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## Isolation and Evaluation of Antagonistic Plant Growth Promoting Rhizospheric Bacteria from Winter Maize

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### Abstract

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The minerals, vitamins, fiber and oil included in maize make it a particularly good source of these nutrients. Plant growth-promoting bacteria have been demonstrated to be useful agents for protecting host plants from pathogens and stress conditions in addition to enhancing plant growth and development. In the current study, bacteria from the rhizosphere of winter maize was isolated and their antagonistic activity against *Rhizoctonia solani* and plant growth-promoting qualities was assessed. Four soil samples yielded a total of thirteen microorganisms, of which eleven showed antagonistic activity towards the disease *Rhizoctonia solani*. Of these eleven isolates, seven could create ammonia, nine could break down inorganic phosphate in a test tube, and eight could dissolve zinc.

**Keywords:** Maize, *Rhizoctonia solani*, antagonism, ammonia production, phosphate and zinc solubilization

## 1. Introduction

It is believed that *Zea mays*, usually referred to as maize, developed from a wild grass in central Mexico about 7000 years ago. Maize was enhanced by Native Americans to become a more nutrient dense food source. With a 72% carbohydrate, 10% protein, and 4% fat content, maize has a 365 Kcal/100 g caloric density. The maize plant yields a vast variety of food and industrial products, including starch, sugars, oil, drinks, glue, industrial alcohol, and fuel ethanol (Ranum *et al.*, 2014). The rhizosphere of maize contains a variety of rhizobacteria types, including *Streptomyces*, *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Azospirillum* Rhizobacteria that promote plant growth either directly or indirectly affect the plants themselves to provide their growth- promoting effects. The disease *Rhizoctonia solani* f. sp. *Sasakii* caused banded leaf and sheath blight (BLSB) in maize is regarded as one of the most significant and a major factor in low yields. First identified on maize in Sri Lanka, banded leaf and sheath blight (BLSB) is caused by *Rhizoctonia solani* f.sp. *sasakii* Exner (Bertus, 1927). *Rhizoctonia solani* is regarded as a significant soil- borne plant pathogenic fungus that reduces crop yields by 20-40% per year lughout the world. Most of the world is home to the bacterium *Rhizoctonia solani*, which may intact a variety of host plants, including maize and cause seed decay, damping off, stem canker, root rot, aerial blight, and seed/cob decay. *Rhizoctonia solani* is able to endure harsh climatic conditions because it builds extremely resilient structures and is a significant source of the illness known as sclerotia. According to Abbas *et al.* (2019), using plant-growth-promoting rhizobacteria (PGPR) and microbial biocontrol agents is one of the safer, longer-lasting ways to control *Rhizoctonia solani*. Through siderophore activities and nitrogen fixation, PGPR can reduce the impact of poor soil on the yield of maize. Additionally, phosphorus is crucial for the growth of maize. *Agrobacterium*, *Burkholderia*, *Bacillus*, *Erwinia*, *Flavobacterium*, *Pantoea*, *Pseudomonas*, *Microbacterium*, *Mycobacterium*, *Rhizobium*, and *Sphingomonas* are among the genera to which PGPR belongs. In addition to producing plant hormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase is another mechanism by which plant-associated microorganisms can promote the growth of plants. It can also improve nutrient absorption by the abilization of inorganic phosphate and zinc oxide as well as the production of ammonia and siderophore (Nadeem *et al.*, 2016; Scagliola *et al.*, 2016; Shaikh and Saraf 2017, Nutaratat *et al.*, 2017).

## 2. Materials and methods

### Bacterial isolates from winter maize rhizosphere: Isolation and purification

Four soil samples were taken for this study from the rhizosphere of several winter maize crops. The method employed to isolate the bacterium was serial dilution. We chose three distinct types of media: nutrient agar (NA), King's B medium (KB), and Jensen's medium (JM) to isolate several bacterial isolates from the rhizosphere of winter maize. Colonies with distinct morphotypes were selected and streaked on new plates, while isolates with the same colony, colour, and morphology were eliminated. Until pure cultures were obtained, these isolates were repeatedly streaked on the appropriate media. On the appropriate media, the slant cultures were created and kept at 4°C. Subculturing was carried out eight weeks apart.

