IJETIR

ISSN (Online) 2583-0554

International Journal of Emerging Technologies and Innovative Research (IJETIR)

Volume 3, Issue 3, March 2023

Impact Factor: 5.731

Screening, Isolation and Biochemical Characterization of Plant Growth Promoting (PGP) Bacteria Colonizing the Rhizosphere of *Zea mays* L.

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Abstract: *Plant growth promoting Rhizobacteria (PGPR) are a group of bacteria that can be found in the rhizosphere, in association with roots which can enhance the growth of plant directly or indirectly. Rhizospheres of crop plants have been well studied with the objective of screening PGPR, play an important role in maintaining ecological balance. Plant growth promoting Rhizobacteria (PGPR) are an attractive eco-friendly alternative to chemical fertilizers in agriculture. The present work explores the characterization of Rhizobium and azotobacter isolated from root nodules of corn (Zea mays). Each isolates were morphologically and biochemically characterized. Each samples were tested for plant growthpro motion assays including indole acetic acid (IAA), ammonia, NaCl variation assay, to select for ones possessing multi-trait plant growth promoting (PGP) properties. The present study shows the antifungal activity of Rhizobacteria and azotobacter against some fungal spp. The Rhizobacteria were gram negative rod shaped and mucous producing. It utilizes glucose and starch as a carbon source.*

Keywords: PGP bacteria, Indole Acetic acid, NaCl assay, Rhizosphere soil, Azotobacter

I. INTRODUCTION

Plant growth-promoting rhizobacteria are bacteria that colonize plant roots and promote plant development by a number of processes, including phosphate solubilization, siderophore generation, HCN production, and biological nitrogen fixation (Vejan et al., 2016).Today, food and environmental quality are the majorissues from the context of global concern, by utilizing the ecologically beneficial bacteria likePGPR will not only reduce the utilization of chemical fertilizers but also promote yield in regular agriculture practices (Verma et al, 2015).The important role is played by plants in selecting and enriching the types ofbacteria by the constituents of their root exudates. The use of bio-fertilizer and bioenhancer such as N2 (nitrogen) fixing bacteria and beneficial microorganism can reduce chemical fertilizer applications and consequently lower production cost. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture(Ştefan , et al 2008).The classical approaches along with biotechnological and molecular tools combined will definitely aid in understanding rhizophorebiology and their efficient interactions with PGPRs. Kamaruzzaman et al., (2020) isolated bacteria from rhizosphere of *Scirpus grossus* growing in the lead contaminated soil. When rhizobia colonize the roots from non-legume plant in a non specific relationship and may behave as PGPR. It has been proven that plant productivity increases when the Rhizobiaare present in rhizosphere. It provides the major biological source of fixed nitrogen in agricultural soils (Shahzad et al 2012).*Azotobacter* is generally regarded as a free-living aerobicnitrogen-fixer. *Azotobacter* strains could affect seed germination and seedling growth in a plant. It hasbeen shown that wheat yield increased up to 30% with *Azotobacter* inoculation (Gholami et al 1992.). Modern agriculture relies on extreme use of chemical fertilizers and pesticides to increase crop production which caused severe adverse effect on soil health and environment(Aktar et al, 2009).

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However, use of PGPR in agriculture in order to enhance the growth of plant via circulating the nutrients in the soil is an ecofriendly strategy to minimize the need of synthetic fertilizers as much as possible(Verma et al, 2015 and Saharan et al, 2011).There are many species of soil bacteria which are reported to promote plant growth by producing growthregulators, inducing root exudation and enhancing the availability of nutrients to plant besides control of soil born plant pathogenic fungi. PGPR may induce plant growth promotion by direct or indirect modes ofaction. The mechanism involved in PGPR-mediated plant growth promotion is directly by production ofplant growth regulators (auxins, cytokines, gibberellins) and facilitation of the uptake of nutrients(nitrogen fixation, solubilisation of phosphorus). The indirect promotion of plant growth occurs whenPGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitorysubstances (antibiotics, antifungal metabolites, iron-chelating siderophores, cell wall-degrading enzymesand competition for sites on roots) or by increasing the natural resistance of the host (induced systemicresistance).

II. MATERIALS AND METHODS

Screening, Isolation and identification of *Azotobacter*species

The soil sample was collected from agricultural field of Dharangaon, Maharashtra, India. The soil samplewas suspended in 10 ml autoclaved distilled water and serially diluted up to 10^{-8}. 0.1 ml of each dilutionwas spread on sterile Ashby's agar plates and plates were incubated at 30^0C for 48-72 hr. After incubationwell isolated colonies were randomly selected on the basis of colony morphology and maintained onsterile Ashby's agar slants. Each isolate was characterised by gram staining and motility.

Morphological Characteristics

Morphological characteristics of bacteria were observed by using Gram staining technique as describedby Arora, 2003 and observed under oil immersion objective of microscope.

