International Journal of Applied and Natural Sciences (IJANS)
ISSN (P): 2319–4014; ISSN (E): 2319–4022
Vol. 10, Issue 2, Jul–Dec 2021; 115–126

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

BIOASSAY TEST FOR DETECTION OF PLANT GROWTH PROMOTING SUBSTANCE IN *BACILLUS POLYMYXA* ISOLATED FROM RHIZOSPHERE OF BRINJAL

Jenifer Lolita C[1] & Keshamma E[2]

[1]*Research Scholar, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India*

[2] *Research Scholar, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India*

ABSTRACT

Background: *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. Among the possible mechanisms, proposed to explain the growth promotion by rhizobacteria are the enhancement of mineral availability and uptake and production of phytohormones, which stimulate plant growth. Many rhizobacteria produce phytohormones, this evidence has been primarily from analysis by bioassay, usually on chemically defined media, always in a system containing a single microorganism.*

Objectives: *To test the presence of plant growth promoting substance by Bacillus polymyxa.*

Materials and Methods: *24 hr bacterial culture of Bacillus polymyxa in liquid nitrogen free Burk's media incubated at 37 °C was used to test for the presence of growth promoting substances. The seeds of brinjal used in these bioassays were obtained from Division of vegetable crops IIHR. Bioassays were conducted to test for the presence of growth promoting substances produced by the bacteria. Bioassays were carried out using ragi (Eleusine coracana) seeds. Sterilized ragi seeds were germinated in sterile petriplates with filter paper (Wattmann No 1) containing either 24 hr bacterial culture filtrate or nitrogen free Burk's media or distilled water. Growth parameters such as germination count, radicle length and plumule length of 72 hr old seedlings were recorded. Brinjal (Solanurn melongena L.) seeds were similarly tested for the germination and growth stimulation properties of the culture filtrate.*

Results: *Bioassays were conducted to test for the presence of growth promoting substances using ragi (Eleusine coraccma) and brinjal (Solanum melongena L.) seeds. The results of the bioassays proved that inoculation with Bacillus polymyxa produced growth-promotion. Among the possible mechanisms, proposed to explain the growth promotion by rhizo bacteria, is the production of phytohormones, which stimulate plant growth. These growth-promoting substances were identified to be cytokinins and gibberellins. The present study showed direct evidence that Bacillus polymyxa inoculation affected the status of cytokinins and gibberellins in brinjal seedlings. The amount of zeatinriboside in culture filtrate of B.polymyxa tested by Immuno detectionwasfoundtobe114.7pg/mole. The plants treated with Bacillus polymyxa had a zeatin riboside concentration of 65.66 $pg \cdot ^{1}$fresh weight of the plant sample as compared with 16.77 $pg \cdot ^{1}$ fresh weight of the plant sample of the controlplants. The amount of gibberellin in the culture supernatant of Bacilluspolymyxa (BBI)was 0.08µg/mg. The plant streated with Bacilluspolymyxa (BBI) showed an increase of 61.08 % in gibberellin concentration over the control plants.*

Conclusion: *The plants treated with Bacillus polymyxa showed an increase of 61.08% in gibberellin concentration over the control plants. The results of the present study are pointers to the fact that growth hormones are produced by Bacillus polymyxa.*

KEYWORDS: *Brinjal, Ragi, Bacillus Polymyxa, Azospirullum, Root Hairs, Gibberellins*

Article History

Received: *11 Nov 2021* **|** ***Revised:*** *12 Nov 2021* **|** ***Accepted:*** *24 Nov 2021*

INTRODUCTION

Many rhizobacteria produce phytohormones, this evidence has been primarily from analysis by bioassay, usually on chemically defined media, always in a system containing a single microorganism. Many studies have revealed that the increase in yield, biomass and other growth parameters has a direct bearing on the use of these diazotrophs as biofertilizers. Inoculation of plants with Azospirillum are known to alter root morphology, increase numerous plant shoot growth parameters and eventually increase the yield of many cereals. (Patriquin et al., 1983) Enhanced mineral uptake in inoculated plants was proposed as a possible mechanism of plant growth enhancement. Many studies have proved that inoculation with rhizobacteria induced enhancement of mineral uptake like nitrates (Kapulnik et al., 1983; Pacovsky et al., 1985; and Morgenstrn and Okon, 1987), and potassium (Lin et al., 1983), and Phosphorus (Sarig and Okon, 1984 Sarig et al., 1988) in plants. Puente and Bashan (1990) observed improvement in seed germination and seedling growth parameters of giant columnar cactus when inoculated with Azospirillum brasilense strains. Interaction studies by Bashan et al. (1990) however revealed no consistent pattern of increased ion content of plant tissues in any bacterial strain-plant combination evaluated (Wheat-Azospirillum; Rice-Azospirillum). They concluded that although Azospirillum was capable of changing the balance and qualities of several minerals, these changes alone were not responsible for significant increase in plant growth and yield in the plants studied.

