Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (6): 1600-1608

(International Peer Reviewed Journal)

Screening Of PGPR Isolates For Plant Growth Promoting Traits From Maize Rhizosphere Soil Samples Of Perambalur District

K. Vinithadali* and K. Muthuselvam

*Department of Microbiology, Annamalai University, Annamalai Nagar, Tamil nadu, - 608002, **INDIA**

Email: kvinitha13@gmail.com

Received on 20th October and finalized on 24th October 2013

ABSTRACT

The present study was undertaken to screen the efficient plant growth promoting rhizobacteria (PGPR) that were isolated form ten maize rhizosphere soil samples of Perambalur district in terms of accessing their ability in production of important plant growth promoting traits viz., phytohormones (indole acetic acid and gibberellic acid) and siderophores (catechol and salicylate types). The results revealed that the Azospirillum (MAZ-6) and Bacillus (MBA-6) isolates obtained from Krishnapuram location and the Pseudomonas (MPS-5) from Veeraganur location were found to be efficient strains in terms of exhibiting plant growth promoting traits than their respective reference strains.

Keywords: *Azospirillum, Bacillus, Pseudomonas*, IAA, GA_3, siderophores.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) play a major role in maintaining the soil ecosystem in the rhizosphere, rhizoplane and phyllosphere that are ultimately beneficial to the plants. Beneficial free living soil bacteria occurring in the plant rhizosphere are usually referred as plant growth promoting bacteria and abbreviated as PGPB [1]. They are also referred as yield increasing bacteria [2]. Rhizospheric bacteria are capable of fixing atmospheric nitrogen, solubilizing phosphorus, iron and producing plant hormones *viz.*, auxins, gibberellins and cytokinins and promote plant growth by directly affecting the metabolism of the plants by providing substances that are usually in short supply. Additionally, they improve plant tolerance to drought, high salinity and metal toxicity through the production of enzyme 1-amino cyclopropane carboxylate deaminase. PGPR prevents the deleterious effects of phytopathogenic microorganisms. They produce antibiotics that harm or inhibit other microbes, but not plants, by limiting the availability of iron to pathogens or by altering the metabolism of the host plant to increase its resistance to pathogen infection.The plant responses to inoculation with PGPR include enhanced nitrogen fixing ability with minimal inoculation, direct increases of various growth parameters like plant dry weight, development and morphology of root system, grain yield, protein and mineral nutrient content, displacement of deleterious and pathogenic rhizosphere microorganisms, increased phosphorus solubilization and enhanced VAM colonization [3].

The mechanisms attributed for plant growth stimulation, is mainly due to improvement of water and mineral uptake [4,5] and production of biologically active substances, such as vitamins, amino acids, Phytohormones [6-8] and antibiotics [9]. The present study was undertaken to screen the efficient PGPR isolates of *Azospirillum, Bacillus* and *Pseudomonas* isolated from maize rhizosphere soil samples of Perambalur district.

MATERIALS AND METHODS

The PGPR isolates from ten locations of Perambalur district were isolated and purified by following standard protocols and designated as MAZ (*Azospirillum*), MBA (*Bacillus*) and MPS (*Pseudomonas*) and serially numbered from 1 to 10. They were screened for certain important plant growth promoting traits *viz.*, ARA activity [10], fixed nitrogen (*Azospirillum* isolates) [11], phosphorus solubilization potential (*Bacillus* isolates) [12-14], phytohormones such as indole acetic acid [15] and gibberellic acid [16] production and siderophore production [17] by all PGPR isolates.
All the reported data are the arithmetic means of 3 replicates. Statistical analysis of all the parameters was carried out through analysis of variance (ANOVA) [18].

