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Original Article

Screening for plant growth promoting bacteria around junagadh

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ABSTRACT

Plant growth promoting bacteria help in improving crop yields and have agricultural and environmental importance. Plant growth promoting rhizobacteria found in the rhizosphere of the plant in association with the plant roots help to enhance plant growth directly or indirectly. In the present study attempt has been made to study bacteria which possess plant growth promoting activities such as nitrogen fixation, phosphate solubilization and indole acetic acid production.. Samples of soil were collected from places near Junagadh, 22 showed nitrogen fixation, 32 isolates showed phosphate solubilization and 9 showed indole acetic acid production from 54 isolates. Of the selected isolates were further analyzed for the production of siderophores. These isolates have the tendency for the use as plant growth promoting rhizobacteria and biofertilizer.

Keywords: Phosphate solubilizers, nitrogen fixation, indole acetic acid, biofertilizer.

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Introduction

Soil ecology is the study of the interactions among soil organisms, and between biotic and abiotic features of the environment of soil. It is particularly concerned with the nutrients cycling, formation and maintained of the pore structure, the spread and strength of pathogens, and the biodiversity of the biological community. Earth may look dead and may thought to have no movement occurring in it. In soil, more than terrestrial ecology occur and have abundance biodiversity on Earth. However, there is microbial life in soil that is inhabiting, multiplying, and has coordination with all ecosystems. Microorganism in soil may contain bacteria, fungi, protozoa, algae and viruses. It is difficult still to understand the function, biodiversity and ecosystem(Bowker et al., 2010). Microorganisms are thought to be an important part in the formation of soil configuration. The physical and chemical effect depends on products of microbial degradation of plants and animals residues; products synthesis by microorganisms during decomposition of organic matter; or humus compounds form in the course of microbial degradation of added plant residues, stable matter, etc. The determination of biodiversity of microorganism in soil has become more essential along with the reaction processing occurring in soil ecosystem(Reed & Martiny, 2007). Microbial population mainly depends on fertility of soil. Many recent studies have many approaches in determining bacterial diversity(Lauber et al., 2009). There is still limited understanding in fungi diversity. Fungi in soil have significant contribution in soil for decomposition, the cycling of materials of soil as well as nutrients and maintain biogeochemical cycles(Bailey et al., 2002). The complexity of the interaction in soil ecosystem is established by the several numbers and diverse interactions among its physiological, chemical and biological components (Buscot, 2005). The essential elements especially the major nutrients (NPK) are considered the most important among nutrients and factors limiting growth and yield of plant

since plant growth are negatively affected by their deficiency. In the present study attempt has been made to isolate plant growth promoting bacteria around Junagadh.

MATERIALS AND METHODS.

1.Soil samples

Soil samples from planted area near Junagadh (GA, GB &GC) and forest (FO) near Junagadh. From each place three samples were collected. The rhizosphere soil around the root was carefully dug up to 30 cm depth collected and transferred into polythene bags. The soil samples were stored in refrigerator till further use.

2.Different media used during investigations.

Different components of various media were weighed, dissolved in appropriate amount of water, pH was adjusted to 7.0 and autoclaved in an autoclave at 1210C (15 psi) for 20 minutes. Compositions of various media used during the present investigation are given below:
Jensen's nitrogen free agar medium(Jensen, 1954), Pikovskaya medium(Pikovskaya, 1948), Ashby's media(Pattani & Yadav, 2014), Nutrient agar medium

3.Colony Morphology:

The morphology of isolated colony of each isolates was described with naked eyes according to Benson's Manual(Benson, 2001). They were streaked on freshly prepared Ashby's agar plates and incubated for 2 days at 28°C.

4.Bacterial cell Morphology and Gram's Reaction:

Gram's staining of isolates was done according to the protocol given by Aneja(Aneja, 2007). Cells taking the colour of counter stain (pink) were considered as Gram negative and those retaining the violet colour of primary stain were considered as Gram positive. Cell shape was also recorded while recording the Grams reaction.

5.Nitrogen Fixing Capability:

An elaborate test was conducted to evaluate nitrogen fixing capability of the isolates. Plates of Ashby's media were inoculated with dilution of soil samples 104, 105, 106 and 107 in triplicates. Plates were incubated at 28°C. Isolates fixing nitrogen showed growth on the medium. Un-inoculated plates in triplicates served as control.