The plant growth promoting influence of Rhizobacteria have been extensively examined (Michiels et al., 1989; Sumner, 1990). Some of the recent research concerning the Azospirillum plant interaction have focused on phytohormone production by the rhizobacteria (Bashan, 1983; Bashan et al 1990; Kucey, 1988; Salmeron et al., 1990) extracted growth promoting substances from Azotobacter chrococcum isolated from roots of Zea mays. These growth-promoting substances were identified to be auxins, gibberellins and cytokinins. Extrapolation from evidence obtained in pure culture systems has led to the hypothesis that one mechanism of this interaction is phytohormonal. Semi qualitative evidence that rhizobacteria produce phytohormones has accumulated from relatively simple systems using chemically defined media or single microbe cultures. Using evidence from bioassays and immunoassay techniques, it has been concluded that Gibberellin (GA) like substances are produced by Azatobacter (Gonalez -Lopez et al., 1986,Janaz et al. 1989) showed that Azospirillum produces gibberellins in pure cultures on chemically defined medium and in co-cultures on straw. Bottini et al. (1989) used gas chromatography and spectrometry to demonstrate the production of GA's in pure cultures in defined liquid media of Azospirillum and Rhizobium.

Nieto and Franken berger (1986) conducted glass house expe1iments to demonstrate the production of cytokinins by the isolated Azotobacter when provided with pretested cytokinins precursors. Many root-associated bacteria are demonstrated cytokinins producers. Zeatin riboside was isolated and characterized by Letham (1974). Study by Holland (1997) are pointers to the fact that the rhizobacteria are the cytokinins producers. Crozier et al. (1988) analysed the presence of IAA and related indoles in culture media of Azospirillum lipoferum and of Azospirillum brasilense. They demonstrated the involvement of IAA in the interaction between *Azospirillum brasilense* and *Panicum miliacewn* roots. Studies by Govindan andPurushothaman (1984) revealed the production of IAA and GA like substances by Azospirillwn.

Omay et al. (1988) studied the factors influencing IAA release by Azospirillum brasilense under batch cultures. Baca et al. (1994) demonstrated IAA production in wild strains of Azospirillum. The present study was carried out to test if the bacterial isolate identified as Bacillus polymyxa could produce phytohormones, which could influence growth enhancement in brinjal.

MATERIALS AND METHODS

24hr bacterial culture of *Bacillus polymyxa* in liquid nitrogen free Burk's media incubated at 37°C was used to test for the presence of growth promoting substances. The seeds of brinjal used in these bioassays were obtained from Division of vegetable crops IIHR. Bioassays were conducted to test for the presence of growth promoting substances produced by the bacteria. Bioassays were carried out using ragi (*Eleusine coracana*) seeds. Sterilized ragi seeds were germinated in sterile petriplates with filter paper (Wattmann No 1) containing either 24 hr bacterial culture filtrate or nitrogen free Burk's media or distilled water. Growth parameters such as germination count, radicle length and plumule length of 72hrold seedlings were recorded. Brinjal (*Solanurn melongena* L.) seeds were similarly tested for the germination and growth stimulation properties of the culture filtrate.

Test for Zeatin Riboside in Pure Culture of Bacillus Polymyxa

Pure culture filtrate of Bacillus polymyxa was tested for the presence of Zeatin riboside by Immuno detection using the antibodies raised against the zeatin riboside in rabbit. 10 ml of sterile nitrogen free Burk's broth was inoculated with *Bacillus polymyxa*. The inoculated tubes were incubated for one week at 37°C. The broth was centrifuged at 5000 rpm and the supernatant was collected and shaken with equal volume of saturated n-butanol in a separatory funnel. This process was repeated thrice. The water fraction was rejected and the butanol fractions were pooled and condensed at 40°C. The condensed fraction was taken in tris buffer.Elisa plates were coated with prediluted solution of antibodies in 200µL of 50 mM coating buffer. These plates were sealed with parafilm and incubated overnight at 4°C. The contents were then decanted off and the plates were washed three times with washing buffer at room temperature. The wells of the plates were filled with 200 µL of blocking buffer and incubated at 37°C for 30 minsthen wells were then washed thrice with washing buffer. To some antibody coated well 100 µL of standard solution of zeatin 1iboside (25µmol) was added. 100 µL of condensed culture filtrate taken in tris buffer were added to three wells. This was followed by the addition of 100 µL of prediluted tracer. 100 µL of water was used in the place of standard to determine the amount of bound tracer. Unspecific binding (NSB) was calculated by using a standard (200mg). The plates were then sealed, mixed gently and incubated at 35°C for two hours. The contents of the well were then discarded and each well was washed thrice with washing buffer. Detection was done using 200 µL of p-nitrophenyl phosphate. Substrate buffer was added to each well and the plates were incubated for 30 min at 37°C in an incubator. The substrate reaction was tested by adding 50 µL of 5N KOH and the plates were read at 450nm. The concentration of zeatin riboside was obtained by comparing the results with the standard curve and values were expressed as pg of zeatin riboside per fresh weight of the pellet.

