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Location of bacteria on the surface and in the interior of root and stem of brinjal (*Solanum melongena* L.) plants

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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 objective of the study was to locate the bacteria present on the surface and in the interior of root and stem 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. The location of bacteria on the surface and in the interior of root and stem of brinjal were observed under light microscope, transmission electron microscope and scanning electron microscope. The bacteria were present on the root cap, root hairs, root tips and region of cell elongation when viewed under light microscope. Light microscopic observations of root and stem sections revealed the presence of bacteria at various depths in the cortex between the epidermis and endodermis occupying the intercellular regions. Bacteria were also observed inside the cortical cells and in the cells of the vascular region. The number of bacteria present in the root region was significantly higher than in the stem. In stem, the bacteria were predominant on the epidermal layer and cortical tissue as observed under transmission electron microscope. Under scanning electron microscope, the bacteria were present as aggregates, each aggregate containing several cells. Fibrillar anchoring of the bacteria to the root hair were observed and the bacteria were present even at the base and on the stem and leaf hair. For the first time the presence of growth promoting bacteria on the rhizosphere and endorhizosphere of brinjal *(Solanum melongena* L.) cultivars was established. The bacteria were localized on the root cap, root hairs, root tips, region of cell elongation, at various depths in the cortex between the epidermis and endodermis occupying the intercellular regions. Bacteria were also present inside the cortical cells, in the vascular region, epidermal layer, cortical tissue, at the base and on the stem and leaf hair.

Keywords **- Bacteria, *Solanum melongena* L., Microscopy, Root, Stem**

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 Bashan and Holguin and Bashan et al.[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. Quimio and Coroza, Quimio and Cadapan isolated Azospirillum

from the rhizosphere of tomatoes, white potatoes and corn.[15,16] Mohandas reported the presence of a new species of Azospirillum bangalorense colonizing the endorhizosphere of tomato.[17] Studies on many graminaceous plants showed the presence of associated bacteria from the interiors of various plant parts.[13,18] Dobereiner and Boddey and Lalande et al. did similar studies relating to isolation of nitrogen fixing bacteria associated with the roots and rhizosphere of maize and other cereals and grasses.[19,20] Brand et al. isolated a root colonizing bacterium, which was characterized as Pseudomomas strain from roots of potato.[21] 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.[22] The isolated associated bacteria in many of these studies was identified as Azospirillwn.[17,18,23-26] Lukin observed spatial distribution of associated microorganisms identified as Azospirillium in rhizosphere of barley plants.[27] Holguin et al. isolated two nitrogen-fixing bacteria from the rhizosphere of mangrove trees, which were characterized as Azospirillium.[28] Hill et al. characterized a nitrogen-fixing bacteria associated with the roots of sweet potato as Azospirillum.[2] 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 and used to see the localization for the presence of bacteria on the surface and in the interior of root and stem of brinjal plants under microscope.

II. 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 and media were used from Himedia.

Location of bacteria by light microscopy

Stem and root bits of three-week old seedlings of brinjal *(Solanum melongena* L.) were excised, washed in running water followed with three washings with sterile distilled water and observed under the light microscope. Thin free hand sections of stem and root were mounted on glass slides, stained with safranine and examined and photographed under light microscope (Olympus BA,) at 10 x and 100x magnification.