6.Phosphate Solubilizing Capability :

Plates of Pikovskaya medium having tri calcium phosphate (TCP) as phosphate source were prepared inoculated with dilution of soil samples 104, 105, 106 and 107 in triplicates. Un-inoculated plate served as the control. Plates were incubated at 28°C for 4 days. A zone of clearance developed around the colony was taken as indicator of phosphate solubilization. Nitrogen fixation and Phosphate solubilization are the characteristics of ecological importance.

7.Estimation of indole acetic acid (IAA)

Salkowski's reagent (50ml of 35% perchloric acid; 10ml of ferric chloride 0.5M) was prepared. The contents were mixed by shaking and allowed to stand at room temperature for 30 min for the development of pink colour due to presence of indole acetic acid. The selected efficient soil isolates were tested for their ability to produce IAA, in the absence and presence of tryptophan. The bacterial isolates were inoculated in 5 ml Jensen's liquid medium), incubated at 28±2°C. Cultures were centrifuged at 3000 rpm for 30 minutes. Two millilitres of the supernatant was mixed with two drops of ortho-phosphoric acid and 2ml of

Salkowski's reagent. Development of pink colour indicated IAA production.(Hartman et al., 1983)

8.Production of ammonia

Grow the isolates in the peptone water (Dye, 1962) in tubes. Incubate the tubes at 30 ºC for 4 days. Add 1ml Nesseler's reagent in each tubes. Presence of faint yellow colour indicates small amount of ammonia and deep yellow to brownish colour indicates maximum production of ammonia.

9.Oxidase test.

Culture of isolates were placed on oxidase disc and appearance of blue colour in 1 minute indicates positive result.(York et al., 2004)

Results and Discussion

1.Isolation of Bacteria.

Bacteria isolated and purified during the study using nutrient agar growth medium. The number of rhizobacterial bacterial cultures obtained from the soil was isolated.

2.Characterization of bacterial Strain:

The colony and cell morphology and the Gram's reaction of the bacterial isolates. The accession number given to the isolates are FO01, FO02, FO03, FO04, GA05, GA06, GA07, GA08, GA09, GA10, GA11, GA12, GA13, GA14, GA15, GB16, GB17, GB18, GB19, GB20, GB21, GC22, GC23, GC24, GC25, GC26, GC27, GC28, GC29, GC30, GC31 and GC32. From samples about 54 bacterial cultures have been isolated of that 22 (both positive in Pikovskaya and Ashby's media) isolates were from samples, GA, GB and GC were from Green city and FO were from Forest. It was observed that Out of 22 isolates 5 were Gram negative cocco bacilli, 3 were Gram negative cocci, 3 were ¬¬¬¬¬Gram negative bacilli, 3 were Gram positive cocco bacilli 5 were Gram positive bacilli and 3 were Gram positive cocci. It may be possible that nutrient agar medium supported more of Gram negative cocco bacilli than the others. Normally gram negative bacilli dominate the rhizosphere, more number of gram negative bacilli than the gram positive ones were reported in soybean plant(Hung & Annapurna, 2004).

3.Nitrogen fixation:

Isolates which showed nitrogen fixation were 22 which showed growth on Ashby's medium and turned the greenish colour of the medium to blue due to the production of ammonia in the medium which leads to the alkalinity due to which colour of the dye turns blue. However, confirmatory test of nitrogenase activity using acetylene reduction assay (ARA) need to be performed to establish their nitrogen fixing capability(Baldani et al., 2014).

4. Phosphate solubilization:

Of the 54 bacterial isolates 32 isolates (FO01, FO02, FO03, FO04, GA05, GA06, GA07, GA08, GA09, GA10, GA11, GA12, GA13, GA14, GA15, GB16, GB17, GB18, GB19, GB20, GB21, GC22, GC23, GC24, GC25, GC26, GC27, GC28, GC29, GC30, GC31 and GC32) solubilized tricalcium phosphate and formed zone of clearance in Pikovskaya's medium. Solubilization of tricalcium phosphate requires either acid production or chelate formation by the bacterium in the medium. Probably other isolates did not produce acid in sufficient amount or chelate to solubilize tricalcium phosphate in the medium. Solubilization of inorganic phosphate by Pseudomonas fluorescens were isolated from soils of Cameroon(Henri et al., 2008). P-solubilizing activity of microorganism is determined by the biochemical ability to produce and release organic acids, which via their carboxylic groups chelate the cations (mainly Ca2+) bound to phosphate converting them into the

soluble forms(Yu et al., 2012)

5.Production of Indole Acetic Acid

Out of 54 bacterial isolates, 9 cultures (GA06, GA07, FO03, GC28, GC29, GC43, GC47, GC50 and GB40) were further checked for the production produced IAA from tryptophan. These broth cultures containing tryptophan showed red coloration on addition of salwoski reagent. The ability to synthesize IAA is an attribute that many bacteria including both plant growth-promoters and phytopathogens possess(Duca et al., 2014).

