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Evaluation and Assessment of Azotobacter Rhizobium and Phosphate Solubilizing Bacteria with Potential Biofertilizer Production

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ABSTRACT

In the confront investigation rhizosphere soil was collected in and around. The rhizosphere soils were serially diluted and pour plated on hand-picked medium of pikovskay's media and winogradsky's media for isolation of bacteria. The isolated bacteria were known supported morphological and organic chemistry tests. The results were compared with Bergy's manual of determinative medicine. Screening is finished by growth promoting bacteria by seed germination. The potency on completely different crop plants in terms of growth and yield each length and biomass. From the results, it had been noted as Azotobacter and actinomycete molecular characterization of microorganism isolates studied by sixteen sRNA sequencing. Azotobacter and Rastolnia sp. were mass polite and utilized in the experiment of plants and in probiotic fishes. The length and weight was evaluated in plant and fish. The isolated bacteria were inoculated in nutrient broth and plant was inoculated in potato grape sugar broth and it's extracted by exploitation fermentation alcohol. Artimia deadliness check was done by exploitation brain shrimp in ninety six titer well plate by hand-picked organisms in bacteria and in fungi. The medication activity check was done by Kirby Boyer methodology in Azotobacter and phosphate solubilizing bacteria.

KEYWORDS: *Evaluation, Assessment, Azotobacter, Rhizobium, phosphate solubilizing bacteria*

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INTRODUCTION

Biofertilizer contains living microorganisms and promotes growth by increasing the supply of primary nutrient (nitrogen and phosphorus) to the host plant. Biofertilizer conjointly provides nutrients needed by the plants and helps to extend the soil quality with natural organism. A number of the useful microbes utilized in biofertilizers square measure N2 –fixing bacteria, phosphate-solubilizing microbes and mycorrhizae that square measure able to fix atmospherical element or solubilize phosphorus within the soil. Microorganisms square measure extensively used as biofertilizers in agricultural practices. The biofertilizers square measure the live or contain latent cell of useful microorganisms that augment the supply of nutrients to the plants. The useful microorganism's square measures bacteria genus, Azotobacters, azospirillum, eubacteria, phosphobacteria and mycorrhiza. Among these Azotobacters and phosphate bacteria play major role within supply of nutrients and within the plant growth promoting activities. These bacteria square measure gift in low populations within the natural surroundings.

The increased by artificial means and incorporated into the agricultural lands within the sort of biofertilizers. A great deal of analysis work is offered on the biofertilizers in agriculture practices element and phosphorus square measure the most important plant nutrients, that square measure said because the master component in crop production. It's a bigger a part of soil phosphorus (approximately 95-99%) is gift within the sort of insoluble form; dominant in basic soil and unable to utilize by the plants.

Plants and microbes species have developed mutualism or mutualist relationships bacteria genus is that the root of legumes host element fixing bacteria which might invade root and obtain sugars from the plant. They convert massive amounts of dinitrogen (N2) from the atmosphere into forms that the plants will use. Some soil bacteria, isolated from the basis region of plants, square measure notable to boost growth of the plants. These useful free- living soil bacteria square measures termed as plant growth promoting rhizobacteria (PGPR). The useful result of PGPR is mediate through either direct or indirect mechanisms.

The PGPR conjointly improve growth by indirect mechanisms like, suppression of microorganism, fungous and worm pathogens, and production of siderophores, HCN, ammonia, antibiotics, volatile metabolites etc. dependent relationship between phosphate solubilizing bacteria (PSB) and plants is synergistic in nature as bacteria offer soluble phosphate and

plants provide root borne carbon compounds (mainly sugars), that may be metabolized for microorganism growth.

Azotobacter naturally fixes atmospherical element within the rhizosphere. There square measure completely different strains of every Azotobacter have varied chemical, biological and different characters. However, some strains have higher element fixing ability than others. Azotobacter uses carbon for its metabolism from easy or compound substances of carbonic in nature.