### Antifungal test of bacterial isolates against *Rhizoctonia solani*

One centimeter from the border of the Petri dish containing PDA, bacterial isolates were streaked. In opposing Petri plates, perpendicular to the bacterial streak, nine mm of *Rhizoctonia solani* mycelium was inserted and cultured for seven days at 28 °C. Control plates were Petri dishes that had only been inoculated with fungus. Using the formula proposed by Skidmore and Dickinson (1976), % Inhibition =  $\frac{\text{Growth of pathogen in control (mm)} - \text{Growth of pathogen in treatment (mm)}}{\text{Growth of pathogen in control (mm)}} \times 100$ . The width of the inhibition zone and mycelia growth of the test pathogen were observed and recorded (Beneduzi *et al.*, 2012).

### Screening of bacterial isolates of winter maize properties of Ammonia production test and Phosphate & Zinc solubilization activity

The ability of each bacterial isolate to produce ammonia in peptone water (Peptone 10 g, NaCl 5 g in 1 litre, pH-7.0) was examined. Using Nessler's reagent, a qualitative assessment of the bacteria's capacity for production was made. The isolated rhizobacteria were raised in 4% peptone broth and cultured at 30°C for seven days. The bacterial solution was then given 0.5 ml of Nessler's reagent. According to Yadav *et al.* (2010), the transition from brown to yellow colour signifies the synthesis of ammonia.

The cultures were grown on double-layered plates so that the phosphate solubilizing ability could be evaluated. The bromophenol blue dye was used in this medium to make the National Botanical Research Institute (NBRI) growth medium and Pikovskaya, S media (PKV). This medium was utilised to isolate the phosphate solubilizer. Spot inoculations of exponentially growing isolates were made on the plates, which were then incubated at 28 °C for 7 days. When a clear hole enclosing the spot-inoculated bacterial colonies was seen after the incubation time, the reaction was deemed positive. By measuring the zone of solubilization surrounding the colonies, the level of solubilization exhibited by each chosen isolate was calculated (Sezen *et al.*, 2016).

By employing the technique outlined by Fasim *et al.*, (2002), a qualitative analysis for ZnO solubilization by isolated rhizobacteria was carried out. Utilizing Tris-minimal salt media containing (g/L), the isolates were tested for their capacity to solubilize zinc. To see the halo zones surrounding the bacterial colonies, the bacterial isolates were seeded on plates and cultured at 30 °C for 7 days. To gauge the level of solubilization achieved by each isolate, the zone of solubilization

surrounding the colonies was measured (Fasim *et al.*, 2002).

### 3. Results and discussion

A diverse and active biological habitat for notable bacteria is the plant rhizosphere. In addition to interacting with plants and promoting growth through the release of growth compounds and enhanced mineral nutrition, these bacteria help combat diseases that prevent plants from growing healthily. The goal of the current study was to identify microorganisms with PGPR activities and antagonistic properties against *Rhizoctonia solani*, the pathogen that causes banded leaf and sheath blight disease in maize. Thirteen bacterial soil isolates in all were selected and refined further. On mixed PDA and Nutrient Agar media, the ability of thirteen bacterial isolates to prevent *Rhizoctonia solani* growth was evaluated. Out of thirteen, 11 isolates showed inhibitory effect on the pathogenic fungus and recorded with medium to bigger clearing zone on the test medium. Most antagonistic effects against *Rhizoctonia solani* as inhibition recorded 87%, 86% and 83%. Rest two isolates showed very low to negative inhibitory effect against the pathogenic fungus (Table-1). The volatile organic compounds produced by strain HS-26 decreased mycelial development in a manner similar to the extracellular antifungal metabolites, with the suppression of *Fusarium oxysporum*, *Bipolaris sorokiniana* and *Rhizoctonia solani* being around 46.30%, 63.86%, and 44%, respectively. (Wang *et al.*, 2019).

