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Isolation and characterization of plant growth promoting microorganisms from Chilli rhizosphere

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ABSTRACT The present study was carried out with the objective to isolate and characterize chilli rhizospheric soil microorganism. The rhizosphere soil samples of Chilli plant were collected from different chilli plant cultivating areas of (Hunsur) Mysore, (Holenarsipura) Hassan regions of Karnataka, India. Bacteria, Azospirillum, Azotobacter, Pseudomonas, Actinomycetes, Rhizobia, and Fungi were isolated by dilution plate count technique employing 7 different media. The isolated microorganisms were characterized by their morphological, cultural and staining properties. Mycorrhizal fungi were identified as Penicillium sp., Fusarium sp., Pythium sp., Cladosporium sp., Tricoderma sp., Curvularia sp., Rhizopus sp., and Aspergillus. Different direct plant growth promoting activities of other 24 isolates viz. phosphate solubilization, siderophore production, IAA production were studied. It was found that out of 24 isolates, 1 bacterial isolate and 1 strain of Pseudomonas showed positive for siderophore production.

KEYWORDS : Rhizosphere, IAA, siderophore, Pseudomonas, Bacillus

Introduction

In temperate areas Capsicum annuum is usually grown as a herbaceous annual crop but ecologically in tropical areas it is a perennial shrub (which may live a few years to a few decades), and it can be grown in climate-controlled greenhouses as a perennial. This species also includes a wide variety of the cultivated non-pungent and pungent Capsicum peppers in some tropical areas and in most temperate areas. Throughout the world, the species C. annuum shows phenotypic diversity in plant habit and especially in sizes, pungency, shapes, colours, and other qualities of the fruits (Andrews, 1995; Bosland, 2000).

This immense agricultural, horticultural and biological diversity has helped to make C. annuum globally important as a fresh and cooked vegetable (e.g. for salads, warm dishes, pickled) and a source of food ingredients for sauces and powders and as a colourant, which is used as well in cosmetics (Andrews, 1995; Bosland, 2000).

Moreover, the species is used medically and medicinally, and provides the ingredient for a repellent or non-lethal deterrent to some animal and human behaviours (Krishna De, 2003; Cordell and Araujo, 1993; Palevitch and Craker, 1995;). They are also cultivated ornamentally especially for their glossy brightly fruits with a wide range of colours.

It also comprises numerous chemicals including fatty oils, carotenoids, capsaicinoids, fibre, protein, vitamins, steam-volatile oil, and mineral elements (Bosland and Votava, 2000; Krishna De, 2003). Many chilli constituents have importance for nutritional value, texture, aroma, flavour, and colour. The ripe fruits are especially rich in vitamin C (Osuna-García et al., 1998; Marin et al., 2004). Of greatest interest, the two chemical groups are the carotenoids and the capsaicinoids. The rich supply of carotenoids contributes to chili peppers' nutritional value and colour. The capsaicinoids are alkaloids that give hot chili peppers their characteristic pungency. (Pérez-Gálvez et al., 2003).

Materials and methods

Soil analysis: The rhizosphere soil samples were collected from different chilli plant cultivating areas: Mysore, Hassan regions of Karnataka, India. Soil samples were collected from chilli plant rhizospheres in order to isolate, characterize and investigate the diversity of plant growth promoting microorganisms associated with the roots of chilli plants. Top soil was removed and soil at 15cms depth was collected. Roots were pulled out soil surrounding the roots were collected in sterile plastic bags and preserved at lower temperature. Analysis for rhizosphere were completed within 2 days of soil sample collection.

Isolation

The soil samples were desiccated, compacted and sieved, from which 1g soil was suspended in suspended in 9mL saline and swen at 150rpm for 20 minutes at 37°C. Supernatant was serially diluted (10-1 to 10-10) in triplicates and inoculated using spread plate technique and pour plate technique on different media such as

a. Yeast Extract Mannitol Agar with Congo Red (CRYEMA) for Rhizobia were incubated at 28°C for 2days.

b. Ashby's Mannitol Agar for Azotobacter were incubated at 28°C for 3-4 days.

c. Nitrogen free Bromothymol Blue (NfB) agar for Azospirillum were incubated at 30°C for 7-8days.

