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Isolation of Bacteria from Rhizosphere, Phylloplane and Caulosphere of Brinjal (*Solanum melongena* L.)

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ABSTRACT

Rhizosphere, phylloplane and caulosphere is the region where a complex community of microbes, mainly bacteria and fungi are present. The microbe- plant interaction in these regions can be beneficial, neutral, variable, or deleterious for plant growth. The bacteria that exert beneficial effects on plant development are termed plant growth promoting bacteria. To isolate the bacteria from rhizosphere, phylloplane and caulosphere of brinjal (*Solanum melongena* L.). The seeds of 16 cultivars of brinjal (*Solanum melongena L.*) viz., *Arka keshav*, *Arka shirish*, *Arka kusumaker*, and IIHR accession numbers 389,386,387,377 Tc, BB44, 391, 433, 434, 427, 447, 448, 476 and 487 that were used in the initial screening experiment were obtained from the Department of Vegetable crops, IIHR, Hessaraghatta, Bangalore. Brinjal (*Solanum melongena L.*) plants of different varieties were collected from seven locations around Bangalore viz., Hessaraghatta, Yelahanka, Kengeri, Madi vala, Hebbal, Tirumalapura and Attibele were also screened for the presence of associative bacteria. Associative microorganisms isolated from the rhizosphere, phylloplane and shoot regions of brinjal (*Solanum melongena L.*), revealed the presence of three morphologically different colonies. 80% of 16 cultivars of the brinjal (*Solanum melongena L.*) screened showed the presence of associative bacterial colonies. In this study diazotrophic BBI were obtained from the rhizoplane, phylloplane and stem of 16 cultivars of brinjal *(Solanum melongena L.*) that were screened. The dominant pearl-colored colonies isolated from all varieties of brinjal plants that were screened was identified and showed maximum nitrogen fixing ability compared with that of the other colonies. The phylloplane of brinjal (*Solanum melongena L.*) plants from seven different locations around Bangalore showed the presence of the dominant pearl-colored colonies. Moderate growth of bacteria was observed in root, stem and leaf bits sterilized up to 35 minutes. Even on surface sterilized roots which were homogenized and inoculated on growth media, dense growth of bacteria was observed there by establishing the presence of bacteria inside the root system. For the first time the presence of growth promoting bacteria on the rhizosphere and endorhizosphere of brinjal (*Solanum Melongena L.*) cultivars was established.

Keywords- Bacteria, Rhizosphere, Phylloplane, Caulosphere, *Solanum melongena* L.

I. INTRODUCTION

Although symbiotic Nitrogen fixation especially legume-rhizobium system has been proved to be the best form of biological Nitrogen fixation, associative nitrogen fixation cannot be ignored. Nitrogen fixation on the rhizoplane, phylloplane and stem have been attributed to the presence of diazotrophic bacteria associated with the roots, stem and leaves of plants [1]. Associative bacteria have been isolated from the rhizoplane, phylloplane and stem of many non-leguminous plants. Many studies have dealt with isolation of associative microorganisms from the roots of cereals, vegetable and fruit crops such as sweet potato [2], arecanut, banana, coconut, cashew, citrus, custard apple, grape, guava, jackfruit, litchi, mango, papaya, pomegranate- ate, phalsa, pepper, and strawberry [3], Spartina altemifolia [4], sugarcane [5], barley [6], wheat, maize, sorghum, millet and rice [7-12]. Root Smiace colonization by Azospipirillurn species in tomato, pepper, cotton and cucumis sativa under normal condition were studied by [13][14]. The bacteria were found efficiently colonizing the root elongation and root hair zones of the above plants. These isolated bacteria had growth promoting properties [15][16]. Isolated Azospirillum from the rhizosphere of tomatoes, white potatoes and corn [17]. Reported the presence of a new species of *Azospirillum bangalorense* colonizing the endorhizosphere of tomato. Studies on many graminaceous plants showed the presence of associated bacteria from the interiors of various plant parts [18-21] did similar studies relating to isolation of nitrogen fixing bacteria associated with the roots and rhizosphere of maize and other cereals and grasses [22]. Isolated a root colonizing bacterium, which was characterized as Pseudomomas strain from roots of potato. Nitrogen fixing organisms were isolated from the roots of many non- leguminous crops such as *Spiacia oleracea*, *Brassica chinensis* and *Brassica rapa* by [23]. The isolated associated bacteria in many of these studies was identified as Azospirillwn [17][19][24-28] observed spatial distribution of associated microorganisms identified as Azospirillium in rhizosphere of barley plants [29]. Isolated two nitrogen-fixing bacteria from

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the rhizosphere of mangrove trees, which were characterized as [2] characterized a nitrogen-fixing bacteria associated with the roots of sweet potato as Azospirillum. In the present study both sterile and unsterile root, leaf and stem bits of 16 cultivars of brinjal (*Solanum melongena* L.) plants were used for the initial screening of associative bacteria. The dominant colonies of bacteria present in the rhizoplane, phylloplane and stem were isolated.

