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

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Abstract: 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, *Rhizoctoniasolani*, Antagonism, Ammonia production, Phosphate and Zinc solubilization

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 nutrientdense 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. Sasakiicaused 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 1ughout 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.*Rhizoctoniasolani* 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 *Rhizoctoniasolani*. 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).

MATERIALS AND METHODS

Bacterial isolates from winter maizerhizosphere: 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 *Rhizoctoniasolani*

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 *Rhizoctoniasolani* 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 Dikinson (1976), % Inhibition = Growth of pathogen in control (mm) - Growth of pathogen in treatment (mm)100 Growth of pathogen in control, 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 Ammoniaproduction 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'sreagent. 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 (NBRIP) 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 ZnOsolubilization 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).

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 *Rhizoctoniasolani*, 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 *Rhizoctoniasolani* 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 *Rhizoctoniasolani* 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 *Rhizoctoniasolani* 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 Nesseler'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 isolatesdisplayed 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 solibilization. In other piece of work Kumari*et al.,* (2018) alsostudied that three bacterialisolates 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 zincsolubilization abilities was carried out on NBRIP medium. Four isolatesshowed higher zinc solubilization activity and fourisolates showed medium zincsolubilization activity. Five isolates showed poor zincsolubilization 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 (Gotet*i et al.*, 2013; Saravanan*et al.*, 2007).

Table1:Screening of rhizobacterial isolates against *Rhizoctoniasolani* 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	+	+++	++

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