Test for Gibberellin in Pure Culture of Bacillus Polymyxa

10 mL of sterile liquid broth of nitrogen free Burk's medium was inoculated with *Bacillus polymyxa*. The inoculated tubes were incubated for one week at 37°C. The broth was centrifuged at 5000 rpm and the supernatant was collected and shaken with equal volume of saturated methanol in a separator funnel and this procedure was repeated thrice. The water fraction was rejected and the methanol fractions were pooled and condensed at 40°C. The condensate was taken in a mobile phase

(100% methanol). Standard GA was prepared in the dilutions of 0-100 ppm and 20ml of each dilution was injected into the HPLCand the retention time of each dilution was noted, and a standard curve was prepared. The readings were co-related on the standard graph and the percentage of GA present in each sample was calculated. The method followed was that of Koshioka et al., 1983.

Hormone in Plants Treated With Bacillus Polymyxa

Zeatin Riboside in Plants Treated with Bacillus Polymyxa

Brinjal (*Solanum melongena* L.) plants inoculated with *Bacillus polymyxa* were grown in clay pots filled with sandy loam soil. Three-week-old seedlings were removed from the soil and the roots were excised. Five grams of fresh root bits were placed in 100ml conical flasks. The roots were ground in 80% ice cold methanol and kept overnight. The solution was filtered after 24 hours and the filtrate was retained. The residue was again agitated with 30mL ice cold methanol and kept overnight. The solution was filtered again after overnight incubation. The filtrate was pooled and condensed under vacuum at 40°C. The condensed material was taken in 5mL double distilled water. The pH of the solution was adjusted to 8.0. This solution was shaken with 70mL water saturated butanol in a separator funnel. The butanol fractions were pooled and condensed under vacuum at 40°C. The condensed sample was taken in Tris buffer. Elisa procedure that was followed was same as previously described. The amount of zeatin riboside was expressed as pg/mg of fresh weight of the sample. The brinjal plants (treated and control) were removed periodically from the pots. The roots were excised and observed to record any changes in root and root hair morphology.

Gibberellin in Plants Treated With Bacillus Polymyxa

The method followed was that of Koshioka et al. (1983). Brinjal (*Solanum melongena* L.) plants inoculated with *Bacillus polymyxa* were grown in clay pots filled with sandy loam soil. Three week old seedlings were removed from the soil and the roots were excised. Five grams of fresh root bits were placed in 100ml conical flasks. The roots were ground in 80% ice cold methanol and kept overnight and the solution was filtered after 24 hours and the filtrate was retained. The residue was again agitated with 30ml ice cold methanol and kept overnight. The solution was filtered. The filtrate was pooled and condensed under vacuum at 40°C. The condensate was taken in a mobile phase (100% methanol). Standard GA was prepared in the dilutions of 0-l00 ppm. 20µL of each dilution was injected into the HPLC and the retention time of each dilution was noted, and a standard curve was prepared. 20µL of sample was injected into the HPLC and the retention time was noted. The readings were correlated on the standard graph and the percent of gibberellin present in the plant was calculated.

RESULTS

Bioassays to Test for the Plant Growth Promoting Substance by Bacillus Polymyxa (BBI)

Plant response to inoculation was evaluated in these bioassays by measuring the germination count and seedling growth. The bioassay using ragi (*Eleusine coracana*) revealed that the culture filtrate triggered germination and enhanced growth of the seedlings. The seedlings treated with the culture filtrate showed an average germination count of 97 % as against 31 % of the control.(Plate-1(a) & (b)) The average radicle length measured 2.9 cms in treated seedlings compared to 1.0 cm of the control seedlings. Visual observations revealed that the treated seedlings developed more root hairs than the controls.

