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# PHYSIOLOGICAL AND BIOCHEMICAL PROFILE OF BACTERIAL ISOLATE FROM RHIZOSPHERE 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. In view of increasing environmental contamination with the deterioration of soil health and due to use of chemical fertilizers for increasing crop productivity, present study was conducted to identify the bacterial isolate 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 using reference strain viz., Bacillus polymyxa strain 10401 obtained from France. The brinjal bacterial isolate (BBI) was characterized by determining it's physiological and biochemical profile. API kits (API SYSTEMS, FRANCE) were used to detect the various sugars fermented by the BBI. Two API kits namely 20 B and 50 CHB were used. The physiological and biochemical profile of the isolated BBI was interpreted using 20B and 50 CHB reference tables supplied with API kits. The bacteria could not ferment urease but could ferment Arabinose, Mannitol, Amidon, Rhamnose, Galactose, sucrose fermentation, fructose fermentation, glycerol, melibiose, and sorbitol only after 48 hrs of incubation using 20B API kit. The results of the 50 CHB revealed that the isolated BBI could ferment Glycerol, L-Arabinose, D-Glucose, D-Fructose, D-Mannose, Dulcitol, Inositol, Mannitol, Amygdaline, Arbutin, Esculin, Salicin, cellobiose, Maltose, Melibiose, Saccharose, Trehalose and Xylitol at 24 hrs and 48 hrs after incubation. In conclusion, the physiological and biochemical profile ofBBI was found to tally with Bacillus polymyxa strain 10401 of France. 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.).*

***KEYWORDS:*** *Brinjal Bacterial Isolate, Physiological Characters, Biochemical Characters, Solanum Melongena L. Bacillus Polymyxa*

## *Article History*

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## INTRODUCTION

Indiscriminate use of chemical fertilizer and pesticides over the last few decades have not one just resulted in the contamination of environment, but also reduced soil fertility in general and soil microbial in particular. Due to the decreasing soil health, emphasis has been given to organic farming and the application of microbial formulations for

increasing crop productivity with concomitant decrease in application of the chemical fertilizer. 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. In Karnataka, brinjal iscultivated in 16,602 hectares of land and has an average yield of 30-35 tons per hectare. 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)[9]isolated and characterized rhizosphere bacteria of sweet potato.[9]Levanony et al. (1987)[7] identified Azospirillum brasiliense in cereal roots using ELISA. Rai and Guar, (1988)[8]characterizedAzotobacterassociated with roots of wheat.[10]Identified rhizobacteria associated with maize as Azospirillwn.[10] Identified Azospirillum brasilense on surface and endosphere of wheat roots by immunogold labelling.[11]Isolated a root colonizing rhizobacteria, which was characterized as Pseudomonas.[12]Identified endophytic bacte1ia of maize and cotton.

Penot et al. 1991.Mclnroy and Kloepper 1991,[13,12]characterized *Azospirillum* associated with maize cultivated in France, using biochemical tests.[14]Isolated associated bacteria from the interiors of many graminaceous plants, many of which were identified as Azospirillum.[15]Observed spatial distribution of associated bacteria identified as Azospirillum brasilense m the rhizosphere of barley plants.[16] isolated and identified rhizobacteria associated with mangrove trees as staphylococcus.[17]identified the isolated bacteria from the rhizosphere of sunflower as Azospirillwn. In this study, an efforts have been made to identify the brinjal bacterial isolate (BBI) from the rhizosphere of brinjal by physiological and biochemical characterisation which reduce the addition of such high dose of nitrogen and phosphatic fertilizers, which promote growth and improve yield upon inoculation.In the present study we aimed to identify the bacterial isolate from rhizosphere of brinjal (*Solanum melongena* L.)

## MATERIALS AND METHODS

The isolated BBI was subcultured in solid and liquid nitrogen free liquid Burk's media. The pure cultures of BBI were used to throughout the study. Identification of the dominantly associated bacterial isolate of brinjal (*Solanum melongena* L.) was done comparing with a reference strain *viz.*, *Bacillus polymyxa* strain 10401 obtained from France.

### Physiological and Biochemical Profile of BBI

The BBI was characterized by determining it's physiological and biochemical profile. API kits (API SYSTEMS, FRANCE) were used to detect the various sugars fermented by the BBI. Two API kits namely 20 Band 50 CHB were used.