6.Production of ammonia.

Cultures 22 were further checked for the production of siderophores and ammonia. Cultures 19 (GA05, FO01, GA07, FO04, GA08, GC23, GC25, GC28, GA11, GA12, GA15, GC45, GC43, GB41, GC47, GC29 GC50, GB40, GC53 and GC54) showed positive results of siderophore production. All the above cultures gave positive results for the ammonia production.

7.Oxidase test

Cultures 22 were preceded for oxidase test. Cultures 22 (GA05, GA06, FO01, FO03, GA07, FO04, GA08, GC23, GC25, GC28, GA11, GA12, GA15, GC45, GC43, GB41, GC47, GC50, GB40, GC53 and GC54) were able to use citrate thus gave positive reaction.

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Table 1: Colony characters of selected isolates.

Accession no.	Shape	Size	Margin	Elevation	Texture	Optical Character	Pigment
GA05	Circular	Large	Genate/ Lobate	Unbonate	Smooth	Opaque	Off White
FO01	Circular	Large	Entire	Convex	Smooth	Opaque	Off White
GA06	Circular	Large	Entire	Flat	Smooth	Opaque	Light Yellowish White
GA07	Circular	Medium	Entire	Flate	Smooth	Opaque	No Pigment
FO03	Circular	Medium	Entire	Flat	Smooth	Opaque	No Pigment
FO04	Circular	Medium	Lobate	Umbonate	Smooth	Opaque	Off White
GA08	Circular	Medium	Entire	Flat	Smooth	Opaque	Off White
GC23	Circular	Large	Crenate	Umbonate	Smooth	Opaque	Off White
GC25	Circular	Medium	Entire	Subcuous	Smooth	Opaque	Orange
GC28	Circular	Large	Entire	Flat	Smooth	Opaque	Off White
GC29	Circular	Large	Auriculate	Flat	Smooth	Opaque	Off White
GA11	Circular	Medium	Entire	Convex	Smooth	Opaque	Orange
GA12	Circular	Large	Entire	Convex	Smooth	Translucent	No Pigment
GA15	Circular	Large	Crenate	Umbilicate	Smooth	Opaque	Off White
GC45	Round	Medium	Entire	convex	Moist	Opaque	Off white
GC43	Round	Medium	Entire	Convex	Moist	Translucent	Colourless
GB41	Round	Medium	Entire	Convex	Moist	Translucent	Colourless
GC47	Round	Large	Entire	Convex	Moist	Opaque	Off white
GC50	Round	Tiny	Entire	Convex	Moist	Translucent	Pinkish
GB40	Round	Tiny	Entire	Convex	Smooth	Opaque	Dark pink
GC53	Irregular	Medium	Indulate	Raised	Moist	Translucent	Colourless
GC54	Round	Medium	Entire	Convex	Dry	Translucent	Colourless

Table 2. Biochemical tests

Accession no.	Nitrogen fixation	Phosphate solubilization	Catalase	Oxidase	NH_3	IAA	Gram Staining	
GA05	+	+	-	+	+	-	-	Cocco bacilli
FO01	+	+	+	+	+	-	-	Cocco bacilli
GA06	+	+	+	+	-	+	+	Bacilli
GA07	+	+	+	+	+	+	+	Cocci
FO03	+	+	+	+	-	+	+	Bacilli
FO04	+	+	+	+	+	-	-	Bacilli
GA08	+	+	+	+	+	-	+	Bacilli
GC23	+	+	+	+	+	-	-	Bacilli
GC25	+	+	+	+	+	-	+	Cocci
GC28	+	+	+	+	+	+	+	Cocco bacilli
GC29	+	+	-	+	-	+	-	Cocci
GA11	+	+	+	+	+	-	-	Cocco bacilli
GA12	+	+	+	+	+	-	+	Bacilli
GA15	+	+	+	+	+	-	-	Cocci
GC45	+	+	-	+	+	-	-	Cocco bacilli
GC43	+	+	+	+	+	+	-	Bacilli
GB41	+	+	+	+	+	-	+	Cocci
GC47	+	+	-	+	+	+	-	Cocco bacilli
GC50	+	+	+	+	+	+	+	Bacilli
GB40	+	+	+	+	+	+	+	Cocco bacilli
GC53	+	+	+	+	+	-	-	Cocci
GC54	+	+	+	+	+	-	+	Cocco bacilli

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