RESULTS AND DISCUSSION

Screening of *Azospirillum* isolates for nitrogenase activity and cell nitrogen content: The *in vitro* nitrogenase activity and cell nitrogen content of *Azospirillum* isolates obtained from maize rhizosphere soils of Perambalur district are presented in table 1. Among the ten isolates, MAZ-6 obtained from rhizosphere soil of Krishnapuram recorded the higher nitrogenase activity of 376.21 n moles of C_2H_4 produced mg^{-1} of protein h^{-1} and cell nitrogen of 42.26 mg g^{-1} of cell weight followed by MAZ-8 with 362.30 and 37.56 as respective values. Whereas, the reference strain *Azospirillum lipoferum* MTCC 2306 produced 355.65 n moles of C_2H_4 produced mg^{-1} of protein h^{-1} and cell nitrogen of 37.49 mg g^{-1} of cell weight. The minimum nitrogenase activity of 156.00 n moles of C_2H_4 mg^{-1} of protein h^{-1} and cell nitrogen content of 26.98 mg $g^{-1}$was recorded in MAZ-3. Based on the nitrogenase activity and cell nitrogen content of the isolates MAZ-6 was selected for further studies.The bacterial diversity in a variety of plants, coming under cereals and legumes were assessed based on the release of enzymes (soil dehydrogenase, phosphatase, nitrogenase, etc), metabolites (siderophores, antifungals, HCN, etc), growth promoters (IAA, ethylene). Based on such primary screening protocols, effective rhizobacteria have been field tested [19].
Nitrogen is a necessary component which is used for the growth of the plant. Plants need a limited amount of nitrogen for their growth. The types of crops also determine the level of nitrogen. Some crops need more nitrogen for their growth while some crops need less amount of nitrogen. Biological nitrogen fixation is estimated to contribute 180×10^6 metric tons/year globally of which 80% comes from symbiotic associations and the rest from free-living or associative systems. The ability to reduce and siphon out such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and archaea. This includes symbiotic nitrogen fixing forms namely: *Rhizobium,* the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees and non-symbiotic (free-living, associative or endophytic) N_2 - fixing forms such as Cyanobacteria, *Azospirillum, Azotobacter, Acetobacter diazotrophicus, Azoarcus,* etc. [20].

Table 1. Screening of *Azospirillum* isolates for nitrogenase activity and cell nitrogen content under *in vitro* conditions

Name of the isolate	Nitrogenase activity*	Cell nitrogen content**
MAZ-1	235.03	37.92
MAZ-2	181.12	28.60
MAZ-3	156.00	26.98

MAZ-4	248.54	36.00
MAZ-5	343.20	38.90
MAZ-6	376.21	42.26
MAZ-7	249.38	29.90
MAZ-8	362.30	37.56
MAZ-9	352.00	36.80
MAZ-10	325.00	31.23
MTCC 2306	355.65	37.49
S.E.	1.77	1.71
C.D. (P=0.05)	3.94	3.65

* n moles of C_2H_4 produced mg^{-1} of cell protein h^{-1}, ** mg g^{-1} cell weight

The rhizosphere of Kallar grass, the strains of *Azospirillum lipoferum* fixed more nitrogen than *Azospirillum brasilense* as evidenced by their nitrogenase activity [21]. Variations in C_2H_4 reduction activities of different strains of *Azospirillum brasilense* isolated from rhizosphere of cotton [22].

Screening of *Azospirillum* isolates of maize rhizosphere soils for phytohormones and siderophore production: The Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential of *Azospirillum* isolates obtained from the maize rhizosphere soil samples of Perambalur district were investigated and the results were furnished in table 2.

It was evidenced that all the ten isolates of *Azospirillum* (MAZ-1 to MAZ-10) were able to produce both phytohormones *viz.,* IAA and GA_3 with varying quantities between them. The phytohormones production ranged from 29.85 to 79.90 µg 25 ml^{-1} broth for IAA and from 2.74 to 8.62 µg 25 ml^{-1} broth for GA_3 was recorded. The *Azospirillum* isolate MAZ-6 produced the maximum amount of 79.87 µg of IAA $25ml^{-1}$ and 8.73 µg of GA_3 $25ml^{-1}$ of nitrogen free malate broth. It was followed by the reference strain MTCC 2306 with 68.22 µg of IAA and 7.80 µg of GA_3 $25ml^{-1}$ and MAZ-4 with 66.07 µg of IAA and 7.26 µg of GA_3 $25ml^{-1}$.