20 B API kit required the use of 24hr culture of BBI in liquid Burk's media. The Bacterial culture was harvested and suspended in 1 ml of physiological saline (pH 7.0). This suspension was used in both 20 B and 50 CHB kits. The incubation tray of 20 B API kit were taken and the galleries were placed in the tray. The tray was closed with the cover and the entire set was incubated at 37°C. The galleries were read at 24 and 48 hrs after incubation. Metabolism produces colour changes that were either spontaneous or revealed by the addition of reagents. Catalase activity was tested by adding 1.5 % hydrogen peroxide. The results obtained were recorded and interpreted using the interpretation table to reveal the

biochemical and physiological profile of the isolated bacteria.

The 50 CHB kit were used to test fermentation of 49 sugars that could be fermented by the BBI. 50 CHB kit had 5 groups of 10 tests each along with an instruction leaflet. The 50 CHB kit required the use of 24 hr culture in physiological saline (pH 7.0) which was transferred to 50 ml of CHB media before being introduced into 50 CHB galleries. The galleries were incubated at 37°C and read after 24 and 48 hrs. Analytical profile index (API) CHB medium required ammonium sulphate (2g/L), yeast extract (0.5g/L), tryptone (lg/L) in phosphate buffer (pH 7.8) and phenol red (0.18 g/L).

## RESULTS

### Physiological and Biochemical Profile of BBI

The physiological and biochemical profile of the isolated BBI was interpreted using 20B and 50 CHB reference tables supplied with API kits. (Plate-1, 2a & 2b) The result of different tests is given in the table 1. The bacteria could not ferment urease but could ferment Arabinose, Mannitol, Amidon, Rhamnose, Galactose, sucrose fermentation, fructose fermentation, glycerol, melibiose, and sorbitol only after 48 hrs of incubation.(Table 1)

The results of the 50 CHB (Plate-2 (a), 2 (b) & Table 2) revealed that the isolated BBI could ferment Glycerol, L-Arabinose, D-Glucose, D-Fructose, D-Mannose, Dulcitol, Inositol, Mannitol, Amygdaline, Arbutin, Esculin, Salicin, cellobiose, Maltose, Melibiose, Saccharose, Trehalose and Xylitol at 24 hrs and 48 hrs after incubation. The isolated bacteria did not have the capacity to ferment Erythritol, 3- Methyl xyloside, L- Sorbose, Sorbitol, X -methyl D-glucosamine, Insulin, Melezites, Glycogen, 3- Gentobiose, Turanose, D-Lyxose, Glyconate, 2-Centoglyconate and 5-Centoglyconate at 24 hrs and 48 hrs. The isolated BBI had the capacity to ferment D-Arabinose, Ribose, D-Xylose, L-Xylose, Galactose, D-Mannose, Rhamnose, X -methyl D Mamoside, N-acetyl glucosamine lactose, Amidon, D-tagatose, D-fucose and Arabitol only after 48 hours of incubation. The above tests along with gram staining, motility and catalase, which were all positive, gave a total of 50 tests which revealed the physiological and biochemical profile of the isolated bacteria. The biochemical profile of the Bacillus polymyxa (BBI) tallied with that of the reference strain *Bacillus polymyxa* 10401 obtained from France.

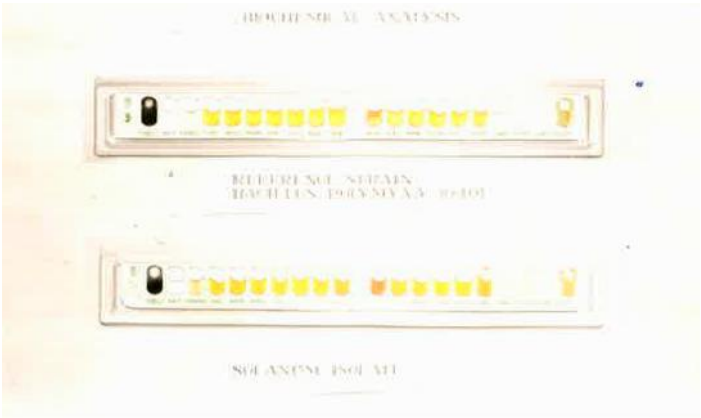

**Figure 1: Plate-1: Different Physiological and Biochemical Tests to Identify Brinjal Bacterial Isolate using 20B API Kit.**

**Figure 2: Plate-2 (a):Different Physiological and Biochemical Tests to Identify Brinjal Bacterial Isolate using 50B CHB API Kit.**

**Figure 3: Plate-2 (b): B. Polumyxa Strain 10401 (France) Showing Identical Physiological and Biochemical Profile as BBI on 50B CHB API Kit.**