Azotobacters square measure aerobic, nonsymbiotic, heterotrophic bacteria with distinctive ability of fixing atmospherical element. The bacteria square measures gram-negative, non-motile, however typically motile by covered flagella. Azatobacters square measure extremely necessary for his or her ability to repair molecular element, contributory to the productivity of any surroundings. They were well-tried through an experiment to repair ten mg of atmospherical element per gram of saccharide consumed. A special cluster of bacteria (phosphate solubilizing bacteria) occurring in ocean water is capable of dissolving insoluble inorganic element into soluble inorganic element. The actual investigation is aimed towards the prospective role of Azotobacter and phosphate solubilizing bacteria as biofertilizer.

Materials and ways Sample assortment
The soil samples were collected from around. The samples were serially diluted and unfold plate methodology was done. The pure cultures were patterned in pikovskay's medium and winogradsky medium. The pure cultures were hold on in forty C for more study.

Pikovskay's medium – during this medium is employed to spot the phosphate solubilizing microorganisms. There in halo zone shaped as positive result.

Winogradsky medium- during this medium is employed to spot the Azotobacter. Two strains are hand-picked for his or her potency of fixing the atmospherical element and or solubilizing the insoluble phosphate. These strains have scope for his or her attainable utility as bio-fertilizers. It's necessary to spot the 2 strains at species levels before their economic utility. This chapter so, deals with the identification of most potent strains supported their cultural, morphological and organic chemistry characteristics.

Cultural Characteristics
The cultural characterization was studied supported morphological and organic chemistry characterization, like abundance of growth; pigmentation, optical characteristics, type size margin and elevation were studied on agar plates.

Germinating seed methods Seed germination test
The selected strains were bioassayed for his or her ability to promote/ inhibit seed plant growth by exploitation the tactic with modifications. Rice seeds were immersed in ethyl alcohol for twenty sec. later on the seeds were in real time washed with sterile water (3X10min) and incubated in zero.1% dichloride of mercury resolution for four minutes. Following this treatment, the seeds were washed repeatedly in excess amounts of sterile water for 5-6 hours on a shaker before seeding them in petrifies containing in their various medium containing at 108 cells ml-1. The seeds were unbroken for one hour within the substance and decanted the medium. 5 replicates of one hundred seed of rice, Phaseolus aureus and herb were maintained for every microorganism species and incubated at thirty o c for three days. When 3 days the proportion of seed germination was measured, shoot length and root length were measured in rice when five days of immunization within the case of Phaseolus aureus and rice, solely root length was measured when three days of immunizations.

Antibacterial Assay
Antibacterial activity was dole out exploitation disc diffusion methodology. The substances was utilized Muller Hinton Agar (MHA). The tests were conducted at three completely different concentration of crude extract (5mg, 2.5mg and 1.5mg/disc). The plates were covered for 24hours at 37oC.

Artemia morbidness Assay
After extraction with ester the crude extract was tested for the branchiopodan toxicity assay that may be a straightforward, speedy and cheap bench prime assay and thought of as a useful gizmo for preliminary assessment of general toxicity (Meyer et al., 1982).

Cultivation of plant life Strains
The plant life strains were inoculated in a very one thousand cubic centimeter flask containing 250 cubic centimeter of potato glucose broth (PDB). Cultivation was performed at temperature below static conditions and daylight looking on the plant life growth finishes on liquid medium was incubated for two weeks.

Isolation of Genomic deoxyribonucleic acid
The Genomic deoxyribonucleic acid was performed in designated microorganism isolates exploitation QIAGEN deoxyribonucleic acid isolation kit (Qiagen). The sequence of 16S rRNA experiment was dole out.

Polymerase Chain Reaction
A try of oligonucleotide primers is intended complementary to a target deoxyribonucleic acid molecule specified they will be extended by a deoxyribonucleic acid enzyme towards one another than the region of the guide delimited the primers are often greatly amplified by winding up cycles of denaturation, primer annealin and chemical process.