Location of bacteria by transmission electron microscopy

Root and stem bits of three weeks old brinjal (*Solanum melongena* L.) were excised and washed with running water followed by three washings with sterile distilled water and fixed in 25% glutaraldehyde and 0.17 M cacodylate buffer for four hours. The root and stem bits were washed with cacodylate buffer and fixed in 0.1 % osmium tetraoxide in 0.1% cacodylate buffer for one hour. They were then dehydrated in ascending graded ethanol series (30%, 50%, 70%, and 100%) followed by ethanol isoamyl acetone mixture (2:1, l:1 and 1:2 ratio) and finally immersed in 100% isoamyl acetone. One hour prior to embedding, infiltration was done using plastic mixture. Embedding of the sample was done after passing them through ascending gradations of acetone plastic mixture (2:1, 1:2, and pure plastic) for one hour each. The embedded sample in plastic mixture were placed in silicon molds and left at room temperature for 3-4 hours followed by placement in an oven at 70°C for 24 hours. The hard blocks that were formed were carefully trimmed and 1 µm sections were cut on an ultramicrotome using glass knife. The ultrathin sections were collected on 400 mesh copper grids precleaned with 1 N HCl and water dried with ethanol. The sections were stained with uranyl acetate and examined by electron microscopy. Photographs were taken using orthochromatic sheet films.

Location of bacteria by scanning electron microscopy

Three-week-old brinjal seedlings raised in sterile soil inoculated with brinjal bacterial isolate (BBI) were removed and washed with sterile distilled water. The roots were cut into 3 inch bits and fixed in 0.l M sterile phosphate buffer (pH 6.1) containing 3% v/v glutaraldehyde per 3 hours. The roots were then serially dehydrated with 30, 50 and 90% chilled acetone in sterile water for 30 minutes each. The roots were then exposed to 100% chilled acetone for one hour. The root segments were dried in lower temperature and gold coated using a diode spluttering system. The gold - coated roots were observed under scanning electron microscope. The gold coating was done after the sample was mounted on aluminium stubs using a double sticking tape. Gold coating was done using a sputter coater (Polarson Equipment Ltd., SEM coating unit E-5000) and the film used was 125 ASA black and white. The film was rolled in developing tank, washed with tap water and developed by shaking continuously for 15-20 minutes. The film was again washed with tap water and the negative was fixed for 10 minutes and washed in running water for one hour.

III. RESULTS

Location of bacteria by light microscopy

Light microscopic studies revealed the presence of associative bacteria on the surface of the root. These bacteria were present on the root cap, root hairs, root tips and region of cell elongation when viewed under light microscope. (Plate-1, 2, 3a and 3b) In surface view of the mature roots, bacteria were observed distributed in aggregations along the epidermis. Dense aggregations were also observed on the root hairs, especially at the base and tip of root hairs in irregularly defined clumps. Apical region of the roots including the root cap region showed the presence of these bacterial aggregates. Light microscopic observations of root and stem sections revealed the presence of bacteria at various depths in the cortex between the epidermis and endodermis occupying the intercellular regions. Bacteria were also observed inside the cortical cells and in the cells of the vascular region. (Plate-4)

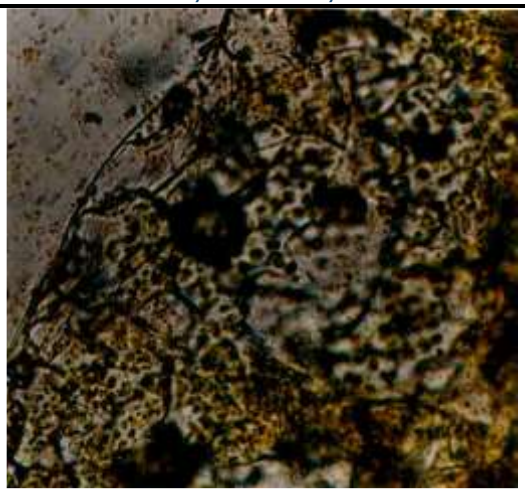

Plate-1

Plate-2

Plate-3a

Plate-3b

Plate-4

Plate-1: Photomicrograph of transverse section of brinjal leaf showing bacterial colonies in the epidermis and mesophyll cells taken under 100x

Plate-2: Photomicrograph of root cap of brinjal (*Solanum melongena* L.) plants treated with *Bacillus polymyxa* (BBI) showing the presence of bacteria taken under 100x.

