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Study on Isolation and Identification of Bacterium from Rhizosphere of Brinjal (*Solanum melongena L.*)

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Abstract: Present study aimed to identify the bacteria isolated from rhizosphere of brinjal (Solanum melongena L.). The pure cultures of bacterial isolate from brinjal (Solanum melongena L.) were used to identify the bacteria. Identification of bacteria was done based on its morphological characteristics using reference strain viz. Bacillus polymyxa strain 10401 was obtained from France. Colony and morphological characters were observed under a light microscope, followed by Gram reaction and motility test. Results revealed that brinjal bacterial isolate had pearly colonies with smooth margin, constant in size, bead like and slightly raised from the surface of the culture plate. The BBI revealed rod shaped sporulating bacteria observed under light microscope. The BBI was Gram positive and turned yellow when L-Aniline 4-nitroanilide hydrochloride solution was added to the bacterial colony indicating the gram-positive nature of BBL. The bacteria were motile with the presence of single polar flagella as observed under a transmission electron microscope. The bacterial isolate which showed nitrogen fixing and phosphate solubilizing properties was identified as Bacillus polymyxa belonging to the class Eubacteriales, family Bacillaceae and genera Bacillus. In conclusion, for the first time the presence and identification of nitrogen fixing and phosphate solubilizing properties having bacteria was identified as Bacillus polymyxa on the rhizosphere of brinjal (Solanum Melongena L.).

Keyword: Morphological characteristics, Solanum Melongena L. Bacillus polymyxa, Brinjal Bacterial Isolate (BBI)

1. INTRODUCTION

In Karnataka, brinjal is cultivated in 16,602 hectares of land and has an average yield of 30-35 tons per hectare. Brinjal (Solanum melongena L.) is a member of the family Solanaceae and a native of India. It is an important vegetable crop of south India. The fruit is rich in vitamin A and vitamin C and is employed in Ayurveda system. The crop needs 369 kg of urea and 80 kg of phosphatic fertilizer per hectare. 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].

There are many reports relating to the characterisation and identification of the nitrogen fixing bacteria associated with a wide variety of grasses and cereal crops. [2-8]. Hill et al., 1983 isolated and characterized rhizosphere bacteria of sweet potato [9]. Levanony et al. (1987) identified Azospirillum brasiliense in cereal roots using ELISA [10]. Rai and Guar, (1988) characterized Azotobacter associated with roots of wheat [8]. Lalande et al. (1989) identified rhizobacteria associated with maize as Azospirillwn [10]. Lalande R (1989) identified Azospirillum brasilense on surface and endosphere of wheat roots by immunogold labelling [10]. Brand et al. (1991) isolated a root colonizing rhizobacteria, which was characterized as Pseudomonas [11]. Mclnroy and Kloepper (1991) identified endophytic bacte1ia of maize and cotton [12].

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Penot et al., 1991 [13], and Mclnroy and Kloepper, 1991 [12], characterized Azospirillum associated with maize cultivated in France, using biochemical tests. Agarwala et al. (1991) isolated associated bacteria from the interiors of many graminaceous plants, many of which were identified as Azospirillum [14]. Lukin (1990) observed spatial distribution of associated bacteria identified as Azospirillum brasilense the rhizosphere of barley plants [15]. Holguin et al. (1992) isolated and identified rhizobacteria associated with mangrove trees as staphylococcus [16]. Fages and Mulard (1986) identified the isolated bacteria from the rhizosphere of sunflower as Azospirillwn [17].

With this viewpoints, in this study, efforts have been made to identify the brinjal bacterial isolate (BBI) from the rhizosphere of brinjal which reduce the addition of such high dose of nitrogen and phosphatic fertilizers, which promote growth and improve yield upon inoculation.

2. MATERIALS AND METHODS

2.1 Morphological characters

The isolated BBI was subcultured in solid and liquid nitrogen free liquid Burk's media. The pure cultures of BBI were used to characterize the bacteria. Identification of the dominantly associated bacterial isolate of brinjal (Solanum melongena L.) was done based on its morphological characteristics using a reference strain viz., Bacillus polymyxa strain 10401 obtained from France.

2.2 Colony characters and light microscopic studies

The bacterial identification required the observation of colony character and morphological characters under light microscopy. Gram reaction characteristics was assessed to identify the bacteria. The colony characters were recorded by observing 24 and 48 hr pure culture of the bacteria on solid Burk's media. The observations recorded were colony colour, margin and shape and the method followed was that of Subba Rao (1983) [18]. A drop of the 24 hr culture was smeared, stained and observed under oil immersion. Both vegetative and sporulating stages were observed.

2.3 Gram staining

Gram staining of the bacteria was done according to the procedure described in Bergey's 19-Manual of Determinative Bacteriology (1984). A drop of the pure culture was smeared on the slide, air-dried and fixed. The air-dried smear was treated sequentially with crystal violet, iodine, absolute alcohol and safranine. The gram reaction of the BBI was confirmed by adding 1% solution of L-aniline, 4-nitroanilide hydrochloride in tris buffer. When a drop of this solution was added on a colony of gram +ve bacteria, it turns yellow.

2.4 Motility test

Stab cultures of the BBI in solid Burk's media were prepared and incubated at 37 °C. 24 hr and 48 hr cultures were examined for motility. Motility was confirmed by suspending a drop of 24hr culture in a cavity slide and observing under a light microscope [18].

