Journal of Phytology 2011, 3(11): 13-18
ISSN: 2075-6240
Available Online: http://journal-phytology.com/

JOURNAL OF PHYTOLOGY

Growth Response of *Ayapana* on inoculation with *Bacillus megaterium* isolated from different soil types of various agroclimatic zones of Karnataka

Sandeep. C*, Thejas M S, Sanjeev Patra, Tejaswini Gowda, R Venkat Raman, Radhika M, Suresh C. K and S.R. Mulla

Department of Biotechnology, University of Agricultural Sciences, GKVK Campus, Bangalore -560065.

Abstract

A study was undertaken to find out the growth response of *Ayapana* on inoculation with *Bacillus megaterium* isolated from different soil types of various agroclimatic zones of Karnataka. *Bacillus megaterium* strains from different soil types of various agroclimatic zones of Karnataka were isolated, identified and confirmed using standard synaptic keys. *Ayapana* was used as a host plant to study the growth response, biomass and nutrient content. Treatments of *Bacillus megaterium* isolates from ten different soil types of various agroclimatic zones of Karnataka were given to seedlings of *Ayapana*. There were significant changes in the plant growth, biomass and nutrient uptake in plants inoculated with *Bacillus megaterium* when compared to control plants. The parameters such as plant height, number of leaves, shoot and root fresh weight, shoot and root dry weight and nutrient uptake were studied. Plants inoculated with *Bacillus megaterium* isolates performed well when compared to uninoculated plants. The heights of plants inoculated with *Bacillus megaterium* isolates were found to be more than uninoculated plants. In plants inoculated with *B. megaterium isolates,* the height, number of leaves, fresh and dry weight of roots and shoots, nitrogen content, P content and chlorophyll content remained higher than the uninoculated plants. Among ten isolates inoculated, Zone 7 isolate recorded significantly high values in almost all growth parameters chosen for the study. The results suggests that plants inoculated with *Bacillus megaterium* isolates showed better growth response, biomass yield and nutrient content when compared to uninoculated plants.

Keywords: *Bacillus megaterium*, *Ayapana*, growth response, nitrogen content and chlorophyll content.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are the soil bacteria that colonize the roots of plants following inoculation onto seed and also enhance plant growth [1]. Phosphorus is one of the 17 chemical elements required for plant growth and reproduction and is often referred to as the “energizer” since it helps store and transfer energy during photosynthesis. It is applied to the soil in the form of phosphatic fertilizers.

Approximately 95–99% of the soil phosphorus is present in the form of insoluble phosphates and hence cannot be utilized by the plants. To increase the availability of phosphorus for plants, large amounts of fertilizer are used on a regular basis [2]. Soils having high pH have the problem of phosphorous availability for plants. In such a situation phosphate solubilizing microorganisms can be useful to reverse the process. Plant growth promoting rhizobacteria (PGPR) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms [3]. Microorganisms are involved in a range of processes that affect the transformation of soil phosphorous and thus are integral part of the soil ‘P’ cycle. In particular soil microorganisms are effective in releasing phosphorous from inorganic and organic pools of total soil phosphorous through solubilization and mineralization [4].

Phosphorous solubilizing microorganisms are ubiquitous in soils and could play an important role in supplying P to the plants, where plant available P content in soil is less. Use of Phosphate Solubilizing Bacteria (PSBs) as bioinoculants will increase the available P in soil, helps to minimize the P-fertilizer application, reduces environmental pollution and promotes sustainable agriculture. PSM is a group of heterotrophic microorganism capable of solubilizing the inorganic P from insoluble sources. Application of Phosphate Solubilizing Microorganism (PSMs) in the field has been reported to increase crop yield. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSB [5]. Phosphate Solubilizing Bacteria are useful for all the crops i.e. Cereals, cash crops, leguminous crops, horticultural crops and vegetables etc.

Karnataka state has different soil type’s viz., red soil, black soil, sandy soil, laterite soil and alluvial soil, and is divided into ten agro climatic zones on the basis of annual rainfall, soil type, cropping pattern and other climatic conditions. The geographical area of Karnataka is classified into ten agro-climatic zones *viz.,* North eastern transition zone, North eastern dry zone, Northern dry zone, Central dry zone, Eastern dry zone, Southern dry zone, Southern transition zone, Northern transition zone, Hilly zone and Coastal zone.