Table 2. Screening of *Azospirillum* isolates for phytohormones and siderophore production

Name of the isolate	**IAA (µg 25 ml^{-1} of broth)**	**GA_3 (µg 25 ml^{-1} of broth)**	**Siderophore production (µg ml^{-1})**	
			Catechol Type	**Salicylate Type**
MAZ-1	30.02	3.16	2.56	5.62
MAZ-2	37.04	3.56	4.78	7.78
MAZ-3	67.80	6.90	4.33	7.87
MAZ-4	66.07	7.26	3.56	7.43
MAZ-5	52.09	5.22	1.34	2.58
MAZ-6	79.87	8.73	4.98	8.56
MAZ-7	53.82	5.12	3.68	4.23
MAZ-8	42.67	3.98	3.20	3.25

MAZ-9	29.85	2.74	2.34	3.97
MAZ-10	35.68	3.97	3.58	6.21
MTTC 2306	68.22	7.80	4.80	7.89
S.E.	0.63	0.32	0.18	0.29
C.D. (P=0.05)	1.26	0.82	0.36	0.59

All the *Azospirillum* isolates produced both catechol and salicylate type of siderophore with varying quantities. The catechol type of siderophore produced by *Azospirillum* isolates ranged from 1.34 to 4.98 μg ml^{-1} and salicylate type ranged from 2.58 to 8.56 μg ml^{-1} of culture broth. The isolate MAZ-6 produced higher quantity of 4.98 and 8.56 μg ml^{-1} of catechol type and salicylate type of siderophore respectively followed by the reference strain MTCC 2306 with 4.80 and 7.89 μg ml^{-1} of catechol and salicylate type of siderophore. The minimum amount 1.34 and 2.58 μg of catechol and salicylate type of siderophore ml^{-1} was respectively produced by the isolate MAZ-5.

Thirty five isolates of N_2-fixing bacteria were screened by [23] to assess the production of plant growth promoting substances. Each strain has different potential in N_2-fixing ability and has difference in physiology and morphology of the colonies and the cells. N_2-fixing bacterial inoculation increased Vetiver growth and development. [24] revealed that the N_2-fixing bacteria (*Azospirillum*) produced plant growth hormone, Indole-3-acetic acid (IAA) at 30-40 μg/ml in the broth media. *Azospirillum* grew well outside and inside the Vetiver root.

Screening of *Bacillus* isolates for phosphate solubilisation potential: The results clearly showed that all ten *Bacillus* isolates obtained from maize rhizosphere soil samples of Perambalur district exhibited clear zones around their growth on Sperber's hydroxy apatite medium. Further, the isolates were screened for release of phosphorus from tricalcium phosphate in Pikovskaya's broth, acid phosphatase activity, change in the pH of the medium and titrable acidity of the culture medium and the results were presented in table 3. All the *Bacillus* isolates (MBA-1 to MBA-10) were solubilised tricalcium phosphates and the amount of phosphorus released from 100 mg of TCP in broth varied considerably. The isolate MBA-6 from Krishnapuram recorded higher phosphorus solubilisation of 30.68 mg followed by MBA-4 from Keelaperambalur which released 25.43 mg of phosphorus from 100 mg of TCP and the reference strain *Bacillus megaterium* MTCC-8073 solubilised about 28.66 mg of phosphorus from 100 mg of TCP. The acid phosphatase activity of the ten isolates ranged from 40.06 to 80.13 n moles of P-nitro phenol released min^{-1} mg^{-1} of cell protein. The maximum amount of phosphatase activity was recorded by MBA-6 (80.13 n moles of P-nitro phenol min^{-1} mg^{-1} of cell protein) followed by the reference strain *Bacillus megaterium* MTCC – 8073 and isolate MBA-4 with 76.63 n moles of P-nitro phenol min^{-1} mg^{-1} of cell protein. Whereas, MBA-3 isolate recorded the minimum amount of phosphatase activity of 40.06 n moles of P-nitro phenol mg^{-1} of cell protein min^{-1}.