**Table 1: Physiological and Biochemical Profile of the BBI using 20B API Kit**

| Substrate | Reference Strain | | Brinjal Bacterial Isolate | |
|---|---|---|---|---|
| | 20 Hrs | 48 Hrs | 20 Hrs | 20 Hrs |
| Gelatin | - | - | - | - |
| NIT | - | - | - | - |
| Phenylgalactoside | + | + | + | + |
| Sucrose | + | + | + | + |
| Arabinose | | + | - | + |
| Mannitol | + | + | + | + |
| Fructose | | + | - | + |
| $NO_2$to$NO_3$ | + | + | + | + |
| Maltose | + | + | + | + |
| AmidonRhamnose | | + | - | + |
| Galactose | | + | - | + |
| Melibiose | + | + | + | + |
| | | + | - | + |
| Sorbitol | + | + | + | + |
| Urease | | | | |
| ND | - | - | - | - |
| H2S | - | - | - | - |
| Gramreaction | + | + | + | + |
| Motility | + | + | + | + |
| Catalase | + | + | + | + |

All observations are visual; - Denotes negative for fermentation; + Denotes positive for fermentation

**Table 2: Physiological and Biochemical Profile of the BBI using 50 CHB API Kit**

| Substrate | Reference strain | | Brinjal Bacterial Isolate | |
|---|---|---|---|---|
| | 20 Hrs | 48 Hrs | 20 Hrs | 48 Hrs |
| Glycerol | - | - | - | - |
| Erythriol | + | - | + | - |
| D.Arabinose | | | | + |
| L.Arabinose | + | + | + | + |
| Ribose | - | + | - | + |
| D.Xylose | - | + | - | + |
| L.XyloseAdonitol | + | + | + | + |
| B.MethylXyloside | - | + | - | + |
| Galactose | - | + | - | + |
| D.Glucose | + | + | + | + |

| | | | | |
|---|---|---|---|---|
| D. Fructose | + | + | + | + |
| D.Mannose | - | + | - | + |
| L.Sorbose | - | - | - | - |
| Rhamnose | - | + | - | + |
| Dulcitol | + | + | + | + |
| Inositol | + | + | + | + |
| Mannitol | + | + | + | + |
| Sorbitol | - | - | - | - |
| *r:x*Methyl.D.Mamoside | - | + | - | + |
| *r:x*Methyl.D.Glucoside | - | - | - | - |
| N-Acetylglucosamine | - | + | - | + |
| Amygdaline | + | + | + | + |
| Arbutin | + | + | + | + |
| Esculin | + | + | + | + |
| Salicin | + | + | + | + |
| Cellobiose | + | + | + | + |
| Maltose | + | + | + | + |
| Lactose | - | + | - | + |
| Melibiose | + | + | + | + |
| Saccharose | + | + | + | + |
| Trehalose | + | + | + | + |
| Insulin | - | - | - | - |
| Melezites | - | - | - | - |
| Amidon | - | + | - | + |
| Glycogen | - | - | - | - |
| Xylitol<br>B.Gentobiose | + | + | + | + |
| D. Lyxose | - | - | - | - |
| D.Tagatose | - | + | - | + |
| D.Fucose | - | + | - | + |
| D.Arabitol | - | + | - | + |
| Glyconate | - | - | - | - |
| 2-Centogluconate | - | - | - | - |
| 5-Centogluconate | - | - | - | - |
| Gram staining | + | + | + | + |
| Motility | + | + | + | + |
| Catalase | + | + | + | + |

All observations are visual; - Denotes negative for fermentation; + Denotes positive for fermentation

## DISCUSSIONS

The physiological and biochemical profile of BBI was found to tally with *Bacillus polymyxa* strain 10401 of France. 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), [18] which includes another phosphate solubilisers *viz*., *Bacillus macerans.* The optimum temperature requirement for the isolate was 37 °C but it showed a long-range temperature tolerance. It could grow well in a pH range of 6.3 to 7.0. The physiological and biochemical profile of the *Bacillus polymyxa* was different at 24 hrs from 48 hrs after inoculation. Growth promoting properties of *Bacillus polymyxa* have been reported by Holl and Chanway (1992)[19] in pine seedlings. These studies further support the present finding of growth promotion by *Bacillus polymyxa* isolated from the roots of brinjal (*Solanum melongena* L.). Similar identification of bacteria isolated from the rhizosphere of different crops such as tomato, by Mohandas (1987)[20] and Penot et al. (1992)[13]respectively. [13]Characterized rhizosphere bacteria associated with roots of maize in France as Azospirillum using similar biochemical tests. Growth

promoting properties of Bacillus polymyxa have been reported by Holl and Chanway (1992),[19] in pine seedlings. These studies further support the present finding of growth promotion by Bacillus polymyxa isolated from the roots of brinjal (*Solanum melongena L.*).