II. METERIALS AND MATHODS

The seeds of 16 cultivars of brinjal (*Solanum melongena* L.) viz., *Arka keshav, Arka shirish, Arka kusumaker,* and IIHR accession numbers 389,386,387,377 Tc, BB44, 391, 433, 434, 427, 447, 448, 476 and 487 that were used in the initial screening experiment were obtained from the Department of Vegetable crops, IIHR, Hessaraghatta, Bangalore. Brinjal (*Solanum melongena* L.) plants of different varieties were collected from seven locations around Bangalore *viz.*, Hessaraghatta, Yelahanka, Kengeri, Madi vala, Hebbal, Tirumalapura and Attibele were also screened for the presence of associative bacteria. The standard laboratory chemicals were used and Bacteriological media used were from Himedia.

The procedure given by Patriquin and [1] was followed to isolate the associative bacteria from the root, stem and leaf of brinjal. The cultivars were grown in '10 x 10cm clay pots filled with sandy loam soil and topped with farm yard manure (FYM) for a period of 2 months. Sixteen brinjal cultivars were screened for the presence of associative microorganisms. The plants were carefully removed from the pots and the roots system was washed with sterile distilled water and was cut into bits measuring 0.5 cms. The bits were divided into two sets, one set was surface sterilized by immersing in 0.1% mercuric chloride for 2-3 minutes followed by washing with distilled water. Both control and surface sterile root bits were inoculated individually into test tubes containing 5 ml of sterile solid nitrogen free Burk's media [8]. The inoculated test tubes were incubated at 35° C for 48 hours. The bacterial growth from the sterile roots was isolated and sub cultured in nitrogen free Burk's media. They were multiplied in TYMB media [30]. Pure cultures were maintained in stab cultures containing nitrogen free Burk's media.

Leaves were separated from the stem, washed in running water and cut into 0.2 cms long strips followed by washing with sterile distilled water thrice. One set was surface sterilized by immersing in 0.1 % mercuric chloride for 2-3 minutes. Both control and surface sterilized leaf bits were inoculated into test tubes containing 5ml of semi solid nitrogen free Burk's media [8], and incubated at 37°C for 48 hours. The bacteria were isolated from sterile leaf tissues and subcultured on nitrogen free Burk's media. Pure cultures were maintained in stab cultures, containing nitrogen free Burk's media. Associated bacteria from the stem of the above mentioned 16 cultivars of brinjal (*Solanum melongena* L.) were isolated using the same procedure that was followed for the isolation of associative bacteria from the rhizosphere. Pure cultures were maintained in stab cultures containing nitrogen free Burk's media.

III. RESULT

Associative microorganisms isolated from the rhizosphere, phylloplane and shoot regions of brinjal (*Solanum melongena* L.), revealed the presence of three morphologically different colonies. (Table-1) 80% of 16 cultivars of the brinjal (*Solanum melongena* L.) screened showed the presence of associative bacterial colonies. (Table-2) In this study diazotrophic brinjal bacterial isolate (BBI) were obtained from the rhizoplane, phylloplane and stem of 16 cultivars of brinjal (*Solanum melongena* L.) that were screened. The dominant pearl-colored colonies isolated from all varieties of brinjal plants that were screened was identified and chosen for the present study as it showed maximum nitrogen fixing ability compared with that of the other colonies. The phylloplane of brinjal (*Solanum melongena* L.) plants from seven different locations around Bangalore showed the presence of the dominant pearl-colored colonies. Moderate growth of bacteria was observed in root, stem and leaf bits sterilized up to 35 minutes (Plate-1). Even on surface sterilized roots which were homogenized and inoculated on growth media, dense growth of bacteria was recorded there by establishing the presence of bacteria inside the root system. (Table-3)

Table 1: Isolation of associative bacteria from sterilized and unsterilized rhizoplane, phylloplane and stem of 16 IIHR varieties of brinjal (*Solanum melongena* L.).