Similar bioassays with brinjal (*Solanum melongena* L.) plants treated with culture filtrate of *Bacillus polymyxa*revealed significantly higher germination count than the controls (Plate-1(a), 1(b) & Plate-2). Seeds tested with the medium showed 30% germination as compared to 25 % of the control. The seeds soaked with the culture filtrate

showed an average germination count of 100 %. All growth parameters showed significant improvement in brinjal plants treated with the culture filtrate *Bacillus polymyxa* than in uninoculated controls. The plant growth parameters recorded in 14 day old plants revealed that the plants treated with Bacillus polymyxa had a significantly higher radicle length and plumule length. (Table 1 and 2) These results revealed that the *Bacillus polymyxa* does produce growth-promoting substances, which is responsible for enhanced growth parameters. Further study was undertaken to characterize these growth- promoting substances.

Figure 1: Plate 1(a): *Elusine Coracana* (Ragi) Seeds Treated with *Bacillus Polymyxa* Culture Filtrate Showing Higher Germination Count Than Controls, in Abioassay. Control Plants in the Right Plate

Figure 2: Plate 1(b): *Solanum Melongena* L. (Brinjal) Seeds Treated with *Bacillus Polymyxa* Culture Filtrate Showing Higher Germination Count Than Controls, in a Bioassay. Control Plants in the Right Plate.

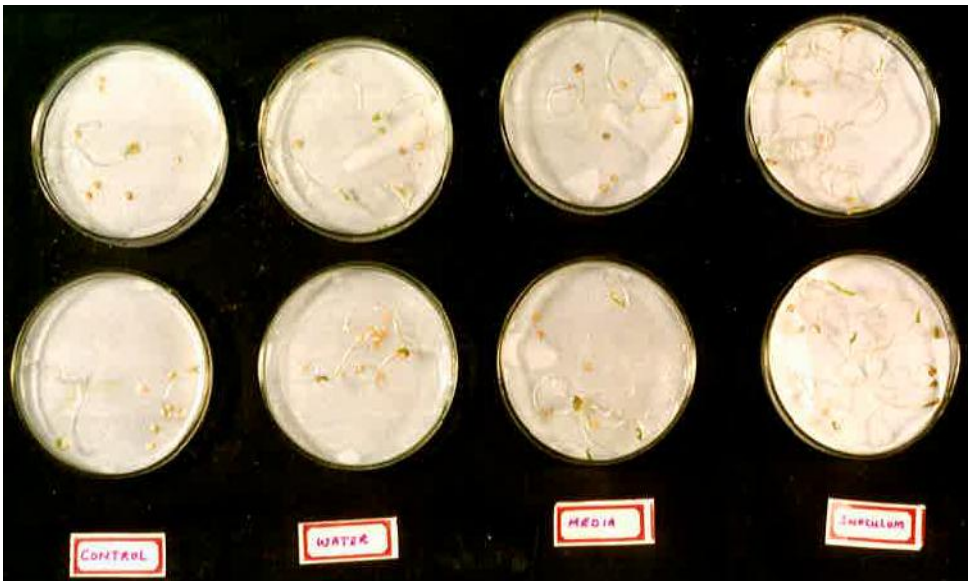


Figure 3: Plate 2: Bioassay of *Bacillus Polymyxa* Culture Filtrate using Brinjal (*Solanum Melongena* L.) Seeds Treated Show Higher Germination Count Than Control. Control Seeds Were Treated with Distilled Water.

Table 1: Germination and Growth Promotion in Ragi (*Eleusine Coracana*) and Brinjal (*Solanum Melongena* L.) Induced By *Bacillus Polymyxa* Culture Filtrate

Treatments	Ragi seeds			Solanum seeds		
	Germination count%	Radicle length (cms)	Plumule length (cms)	Germination count%	Radicle length (cms)	Plumule length (cms)
Control	25	0.01	1.11	31	0.02	0.02
Water soaked	30	0.02	1.19	47	0.03	1.14
Media soaked	45	0.02	1.19	47	0.03	1.16
Culture filtrate	100	0.04	1.31	97	0.05	1.27

Mean of 15 plates, Reading after two weeks

Table 2: Germination and Growth Promotion in Ragi (*Eleusine Coracana*) and Brinjal (*Solanum Melongena* L.) Induced By *Bacillus Polymyxa* Culture Filtrate

