilities of the isolates were further evaluated.

2.2. Assay for biocontrol attributes and plant growth promotion

2.2.1 Production of ammonia

Freshly grown cultures were inoculated into 10 mL of peptone water and incubated for 48 to 72 hr at 37°C. Following incubation, 0.5 mL of Nessler's reagent was added to each tube, and brown to yellow color indicative of ammonia production, was observed *(Cappuccino and Sherman, 1992).*

2.2.2 Production of Indole acetic acid

The isolates were cultured for 5 to 7 days at 30°C in nutritional broth supplemented with tryptophan (2 mg/ml). After incubation cultures were centrifuged for 30 minutes at 3000 rpm. Orthophosphoric acid (2 drops) and four milliliters of the Salkowski's reagent solution were combined with the supernatant (2 milliliters). The emergence of the pink color signifies the production of IAA *(Bric et ai. 2004).*

2.2.3 Siderophore production

Utilizing blue agar plates incorporated with the dye chrome azurol S (CAS) (60.5 mg dissolved in 50 mL of sterile distilled water and mixed with 10 mL iron (III) solution (1 mM FeCl3.6H2O, 10 mM HCl). This solution was added slowly to 72.9 mg hexa-decyl-tri methyl ammonium bromide (HDTMA) dissolved in 40 mL water. The resultant dark blue liquid was autoclaved at 121ºC for 15 min. This solution was slowly added to the nutrient agar media), following the method described by *Schwyn and Neiiands' (1987)* which is a universal technique was used to detect siderophore production. On blue agar, the colonies' orange halos indicated the presence of siderophores.

2.3. Cell wall degrading Enzymes

2.3.1 Protease Activity

Skim milk agar plates was utilized to detect protease activity. The isolates were inoculated and then incubated at 37°C for 24 to 48 hrs. The activity was determined by observing a clear halo of hydrolysis around the colony *(Smibert and Krieg, 1994).*

2.3.2 Ceiiuiase Activity

Strains were cultured on M9 medium supplemented with cellulose (10 g/L) and yeast extract (1.2 g/L), then incubated for 8 days. After the incubation period, Gram's Iodine solution was applied to observe the zone of hydrolysis. Clear zones indicate positive for cellulase production *(Catteian et ai. 1999).*

2.3.3. Pectinase Activity

Strains were cultured on M9 medium along with pectin (4.8 g/L) and of yeast extract (1.2 g/L), then incubate for 3 days. Following incubation, Gram's Iodine solution was overlaid to visualize the zone of hydrolysis. Colonies showing clear zone were indicated as pectin producer *(Catteian et ai. 1999).*

2.3.4. Antagonistic Activity against the Pathogenic Fungi

Every bacterial isolates was streaked one side of a Capek dox agar plate, with a 5mm mycelia mat of the soil-borne fungus, *Fusarium moniiiforme* on the opposite side. The plate was then incubated for 7 to 14 days at 28°C. Inhibition zone that developed between the bacterial and fungal isolates during cultivation demonstrated the antagonistic action of the bacterial isolates against the fungal isolates *(Ayyadurai et ai. 2007).*

3. Results & Discussion

3.1 Ammonia Production

All green plants need nitrogen as one of their main nutrients, however, most biological systems can't use nitrogen without modification. Ammonia is produced during the nitrogen fixation process from molecular nitrogen and soil's nitrogen-fixing bacteria are responsible for this process *(Mbai et ai. 2013).* Hence the production of ammonia is an important trait that can determine the PGPR potential of a bacterium. Of the 54 strains that were tested, 50 of them turned yellow to brown when Nessler's reagent was added *(Figure 1),* indicating that ammonia may have been produced.

3.2. Indole Acetic Acid production

The most important auxin in plants, IAA regulates a variety of critical physiological functions such as tissue differentiation, cell growth and division, and light-responsiveness *(Frey-Kiett et ai. 2005).* The principal impact of indole acetic acid produced by PGPR on the root system is to increase the size, quantity, and ramifications of adventitious roots. This allows the roots to utilize a greater volume of soil, which supplies the plant with a significant amount of nutrients.