Screening of all the winter maize bacterial isolates was carried out for ammonia production in peptone water medium in the presence of Nessler's reagent. The isolates showed light brown colour (medium amount of ammonia) to deep brownish coloration (maximum production). Among rhizospheric bacterial isolates, six isolate showed deep brown colour which indicated high ammonia production, while three isolates exhibited medium level ammonia production. The four isolates tested very poor for ammonia production (Table-2). Similar results observed by Geetha *et al.*, (2014) all the six bacterial isolates found positive for ammonia production by the PGPR is useful for plant growth promotion.

The qualitative screening of 13 bacterial isolates for phosphate solubilization abilities was carried out on Pikovaskaya medium the five isolates displayed higher phosphate solubilization activity and four isolates showed medium phosphate solubilization activity. Four isolates showed poor phosphate solubilization activity (Table-2). Our results correlate with findings of Chen *et al.*, (2022), as they found fluorescent *Pseudomonas* and *Bacillus* was positive for phosphate solubilization. In other piece of work Kumari *et al.*, (2018) also studied that three bacterial isolates namely BHU B13-398, PF3-SER396 and BHU M showed significant phosphate solubilization. Our experiment results are better supported by earlier findings.

The qualitative screening of 13 bacterial isolates for zinc solubilization abilities was carried out on NBRIP medium. Four isolates showed higher zinc solubilization activity and four isolates showed medium zinc solubilization activity. Five isolates showed poor zinc solubilization activity (Table-2). The application of Zinc fertilizers to the agricultural soil does not have an impact on plant growth as these fertilizers become unavailable in a short period. These bacteria are of great interest as they have been proposed as inoculants for agriculture (Goteti *et al.*, 2013; Saravanan *et al.*, 2007).

**TABLE1:SCREENING OF RHIZOBACTERIAL ISOLATES AGAINST *RHIZOCTONIA SOLANI* OF MAIZE QUALITATIVELY**

| Isolate | Inhibition zone (cm) | Distance (cm) | Inhibition % |
|---------|----------------------|---------------|--------------|
| 1.      | 1.6                  | 3             | 53.33        |
| 2.      | 1.51                 | 3             | 50.33        |
| 3.      | 1.70                 | 3             | 56.66        |
| 4.      | 1.80                 | 3             | 60.00        |
| 5.      | 1.96                 | 3             | 66.33        |
| 6.      | 1.98                 | 3             | 66.00        |
| 7.      | 2.57                 | 3             | 85.66        |
| 8.      | 1.9                  | 3             | 66.33        |
| 9.      | 2.6                  | 3             | 86.66        |
| 10.     | 2.1                  | 3             | 70.00        |
| 11.     | 2.2                  | 3             | 73.33        |
| 12.     | 2.31                 | 3             | 77.00        |
| 13.     | 2.5                  | 3             | 83.33        |

**TABLE 2: SCREENING OF MAIZE RHIZOSPHERIC BACTERIA AND PGP ACTIVITY QUALITATIVELY**

| Isolate No. | Ammonia production | Phosphate solubilization | Zinc solubilization |
|-------------|--------------------|--------------------------|---------------------|
| Control     | -                  | -                        | -                   |
| 1.          | +                  | +++                      | +                   |
| 2.          | ++                 | ++                       | ++                  |
| 3.          | +++                | +                        | +                   |
| 4.          | +++                | +                        | +                   |
| 5.          | +++                | +++                      | +++                 |
| 6.          | +++                | ++                       | +++                 |
| 7.          | ++                 | +                        | ++                  |
| 8.          | +++                | +                        | +                   |
| 9.          | ++                 | ++                       | ++                  |
| 10.         | +                  | ++                       | +                   |
| 11.         | +                  | +++                      | +++                 |
| 12.         | +++                | +++                      | +++                 |
| 13.         | +                  | +++                      | ++                  |