Biochemical Tests

The isolated sample was biochemically characterized viz, Catalase Test, Indole Production Test,Methyl Red Test, Vogas Proskaure Test, Citrate Utilisation Test as described by (Lowe et al,1962), Starchhydrolysis Test and motility test as mentioned by Arora,2003.

Isolation of *Rhizobium* from Corn roots

The fresh root nodules of Corn were collected from the plants grown in the field from MalkapurDist- Buldana, Maharashtra. The collected nodules were surface sterilized with 75 % and 0.1% ethanol andmercuric chloride respectively and washed thoroughly with distilled water. The root nodules were crushedand the suspension was streak on YEM (Yeast Extract Mannitol pH 7.0) agar plates and incubated at29.4°C for two days (Aneja 2003). After two days of incubation, *Rhizobium* colonies were obtained.Isolates colonies were used for morphological and biochemical tests.

Morphological Characteristics

The morphological characteristics comprised colony morphology, colony morphology parameters werediameter, form, transparency and colour Aneja,2003. Motility and Gram staining was performed toevaluate type of strain.

NaCl variation Assay

Rhizobium cultures were grown in triplicates on YEM medium of different concentrations of NaClranging from 0.1 to 1.0% (w/v). Growth was determined by measuring the optical density (O.D) at 600nmafter 48 hours of inoculation.

Gelatin Hydrolysis

To determine capability of *Rhizobium,* to determine gelatinase enzyme as use gelatin as media source.Degradation of gelatin indicates the presence of gelatinase enzyme (Aneja, 2003). The actively growingculture were inoculated in

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nutrient gelatin medium (5g/L peptone, 3g/L beef extract, 12g/L gelatine) andincubated for 48 hours. On subjecting the growing culture to low temperature treatment at 4o C for 30 to60 minutes, the cultures which produce gelatinase remain liquefied while others due to presence of gelatinbecome solid.

Starch Hydrolysis

The test was performed to determine capability of *Rhizobium* to use starch as a carbon source (deOliveira, 2007). Starch agar medium (5g/L peptone, 3g/L potato starch, 3g/L beef extract, 15g/L agar, pH 7.0) were inoculated with culture and incubated at 29.5oC for 48 hours. In the presence of starch theproduction of extra cellular enzymes occurs indicating the potential of the organism to use starch ascarbon source. Drops of Iodine solution (0.1 N) were flooded on 48 hours old incubated Petri plates.Formation of blue colour indicates non ionization of starch and vice versa.

Catalase Test

This test was performed by adding 2-3 drops of 3% hydrogen peroxide in fresh YEM broth cultures ofisolates. Transfer a colony on microscope slide and add the drop of 3% hydrogen peroxide. If catalase is present, the hydrogen peroxide is broken down into the water and oxygen, which result in the immediateformation of gas bubbles.

Antagonistic activity of *Azatobacter*and *Rhizobium*

The *Azatobacter* isolates were grown at 30^0C on a rotary shaker (170rpm) in 250 ml flasks with 100 mlproduction medium containing (g/l^{-1}): Sucrose, 20; Yeast extract, 0.5; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.2;$FeCl_3$, 0.0016 and Na_2MoO_4, 0.001 for 72 Hrs. The bacterial cells were separated by centrifugation at10,000rpm for 30 min. Agar well diffusion method was used to check the presence of antifungalmetabolites. The 0.1 ml suspension of *Fusarium oxysporum* was spreaded on potato dextrose agar plates.The 0.1 ml of cell free supernatant (CFS) was added in well and the plates were incubated at 30^0C andobserved the zone of inhibition around the well.

Morphological Characteristics

The bacterial colonies were observed under oil immersion objective of microscope. Similarly usingGram staining technique was also performed.

Biochemical Tests

All the isolated organisms were biochemically characterized through different biochemical tests viz,Catalase Test, Indole Production Test, Methyl Red Test, Vogas Proskaure Test, Citrate Utilisation test,Starch hydrolysis test as mentioned by (Arora,2003).

Sugar Fermentation Tests:

The isolates were also examined for fermentation of the various sugars including Glucose, Mannitol,Galactose, and Maltose. 1% aqueous stock solution of the test sugars were prepared in small tubes whilefor sialicin 4%sugar solution was prepared and sterilized as mentioned by (Hugh, 1953).

Antagonistic activity of *Rhizobium*

Antagonistic activity of *Rhizobium* was determined by method mention for azatobacter.

III. RESULTS AND DISCUSSION

Isolation of Sample 1species

Isolated Sample 1 colony of bacteriawas selected on the basis of their colony morphology. The isolated Sample 1was gram negative, motile, produce large mucoid, opaque and yellow colour colonies on Ashby's agarplate.