## CONCLUSIONS

The study identified the bacterial isolate as *Bacillus polymyxa* which possess several plant growths promoting traits. Thus, the use of plant promoting traits as 'bio fertilizers' is a novel approach to replace chemical fertilizers and pesticides for sustainable agriculture in India. Since these organisms can be effective and perform close to its optimum potential.

## REFERENCES


1. *Dobereiner J. Forage grasses and grain crops. Methods for evaluating biological nitrogen fixation. 1980:535-6.*
2. *Dobereiner J, Marriel IE, Nery M. Ecological distribution of Spirillum lipoferum Beijerinck. Canadian Journal of Microbiology. 1976;22(10):1464-73.*
3. *Tarrand JJ, Krieg NR, Döbereiner J. A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerinck) comb. nov. and Azospirillum brasilense sp. nov. Canadian journal of microbiology. 1978;24(8):967-80.*
4. *van Berkum P, Sloger C. Immediate acetylene reduction by excised grass roots not previously preincubated at low oxygen tensions. Plant Physiology. 1979;64(5):739-43.*
5. *Van Berkum P, Bohlool B. Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbiological reviews. 1980;44(3):491-517.*
6. *Silva DM, Ruschel AP, Matsui E, Lima Nogueira ND, Vose PB. Determination of the activity of N 2-fixing bacteria in sugarcane roots and bean nodules using tritiated acetylene reduction technique and electron microautoradiography. In Associative Nitrogen fixation Eds. 1981:145-152.*
7. *Levanony H, Bashan Y, Kahana ZE. Enzyme-linked immunosorbent assay for specific identification and enumeration of Azospirillum brasilense Cd. in cereal roots. Applied and Environmental Microbiology. 1987;53(2):358-64.*
8. *Rai SN, Gaur AC. Characterization of Azotobacter spp. and effect of Azotobacter and Azospirillum as inoculant on the yield and N-uptake of wheat crop. Plant and Soil. 1988;109(1):131-4.*
9. *Hill WA, Bacon-Hill P, Crossman SM, Stevens C. Characterization of N2-fixing bacteria associated with sweet potato roots. Canadian journal of microbiology. 1983 ;29(8):860-2.*
10. *Lalande, R., Bissonnette, N., Coutlee, D. and Antoun, H. (1989). Plant and Soil (Netherlands). 1989;117 (2): 207-218.*
11. *Brand I, Lugtenberg BJ, Glandorf DC, Bakker PA, Schippers B, De Weger LA. Isolation and characterization of a superior potato root-colonizing Pseudomonas strain. Bulletin OILB SROP (France). 1991.*
12. *McInroy JA, Kloepper JW. Analysis of population densities and identification of endophyte bacteria of maize and cotton in the field. Bulletin OILB SROP (France). OILBSIRP. 1991:328- 331.*
13. *Penot I, Berges N, Guinguene C, Fages J. Characterization of Azospirillum associated with maize (Zea mays) in France, using biochemical tests and plasmid profiles. Canadian Journal of Microbiology. 1992;38(8):798-803.*
14. *Agarwala-Dutt R, Tilak KV, Rana JP. Isolation of Azospirillum from the interior of various parts of some graminaceous plants. Zentralblatt für Mikrobiologie. 1991;146(3):217-9.*

15. *Lukin S, Kozhevin P, Zviagintsev D. Spatial distribution of Azospirillum brasilense cells in the rhizosphere of barley plants. Mikrobiologiâ (Moskva, 1932). 1990(6):1090-3.*
16. *Holguin G, Guzman MA, Bashan Y. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: Their isolation, identification and in-vitro interaction with rhizosphere Staphylococcus sp. FEMS Microbiology Letters. 1992;101(3):207-16.*
17. *Fages J, Mulard D. Isolation of rhizosphere bacteria and their effect on Zea mays in pots [Azospirillum lipoferum, Enterobacter cloacae, Pseudomonas diminuta]. Agronomie (France). Agronomie (France). 1988;1108(4):309-314.*
18. *Krieg NR, Holt JG. Bergey's manual of systematic bacteriology. Yi Hsien Publishing Co. 1984.*
19. *Holl FB, Chanway CP. Rhizosphere colonization and seedling growth promotion of lodgepole pine by Bacillus polymyxa. Canadian Journal of Microbiology. 1992;38(4):303-8.*
20. *Mohandas S. Field response of tomato (Lycopersicon esculentum Mill 'Pusa Ruby') to inoculation with a VA mycorrhizal fungus Glomus fasiculatum and with Azotobacter vinelandii. Plant and soil. 1987;98(2):295-7.*