Bacillus Megaterium is a Gram Positive, Rod Shaped Endospore-Forming Bacteria. It is a P solubilizing bacteria which has got PGPR activity also. This plant growth promoting rhizobacteria (PGPR) has got the capability of solubilising the insoluble phosphate

Received: Aug 18, 2011; Revised: Oct 15, 2011; Accepted: Nov 20, 2011.

*Corresponding Author

Sandeep. C
Department of Biotechnology, University of Agricultural Sciences,
GKVK Campus, Bangalore -560065.

Tel: +91-9886778079; Fax: +91-803330277
Email: sandeep.c.naidu@gmail.com

in the soil and make them available to the plants.

Ayapana is an ornamental perennial herb with aromatic leaves that grows 20 to 30 cm high. Reduces nausea, stops bleeding, antiseptic cleanses blood, calms coughs reduces fever, protects liver promotes sweating, prevents ulcers thins blood, kills cancer cells, heals wounds. *Ayapana* is in the large Asteraceae plant family (which is also called the sunflower or daisy family). The Asteraceae is the second largest family in its division with some 1,100 genera and over 20,000 recognized species. Two common and well known North American medicinal plant species in the family are boneset (*Eupatorium perfoliatum*) and Joe-Pye-weed *(Eupatorium purpureum*). *Ayapana* is a rich source of naturally occurring coumarin chemicals. Coumarins are chemical compounds found in many plants and which usually have a sweet scent—much like newly-mown hay. Coumarin has clinical value as the precursor for several anticoagulant drugs; most notably,one widely prescribed drug called *warfarin*. Two of ayapana's coumarin chemicals are called *ayapanin* and *ayapin* which were first discovered in the late 1930s.

In the present study isolation and identification of *Bacillus Megaterium* from different soil types of various agroclimatic zones of Karnataka was carried out. Growth response, biomass and nutrient content of *Ayapana* inoculated with *Bacillus Megaterium* isolated from different soil types of various agroclimatic zones of Karnataka was studied under green house conditions.

MATERIALS AND METHODS

The experiments to study "Growth Response of *Ayapana* on inoculation with *Bacillus megaterium* isolated from different soil types of various agroclimatic zones of Karnataka" were conducted at the Department of Plant Biotechnology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore, Karnataka, India. The material used and methods followed are described below.

Collection of soil samples from different agro climatic zones of Karnataka

Karnataka state is divided into ten agro climatic zones on the basis of annual rainfall, soil type, cropping pattern and other climatic conditions.

Soil sampling

Four soil samples of 500 grams each were collected randomly from top six-inch layer of soil from each agro climatic zone and packed in polyethylene bag. They were transferred to Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, for further studies.

Processing of soil samples

The soil samples collected from each zone were dried inside the laboratory at 28^{o}C. Four soil samples collected from each zone were mixed well to get a pooled soil sample for a zone. Totally ten soil samples was obtained for the study. Each soil sample was sieved through 1000µ mesh to remove the bigger soil particles and debris. The sieved soil samples were used for the spore isolation.

Isolation of *Bacillus megaterium* from different zones

Bacillus megaterium was isolated from the soils collected from different zones, by growing in glucose mineral agar media. For isolation, cell material was checked microscopically for the presence of typical cells of Bacillus megaterium and purified on nutrient agar. *Bacillus megaterium* was isolated by heating the soil sample to kill non spore forming mesophiles, then the dilutions were made upto 10^{-3} and 10^{-4}. It was then plated on glucose mineral base agar medium. It was incubated at 30^{0}C for 2 days. Then all the isolates were subjected to various tests for confirming their identity.

Identification of *Bacillus megaterium*

The different tests for identification were carried as per Bergy's manual of Determinative Bacteriology.

Colony morphology and microscopic examination

All the check isolates and standard strains formed completely white, round, smooth and shiny colonies. During microscopic examination all the isolates were found to be gram positive rods. Presence of endospores was confirmed by endospore staining.