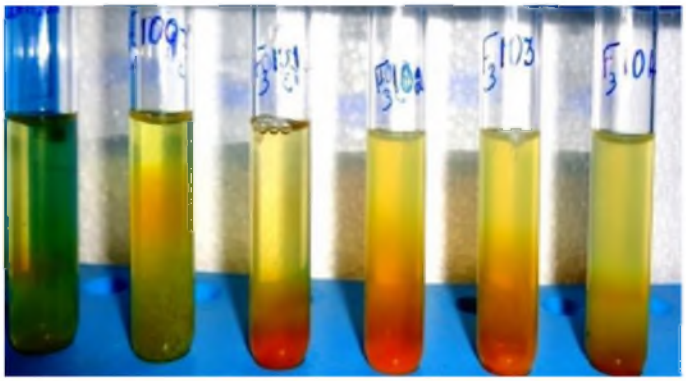

Figure 1: *Ammonia Production Test*

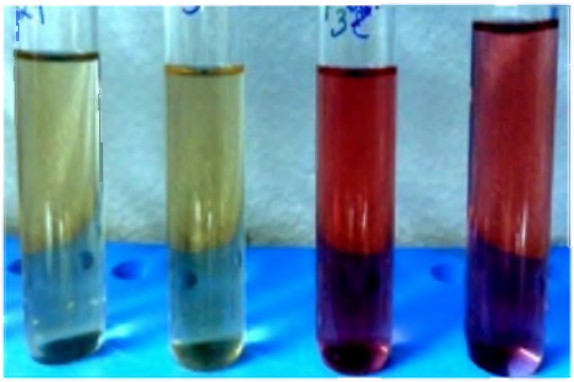

Figure 2: *IAA Production Test*

Out of 54 isolates analyzed, 38 isolates showed a positive result for IAA production *(Figure 2).*

3.3 Production of Siderophores

In the rhizosphere, siderophores bind to iron (Fe3+), rendering it inaccessible to phytopathogens and safeguarding the health of the plants. 14 of the 54 isolates that were chosen had the CAS-blue agar test confirm that they produced siderophores *(Figure 3)*. The characteristic color for the reaction where siderophores remove iron from CAS that is documented in the literature indicates the generation of siderophores *(Schwyn and Neilands 1987)*.

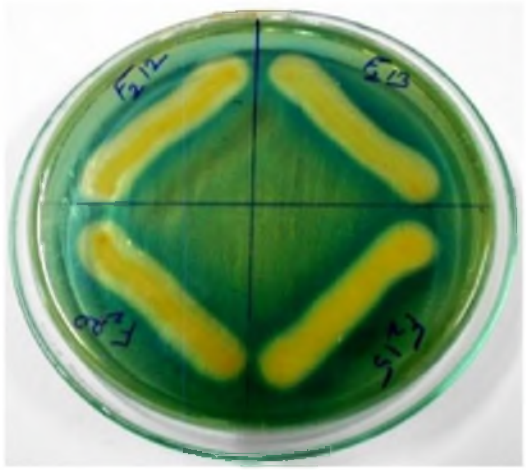

Figure 3: *Production of Siderophores*

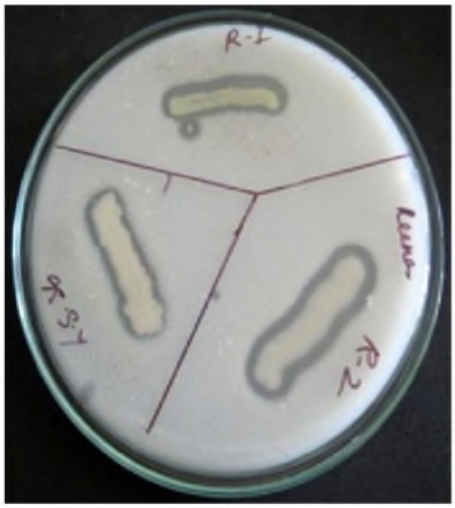

Figure 5 : *Cellulase Activity*

3.4 Cell wall degrading Enzymes

3.4.1 Protease Activity

Protease is an extracellular enzyme that is known to hydrolyze the polymeric compounds of microbial cell wall. Protease producing bacteria displaces other bacteria if they are large in number and also inhibits other bacteria from growing rapidly. Out of the 54 strains, 26 isolates produced distinct zones surrounding the colony on skim milk agar media, indicating proteolytic activity *(Figure 4)*.