The titrable acidity of *Bacillus* isolates (MBA-1 to MBA-10) were ranged from 2.48 to 643 in the culture medium after 7 days growth. The reference strain *Bacillus megaterium* MTCC-8073 recorded titrable acidity of 5.03. Whereas, the isolate MBA-7 recorded titrable acidity of 6.43 as the higher value. The change in pH of the culture medium after 7 days of growth varied from 3.08 to 6.32. The MBA-6 isolate from Krishnapuram showed significant reduction in a pH of 3.08.

Table 3. Screening of *Bacillus* isolates for phosphate solubilisation efficiency

Name of the isolate	Phosphorous solubilized*	Acid phosphatase activity**	Titrable Acidity (g/l)	pH of the culture filtrate
MBA-1	24.67	65.87	4.41	3.37
MBA-2	18.71	50.34	3.64	3.12
MBA-3	8.33	40.06	2.48	4.38
MBA-4	25.43	70.63	5.18	5.29
MBA-5	10.48	50.68	2.96	3.86
MBA-6	30.68	80.13	3.36	3.08
MBA-7	15.24	56.13	6.43	6.32
MBA-8	9.87	48.36	2.68	3.73
MBA-9	12.36	59.78	3.03	3.43
MBA-10	24.33	73.28	2.46	3.96
MTCC – 8073	28.67	77.18	5.03	4.93
S.E.	1.01	0.41	0.22	0.13
C.D. (P=0.05)	2.13	0.88	0.47	0.36

***** mg of P released from 100 mg of tricalcium phosphate,
****** n moles of p-nitro phenol released in min^{-1} mg^{-1} of cell protein.

Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to change insoluble phosphate in soil into soluble form by secreting organic acids, such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. Since, plants utilize only inorganic phosphorus, organic phosphorus compounds must first be hydrolyzed by phosphorus enzyme which mostly originates from plant roots, through the action of fungi and bacteria [25].

The role of microorganisms in solubilizing insoluble phosphate in soil and making it available to plants is well known [26]. Phosphate solubilizing microorganisms include several genera of bacteria *viz*., *Bacillus, Pseudomonas, Klebsiella* and *Serratia* [27-29]. Among bacteria, most efficient phosphate solubilizing bacteria belonged to the genera *Bacillus* and *Pseudomonas* [29]. The phosphate solubilizing bacterial isolates were screened based on the ability to release soluble phosphorous from apatite in the culture medium [30, 31].

A positive correlation between the degree of P solubilization and pH reduction was observed. The pH of the culture filtrate of phosphate solubilizing *Bacillus* varied from 4.0 to 6.5 and the amount of phosphate solubilization is directly correlated with decrease in pH of the culture medium [32, 33]. Phosphate solubilizing bacteria reduced the pH of the medium consequent of the release of organic acids. This acid solubilization mechanism of phosphates was clearly reported by [29]. Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well aerated soil although it is easily precipitated in iron-oxide forms [34].

Screening of *Bacillus* isolates for phytohormones and siderophore production : The Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential of *Bacillus* isolates obtained from the rhizosphere of Maize were studied and the results were given in table 4. The study clearly revealed that all the ten *Bacillus* isolates were able to produce phytohormones and siderophore with varying amounts among them. The IAA production ranged from 18.04 to 60.04 µg 25 ml^{-1} of broth and GA_3 production ranged from 1.78 to 5.56 µg 25 ml^{-1} of broth levels. The MBA-6 isolate from Krishnapuram recorded higher levels of 60.04 and 5.56 µg 25 ml^{-1} of broth of IAA and GA_3 respectively. It was observed that the reference strain MTCC-8073 was produced lesser quantities of 56.23 and 5.21 µg 25 ml^{-1} of broth of IAA and GA_3 respectively. Similar to phytohormones production, the siderophore contents were higher in the same isolate MBA-6 as 7.43 and 7.06 µg 25 ml^{-1} of broth of catechol type and salicylate type siderophore. It was followed by the reference strain MTCC-8073 with 6.39 and 6.48 µg 25 ml^{-1} of broth as respective values.