Treatments	Ragi seeds			Brinjal seeds		
	Germination count%	Radicle length (cms)	Plumule length (cms)	Germination count%	Radicle length (cms)	Plumule length (cms)
Control	11	1.0	2.34	12	1.7	2.41
Water soaked	20	1.9	2.37	23	2.0	2.47
Media soaked	22	2.0	2.42	36	2.2	2.45
Culture filtrate	100	2.9	3.12	100	3.1	3.28

Mean of 15 plates, Reading after two weeks

Hormone Production in Pure Culture of *Bacillus Polymyxa*

Zeatinribo Side in Pure Culture of *Bacillus Polymyxa*

The amount of zeatin riboside present in the culture filtrate of *Bacillus polymyxa*was 114.7 pg/mole. When theconcentration was diluted ten times the amount of Zeatin riboside was 111.1 pg/mole. This concentration of Zeatin riboside can influence the growth parameters of the treated seeds as observed in the bioassays (Table 3).

Table 3: Amount of Zeatin Riboside Concentration in Culture Filtrate of *Bacillus Polymyxa*

Sample	Concentration	Absorbance	Concentration pg ml^{-1}
	1/1	0.073	114.7
2	1/10	0.724	111.1
3	1/25		
standard	25 pg	0.333	25

Mean of Three Readings

Gibberellin in Pure Culture of *Bacillus polymyxa*

The amount of Gibberellin in 10 mL of the culture of *Bacillus polymyxa*was 0.08µg/mL of the culture supernatant.

Hormones in Plants Treated With *Bacillus Polymyxa* (Bbi)

Zeatin Riboside in Plants Treated With *Bacillus Polymyxa*

The plants treated with Bacillus polymyxa had a zeatin riboside concentration of 65.66 pl g-1 fresh weight of the plant sample as compared with 16.77 ppm/g fresh weight of the plant sample of the control plants (Table 4).

Table 4: Amount of Zeatin Riboside Concentration in Culture Filtrate of *Bacillus Polymyxa*

Sample	Weight of sample	Sample used for ELISA (mg)	Mean	Zeatin rhiboside pgms fresh wt.
Control	2gm	100	0.975	16.77
Azospirillum and *Bacillus polymyxa* treated plants	2gm	100	0.490	33.37
Bacillus polymyxa treated plants	2gm	100	0.249	65.66
Azospirillum treated plants	2gm	100	0.660	24.77

Mean of Three Readings

The root of the brinjal (*Solanum melongena* L.) plants treated with *Bacillus polymyxa* showed a greater number of root hairs than the controls. The root hairs of treated plants showed certain morphological changes such as elongation, branching, etc. (Plates 3 to Plate 6).

Figure 4: Plate 3: *Bacillus Polymyxa* Inoculated Brinjal (*Solanum Melongena* L.) Seedlings Showing Induction of Root Hair Where Bacteria Become Established

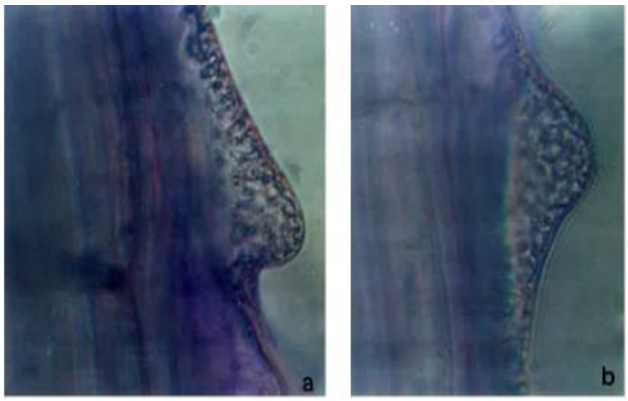
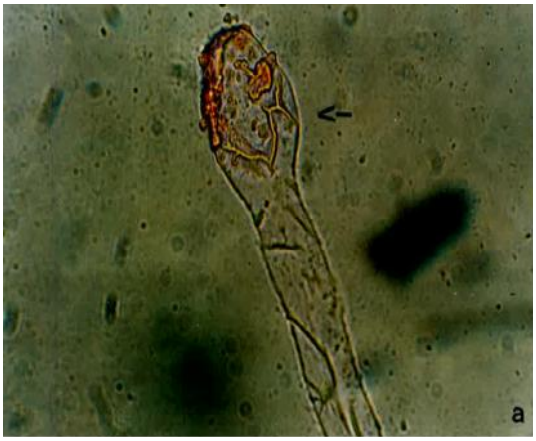


Figure 5: Plate 4: Induction of Root Hairs in Region Where Bacteria Become Established in *Bacillus Polymyxa* Brinjal (*Solanum Melongena* L.) Seedlings. Root Hairs of Brinjal (*Solanum Melongena* L.) Seedlings Treated With Bacillus Showing Swelling at the Tip of the Root Hair (a and b).