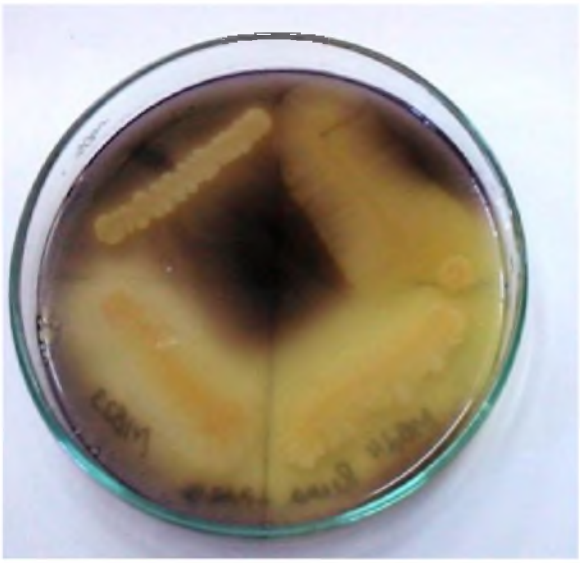

Figure 4: *Protease Activity*

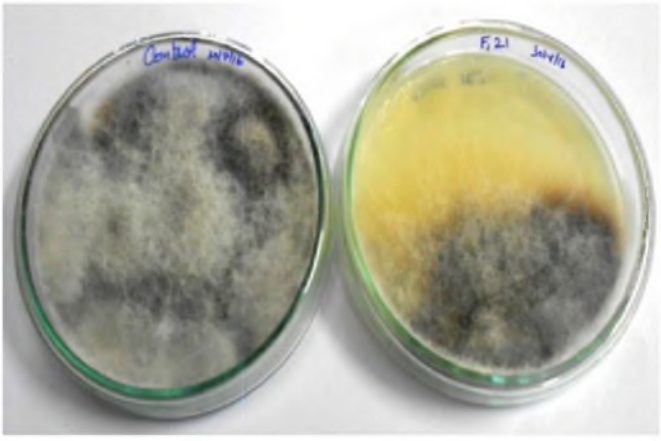

Figure 6 : *Antagonistic activity towards Fusarium moniliforme*

Table 1: *Characterization of the bacterial isolates for plant growth promoting and biocontrol traits*

Isolate No.	NH3	IAA	Sid	Protease	Ant studies	Cellulase	Pectinase
F1 1	-	+	+	-	++	-	-
F1 2	++	+	+	+	++	+	+
F1 3	++	+	+	-	++	-	+
F1 4	+	+	+	+	++	+	-
F1 5	+	+	+	+	++	++	+
F1 6	++	+	+	+	++	-	-
F1 7	++	+	+	-	-	-	-
F1 8	++	+	+	+	++	-	-
F1 9	++	++	++	+	-	-	-
F1 10	++	++	-	+	-	+++	+
F1 11	++	+	-	+	-	-	-
F1 12	+	+	-	+	++	+++	+
F1 13	++	-	-	+	++	-	-
F1 14	-	-	-	-	++	-	-
F1 15	++	+	-	+	++	+	+
F1 16	++	-	-	-	-	-	-
F1 17	+	++	+	-	-	-	-
F1 18	+	++	+	-	-	-	-
F1 19	++	+	+	+	++	+++	+

Table 1 (Contd.): *Characterization of the bacterial isolates for plant growth promoting and biocontrol traits*