Plate-3a: Photomicrograph of the basal region of root hair of brinjal (*Solanum melongena* L.) plants treated with *Bacillus polymyxa* (BBI) taken under 100x

Plate-3b: Photomicrograph showing the presence of bacteria on the lateral region of root hair of brinjal (*Solanum melongena* L.) plants treated with *Bacillus polymyxa* (BBI) taken under 100x

Plate-4: Photomicrograph of transverse section of stem of brinjal (*Solanum melongena* L.) plants the treated with *Bacillus polymyxa* (BBI) showing the presence of bacteria in the intercellular spaces taken under 100x

Location of bacteria by transmission electron microscopy

Transmission electron microscopic studies revealed the presence of the BBI occupying the intercellular and intracellular spaces of the cortical regions and in the stellar regions of the stem and root. They appeared as dark patches in the intercellular spaces of the cell. (Plate-5) The number of bacteria present in the root region was significantly higher than in the stem. In stem, the bacteria were predominant on the epidermal layer and cortical tissue.

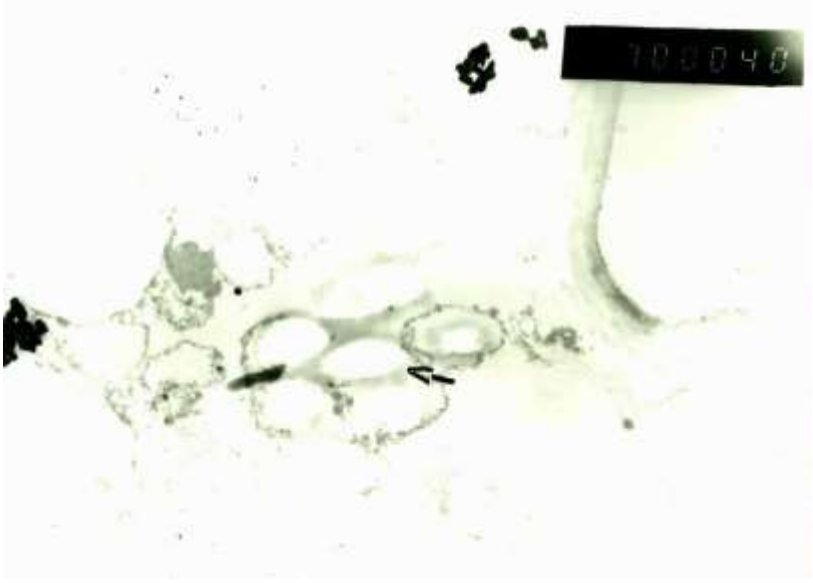

Plate-5: Section of stem of brinjal (*Solanum melongena* L.) treated with *Bacillus polymyxa* (BBI) showing the presence of in the intercellular spaces surface bacteria taken under 200x of transmission electron

Location of bacteria by scanning electron microscopy

Scanning electron microscopic studies revealed the presence of the BBI on the stem, root and root (Plate-6, 7 and 8). They were mostly concentrated on the elongation and root hair zones. On the older parts of the root, the cells were sparsely distributed. The bacteria were concentrated around the root hairs. The bacteria were present as aggregates, each aggregate containing several cells. Fibrillar anchoring of the bacteria to the root hair were observed (Plate-14). The bacteria were present even at the base and on the stem and leaf hair.