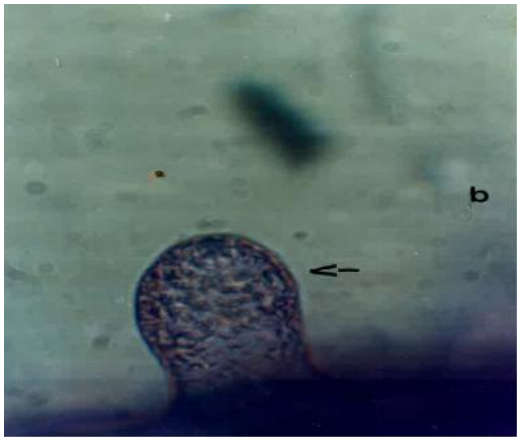


Figure 6: Plate 5 (a): Root Hairs of Brinjal (*Solanum Melongena* L.) Seedlings Treated with Bacillus Polymyxa (Bbi) Showing Swelling at the Tip of Root Hair

Figure 7: Plate 5 (b): Root Hairs of Brinjal (*Solanum Melongena* L.) Seedlings Treated with Bacillus Polymyxa (Bbi) Showing Swelling at the Tip of Root Hair

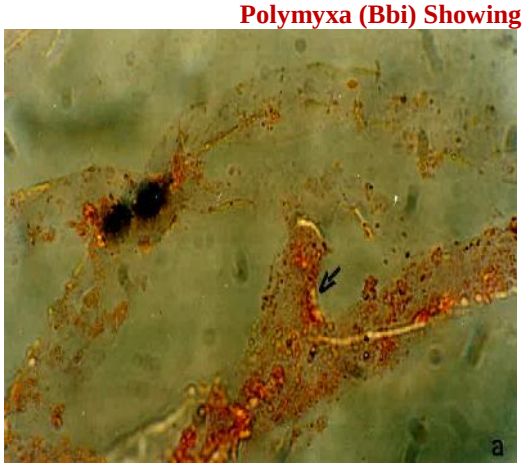


Figure 8: Plate 6 (a): Root Hairs of Brinjal (*Solanum Melongena* L.) Plants Grown in *Bacillus Polymyxa* Inoculated Soil Showing Induction of Branching in the Root Hair

Figure 9: Plate 6 (b): Root Hairs (*Solanum Melongena* L.) Seedling In *Bacillus Polymyxa* Treated Soil

Gibberellin in Plants Treated with *Bacillus Polymyxa*

The plants treated with *Bacillus polymyx*a showed 61.08% an increase in gibberellin concentration over the control plants.

DISCUSSIONS

Studies were cited out to evaluate the extent of influence of *Bacillus polyrnyxa* on the growth of brinjal plants and to test for the presence of growth promoting substance released by the bacteria. The results of the bioassays proved that the *Bacillus polymyxa* produced growth-promoting substances that enhanced germination count and other growth parameters. These growth-promoting substances were identified to be cytokinin and gibberellins. The present study showed direct evidence that Bacillus polymyxa inoculation affected the status of cytokinins and gibberellins in brinjal seedlings. Many studies offer explanations to account for the beneficial action of associative microorganisms. Non symbiotic nitrogen fixing bacte1ia are known to produce growth promoting substances which contribute to enhanced root growth as demonstrated by the studies of Azcon and Barea (1975). Some people are of the opinion that the nitrogen fixing ability of the microorganisms are solely responsible for the growth promotion. Other studies are pointers to the fact that growth-promoting substances produced by these microorganisms are responsible for the growth promotion. The result of the present investigations provide evidence that growth promoting substances released by the bacteria are primarily

responsible for the significantly higher germination count and biomass such as longer plumule and radicle length, increase in the number of root hairs and branching of root hairs. The bioassays were pointers to the presence of growth promoting substances released into the media by the *Bacillus polymyxa* which enhance root growth and thus increase the soil volume explored by the roots, which in turn enhance the mineral uptake and growth parameters. This is in turn helped by the increased soil nitrogen and phosphorus made available by the action of the microorganisms.