Physiological tests for *Bacillus megaterium*

All the physiological tests mentioned were conducted in duplicate for each isolate.

Gelatin liquifaction

Gelatin liquefaction test was performed according to the method described by Blazevic and Ederer [6]. Petriplates containing gelatin agar were spotted with overnight grown bacterial culture at 30^{0}C and incubated for 3 days. The plates were then flooded with 12% $HgCl_2$ solution and allowed to stand for 20 minutes and observed for clear zones around the growth of bacterium to indicate gelatin liquefaction.

Hydrolysis of starch

Hydrolysis of starch was performed according to the method described by Eekford [7]. Starch agar was prepared by spending 1gm of starch powder in 10 ml cold distilled water mixed with 90 ml of nutrient agar and autoclaved at 121^{0}C for 20 minutes. Petriplates containing starch agar were inoculated with test cultures and incubated at 30^{0}C for 3 days. After incubation the plates were flooded with Lugol's iodine, allowed to stand for 15 – 30 minutes and observed for clear zones around the colony to indicate hydrolysis of starch.

Casein hydrolysis

Casein hydrolysis was performed according to the method described by Seely and Vandemark [8]. Petriplates of skim milk agar was streaked with test cultures and incubated at 30^{0}C for observing clear zone against black background.

Acid gas production

Acid gas production test was performed according to the method described by Seely and Vandemark [9]. Bacterial isolates were tested for acid and gas production by inoculating 5 ml of the sterile glucose broth with bromocresol purple (15 ml /l of 0.04% solution as pH indicator) in test tubes containing Durham's tube. The tubes were incubated for seven days at 30^0C. Accumulation of gas in these Durham's tube was taken positive for gas production and change in colour of the medium to yellow was taken as positive for acid production.

Catalase test

Catalase test was performed according to the method described by Blazevic and Ederer [10]. Nutrient slants were incubated at 30^0C for 24 hrs. After incubation these tubes were flooded with 1ml of 3% H_2O_2 and observed for gas bubbles. Occurrence of gas bubbles was scored positive for catalase test.

V-P Reaction

Sterilized V-P broth was dispensed in test tubes tested for acetyl methyl carbinol production after incubation for 3,5,7 days by mixing 3 ml of 40%(w/v) sodium hydroxide with cultures and adding 0.5 mg of creatnine. The tubes were observed for the production of red colour after 30 minutes at room temperature.

Solubilization of insoluble phosphate by *Bacillus megaterium* in Sperber's medium

The overnight cultures of the *Bacillus megaterium* isolates were spotted on sperber's medium to observe the zone of solubilization by the isolates. The plates were incubated at 30^0C for 36 hrs, observed and measured the zone of solubilization produced by these isolates.

Inoculum preparation

The isolated colonies of *Bacillus megaterium* maintained on the Nutrient agar slants was inoculated in 250 ml conical flask containing 100 ml Nutrient broth and incubated at 30^0C under shaking at 100 rpm for six days. The grown cultures were homogenized and 15ml each culture ($15.2x10^8$cfu/ml) inoculated to each pot.

Pot Experiment

Bacillus megaterium isolates were grown separately in a 250 ml flask containing 100ml nutrient agar for 2 days. The grown cultures were homogenized and 15 ml of each of the solution was given to each pot. The geographical area of Karnataka is classified into ten agro-climatic zones *viz.,* North eastern transition zone (NETA-1), North eastern dry zone (NEDA-1), Northern dry zone (NDA-1), Central dry zone (CDA-1), Eastern dry zone (EDA-1), Southern dry zone (SDA-1), Southern transition zone (STA-1), Northern transition zone (NTA-1), Hilly zone (HA-1) and Coastal zone (CA-1). C - Control (uninoculated plant), T_1– *Bacillus megaterium* isolate from Zone 1, T_2 - *Bacillus megaterium* isolate from Zone 2, T_3 - *Bacillus megaterium* isolate from Zone 3, T_4 - *Bacillus megaterium* isolate from Zone 4, T_5 - *Bacillus megaterium* isolate from Zone 5, T_6 - *Bacillus megaterium* isolate from Zone 6, T_7 - *Bacillus megaterium* isolate from Zone 7, T_8 - *Bacillus megaterium* isolate from Zone 8, T_9- *Bacillus megaterium* isolate from Zone 9, T_{10}- *Bacillus megaterium* isolate from Zone 10.