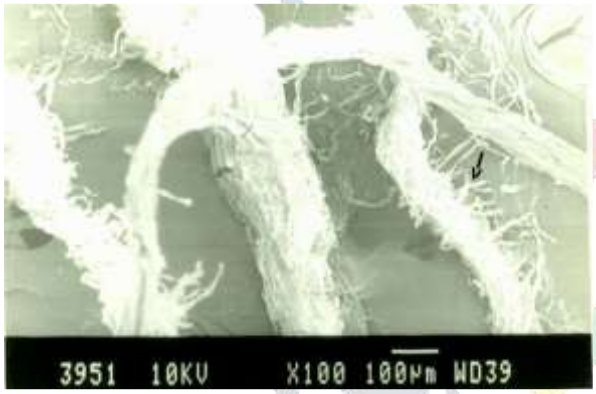

Plate-6

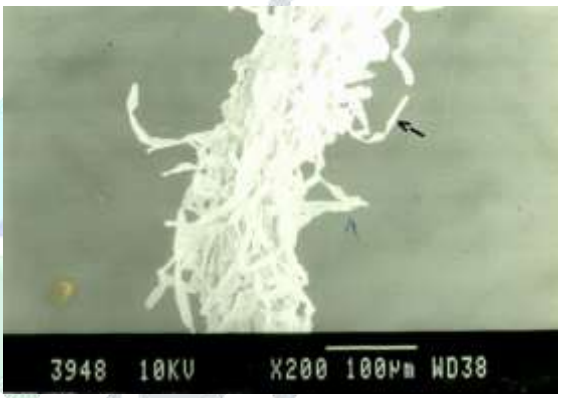

Plate-7

Plate-6: Section of root of brinjal (*Solanum melongena* L.) treated with *Bacillus polymyxa* (BBI) showing the presence of surface bacteria taken under 100x of scanning electron microscope at 10 kV

Plate-7: Section of root of brinjal (*Solanum melongena* L.) treated with *Bacillus polymyxa* (BBI) showing the presence of surface bacteria taken under 200x of scanning electron microscope at 10 kV

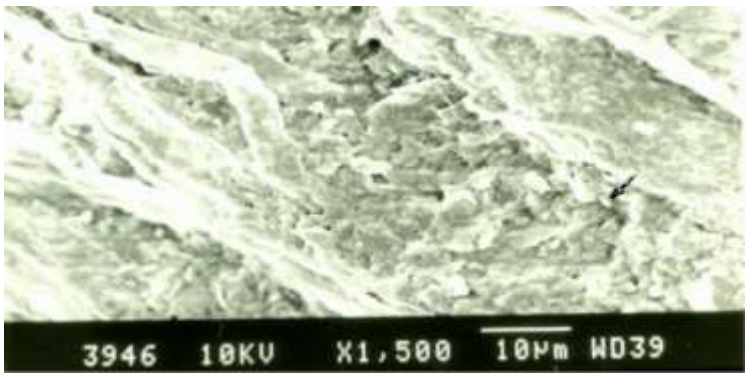

Plate-8

Plate-8: Section of stem of brinjal (*Solanum melongena* L.) treated with *Bacillus polymyxa* (BBI) showing the presence of surface bacteria taken under 500x of scanning electron microscope at 10 kV

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 Gamo, Garno and Ahn 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.[29,30] 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. 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.[13,31] Similar isolations have been reported from roots of cereals, grasses and plantation crops.[8,32]

Dominant colonies of bacteria occupying the endorhizosphere *i.e.*, the intercellular and intracellular spaces of the cortical region of stem and root revealed a type of symbiosis between brinjal plant and the microbe. Bacillus polyrnyxa is a dominant colonizing bacterium, present in all cultivars of brinjal (*Solanum melongena* L) that were screened. The growth of these associative bacteria from the sterile root and stem bits even after 20 minutes of surface sterilization indicated that these bacteria were deep seated within the plant tissues. This inference was further confirmed by light microscope and electron microscopic studies, which revealed that these endosymbionts occupied interiors of plant tissues. Umali Gracia et al. used electron microscopy to demonstrate similar spread of azospirillum infection in the middle lamellae of cortical tissues of guinea grass.[33] Silva et al. used electron micro autoradiography to show the presence of bacteroid like growth in sugar cane roots.[34] Sukhada established the endorhizosphere colonization of azospirillum in tomato roots by transmission electron microscopic studies.[17] Studies by Mclnroy and Kloepper, Levanony et al. and Patriquin and Dobereiner confirmed the presence of bacteria in the endorhizosphere of maize by light microscopy.[11,35,36]