IIHR Acc. Number	Rhizoplane		Phylloplane		Stem	
	Unsterilised	Sterilised	Unsterilised	Sterilised	Unsterilised	Sterilised
389	+	• -+ •	+ •	+	+ •	+ •
386	+	+	+ •	+	+ •	+
387	+ •	+	+ •	+	+ -	+ -
377 TC	+ -	+	+ •	+ •	+	+
Arka keshav	+	• -+ •	+ •	+	+ •	+
Arka shirish	+ •	+ •	+ •	+	+ •	+ •

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Arka kusumakar	• -	+ •	+ •	+ •	+ •	+ •
8844	+ • -	+ •	+ •	+ •	+ •	+ •
391	+ -	+	+	+	+ • -	+
433	+	+	+	+	+ –	+
434	+ •	+ •	+ •	+	+ –	+ •
427	+ • -	+ •	+ •	+	+ •	+ •
447	• -	•	+	+	+ •	+ •
448	+	+	+	+ •	+ •	+
476	+ •	+	+	+	+	+
487	+ •	+ •	+ •	+ •	+ •	+ •

+ Indicates pearl coloured
♦ - Indicates cream-coloured colonies
- Indicates cream-coloured colonies

Table 2: Bacterial growth on brinjal root, stem and leaf sterilized for different time periods

Sterilization (min)	Bacterial growth on		
	Stem	Root	Leaf
2	++	++++	+++
5	++	++++	+++
10	+	+++	++
15	+	++	+
20	-	+	
30			
60			

++++ very high, +++ high, ++ moderate, + low, - nil

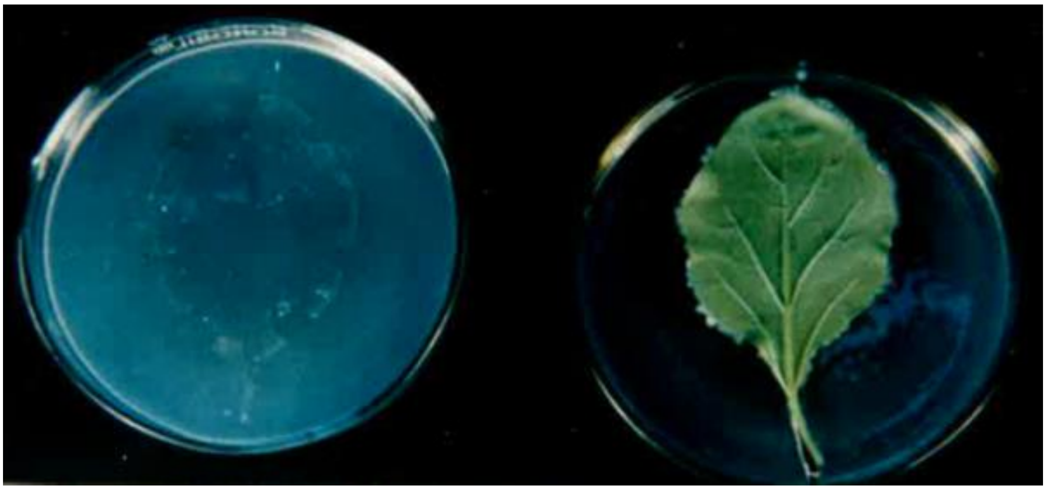

Plate 1: Phyllosphere bacteria in brinjal CV. Arka Keshav

Table 3: Isolation of Associative Bacteria from sterilized rhizoplane, phylloplane and stem of brinjal (*Solanum melongena* L.) plants collected from different locations around Bangalore.

Locations	Bacterial growth on		
	Stem	Root	Leaf
Attibele	+	+	+
Hebbal	+	+	+

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Hessaraghatta	+	+	+
Kengeri	+	+	+
Madivala	+	+	+
Tirumalapura	+	+	+
Yalahanka	+	+	+

+ - indicates pearl colored

IV. DISCUSSION

The presence of growth promoting bacteria on the rhizosphere and endorhizosphere of brinjal (*Solanum Melongena* L.) cultivars was established in the present study for the first time. Though [31][32] in a preliminary study isolated acetylene-reducing bacteria from the rhizosphere of eggplant they did not unequivocally prove their presence in the endorhizosphere or their growth promoting ability. In this study the dominant colonies of associative bacteria were recorded on the surface of stem, root and leaves of brinjal. Studies revealed that these bacteria developed a symbiotic relationship with the root system of the plant colonizing the intercellular and intracellular spaces of the cortex and the stem and the leaf through the conducting tissues. Growth promoting bacte1ia have been isolated in vegetables like tomato, cabbage, spinach, winged bean, capsicum and sweet potato [13][33]. observed aggregates of bacteria on the surface and endosphere of root hair, root cap and elongation zones of tomato using scanning rhizobacteria. Similar isolations have been reported from roots of cereals, grasses and plantation crops [8].

V. CONCLUSION

The study isolated bacteria which possess several plant growths promoting traits. This reveals the potential of these strains for biofertilizer application and commercial use as biocontrol agents in the field. Thus, these isolates can perform close to its optimum potential. Future studies concerning commercial and field applications of integrated stable bio-formulations as effective biocontrol are needed.

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