Result and Discussion
Isolation of bacteria from rhizosphere soil
The rhizosphere soils were collected from around. The rhizosphere soils were used for isolation of bacteria from its soil. Zone formation happens within the phosphate solubilizing bacteria. The full arable microorganism count of the whole soil was found to be a pair of.48 X 10-3 CFU/ml and three.19 X 10^{-3} CFU/ml in Azotobacter and phosphate solubilizing bacteria.

Different colony characteristics of the microorganism strains were determined on the surface of the Pikovskay's medium and winogradsky's medium that was shown in (Figure.1 and 1.1). The isolated colonies were pure refined and hold on at 4 C for more use.

Identification of the isolated bacteria
The organisms were known supported morphological and organic chemistry tests (Table 1) consistent with the Bergey's

manual of derminative medicine. Belong to the genus Azotobacter and Ralstonia severally. Determinations of organic chemistry characterization of bacteria isolated from rhizosphere soil were known as Azotobacter and ralstonia.

Evaluation supported Seed Germination in Phaseolus aureus and Rice

Seed germination in paddy was four-hundredth and Phaseolus aureus was eightieth up to speed. However, the seeds inoculated with bacteria exhibited Associate in nursing increased germination. The germination varied from twenty to hour in rice and eighty to 100 percent in Phaseolus aureus looking on the strains Azotobacter used and twenty to hour in rice and eighty to ninetieth in Phaseolus aureus looking on the strains of phosphate solubilizing bacteria. (Table a pair of and Figure a pair of and a couple of.1)

Root Length in Rice and Phaseolus aureus

The phosphobacterial strain inflated the basis nodules by five hundredth but Azotobacter strains decrease the basis nodules by twenty ninth in rice. The Azotobacter strain inflated the basis nodules by thirty four.75% in Phaseolus aureus but phosphobacterial decrease the basis nodules by twenty ninth in Phaseolus aureus. (Table a pair of and Figure three up to three.3)

Growth measure of Shoot in Phaseolus aureus and Rice

The phosphobacterial strain inflated the shoot by hour. However, Azotobacter strains decrease the shoot by five hundredth in Phaseolus aureus. The Azotobacter strain shriveled the shoot by half-hour in rice. Still, phosphobacterial increase the charge by hour in rice. (Table3)

Antibacterial Activity Test

In antibacterial drug activity take a look at disc diffusion methodology is applied. Erythromycins, Chloromycetin, Nalidicic acid are unit sensitive in phosphate solubilizing bacteria. Chloromycetin, Nalidicic acids area unit sensitive and E-Mycin is resistant in Azotobacter (Table.4 and Figure.4).

Artimia Morbidness Test (Phosphate Solubilizing bacteria and Azotobacter)

Phosphate solubilizing bacteria and Azotobacter area unit utilized in the artimia morbidness take a look at the 2 strains area unit inoculated in nutrient broth unbroken for twenty-four hours incubation. Equal quantity of ester is supplemental and extracted. Compare to Azotobacter phosphate solubilizing bacteria is nice.

Identification of Fungi

In order to select the most promising fungal strains, the isolated fungi were cultivated in a small scale level in potato dextrose broth in an Erlenmeyer flask to gain enough extract for the bioactivity screening. A total of 10 strains were isolated and identified on the basis of their morphological characteristics (Table5). Based on the results of enzyme production, all the fungal strains, Mucor sp., Alternaria sp., Aspergillus terreus, Aspergillus flavipes, and Aspergillus niger were identified and chosen for their biological activities using different bioassay systems, and extract of fungus(Figure5).