V. Conclusion

The numbers of bacteria were more on the epidermal regions of rhizosphere, phylloplane and stem. Scanning Electron microscopic studies proved the presence of these bacteria on the roots. stem and leaves of brinjal (*Solanum melongena* L.) plants. The bacteria were capable of efficiently colonizing the elongation zone, root hair, root cap and the region of cell division. This reveals the potential of these bacteria for biofertilizer application and commercial use as biocontrol agents in the field. Future studies concerning commercial and field applications of integrated stable bio-formulations as effective biocontrol are needed.

VI. References

1. Dobereiner, J. (1980). Forage grasses and grain crops. Methods for evaluating biological nitrogen fixation, 535-536.
2. Hill, W. A., Bacon-Hill, P., Crossman, S. M., & Stevens, C. (1983). Characterization of N2-fixing bacteria associated with sweet potato roots. Canadian journal of microbiology, 29(8), 860-862.
3. Nair, S. K., & Rao, N. S. (1977). Microbiology of the root region of coconut and cacao under mixed cropping. Plant and Soil, 46(3), 511-519.
4. McClung, C. R., van Berkum, P., Davis, R. E., & Sloger, C. (1983). Enumeration and localization of N2-fixing bacteria associated with roots of *Spartina alterniflora* Loisel. Applied and Environmental Microbiology, 45(6), 1914-1920.
5. Graciolli, L. A. (1981). Microorganisms in the phyllosphere and rhizosphere of sugarcane. Associative N_2 Fixation, 91-101.
6. Pohlman, A. A., & McColl, J. G. (1982). Nitrogen fixation in the rhizosphere and rhizoplane of barley. Plant and Soil, 69(3), 341-352.
7. Dart, P. J., & Wani, S. P. (1982). Non-symbiotic nitrogen fixation and soil fertility.
8. Rao, N. S. (1983). Nitrogen-fixing bacteria associated with plantation and orchard plants. Canadian journal of microbiology, 29(8), 863-866.
9. Klucas, R. V., Pedersen, W., Shearman, R. C., & Wood, L. V. (1981). Nitrogen fixation associated with winter wheat, sorghum, and Kentucky bluegrass [*Triticum aestivum*, *sorghum bicolor*, *Poa pratensis*].
10. Kumari, M. L., Kavimandan, S. K., & Subba Rao, N. S. (1976). Occurrence of nitrogen fixing Spirillum in roots of rice, sorghum, maize & other plants. Indian Journal of Experimental Biology, 14(5), 638-639.
11. Patriquin, D. G., & Döbereiner, J. (1978). Light microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. Canadian journal of microbiology, 24(6), 734-742.
12. Patriquin, D. G., Döbereiner, J., & Jain, D. K. (1983). Sites and processes of association between diazotrophs and grasses. Canadian Journal of Microbiology, 29(8), 900-915.
13. Bashan, Y., & Holguin, G. (1993). Anchoring of *Azospirillum brasilense* to hydrophobic polystyrene and wheat roots. Microbiology, 139(2), 379-385.
14. Bashan, Y., Levanony, H., & Whitmoyer, R. E. (1991). Root surface colonization of non-cereal crop plants by pleomorphic *Azospirillum brasilense* Cd. Microbiology, 137(1), 187-196.
15. Quimio, A. J., & Coroza, A. G. (1986). Plant growth promoting activity of rhizobacteria on tomato, white potato and corn [Philippines]. Supplement No. 1.
16. Quimio, A. J., & Cadapan, E. T. (1988). Field performance of selected plant growth promoting rhizobacteria (PGPR) on corn, sorghum and cowpea. Philippine Journal of Crop Science.
17. Mohandas, S. (1987). 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, 98(2), 295-297.
18. Bashan, Y., Harrison, S. K., & Whitmoyer, R. E. (1990). Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Applied and Environmental Microbiology, 56(3), 769-775.
19. Boddey, R. M., & Dobereiner, J. (1988). Nitrogen fixation associated with grasses and cereals: recent results and perspectives for future research. Plant and soil, 108(1), 53-65.
20. Lalande, R., Bissonnette, N., Coutlee, D. and Antoun, H. (1989). Plant and Soil (Netherlands). 117 (2), 207-218.