d. Modified Rose Bengal Agar (MRBA) for fungal counts were incubated at room temperature for 3-5days.

e. Pikovskaya's Agar for isolating of phosphate solubilizers were incubated at 30°C for 3-7days.

f. Soil Extract Agar(SEA) medium for Actinomycetes were incubated at 30°C for 8-12days

g. King's BV(KB) medium for Pseudomonas were incubated at 37°C for 24-48 hrs.

h. Nutrient Agar(NA) for nonspecific organisms were incubated at 37°C for 24-48 hrs.(Aneja, K.R. 2001)

Microscopic study

After the incubation period, plates were checked for the growth of PGPR on different mediarespectively. Fungal identification was done by observing the macroscopic and microscopic structures (Bajjal et al., 1980). Microscopic observations were performed to investigate some characteristics of bacteria, Rhizobia, Fungi, Pseudomonas, Azotobacter, Azospirillum, Actinomycetes and Phosphate solubilizers. Pure cultures of 4 Fast growing colonies were prepared and subjected to Gram staining, H2O2, phosphate solubilization, siderophore and IAA production.

Screening of plant growth promoting microorganism:

Phosphate- solublization: Phosphate- solublization was detected qualitatively by spot inoculation of isolates on Pikovskaya medium. After incubation at room temperature for 48 hours a clear zone around colony was used as indicator for positive phosphate solublization.

Gram staining : A smear of the selected strain was prepared on a

clean glass slide and the smear was allowed to air-dry and then heat fixed. The heat-fixed smear was flooded with crystal violet and after one minute, it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with 95 % ethyl alcohol, washed with water and then counter-stained with safranine for 30s. After washing with water, the smear was dried and examined under oil immersion (100 x).Microscopic observation (x 10 and x 100 magnification) after Gram-staining revealed the shape of the microorganism. Other morphological characteristics such as colony features, type of areal hyphae, growth of vegetative hyphae, fragmentation pattern and spore formation are detailed in Table 1-6. Microscopic analysis of fungi: After the incubation period, plates were checked for the growth of fungi. Fungal identification was done by observing the macroscopic and microscopic structures (Bajjal et al., 1980) Siderophore production: It was assayed according to (Schwyne and Neilands., 1987). Isolates producing orange halo zone around growth on Chrome azurol S agar (CAS) after 48-72 hours of incubation were considered as positive.

Determination of IAA: Determination of IAA production by phosphate solubilizing bacteria after 4 days was done by growing the cultures in Jensons Media containing 100 mg L-t tryptophan at 30^{0}C. Cells were centrifuged and IAA was determined in the supernatant by Salkowski's method (Tang and Bonner., 1974).

Result and discussion

24 different isolates mentioned table 1-6 were isolated from the rhizosphere soil of chilli crop of Hassan and Mysore districts, Karnataka, India. All 24 isolates were characterized based on their morphological features such as shape, margins, colour, and Gram reaction. All 24 isolates were tested for phosphate solubilization, catalase, Indole acetic acid and siderophore production (table 1-6).
Among the 4 isolates of bacteria (table 1), B1 was rod shaped, negative for gram reaction and IAA production but positive for phosphate solubilization. B2 was rod shaped, positive for gram reaction and siderophore production but negative for phosphate solubilization and IAA production. B3 was cocci, negative for gram reaction and IAA production but positive for phosphate solubilization. B4 was cocci, negative for gram reaction and IAA production but positive for phosphate solubilization. Only B4 showed positive on CAS agar plates.

Among the 4 isolates of Azospirillum (table 2), Azo1 was vibroid, negative for gram reaction, phosphate solubilization and IAA production but catalase positive. Azo 2 was rod shaped, negative for gram reaction, phosphate solubilization and IAA production but catalase positive. Azo 3 was rod shaped, negative for gram reaction and catalase test, but positive for phosphate solubilization and IAA production. Azo 4 was rod shaped, negative for gram reaction, phosphate solubilization and IAA production but catalase positive. Neither of them showed positive for siderophore production.

