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Establishing and Quantifying the Phosphate Solubilizing Potential of the Brinjal Bacterial Isolate

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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 quantify the amount of phosphate solubilizing bacteria from rhizosphere, phylloplane and caulosphere of brinjal (*Solanum melongena* L.). Materials and methods: 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 phosphate solubilizing bacteria. Nitrogenase activity was estimated by acetylene reduction assay and analyzed by gas chromatography. The amount of nitrogen fixed brinjal bacterial isolate was quantified by micro Kjeldhal method. The amount of nitrogen fixed by the BBI was equivalent to 23.5 nm of C_2H_2 reduced/tube/hour. The amount of nitrogen fixed by the BBI showed a steady increase upto three days (75 nm of C_2H_2 reduced/tube/hour) after which there was a decline in the amount of nitrogen fixed by the microbe. Phosphate solubilization by the bacteria isolated from brinjal is highly beneficial to the crop, as it would always make more phosphate available to the crop. This phosphate solubilizing potential could be harnessed to reduce the input of inorganic fertilizers. For the first time the presence of phosphate solubilizing bacteria on the rhizosphere and endorhizosphere of brinjal (*Solanum Melongena* L.) cultivars was established.

Introduction

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 (Hill *et al.*, 1983), arecanut, banana, coconut, cashew, citrus, custard apple, grape, guava, jackfruit, litchi, mango, papaya, pomegranate-ate, phalsa, pepper, and strawberry (Nair and Subba Rao, 1977), Spartina altemifolia (McClung *et al.*, 1983), sugarcane (Gracioli, 1981), barley (Pohlman and McColl, 1982), wheat, maize, sorghum, millet and rice (Dart and Wani, 1982 and Rao 1983; Klucas *et al.*, 1981; Kumari *et al.*, 1976; Patriquin and Dobereiner, 1978, Patriquin *et al.*, 1983). Nitrogen fixing organisms were isolated from the roots of many non- leguminous crops such as *Spiacia oleracea*, *Brassica chinensis* and *Brassica rapa* by Ahn *et al.*, (Ahn *et al.*, 1990).

In addition to nitrogen, phosphorus is a vital nutrient for plants and microorganisms. Superphosphate is one of the common forms of phosphatic fertilizers. These fertilizers are prone to get fixed in the soil. Rock phosphates are the basic raw material for phosphatic fertilizers. Direct application of rock phosphates is limited to acidic soils, while in other soils, the applied phosphate becomes insoluble within a short time because monocalcium phosphates are converted quickly to dicalcium phosphates which is unavailable to the plants. Under such conditions large amounts of phosphorus is fixed in the soil, which is unavailable to the plants. Several soil bacteria and a few species of fungi possess the ability to bring insoluble phosphates in the soil to soluble forms by secreting organic acids. These acids lower soil pH and bring about dissolution of bound forms of phosphates. Phosphate solubilizing bacteria solubilize about 50 to 60% of fixed phosphorus in the soil. Biofertilizers are living organisms used as fertilizers either to fix atmospheric nitrogen or to solubilize mineral nutrients like phosphorus. Barrow (Barrow 1980) reviewed and evaluated the utilization of residual phosphorus in soils. Boero and Thien and Alagwadi and Gaur, tested the phosphatase activity and phosphorus availability in the rhizosphere of corn roots (Boero & Thien, 1979; Alagawadi, 1992). As early as 1970 itself Dobereiner established in addition to fixing nitrogen some associated bacteria found to be phosphate solubilizing as well, hence restoring the fertility of the soil in an ecologically balanced way without altering the natural environment (Dobereiner *et al.*, 1970). The phosphate solubilizers invariably increase the uptake of phosphorus and nitrogen thereby increasing the yield of the crop. Some phosphate solubilizing bacteria are *Bacillus circulans*. *Bacillus subtilis* etc. In this present study the qualitative and quantitative studies for the occurrence of phosphate solubilizing bacteria from the rhizoplane, phylloplane and stem of brinjal (*Solanum melongena* L.) plants were undertaken.