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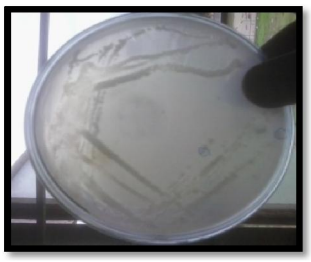

Fig 1: Isolated colony of bacteria of Sample 1

Colony Characteristics:

Table 1: Colony Characteristics of sample 1 bacteria

Sr. No	Colony Characters	Characteristics
1.	Shape	Circular
2.	Size of colony	2.5 mm
3.	Colour	Yellow to green
4.	Elevation	Convex
5.	Margin	Entire
6.	Opacity	Opaque
7.	Motility	Motile
8.	Gram character	Gram negative

Zone of inhibition in antagonistic activity of Sample 1:

The isolated Sample 1 has showed maximum zone of inhibition against *Fusarium* sp. in antagonisticactivities.

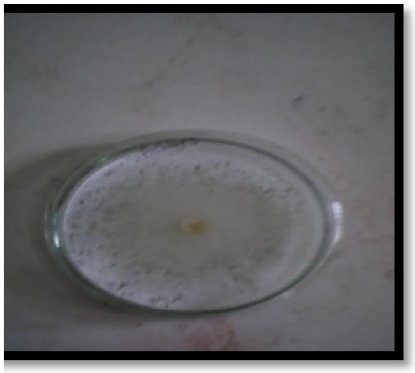

Fig 2: Antagonistic activity of Sample 1 bacteria

Isolation of Sample 2species

Colonies of sample 2 bacteria was obtained on YEM agar medium after incubation at 29.4°C for twodays. Isolated sample 2 bacteria were selected on the basis of their colony morphology. The isolated sample 2 bacteria were gram negative, motile, producing large mucoid, opaque and yellow colour colonies onyeast mannitol agar plate.

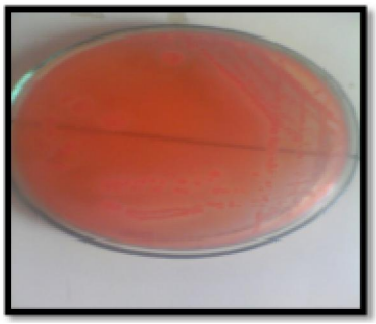

Fig 3: Isolated Sample 2colony of bacteria

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Table 2: Colony Characteristics of sample 2 bacteria

Sr. No	Colony Characters	Characteristics
1.	Shape	Circular
2.	Size of colony	03 mm
3.	Colour	White to pink
4.	Elevation	Convex
5.	Margin	Entire
6.	Opacity	Opaque
7.	Motility	Motile
8.	Gram character	Gram negative

Zone of inhibition in antagonistic activity of sample 2:

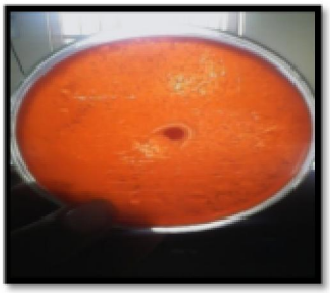

Fig 4: Antagonistic activity of Sample 2 bacteria

The isolated sample 2 bacteria as showed maximum zone of inhibition against *Fusarium sp.* in antagonistic activities.

NaCl variation assay of sample 2

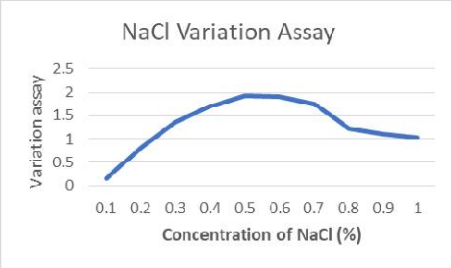

Fig 5: Effect of NaCl % on sample 2 bacteria

Biochemical and Sugar Fermentation tests of sample 2 bacteria

Table 2: Biochemical and Sugar Fermentation test for Sample 2

Sr. No	Tests Performed	Results
1.	Catalase test	Positive
2.	Motility test Positive	Positive
3.	Methyl red test	Negative
4.	Voges- proskauer test	Negative
5.	Indole test	Negative
6.	Glucose	Positive
7.	Galactose	Positive
8.	Maltose	Positive
9.	Mannitol	Positive

Morphological and sugar fermentation tests for identification of Sample 1species

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Table 3: Morphological and sugar fermentation tests for sample 1 species

Sr. No	Tests
1.	Grams nature
2.	Motility
3.	Pigmentation
4.	Amylase production
5.	Mannitol Negative
6.	Galactose Negative
7.	Maltose Negative

IV. CONCLUSION

From the present study, the screening and isolation of sample 1 and 2 bacteria on their selective medium. On the basis of morphological, biochemical and sugar fermentation test, the resulted isolates sample 1 maybe Azotobacter and sample 2 maybe Rhizobium spp. of bacteria. It will be confirmed by the DNA sequencing in near future. We analysed the various plant growth promoting assays for their growth promoting activity. It can be concluded that the *Rhizobacteria* showed bio control characteristics. *In vitro* fungal growthinhibition assay showed antagonism against *Fusarium* spp.

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