Effect of *Bacillus megaterium* on growth of *Ayapana*

The main objective behind this experiment was to study the response of *Ayapana* to inoculation with different *Bacillius megaterium* isolates.

Plant growth parameters

The observations with respect to the growth parameters including plant height, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, nitrogen, phosphorus and chlorophyll content were recorded at different periodical intervals till vegetative stage. However the data presented here is 60 days after treatement (DAT).

The plant height was measured from the soil surface to the tip of the growing point at 60 DAT. The numbers of fully opened leaves were recorded at 60 DAT. The harvested plants were weighed and then the root fresh weight was recorded and expressed as grams per plant. The harvested roots were dried in an oven at 60^0C for 2 days to attain constant weight and then the root dry weight was recorded and expressed as grams per plant. The harvested plants were weighed and then the shoot fresh weight was recorded and expressed as grams per plant. The harvested plants were dried in an oven at 60^0C for 4 days to attain constant weight and then the dry weight was recorded and expressed as grams per plant.

Biochemical studies of plants inoculated with *Bacillus megaterium*

The nitrogen estimation for root and shoot was carried out by Micro–Kjeldahl method [11]. Plant phosphorus concentration was estimated colorimetrically following the vanadomolybdate yellow colour method [12]. Total chlorophyll content of the leaf was estimated following DMSO method [13]. The data obtained from the experiments were subjected to one-way analysis of variance for completely randomized design (CRD) using MSTAT-C software. The treatment means were separated by Duncan's Multiple Range test (DMRT) a 5% level of significance [14].

RESULTS AND DISCUSSION

The experiments were conducted at the Department of Plant Biotechnology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore, Karnataka, India. The results obtained are presented below.

Isolation and identification of Bacillus megaterium

The bacterial cultures were isolated from ten agro climatic zones of Karnataka. The cell material was checked microscopically for the presence of typical cells of *Bacillus megaterium* and purified on nutrient agar plates. Then all the isolates were subjected to various tests for confirming their identity.

Colony morphology and microscopic examination

All the check isolates and standard strains formed completely white, round, smooth and shiny colonies. During microscopic observation all the isolates were found to be gram positive rods. Presence of endospores was confirmed by endospore staining. Then all the isolates were subjected to various tests for confirming their identity [15].

Physiological tests

All the physiological tests performed are presented in the table 1.

Table 1. Physiological Tests for *Bacillus megaterium* isolates

Zones	VP Test	Anaerobic Growth	Acid from Glucose	Gas from glucose	Hydrolysis of casein	Hydrolysis of starch	Hydrolysis of gelatin	Production of solubilising zones in sperbers media
C	-	-	+	-	-	-	+	+
C	-	-	+	-	-	-	+	+
T_1	-	-	+	-	-	-	+	+
T_2	-	-	+	-	-	-	+	+
T_3	-	-	+	-	-	-	+	+
T_4	-	-	+	-	-	-	+	+
T_5	-	-	+	-	-	-	+	+
T_6	-	-	+	-	-	-	+	+
T_7	-	-	+	-	-	-	+	+
T_8	-	-	+	-	-	-	+	+
T_9	-	-	+	-	-	-	+	+
T_{10}	-	-	+	-	-	-	+	+

+ = Positive and **-** = Negative, T_1 to T_{10}: Treatments for isolates from zone 1 to 10

Phosphate solubilising efficiency of different isolates of Bacillus megaterium

Phosphate solubilizing microorganisms are ubiquitous in nature, their number vary with type of soil, climate, vegetation etc [16]. In the present investigation an attempt has been made to isolate *Bacillus megaterium,* a phosphate solubilizer and also a PGPR, from different soil types present in various agroclimatic soils of Karnataka. The efficiency of P solubilization by *Bacillus magaterium* was studied in green house experiment using all *Bacillus megaterium* isolates to make a preliminary selection of efficient isolate using *Ayapana* as indicator plant and the strains were designated as better based on the zone of solubilization. Good P solubilizing zone is an indication of utilization of insoluble Phosphorus and also PGPR activity thereby improving the plant growth.