3. RESULTS

3.1 Morphological characters

3.1.1 Colony characters and light microscopic studies

The BBI had pearly colonies, which were constant in size, bead like, and slightly raised from the surface of the culture plate (Plate-1). The colonies had smooth margin. Colonies of BBI showed good growth within 24 hours of inoculation on nitrogen free Burk's media. The light microscopic observations of the pure culture of the isolated associative BBI revealed it to be rod shaped sporulating bacteria (Plate-2).

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Plate-1: Colonies of brinjal bacterial isolate developed from brinjal (Solanum melongena L.) leaf washings on nitrogen. free Burk's media.

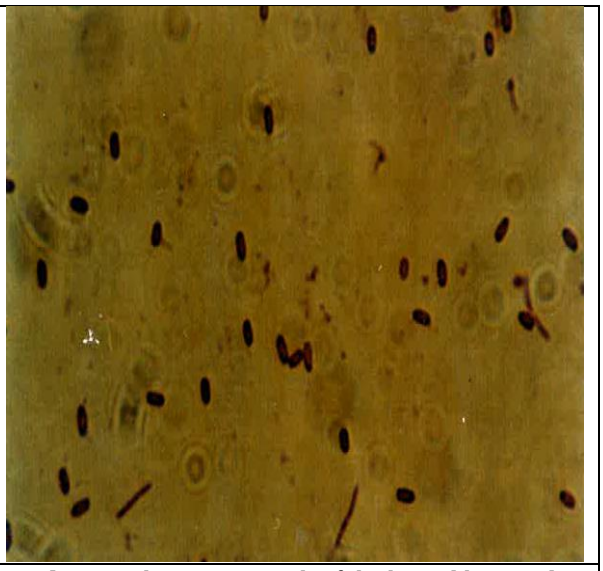

Plate-2: Photomicrograph of the brinjal bacterial isolate (BBL) stained with safranine

3.1.2 Gram staining

Gram staining of the bacteria revealed it to be gram positive which retained the crystal violet stain (Plate-3). L-Aniline 4-nitroanilide hydrochloride solution added to the bacterial colony turned yellow indicating the gram-positive nature of BBL.

3.1.3 Motility test

The spread of the bacteria in stab culture indicated the motility of the bacteria, observation by the hanging drop method and also confirmed the motility of BBL pure culture under transmission electron microscope (TEM) which revealed the presence of single polar flagella (Plate 4).

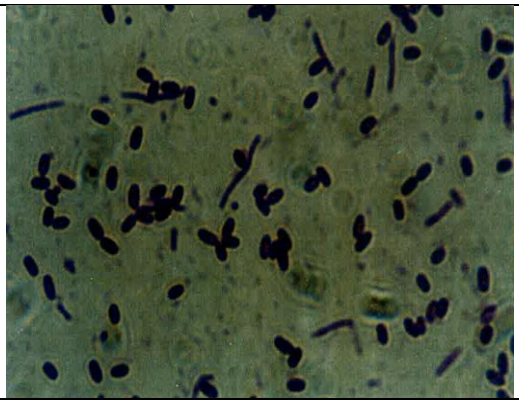

Plate-3: Photomicrograph showing Gram reaction of brinjal bacterial isolate (Solanum melongena L.)

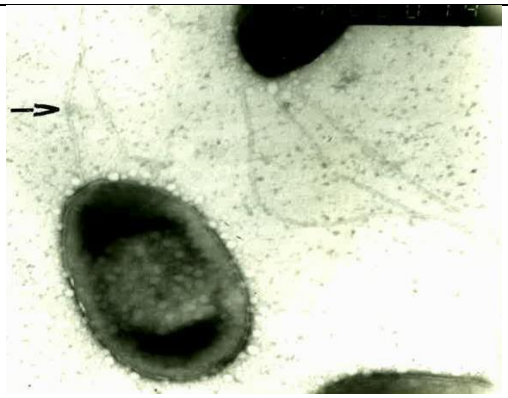

Plate-4: Single cell of BBL showing polar flagella under Transmission electron microscope

4. DISCUSSION

The bacterial isolate which showed nitrogen fixing and phosphate solubilizing properties was identified as Bacillus polymyxa belonging to the class Eubacteriales, family Bacillaceae and genera Bacillus (Bergey's Manual of determinative bacteriology 8th edition), which includes another phosphate solubilisers viz, Bacillus macerans. Identification was done based on its morphological characters. The colonies of Bacillus polymyxa were pearly and bead like in appearance, slightly raised from the surface of the culture plate and a had smooth margin. The isolate showed good growth within 24 hrs of inoculation. Bacillus polymyxa appeared to be rod shaped under light microscope. Growth promoting properties of Bacillus polymyxa have been reported by 20-Holl and Chanway (1997) in pine seedlings. These

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studies further support the present finding of growth promotion by Bacillus polymyxa isolated from the roots of brinjal (Solanum melongena L.).

5. CONCLUSION

The study identified the bacterial isolate as Bacillus polymyxa which possess several plant growths promoting traits. This reveals the potential of this strain for biofertilizer application and commercial use as biocontrol agents in the field. Thus, this strain 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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