21. Brand, I., Lugtenberg, B. J. J., Glandorf, D. C. M., Bakker, P. A. H. M., Schippers, B., & De Weger, L. A. (1991). Isolation and characterization of a superior potato root-colonizing Pseudomonas strain. Bulletin OILB SROP (France).
22. Ahn, S. B., Gamo, H., & Yuk, C. S. (1990). Isolation of N 2-fixing Microorganism from the Root of Non-leguminous Crops. Korean Journal of Soil Science and Fertilizer, 23(1), 67-72.
23. Tyler, M. E., Milam, J. R., Smith, R. L., Schank, S. C., & Zuberer, D. A. (1979). Isolation of Azospirillum from diverse geographic regions. Canadian Journal of Microbiology, 25(6), 693-697.
24. Malik, K. A., & Bilal, R. (1989). Survival and colonization of inoculated bacteria in kallar grass rhizosphere and quantification of N 2-fixation. In Nitrogen Fixation with Non-Legumes (pp. 301-310). Springer, Dordrecht.
25. Bilal, R., Rasul, G., Arshad, M., & Malik, K. A. (1993). Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter spp.* on root surface of grasses. World Journal of Microbiology and Biotechnology, 9(1), 63-69.
26. Agarwala-Dutt, R., Tilak, K. V. B. R., & Rana, J. P. S. (1991). Isolation of Azospirillum from the interior of various parts of some graminaceous plants. Zentralblatt für Mikrobiologie, 146(3), 217-219.
27. Lukin, S., Kozhevin, P., & Zviagintsev, D. (1990). Spatial distribution of *Azospirillum brasilense* cells in the rhizosphere of barley plants. Mikrobiologiâ (Moskva, 1932), (6), 1090-1093.
28. Holguin, G., Guzman, M. A., & Bashan, Y. (1992). 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, 101(3), 207-216.
29. Gamo, T. (1991). *Azospirillum spp.* from crop roots: a promoter of plant growth. JARQ. Japan Agricultural Research Quarterly, 24(4), 253-259.
30. Gamo, T., & Ahn, S. B. (1991). Growth-promoting *Azospirillum spp.* isolated from the roots of several non-gramineous crops in Japan. Soil Science and Plant Nutrition, 37(3), 455-461.
31. Bashan, Y., Singh, M., & Levanony, H. (1989). Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation. Canadian Journal of Botany, 67(8), 2429-2434.
32. Brandao, E. M. (1989). Isolation and selection of plant growth-promoting rhizobacteria in maize.
33. Umali-Garcia, M., Hubbell, D. H., Gaskins, M. H., & Dazzo, F. B. (1981). Adsorption and mode of entry of Azospirillum brasilense to grass roots [Nitrogen fixation].
34. Silva, D. M., Ruschel, A. P., Matsui, E., Nogueira, N. D. L., & Vose, P. B. (1981). Determination of potential N2 [nitrogen]-fixing activity of bacteria in sugarcane roots and bean nodules using tritiated acetylene reduction technique and electron microautoradiography.
35. McInroy, J. A., & Kloepper, J. W. (1991). Analysis of population densities and identification of endophyte bacteria of maize and cotton in the field. Bulletin OILB SROP (France).
36. Levanony, H., Bashan, Y., & Kahana, Z. E. (1987). Enzyme-linked immunosorbent assay for specific identification and enumeration of *Azospirillum brasilense* Cd. in cereal roots. Applied and Environmental Microbiology, 53(2), 358-364.