The Phosphate solubilising efficiency of different isolates of *Bacillius megaterium* was tested on modified Sperber's medium. All the isolates found to have good solubilising ability. Zone 7, Zone 3 and Zone 4 showed very good solubilising ability in Sperber's media. A study conducted by Rodrguez and co-workers showed that the mechanism of P solubilization is due to production of several organic acids who isolated and enumerated P solubilizers based on P solubilizing zone with different sources of insoluble Phosphorous [17].

Response of *Ayappana* to inoculation of *Bacillus megaterium* isolates

The main objective behind this experiment was to study the response of *Ayappana* to inoculation with different *Bacillius megaterium* isolates. Sand: soil mixture in the ratio of 1:1 v/v was filled into pots of uniform size. Planting holes were made at the centre of the pots to enable the inoculation of *Bacillius megaterium* isolates and 15 ml inoculum representing each zone *Bacillius megaterium* isolate was separately added to the pot as per the treatment allocation. The plant height, number of leaves and number of branches is presented in table 2.

Screening studies were conducted using *Ayapana* grown in pots under green house conditions. Studies revealed that in general the plants inoculated with *Bacillus megaterium* isolates showed higher growth when compared to uninoculated control. Zopade [18] reported that increase in plant growth by inoculation with biofertilizers might be due to micro-element and plant growth regulator content in the biofertilizers. The height of the inoculated plants remained always greater than the control. However the heights differed significantly among the plants inoculated with various isolates. The least plant height was recorded in control. The *Ayapana* at 60th DAT (Days after treatment) the maximum plant height (32.5 cm) was recorded in zone-7 isolate treated plants and the lowest plant height (14.5 cm) in *Ayapana* was attributed to control. At 60th DAT the maximum number of leaves (42/plant) was recorded in zone-7 isolate treated plants and the lowest number of leaves (21/plant) in *Ayapana* was recorded in control. The *Ayapana* at 60th DAT the maximum number of branches (7/plant) was recorded in zone-8 isolate treated plants and the lowest number of branches (2/plant) in *Ayapana* was noticed control. Similar results were obtained when combined inoculation of Rhizobium, a phosphate solubilizing *Bacillus megaterium sub sp. phospaticum* strain-PB and a biocontrol fungus *Trichoderma spp*. showed increased germination, nutrient uptake, plant height, number of branches, nodulation, pea yield, and total biomass of chickpea compared to either individual inoculations or in uninoculated control in chickpea as per the studies conducted by Rudresh and co-workers [19].

The fresh weight and dry weight of the plants harvested at 60 days after treatment are presented in table 2. The total fresh weight and dry weight of the plants inoculated with *Bacillus megaterium* isolates were higher than control. The total biomass per plant was significantly influenced by the application of *Bacillus megaterium* isolates in *Ayapana.* The shoot and root fresh weight and dry weight are presented in table 2. In *Ayapana*, among different treatments, the zone 7 isolate treatment recorded maximum total biomass. The highest total fresh weight (14.58g/plant) and dry weight (4.35 g/plant) was recorded in zone 7 isolate and the lowest total biomass was noticed in control. The lowest fresh weight (7.57g/plant) and dry

weight (1.40 g/plant fresh) was recorded in uninoculated control plants. Similar results were obtained by Bashrath Ali and co-workers on inoculations with *Bacillus spp.* which increased shoot fresh weight of *vigna radiata* [20].