Antibacterial Activity Test in Fungi

In nutrient agar plate by using well cutter well is cut around the plate. The pathogenic bacteria such as Bacillus subtilis, Staphylococcus aerus and Salmonella paratyphi were swapped in the nutrient agar plates. Then the extract of fungus such as Aspergillus niger, Alternaria sp., Aspergillus flavipes, Mucor sp., Aspergillus terreus, Aspergillus flavus were inoculated in the petriplates. The zones were formed around the well. (Figure5.1)

Artimia Lethality Assay in Fungi

Aspergillus Niger and Aspergillus flavus are good in Artimia lethality assay in fungi.

16 sRNA Sequencing

The bacterial species was identified as rastonia species the PCR amplification profile of selected strain. The condition is about 1% electrophoresis (Figure 6). The genomic DNA of two bacterial isolates in figure (6.1)

5'TGCCCCACATGATAACGGTTCCCTT
GCAACTCTCACGCAGTGGGGGAG
CCTTGCTTCCCTGCCAGCGAGTGGCG
AACCGGTTATTAATCATCAGTAATGC
CCTGTAGTGGGGGATAACTAGTCGAA
AGATTAACTAATACCGCAAACACCTG
AGGGTGAAAGTGGGCGACCGCAGGC
CTCATGCTATATGAGCGGCGATGTCT
GATTACCTAGTTGGTGGGGTAAAGGC
CTACCAAGGCGACCATCAGTAGCTGG
TCTGACAGGACGATCAGCCACACTGG
GACTGACACACGGCCCACACTCCTAC
GGGAGGCAGCAGTGGGGAATTTTGG
ACATGGGCGAAACCCTGATCCAGCA
ATGACGCGTGTGTGAAGAAGGCCTTC
GGGTTGTAAAGCACTTTTGTCCGGAA
AGAAATGGCTCTGGTTAATACCCGGA
GTTGATGACGGTACCGGAAGAATAAC
GACCGGCTAACTCCGTGCCAGCAGCC
GCCGTAATACGTAGGGTCCAAGCGTT
AATCGGAATTACTGGGCATAAAGCGT
GCGCAGGCGGTTGTGC3'. Icl64817

Ralstonia sps M1 16 s ribosomal RNA partially sequenced PCR amplification profile of selected strain and genomic DNA. It has 1500 base pair sequence. Ralstonia maninitolylitica strain is found by sequencingand biochemical characterization.

In conclusion, Out of 10 strains, the best strains of *Azotobacter* and phosphate solubilizing bacteria were selected for their high beneficial activities. Further study was designed for characterization of the bacterial strains. Two selected strains were identifiedat species level based on their morphological, culture and biochemical characteristics. Further experiment was designed to test the utility of the identified strains as biofertilizer for rhizosphere soil.

Table.1 Cultural and biochemical characteristics of the selected bacterial strains

S.NO	Characteristics	AZO	PSB
1	Gram staining	Gram –negative	Gram-negative
2	Bacterial shape	Ovoid	Rods
Culture characteristics on agar slants			
3	Abundance of growth	Moderate	Moderate
4	optical characteristics	Transparent	Opaque
5	cultural characteristics on nutrient agar plates		
6	Size	Small	Small
7	Elevation	Convex	Flat
	Appearance on nutrient broth cultures	Sediment	Pellicle
8	Motility	Motile	Motile
Intra cellular enzyme			
9	**Carbohydrate Fermentation**		
10	Sucrose	-	-
11	Fructose	-	-
12	Maltose	-	-
13	Mannitol	-	-
14	Hydrogen sulphide production	Not tested	Not tested
15	Nitrate reduction	-	-
	IMViC test		
16	Indole production	-	-
17	Methyl red reaction	-	+
18	Voges proskauer Reaction	-	-
19	Citrate utilization	+	+
20	Urease activity	-	-
21	Catalase activity	+	+
22	Oxidase activity	+	+
Extra cellular enzyme			
23	Starch hydrolysis	+	+
24	Lipid hydrolysis	+	-
25	Gelatin hydrolysis	+	-

Table.2 Evaluation based on seed germination and root nodules in green gram and rice