Screening of *Pseudomonas* isolates for phytohormones and siderophore production: The results of the present study revealed that all the ten *Pseudomonas* isolates (MPS-1 to MPS-10) were able to produce substantial quantities of phytohormones and siderophore with variation in the amount among them. The higher amount of indole acetic acid was recorded in the Veeraganur isolate (MPS-5) with 65.49 µg 25 ml^{-1} of broth. It was followed by the reference strain MTCC-9768 with 63.24 µg 25 ml^{-1} of broth and MPS-7 isolate from Gangaivelli with 60.48 µg 25 ml^{-1} of broth levels. With regard to Gibberellic acid production, the MPS-7 isolate from Gangaivelli recorded 7.49 µg 25 ml^{-1} of broth as higher value followed by MPS-5 isolate from Veeraganur with 7.28 µg 25 ml^{-1} of broth and the reference strain MTCC-9768 with 6.84 µg 25 ml^{-1} of broth.

Table 4. Screening of *Bacillus* isolates for phytohormones and siderophore production

Name of the isolate	IAA (µg 25 ml^{-1} of broth)	GA_3 (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
			Catechol Type	Salicylate Type
MBA-1	24.36	2.41	5.26	5.74
MBA-2	34.34	1.78	3.23	3.18
MBA-3	24.27	2.89	3.13	4.04
MBA-4	43.36	3.24	2.18	3.65
MBA-5	55.64	5.28	5.36	4.43
MBA-6	60.03	5.56	743	706
MBA-7	56.04	5.23	6.48	6.13
MBA-8	18.04	2.58	4.38	4.06
MBA-9	20.34	2.46	5.36	4.85
MBA-10	30.06	3.21	5.24	4.84
MTCC - 8073	56.23	5.21	6.39	6.48
S.E.	1.84	0.19	0.28	0.25
C.D. (P=0.05)	3.90	043	0.61	0.55

The *Pseudomonas* isolate from Veeraganur location (MPS-5) recorded higher amounts of siderophore as 8.34 and 8.78 µg ml^{-1} of catechol and salicylate types respectively. It was followed by the reference strain MTCC-9768 with 7.63 and 8.23 µg ml^{-1} as corresponding values (table 5).

Bacterial siderophore (Pseudobactin and ferrioxiamine B) were inefficient as iron sources for plants and the rhizosphere siderophore producing bacteria can be in competition with the plant for iron. In fact, the vast majority of research on microbial siderophore in the rhizosphere is associated with their biocontrol activities due to their competitive effects with plant pathogens [35].

Table 5. Screening of *Pseudomonas* isolates for phytohormones and siderophore production

Name of the isolate	IAA (µg 25 ml^{-1} of broth)	GA_3 (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
			Catechol Type	Salicylate Type
MPS-1	30.89	6.23	4.47	4.84
MPS-2	28.43	2.89	4.78	5.33
MPS-3	20.66	3.64	3.43	5.88
MPS-4	34.33	4.49	5.48	6.72
MPS-5	65.49	7.28	8.34	878
MPS-6	38.67	5.34	6.68	7.92
MPS-7	60.48	7.49	3.34	7.01
MPS-8	51.43	5.83	4.58	4.67
MPS-9	48.78	3.21	5.78	3.65
MPS-10	54.66	6.43	5.48	6.34
MTCC - 9768	63.24	6.84	7.63	8.23
S.E.	0.62	0.21	0.27	0.23
C.D. (P=0.05)	1.33	0.54	0.55	0.47

All the isolates of *Azospirillum*, *Bacillus* and *Pseudomonas* produced appreciable quantities of siderophore. Among the agriculturally beneficial microorganisms, *Pseudomonas fluorescens* SP-10 secreted highest amount of both catechol and salicylate type of siderophore followed by *Bacillus* isolates and *Azospirillum* isolates. Production of siderophore by agriculturally beneficial isolates and its role in Fe mobilization was reported by several workers [36-38] of the two species of *Azospirillum*, *Azospirillum lipoferum* produced higher amount of siderophore than *Azospirillum brasilense*. It was reported earlier by [39] that *Azospirillum lipoferum* secreted higher quantities of siderophore than other *Azospirillum* species.