Plant growth promoting influence of microorganisms have been extensively examined (Michiels et al., 1989; Sumner, 1990) and it has been proved that bacteria like Bradyrhizobium, Azotobacter, Arthrobacter, Streptomyces and Frcmkia species produce cytokinins (Zeatin). Cytokinins are a class of compounds that stimulate cell division in plants. The role played by these microbes associated with roots and their contribution to root function as cytokinin producers are well documented (Holland, 1997; Corpe, 1989 and Rodriguez Caceres, 1991). The gene responsible for cytokinin production has not yet been isolated from any plant systems.(Binns 1994) Chen and Petschow 1978; Chen and Melitz, 1979; Chen et al., 1985 and Chen, 1987) have reported cytokinins production in tobacco, and peas. Holland (1997) has hypothesized that cytokinins are produced by the microbial symbionts and not by the plants. Our studies also demonstrated higher concentration of cytokinins in BBI associated Brinjal plants. But some more research is needed to state for sure that higher plants do not produce cytokinins and solely depend on endophytic associative bacteria forcytokinins synthesis.

The present study also proved qualitatively that *Bacillus polymyxa*produced gibberellin in pure cultures on chemically defined media. However, this does not provide enough evidence for the nature or the extent of interaction between the gibberellins produced by the microbe *Bacillus polymyxa* and the host plant. But these results are in conformity with studies of *Bottini et al*., 1989 that Azospirillum lipoferum produces gibberellins and the studies of Bashan et al., 1990 that enhanced growth observed after inoculation with *Azospirillum brasilience* was not necessarily due to mineral uptake. The present study is also a pointer to the fact that the microorganisms associated with plant roots are responsible for the presence of phytohormones, which enhance the plant growth. Of late, since no gene responsible for the synthesis of cytokinins and gibberellins have been isolated from the plant systems, it has been assumed that the microorganisms associated with the plant roots are responsible for the production of these hormones. Our study clearly demonstrates that *Bacillus polymyxa* associated with Brinjal (*Solanum melongena L.*) secretes free cytokinins (Zeatin riboside) and Gibberellins, that enhances the growth of brinjal plants.

CONCLUSIONS

The results of the present study are pointers to the fact that growth hormones are produced by *Bacillus polymyxa*. These growth-promoting hormones enhance root growth, which increase the soil volume explored by the roots. This increases the mineral uptake that along with increased soil nitrogen and phosphorus made available by the action of the microorganisms enhances growth and yield.

REFERENCES

1. *Patriquin DG, Döbereiner J, Jain DK. Sitesand processes of association between diazotrophs and grasses. Canadian Journal of Microbiology. 1983; 29(8):900-15.*
2. *Kapulnik Y, Sarig S, Nur I, Okon Y. Effect of Azospirillum inoculation on yield of field-grown wheat. Canadian Journal of Microbiology. 1983; 29(8):895-9.*