S.NO	GREEN GRAM		RICE	
Strains	Seed germination (%)	Root nodules (mm)	Seed germination (%)	Root nodules (mm)
Control	80	30.25	40	27.15
Azo 1	100	28.8	20	23
Azo2	90	20.6	30	14.3
Azo3	80	34.75	60	14
Azo4	100	16	20	29
.Psb1	80	25.75	20	13.5
Psb2	80	34.25	60	12
Psb3	90	22.6	20	10.16
Psb4	80	30	-	-
Psb5	90	21.3	20	50
Psb6	80	36.25	50	37.8

Table.3 Growth measurement of green gram and rice

	Green gram		Rice	
Strains	Millimeter	Percentage	Millimeter	Percentage
Control	99	40	62.4	50
Azo 1	-	-	62.6	30
Azo 2	90	20	47.5	20
Azo 3	103.66	60	94.6	30
Azo 4	73.88	50	63	30
Psb 1	75	10	58.5	20
Psb 2	107.8	50	84	60
Psb 3	104.6	30	117	40
Psb 4	94.4	50	50	10
Psb 5	91.6	30	41	10
Psb 6	-	-	89.8	60

Table.4 Antibacterial activity test

S. No	Antibiotic discs	Phosphate Solubilizing Bacteria	Mm	*Azotobacter*	Mm
1	Erythromycin	Sensitive	13	Resistant	-
2	Chloramphenicol	Sensitive	15	Sensitive	8
3	Nalidiric acid	Sensitive	14	Sensitive	16

Table.5 Artimia lethality assay in fungi

S. No.	Strain code	Name of the Fungi
1	R-f-leaf 1	Aspergillus niger
2	R-f-stem 3	Aspergillus niger
3	A- stem 1	Alternaria sp.
4	A-f-leaf 3	Aspergillus flavipes
5	R- root 1	Mucor sp.
6	A-F-leaf 1	Aspergillus terreus
7	R-f-stem 2	Aspergillus niger
8	A-f-leaf 2	Aspergillus niger
9	A-f-root 1	Aspergillus flavus
10	R-f-stem 1	Aspergillus flavus

Figure 1: Pure culture of microorganisms from root soil inselected media(Pikovskay'sMedia)

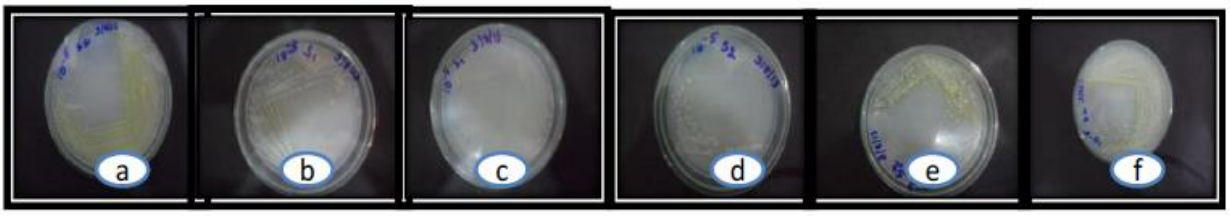

Figure 1.1: Pure culture of microorganisms from root soil in selected media (winogradsky's)

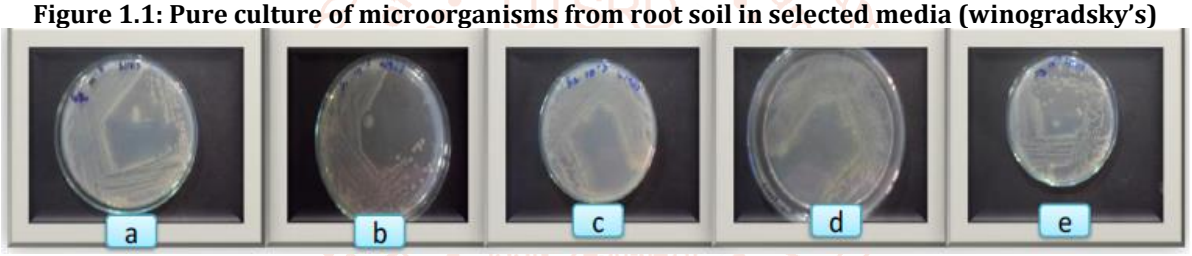

Figure 2: Seed Germination (Green Gram) Figure: 2.1 Seed Germination (Rice)