APPLICATIONS

The screened plant growth promoting rhizobacterial isolates can prove to be an efficient alternative to chemical fertilizers for the enhanced growth and development of maize plant.

CONCLUSIONS

The present study clearly concluded that the PGPR organisms such as *Azospirillum* (MAZ-6) and *Bacillus* (MBA-6) isolated from Krishnapuram location and the *Pseudomonas* (MPS-5) isolated from Veeraganur location were found to be efficient strains in terms of exhibiting plant growth promoting traits **(**ARA activity, fixed nitrogen, Phosphorus solubilization potential, phytohormones such as indole acetic acid and gibberellic acid production and siderophore production) than their respective reference strains.

REFERENCES

[1] J. W. Kloepper, R. Litshiz, Zablotowicz, *Tibtech*, **1989**, 7, 39 - 44.
[2] W. H. Tang, M. H. Ryder, P. M. Stephens and G. D. Bowde (Eds.), Yield increasing bacteria (YIB) and biocontrol of sheath blight of rice. In: Improving Plant Productivity with Rhizosphere Bacteria, Common Wealth Scientific and Industrial Research Organization, Adelaide, Australia. **1994**, pp. 267-278.
[3] R. J. Rennie, J. R. De Freitas, A. P. Ruschel, P. B. Vose, *Can. J. Microbiol.*, **1981**, 28, 462-467.
[4] Y. Bashan, H. Levanony, *Plant Soil*, **1991**, 137, 99-103.
[5] H. Bertrand, C. Plassard, X. Pinochet, B. Toraine, P. Normand, J.C. Cleyet Marel, *Can. J. Microbiol.*, **2000**, 46, 229-236.
[6] I. E. Garcia De Salamone, R. K. Hynes, L.M. Nelson, *Can. J. Microbiol.*, **2001**, 47, 404-411.
[7] B. R. Glick, *Can. J. Microbiol.*, **1995**, 41, 109-177.
[8] F. Persello-Cartiequx, L. Nussaume, C. Robaglia, *Plant Cell Environ.*, **2003**, 26, 189-199.
[9] M. N. Giacomodonato, M. J. PeHinari, G. R. Souto, B. S. Mendez, N. I. Lopez, *World J. Microbiol. Biotechnol.*, **2001**, 17, 51-55.
[10] F. J. Bergersen, Methods for Evaluating Biological nitrogen fixation, John Wiley and Sons, New York, **1980**, p. 702.
[11] E.C. Humphries, Mineral components and ash analysis. Modern method of plant analysis. Springer Verlag, Berlin, **1956**, 1, 468-502.
[12] R. I. Pikovskaya, *Microbiologiya*, **1948**, 17, 362–370.
[13] S. R. Olsen, C. U. Cole, F. S. Watenabe, L. Peon, Estimation of available phosphous in soils by extraction with sodium biocarbonate, USDA, U.S. Govt printing office, Washington D.C., **1954**, pp. 40-45.
[14] R. K. Morton, Transphosphorylation by phosphates. In: Methods in Enzmology Vol. 2, Colowick, S. P and Kaplan (Eds.), Acadamic Press, New York, **1955**, pp. 536 - 559.
[15] S. A. Gorden, L.G. Paleg, *Physiol. Plantarum.*, **1957**, 10, 347-348.
[16] A. Borrow, P. W. Brain, U. E. Chester, P. J. Curtis, H. G. Hemming, E. C. Jeffereys, R. B. Lloyd, I. S., Nixon, G. L. F. Norris, N. Radley, *J. Food Sci. Agric.*, **1955**, 6, 340-348.
[17] M. Reeves, P. L.Neilands, A. Ballows, *J. Bacteriol.*, **1983**, 154, 324-329.
[18] K.A. Gomez, A.A. Gomez, Statistical procedures for agricultural research, Wiley, NY, **1984**, 2nd Ed., 680.
[19] N. Bhavdish, A. Johri, A. Sharma, J. S. Virdi, Rhizobacterial diversity in India and influence on soil and plant health. Biotechnology in India, Springer Verlag, Berlin, **2003**, pp. 49-89.
[20] K. V. B. R. Tilak, N. S. Subba Rao, *Fert. News*, **1978**, 23, 25-28.
[21] T. Hurek, B. Reinhold, G. Nieman, T. Fendric, W. Klingmuller (Ed.) Nitrogen dependent growth of *Azospirillum* sp. in batch culture at low concentration of oxygen, In: Azospirillum IV - Genetics, *physiology and Ecology*. Springer-Verlag. Berlin, **1988**, pp. 115-121.
[22] M. Fayez, Z.Y. Daw, *Biological Fertillizers and Soils*, **1987**, 4, 91-95.