Among the 4 isolates of Azotobacter (table 3), all were Rod shaped and gram negative. Azt1 showed negative result for phosphate solubilization and IAA production but catalase positive. Azt2 and Azt4 showed negative for IAA production but positive for catalase and phosphate solubilization. Azt3 showed negative for catalase test, but positive for phosphate solubilization and IAA production. Neither of them showed positive for siderophore production.

All the 4 isolates of Rhizobium (table 4), were rod shaped and catalase positive. Rhz 1, Rhz 2, and Rhz 4 showed positive for phosphate solubilization and IAA production but Rhz 3 showed negative for Phosphate solubilization and IAA production. Neither of them showed positive for siderophore production.

All the 4 isolates of Actinomycetes (table 5), were rod shaped, among which Act 1 was gram positive, catalase positive; Act 2 and Act 4 was gram negative; Act 4 showed positive for all the test and none of them were siderophore producing organism.

All 4 isolates of Pseudomonas (table 6), were gram negative, catalase positive and phosphate solubilizing, among which only Ps 3 was IAA producing and Ps 4 showed clear zone on CAS agar media.
In the present study, Plant growth promoting Rhizobacteria such as gram negative Cocci, Rhizobium sp., Actinomycetes sp., Azotobacter sp., Azospirillum sp.(Figures in table 8) and Mycorrhizal fungi were successfully isolated. Mycorrhizal fungi identified were Penicillium sp., Fusarium sp., Pythium sp., Cladosporium sp., Tricoderma sp., Curvularia sp., Rhizopus sp., and Aspergillus sp.,(Table 7). All the Mycorrhizal fungi were tested for phosphate solubilization of which all showed a positive result indicating their ability to solubilize phosphate.

Table 1 – Morphological characteristics of Bacteria

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Cell shape	Gram reaction	P	IAA	CAS
B1	Round	Rough	White	Flat	Undulate	Rod	Gram negative	+	-	-
B2	Irregular	Smooth	Creamish	Convex	Wavy	Rod	Gram positive	-	-	+
B3	Round	Smooth	Yellowish	Convex	Entire	Cocci	Gram negative	+	-	-
B4	Round	Smooth	White	Convex	Entire	Cocci	Gram negative	+	-	-

Table 2 – Morphological characteristics of Azospirillum

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Gram reaction	Cell shape	Catalase	P Solubilization	IAA
Azo1	Round	Rough	Pale yellow	Flat	Undulate	Gram negative	Vibroid	+	-	-
Azo 2	Irregular	Smooth	White	Convex	Wavy	Gram negative	Rod	+	-	-
Azo 3	Round	Smooth	Yellowish	Flat	Entire	Gram negative	Rod	-	+	+
Azo 4	Round	Smooth	White	Flat	Entire	Gram negative	Rod	+	-	-

Table 3 – Morphological characteristics of Azotobacter

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Cell shape	Gram reaction	Catalase	P Solubilization	IAA
Azt1	Round	Rough	Pale yellow	Convex	Undulate	Rod	Gram negative	+	-	-
Azt 2	Round	Mucoid	White	Flat	Entire	Rod	Gram negative	+	+	-
Azt 3	Irregular	Smooth	Light brown	Flat	Wavy	Rod	Gram negative	-	+	+
Azt 4	Round	Smooth	Cream	Convex	Entire	Rod	Gram negative	+	+	-

Table 4 – Morphological characteristics of Rhizobium

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Cell shape	Gram reaction	Catalase	P Solubilization	IAA
Rhz1	Irregular	Smooth	White	Convex	Undulate	Rod	Gram negative	+	+	+
Rhz2	Round	Mucoid	White	Flat	Entire	Rod	Gram negative	+	+	+
Rhz3	Round	Smooth	Cream	Convex	Entire	Rod	Gram positive	+	-	-
Rhz4	Irregular	Smooth	Yellow	Flat	Wavy	Rod	Gram negative	+	+	+

Table 5 – Morphological characteristics of Actinomycetes

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Gram reaction	Cell shape	Catalase	P Solubilization	IAA
Act1	Irregular	Smooth	Creamish	Flat	Wavy	Gram positive	Rod	+	-	-
Act 2	Irregular	Smooth	White	Flat	Wavy	Gram negative	Rod	-	-	-
Act 3	Circular	Smooth	Orange	Convex	Entire	Gram positive	Rod	+	+	+
Act 4	Irregular	Smooth	White	Flat	Wavy	Gram negative	Rod	-	-	-