Materials and Methods

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 Dobereiner was followed to isolate the associative bacteria from the root, stem and leaf of brinjal. The bacterial growth from the sterile roots was isolated and sub cultured in nitrogen free Burk's media (Patriquin and Dobereiner, 1978, Patriquin *et al.*, 1983) and they were multiplied in TYMB media. Both sterile and unsterile root, leaf and stem bits 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 and subcultured. The pure cultures of the bacterial isolate were screened for phosphate solubilizing potential.

Establishing the phosphate solubilizing potential of the brinjal bacterial isolate (BBI)

Pikovskaya media containing tricalcium phosphate was used to test the phosphate solubilizing potential of the BBI 1ml of 48-hour pure culture of the dominant brinjal bacterial isolate (BBI) cultured in liquid nitrogen free Burk's media was poured into petriplates containing 15 ml of solid Pikovskaya media when cool and allowed to solidify. The inoculated plates were incubated at 37°C for 48 hours (Rao, 1983). A known strain of phosphate solubilisers (*B. megatherium*) served as a control. During growth of the phosphate solubilizing bacteria, tricalcium phosphate is cleaved and calcium gets deposited on the agar leaving clear zones in the petriplates. The deposition of calcium on the agar plates and formation of a clear zone, which was the indication of phosphate solubilization was recorded in BBI containing petriplates and compared with petriplates containing *Bacillus megatherium*.

Quantifying the amount of phosphorus solubilized by the BBI

Sterile test tubes containing 1ml each of liquid Pikovskaya media were inoculated with 0.1 ml of 48-hour culture of BBI and incubated at 37°C for 24 hours, 48 hours, one week, 15 days and one-month intervals(Rao, 1983).

The pH of the media was recorded at the end of the incubation period. A standard phosphate solution was prepared by dissolving 4.38g of predried potassium hydrogen phosphate in distilled water and the volume was made up to 1000 ml. This solution was diluted 100 times to give a dilution of 10 mg/mL followed by serial dilutions ranging from 0.0-1.0 mg/mL of phosphate at an interval of 0.1 mg/mL of phosphate was prepared. To5ml of each dilution 5ml of ammonium molybdate and 1ml of stannous chloride were added. Colour developed after 5minutes and was readat690nmona Spectrophotometer (Emerson spectrometer 2000). 0mg/mL concentration of phosphorus was used as a blank. Intensity of colour was read on the spectrophotometrically within 12 minutes of addition of last reagent. A standard graph was prepared by plotting concentration against percent transmission of standard phosphate. The concentration of phosphorus solubilized by the BBI was calculated from this standard graph.

Results and Discussion

Establishing the phosphate solubilizing potential of the brinjal bacterial isolate (BBI)

The phosphate solubilizing potential of the BBI was established by culturing it in petriplates containing solid Pikovskaya media. After 48 hours a clear zone was observed in the culture plates formed due to solubilization of tricalcium phosphate. Bacillus megatherium a known phosphate solubilisers was used as a control. (Plate-1a & 1b) The diameter of the clear zone in plates containing BBI was 5.5 cms and the control plates showed a diameter of 5.0 cms.

Quantifying the amount of phosphorus solubilized by the BBI

The BBI solubilized 0.019 mg/mL of phosphorus in 48 hours and 0.039 mg/mL in 72 hours. The amount of phosphorus fixed by the isolate showed a steady increase upto seven days i.e., 0.156-mg/mL after which there was a decline in the amount of phosphorus solubilized by the microbe. The available phosphorus over a period of time from 24 hours to seven days is depicted in figure 1.