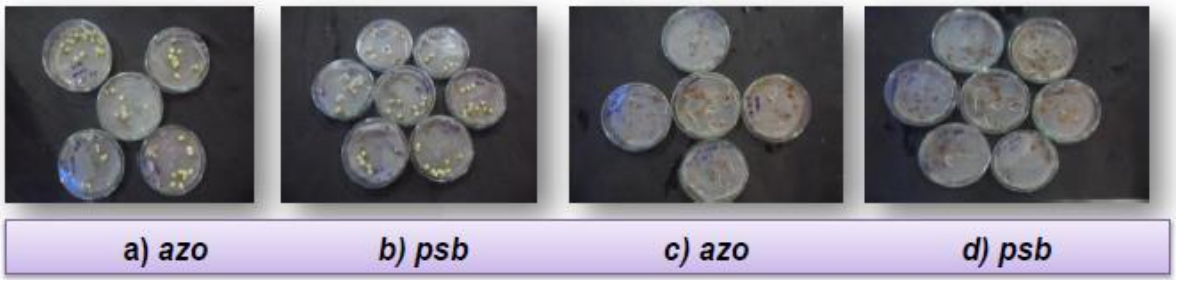

Figure: 3: Plant growth **Figure:3:1: Plant**

Figure 3.2:BrinjalFigure:3.3:Vermicompost Figure4:AntibacterialactivityFigure 5: Extract of fungus

Fig: 5.1: Activity test in fungi **Fig: 6:PCR** **Fig: 6:1: G-DNA**

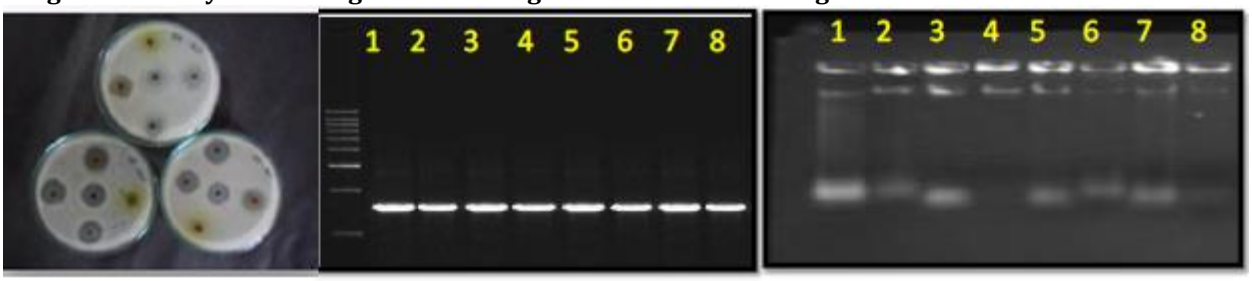

Fig 6: PCR amplification profile of selected strain **Conditions: 1% agarose gel electrophoresis Lane M: 1KBDNALadder;1:Sample)1KBDNALadder(bp):1000,2000,3000,4000,5000,6000,7000,8000,9000;*Fig 6:1: Genomic DNA of selected two bacterial Isolates.***

The bacterial strains especially were proved to be potent biofertilizers for raising green gram and rice. Further experiment was designed to study their probiotic potential in the fishes. The bacterial strains especially *ralstonia* beneficial effect on the fish culture tank, by enhancing the growth and production of fish. Mass culture was prepared. The mortality in % is determined after the formula (A-N-B) $Z^{-1}100$, with A = number of dead larva after 24 h; N = number of dead larva before the addition of test compound; B = number of dead larva in the negative control; Z = total number of larva. A mortality of *Artemia salina* at 10µg/mL is interpreted as high cytotoxic in bacteria and fungal extract diluted in DMSO.