3. *Pacovsky RS, Paul EA, Bethlenfalvay GJ. Nutrition of sorghum plants fertilized with nitrogen or inoculated with Azospirillum brasilense. Plant and Soil. 1985; 85(1):145-8.*
4. *Morgenstern E, Okon Y. Promotion of plant growth and NO3- and Rb+ uptake in Sorghum bicolor× Sorghum sudanense inoculated with Azospirillum brasilense- Cd. Arid Land Research and Management. 1987; 1(4):211-7.*
5. *Lin W, Okon Y, Hardy RW. Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillum brasilense. Applied and Environmental Microbiology. 1983; 45(6):1775-9.*
6. *Sarig S, Kapulnik Y, Nur I, Okon Y. Response of non-irrigated Sorghum bicolor to Azospirillum inoculation. Experimental Agriculture. 1984; 20(1):59-66.*
7. *Sarig S, Blum A, Okon Y. Improvement of the water status and yield of field-grown grain sorghum (Sorghum bicolor) by inoculation with Azospirillum brasilense. The Journal of Agricultural Science. 1988; 110(2):271-7.*
8. *Bashan Y, Harrison SK, Whitmoyer RE. 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. 1990; 56(3):769-75.*
9. *Michiels K, Vanderleyden J, Van Gool A. Azospirillum—plant root associations: a review. Biology and Fertility of Soils. 1989; 8(4):356-68.*
10. *Sumner ME. Crop responses to Azospirillum inoculation. InAdvances in Soil Science 12 1990 (pp. 53-123). Springer, New York, NY.*
11. *Bashan Y. Enhancement of wheat root colonization and plant development by Azospirillum brasilense Cd. following temporary depression of rhizosphere microflora. Applied and Environmental Microbiology. 1986; 51(5):1067-71.*
12. *Kucey RM. Alteration of size of wheat root systems and nitrogen fixation by associative nitrogen-fixing bacteria measured under field conditions. Canadian journal of microbiology. 1988;34(6):735-9.*
13. *Salmeron V, Martinez-Toledo MV, Gonzalez-Lopez J. Nitrogen fixation and production of auxins, gibberellins and cytokinins by an Azotobacter chroococcum strain isolated from the root of Zea mays in the presence of insoluble phosphate. Chemosphere. 1990; 20(3-4):417-22.*
14. *Gonzalez-Lopez J, Salmeron V, Martinez-Toledo MV, Fallesteros F, Ramos-Cormenzana A. Production of auxins, gibberellins and cytokinins by Azotobacter vinelandii ATCC 12837 in chemically-defined media and dialysed soil media. Soil biology & biochemistry. 1986; 18(1):119-20.*
15. *Janzen RA, Rood SB, Dormaar JF, McGill WB. Azospirillum brasilense produces gibberellin in pure culture on chemically-defined medium and in co-culture on straw. Soil Biology and Biochemistry. 1992; 24(10):1061-4.*
16. *Bottini R, Fulchieri M, Pearce D, Pharis RP. Identification of gibberellins A1, A3, and iso-A3 in cultures of Azospirillum lipoferum. Plant Physiology. 1989; 90(1):45-7.*
17. *Nieto KF, Frankenberger WT. Influence of adenine, isopentyl alcohol and Azotobacter chroococcum on the vegetative growth of Zea mays. Plant and soil. 1991; 135(2):213-21.*
18. *Letham DS. Regulators of cell division in plant tissues. Planta. 1974; 118(4):361-4.*
19. *Holland MA. Occam's Razor Applied to Hormonology (Are Cytokinins Produced by Plants?). Plant Physiology. 1997;115(3):865.*
20. *Crozier A, Arruda P, Jasmim JM, Monteiro AM, Sandberg G. Analysis of indole-3-acetic acid and related indoles in culture medium from Azospirillum lipoferum and Azospirillum brasilense. Applied and Environmental Microbiology. 1988; 54(11):2833-7.*

21. *Govindan M, Purushothaman D. Production of phytohormones by the nitrogen fixing bacterium, Azospirillum. Agricultural Research journal of Kerala. 1984; 22(2): 133-138.*
22. *Omay SH, Schmidt WA, Martin P, Bangerth F. Indoleacetic acid production by the rhizosphere bacterium Azospirillum brasilense Cd under in vitro conditions. Canadian journal of microbiology. 1993; 39(2):187-92.*
23. *Baca BE, Soto-Urzua L, Xochihua-Corona YG, Cuervo-Garcia A. Characterization of two aromatic amino acid aminotransferases and production of indoleacetic acid in Azospirillum strains. Soil Biology and Biochemistry. 1994; 26(1):57-63.*
24. *Koshioka M, Harada J, Takeno K, Noma M, Sassa T, Ogiyama K, Taylor JS, Rood SB, Legge RL, Pharis RP. Reversed-phase C18 high-performance liquid chromatography of acidic and conjugated gibberellins. Journal of Chromatography A. 1983; 256:101-15.*
25. *Azcón R, Barea JM. Synthesis of auxins, gibberellins and cytokinins by Azotobacter vinelandii and Azotobacter beijerinckii related to effects produced on tomato plants. Plant and Soil. 1975; 43(1):609-19.*
26. *Corpe WA, Rheem S. Ecology of the methylotrophic bacteria on living leaf surfaces. FEMS Microbiology Ecology. 1989;5(4):243-9.*
27. *Rodríguez-Barrueco C, Cervantes E, Subbarao NS, Rodríguez-Caceres E. Growth promoting effect of Azospirillum brasilense on Casuarina cunninghamiana Miq. Seedlings. Plant and soil. 1991; 135(1):121-4.*
28. *Binns, A. N. Annu Rev Plant physiology and plant mol. Biol. 1994; 45: 173-176.*
29. *Chen CM, Petschow B. Cytokinin biosynthesis in cultured rootless tobacco plants. Plant Physiology. 1978; 62(6):861-5.*
30. *Chen CM, Melitz DK. Cytokinin biosynthesis in a cell- free system from cytokinin- autotrophic tobacco tissue cultures. Febs Letters. 1979; 107(1):15-20.*
31. *Chen CM, Ertl JR, Leisner SM, Chang CC. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology. 1985; 78(3):510-3.*
32. *Chen CM. Characterization of cytokinins and related compounds by HPLC. In High Performance Liquid Chromatography in Plant Sciences 1987 (pp. 23-38). Springer, Berlin, Heidelberg.*