Table 6 – Morphological characteristics of Pseudomonas

Isolates	Shape	Surface texture	Colour	Elevation	Margin	Cell shape	Gram Reaction	Catalase	P Solubilization	IAA	Siderophore
Ps 1	Irregular	Rough	Light brownish	Flat	Wavy	Rod	-	+	+	-	-
Ps 2	Punctiform	Smooth	White	Convex	Entire	Rod	-	+	+	-	-
Ps 3	Circular	Smooth	Creamish	Convex	Entire	Rod	-	+	+	+	-
Ps 4	Circular	Smooth	Yellow	Convex	Entire	Rod	-	+	+	-	+

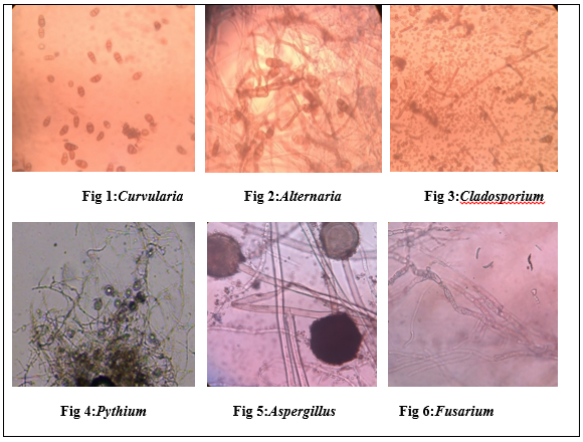

Fig 1:*Curvularia* Fig 2:*Alternaria* Fig 3:*Cladosporium*

Fig 4:*Pythium* Fig 5:*Aspergillus* Fig 6:*Fusarium*

Table 7: Mycorrhizal Fungi

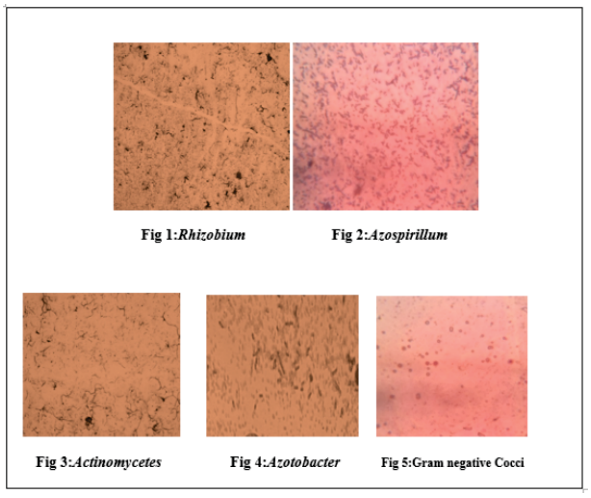

Fig 1:*Rhizobium* Fig 2:*Azospirillum*

Fig 3:*Actinomycetes* Fig 4:*Azotobacter* Fig 5:Gram negative Cocci

Table 8: Rhizospheric microorganisms

Conclusion

It can be concluded that there is diverse soil micro flora well adapted to the environment. The PGPR isolate are very efficient Phosphate solubilizer and could be very effective to be used as bio-inoculant to induce plant growth under drought condition as well as in phosphorous deficient soil. The PGPR Ps4 were the prominent siderophore producers can be considered as biocontrol agent against plant pathogens.

These observations reserve the fact that the rhizospheric region of chilli plant harbors a wide range of microorganisms which are highly antagonistic through their secondary metabolites like siderophore, to one or more rhizospheric microorganism and IAA also show an effective promotion of chilli plant growth. Hence, the use of rhizospheric microorganisms to promote chilli plant growth offers an attractive way to replace chemical fertilizers and reduce the use of pest control agents.

In this study, an increase in the plant growth can be attributed to the ability of the isolate to produce IAA, as IAA positively influences root growth and development, thereby enhancing nutrient uptake (F. Ahmad et al., 2008). It is a well-established fact that improved phosphorous nutrition influences overall plant growth and root development parameters could be attributed to the enhancement of the root growth and development.

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