The selected strains were bioassayed in seedling growth. The percentage of seed germination was measured; shoot length and root lengths were measured in rice and green gram. Antibacterial activity was carried out using disc diffusion method by Muller Hinton Agar (MHA) to find sensitive andresistant.Zone of inhibition was recorded in mm and the experiment was performed in duplicate. In bacteria after extraction with ethyl acetate the crude extract was tested for the *Artemia salina* toxicity assay, which is a simple, rapid and inexpensive bench top assay and considered as a useful tool for preliminary assessment of general toxicity. The best culture is selected from bacteria and fungi.

The mass culture of *Azotobacter* and phosphate solubilizing bacteria are used as biofertilizer in green gram, rice and probiotics of fish. The weight and length of probiotic fish is measured from initial stage, 1st week, 2nd week and 3rd week. The fungal strains are isolated from root, stem, and leaf. *Then the extract of fungus such as Aspergillus niger, Alternaria sp. Aspergillus flavipes, Mucor sp., Aspergillus terreus, Aspergillus flavus* were inoculated in the petriplates leaf. In fungi after extraction with ethyl acetate the crude extract was tested for the *Artemia salina* toxicity assay, which is a simple, rapidandinexpensive bench top assay and considered as a useful tool for preliminary assessment of generaltoxicity.

References

[1] Alexander, M. 1978. "Introduction to soil Microbiology" IInd E. Wiley Eastern Limited, Delhi, pp 467.

[2] Becking, J.H. 1971. "Biological nitrogen fixation and its economic significance". In: Nitrogen-15 in sil plants studies IAEA-Pl-341/14. Vienna, *Int. Atomic Energy Agency,* pp.189-222.

[3] Chandrasekaran, S. 1966. "Studies on the interrelationship between plants and soil microorganisms in respect to phosphorous solubilization". Ph.D. Thesis, Annamalai University, Parangipettai, India, pp. 190.

[4] Glick, B.R. 1995. "Enhancement of plant growth of free living bacteria". *Can. J. Microbial.,* No. 41, pp.109-117.

[5] Lee, K.D., Bai, Y., Smith, D., Han, H.S.,and Supanjani, S. 2005. "Isolation of plant growth promoting endophytic bacteria from bean nodule". *Res. J. Agric. Biol. Sci.*, No. 1(3): pp. 232- 236.

[6] Perez, E., Sulbaran, M., Bali, M.M. and Yarzabal, L.A. 2007. "Isolation and characterization of mineral phosphate- solubilizing bacteria naturally colonizing a limonitic crust in the southeastern Venezuelan region. *Soil Biol. Biochem.,* No. 39, pp.2905-2914.

[7] Shende, S.T., Apte, R.G., Singh. 1977. "Influence of *Azotobacter* on germination of rice and cottonseeds". *Curr. Sci.,* No. 46(19):pp.675-676.

[8] Subba Rao. N.S. 1997. Biofertilizers in agriculture and forestry. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 242.

[9] Subba, R.N.S. 1999. *Soil Microbiol.,* Vol 4, USA: Science Publishers Inc.

[10] Vessey, J.K. 2003. "Plant growth promoting rhizobacteria as biofertilizers". *Plant Soil,* No.255, pp. 571–586.

[11] Zahran, H.H. 1999. "Rhizobium-legume symbiosis and Nitrogen-fixation under severe conditions and in an arid climate". *Microbiol. Mol. Biol. Rev.,* pp. 968-989.

[12] Zaidi, A., Mohammad, S. 2006. "Co- inoculation effects of phosphate solubilizing micro-organisms and glomus fasciculatum on green grambrady rhizobium symbiosis", *Agri. Sci.,* No. 30, pp.223-230.