#### 4. References

- [1] Abbas, A., Khan, S. U., Khan, W. U., Saleh, T. A., Khan, M. H. U., Ullah, S., Ali, A. and Ikram, M. (2019). Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: Alternatives to chemical pesticides. *Comptes Rendus Biologies*, 342(5-6), 124-135.
- [2] Beneduzi, A., Ambrosini, A., and Passaglia, L. M. (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and molecular biology*, 35, 1044-1051.
- [3] Bertus, L. S. (1927). A sclerotial disease of Maize (*Zea mays* L.) due to *Rhizoctonia solani* Kühn. A sclerotial disease of Maize (*Zea mays* L.) due to *Rhizoctonia solani* Kühn. P-44-46.
- [4] Chen, S.X. Kie, Z.X. and Shen, P. (2022). Isolation, Identification and over siderophore production of *Pseudomonas*. *J. Biol.*, 46(5): 691-695.
- [5] Fasim, F., Ahmed, N., Parsons, R., and Gadd, G. M. (2002). Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS microbiology letters*, 213(1), 1-6.
- [6] Geetha, K., Venkatesham, E., Hindumathi, A., and Bhadrach, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vignaradiata*(L.) R. Wilczek. *Int.JCurr.Microbiol.App.Sci*. 3(6):799-809.
- [7] Goteti, P. K., Emmanuel, L. D. A., Desai, S., and Shaik, M. H. A. (2013). Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). *International journal of microbiology*, 2013.
- [8] Kumari, P., Meena, M., Gupta, P., Dubey, M. K., Nath, G., and Upadhyay, R. S. (2018). Plant growth promoting rhizobacteria and their biopriming for growth promotion in mung bean (*Vignaradiata* (L.) R. Wilczek). *Biocatalysis and agricultural biotechnology*, 16, 163-171.
- [9] Nadeem, S. M., Ahmad, M., Naveed, M., Imran, M., Zahir, Z. A. and Crowley, D. E. (2016). Relationship between in vitro characterization and comparative efficacy of plant growth-promoting rhizobacteria for improving cucumber salt tolerance. *Archives of microbiology*, 198, 379-387.
- [10] Nutaratat, P., Monprasit, A. and Srisuk, N. (2017). High-yield production of indole-3-acetic acid by *Enterobacter* sp. DMKU-RP206, a rice phyllosphere bacterium that possesses plant growth-promoting traits. *3 Biotech*, 7, 1-15.
- [11] Ranum, P., Pena-Rosas, J. P. and Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York academy of sciences*, 1312(1), 105-112.
- [12] Saravanan, V. S., Madhaiyan, M., and Thangaraju, M. (2007). Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere*, 66(9), 1794-1798.
- [13] Scagliola, M., Pii, Y., Mimmo, T., Cesco, S., Ricciuti, P. and Crecchio, C. (2016). Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicon* L.) grown under Fe sufficiency and deficiency. *Plant physiology and biochemistry*, 107, 187-196.
- [14] Sezen, A., Ozdal, M., Kubra, K. O. C., and Algur, O. F. (2016). Isolation and characterization of plant growth promoting rhizobacteria (PGPR) and their effects on improving growth of wheat. *Journal of Applied Biological Sciences*, 10(1),

41-46.

- [15] Shaikh, S. and Saraf, M. (2017). Biofortification of *Triticum aestivum* through the inoculation of zinc solubilizing plant growth promoting rhizobacteria in field experiment. *Biocatalysis and Agricultural Biotechnology*, 9, 120-126.
- [16] Skidmore, A.M. and Dickinson, C.H. (1976). Colony interaction and hyphal interference between *Septorianodorum* and phylloplane fungi. *Transactions and Journal of the British Ceramic Society*.66 : 57-74.
- [17] Wang, X., Li, Q., Sui, J., Zhang, J., Liu, Z., Du, J., Xu, R., Zhou, Y. and Liu, X. (2019). Isolation and characterization of antagonistic bacteria *Paenibacillus jamilae* HS-26 and their effects on plant growth. *Bio Med Research International*. P-13.
- [18] Yadav, S. K., Srivastava, A., Srivastava, A., Thomas, M. A., Agarwal, J., Pandey, C. M., Lal, R., Yaccha, S. K., Saraswat, V. K. and Gupta, R. K. (2010). Encephalopathy assessment in children with extra-hepatic portal vein obstruction with MR, psychometry and critical flicker frequency. *Journal of hepatology*, 52(3), 348-354.