Plate.1

a **b**

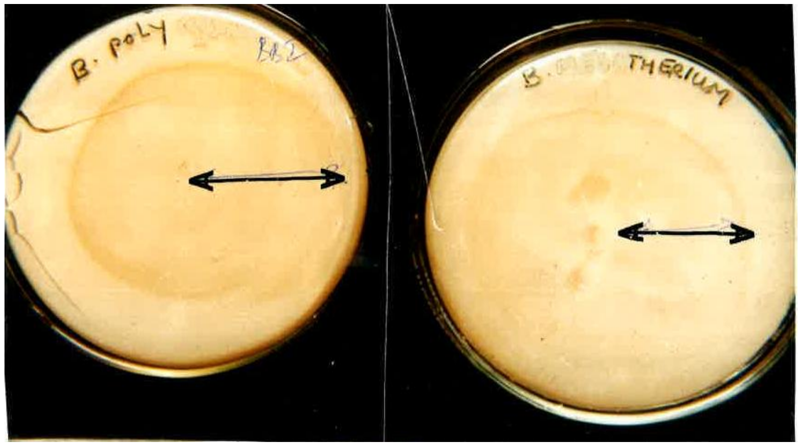
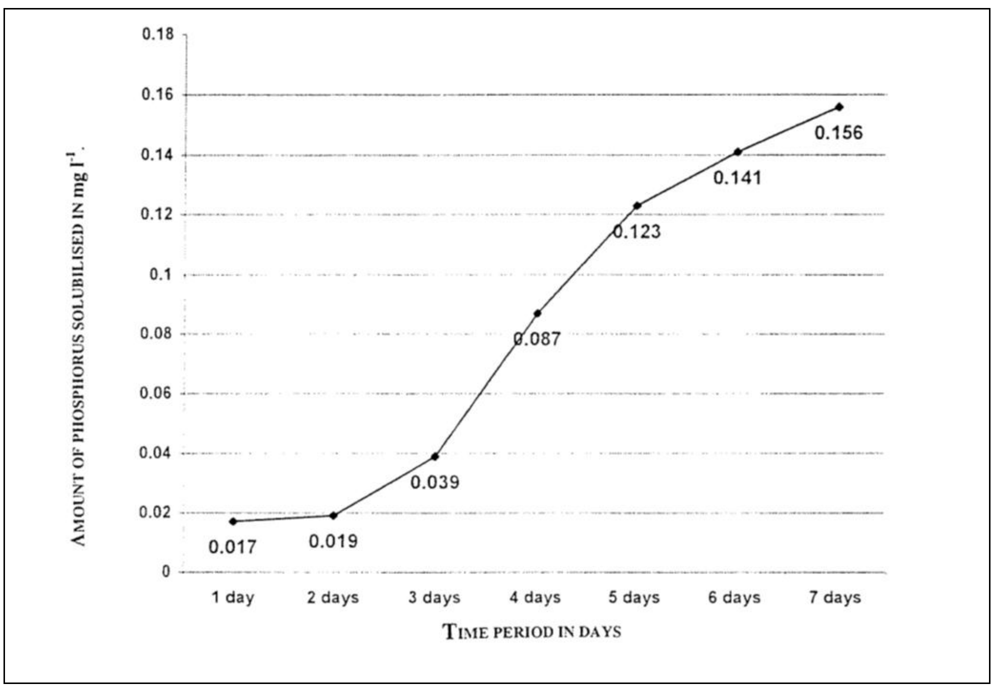


Fig.1 Amount of phosphorus solubilized by the brinjal bacterial isolate in Pikovskaya medium



Growth promoting bacteria have been isolated in vegetables like tomato, cabbage, spinach, winged bean, capsicum and sweet potato. Bashan and Holguin, and Bashan *et al.*, observed aggregates of bacteria on the surface and endosphere of root hair, root cap and elongation zones of tomato using scanning rhizobacteria (Bashan and Holguin, 1993; Bashan *et al.,* 1989). Similar isolations have been reported from roots of cereals, grasses and plantation crops (Rao, 1983; Brandao 1989; Coego *et al.*, 1992 and El Mokadem *et al.,* 1992). Phosphate solubilization by the bacteria isolated from brinjal is highly beneficial to the crop, as it would always make more phosphate available to the crop.

This and phosphate solubilizing potential could be harnessed to reduce the input of inorganic fertilizers.

The present study clearly indicates the potential of efficient phosphate solubilizing isolates from rhizosphere, phylloplane and caulosphere of *Solanum melongena* L. The use of phosphate solubilizing bacterial isolate as inoculants biofertilizer could be an efficient approach to replace chemical fertilizers and pesticides for sustainable cultivation. Further studies are required involving detailed characterization of molecular and functional properties of these phosphate solubilizing bacteria for their applications in the field.

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