[23] S. Siripin, A. Thirathorn, A. Pintarake, T. Yathaputanon, Use of ^{15}N isotope dilution method fixing bacterial inoculation on the growth of vetiver grass. In: Abstract of poster papers, ICV-2, **2000**, p. 30.
[24] S. Patiyuth, B. Tangcham, S. Muanjang, Studies on N_2-fixing bacteria association with Vetiver 1. Biosynthesis of plant growth hormone by *Azospirillum* 2 use of the gus A gene to study *Azospirillum*. In: Abstract on Poster Papers, ICV-2, **2000**, p. 20.
[25] V. Sunantha Posuk, Study on soil microbial biodiversity in rhizosphere of Sunflower in degrading soil. In: Abstract of poster papers, ICV-2, **2000**. p. 24.
[26] B. S. Kundu, A. C. Gaur, *Plant Soil*, **1984**, 79, 227-234.
[27] J. P. Nakas, W. D. Gould, D. A. Klein, *Soil Biol. Biochem.*, **1987**, 19, 13-18.
[28] M. S. Strom, S. Lory, *J. Bacteriol.*, **1987**, 169, 3181-3188.
[29] A. Dave, H. H. Patel, *Ind. J. Microbiol.*, **1999**, 39, 161-164.
[30] S. Banik, B. K. Dey, *Plant Soil*, **1982**, 69, 353-364.
[31] M. A. Whitelaw, T. J. Harden, K. R. Helyar, *Soil Biol. Biochem.*, **1999**, 31, 655-665.
[32] D. Arora, A. C. Gaur. *Ind. J. Microbiol.*, **1978**, 10, p. 315.
[33] D. Arora, A. C. Gaur, *Ind. J. Exp. Biol.*, **1979**, 17, 1258-1261.
[34] F. B. Salisbury, C. W. Ross, Plant Physiology, Wadsworth Pub. Co., Belmont, USA, **1992**, p. 682.
[35] M. Hefte, M. Vande Woestyne , W. Verstrate, J. A. Manthey, D. E. Crowley, D. G. Lester (Ed.), Role of siderophore in plant growth promotion and plant protection by fluorescent Pseudomonas. In: Biochemistry of metal micronutrients in the rhizosphere, Lewis Publishers, Boca Raton, FL, USA, **1994**, pp. 81-92.
[36] B. Saxena, M. Modi, V. V. Modi, *J. Gen. Microbiol.*, **1986**, 132, 2219-2224.
[37] D. B. S. Kumar, H. C. Dube, *Ind. J. Exp. Biol.*, **1991**, 29, 365-370.
[38] A. Sharma, B. N. Johri, *Microbiol. Res.*, **2003**, 158, 243-248.
[39] R. Sridar, Ph.D. Thesis, TNAU, Coimbatore, **1996**.