Isolate No	NH3	IAA	Sid	Protease	Ant studies	Cellulase	Pectinase
F1 20	++	+	+	+	++	-	-
F1 21	++	+	+	+	++	++	+
F1 22	++	-	-	-	++	-	-
F1 23	++	+	-	+	-	-	-
F1 24	++	+	-	+	-	-	-
F1 25	-	-	--	-	-	-	-
F1 26	++	+	+	+	-	-	-
F1 27	++	+	-	+	-	-	-
F1 28	++	+	-	+	-	-	-
F1 29	++	-	-	-	-	-	-
F1 30	++	-	-	-	-	-	-
F1 31	++	+	+	+	-	-	-
F1 32	++	+	-	+	-	-	-
F1 33	+	+	-	-	-	-	-
F1 34	++	++	-	+	-	++	-
F1 35	++	++	-	+	-	-	-
F1 36	++	+	-	-	-	-	-
F1 37	++	+	-	-	-	-	-
F1 38	++	+	-	-	-	-	-

Table 1 (Contd.): *Characterization of the bacterial isolates for plant growth promoting and biocontrol traits*

Isolate No	NH3	IAA	Sid	Protease	Ant studies	Cellulase	Pectinase
F1 39	++	+	-	-	-	-	-
F1 40	++	++	-	-	++	++	-
F1 41	+	++	-	-	++	-	-
F1 42	++	++	-	+	-	-	+
F1 43	++	-	-	+	-	-	-
F1 44	++	-	-	+	-	-	-
F1 45	++	-	-	-	-	-	-
F1 46	+	-	-	-	++	++	-
F1 47	+	-	-	-	++	++	-
F1 48	+	-	-	-	++	-	-
F1 49	+	-	-	-	++	-	-
F1 50	++	-	-	-	++	-	-
F1 51	++	+	-	-	++	-	-
F1 52	-	+	-	-	++	-	-
F1 53	-	-	-	-	++	-	-
F1 54	++	-	-	-	++	-	-

3.4.2 Cellulase Activity

Cellulose has a tight structure that needs to be hydrolyzed by enzymes. Certain cellulases break down celluloses and produce the glucose component, including 1, 4-glucosidase, exo1, 4-d-glucanase, exocellobiohydrolase, and others *(Chaiharn et al. 2008).* Many studies exist about the synthesis of lytic enzymes by microorganisms (*Huang and Chen, 2004; Gupta et al. 2006).* Out of the 54 isolates, 11 isolates showed a clear zone upon the addition of Gram's iodine. It may indicate the role of these microorganisms in biocontrol *(Figure 5).*

3.4.3 Pectinase Activity

A class of enzymes known as pectinase is known to catalyze the depolymerization and deesterification reactions that break down pectic compounds. These enzymes play a part in shielding plants from pathogen-induced infections. One key method of fungal suppression that these enzymes can do is break down the fungal cell wall. Out of the 54 isolates screened, 8 isolates exhibited a good zone of clearance after the addition of Gram's iodine.

3.4.4 Test for Antagonism

One common in vitro technique for initial screening of biological control agents is the dual culture test *(Desal et al. 2002).* The development of inhibitory zones between the bacterial and fungal isolates verified the antagonistic effects *(Figure 6).* Out of the 54 isolates, 26 isolates had good antagonistic activity against Fusarium moniliforme.
The results of plant growth promotion and biocontrol traits has been tabulated in *Table 1.*

4. Conclusion

A total of 54 rhizospheric bacteria that were isolated from various maize-growing locations were examined for potential health benefits as PGPB. These isolates were subjected to experiments to ascertain their enzymatic, auxin-producing, and siderophore-producing properties. The biochemical and enzymatic activities exhibited by these PGPB isolates against *Fusarium moniliforme*, a prevalent soil pathogenic fungus, confirm their potential utility in enhancing fungal resistance in higher plants. To determine these microbes' function as an effective PGPR, molecular methods are necessary. The field of biofertilization and biological control will pay more attention to the native bacteria linked to maize plants because of their multipurpose qualities. The biodiversity of these bacteria will be better understood, and strategies for using these strains as inoculants in organic and sustainable agriculture will be developed.

Conflict of Interest Statement

No conflict of interest is declared by the authors

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