Table 2 - Growth parameters of *Ayapana* influenced by *Bacillus megaterium* isolates

Zones	Plant height(cm) 60 DAT	Number of Leaves/plant 60 DAT	Number of Branches (cm) 60 DAT	Fresh weight (g/plant) 60 DAT			Dry weight (g/plant) 60 DAT		
				Shoot	Root	Total	Shoot	Root	Total
C	14.5	21	2	4.56	3.01	7.57	0.80	0.60	1.40
T_1	22.68	30	4	6.91	5.12	12.03	1.12	0.98	2.10
T_2	29.72	29	4	7.21	5.90	13.11	1.24	0.91	2.15
T_3	24.0	31	4	6.98	5.77	12.75	1.22	0.89	2.11
T_4	28.11	27	5	7.11	6.0	13.11	1.98	1.12	3.10
T_5	28.50	33	5	7.60	6.45	14.05	2.12	1.02	3.14
T_6	29.90	33	4	6.99	5.11	12.10	1.10	0.90	2.0
T_7	32.5	42	6	7.92	6.66	14.58	2.23	2.12	4.35
T_8	31.2	39	7	7.11	5.88	12.99	0.98	0.77	1.75
T_9	29.55	30	4	7.05	5.81	12.86	1.0	0.88	1.88
T_{10}	20.78	28	3	6.0	4.5	10.5	0.91	0.69	1.60
SEM±	0.158	1.194	0.229	0.185	0.150	0.091	0.119	0.146	0.387
CD at 5%	0.466	3.523	0.677	0.546	0.444	0.269	0.353	0.430	1.143

DAT: Days after treatment , T_1 to T_{10}: Treatments for isolates from zone 1 to 10

Biochemical studies of *Ayapana* plants inoculated with *Bacillus megaterium* isolates

The total phosphorus content in *Ayapana* is presented in table 3. In *Ayapana*, total phosphorous content of the plants inoculated with *Bacillus megaterium* isolates differed significantly among various isolates. The maximum P content (5.68 mg/plant dry wt) was observed in the plant inoculated with isolate of zone 7 while minimum phosphorous content (2.22 mg/plant dry wt) was observed in the control. Similar report of increasing P uptake and dry weight of plants through inoculation of phosphate solubilizing organisms was made by Gerestson [21].

Table 3 . Biochemical parameters of *Ayapana* influenced by *Bacillus megaterium* isolates

Zones	*Ayapana*	
	Total Nitrogen Content (mg/plant dry wt)	Total Phosphorous Content (mg/plant dry wt)
C	20.12	2.22
T_1	22.41	3.22
T_2	27.90	4.59
T_3	23.03	3.89
T_4	28.45	4.66
T_5	28.10	4.77
T_6	28.56	4.98
T_7	30.71	5.68
T_8	29.90	5.50
T_9	28.80	5.23
T_{10}	22.34	3.10
SEM±	0.234	0.070
CD at 5%	0.691	0.208

T_1 to T_{10}: Treatments for isolates from zone 1 to 10

However the total nitrogen content in *Ayapana* differed significantly among the plants inoculated with various *B. megaterium* isolates which is presented in table 3. In *Ayapana*, plants inoculated with zone 7 isolate recorded the highest nitrogen content (30.71 mg/plant dry wt). The lowest nitrogen content (20.12 mg/plant dry wt) was noticed in uninoculated control when compared to all other treatments. A similar result was observed by Hesham, when wheat inoculated with mixed inocula of *B. megaterium* and *A. lipoferum* exhibited high shoot dry weight, total nitrogen (N) yield and the shoot phosphorus content increased by 37 and 53 % compared to the plants inoculated with *A. lipoferum* and uninoculated ones, used as control, respectively [22].

However the chlorophyll content in *Ayapana* differed significantly among the plants inoculated with various *B. megaterium* isolates which is presented in table 4. In *Ayapana*, highest total content of chlorophyll was recorded in plants inoculated with zone 7 (1.70 mg/g fw) followed by zone 8(1.65 mg/g fw) and the lowest total chlorophyll content (0.97 mg/g fw) was recorded in control plants.

Results of the present study revealed enhanced plant growth, biomass and nutrient content of *Ayapana* due to inoculation with *Bacillus megaterium* strains isolated from different agro-climatic zones of Karnataka and the zone 7 isolate was found to be the most efficient strain compared to other strains in promoting plant growth, biomass and nutrient uptake.

Table 4. Total Chlorophyll Content of *Ayapana* influenced by *Bacillus megaterium* isolates

Zones	Total Chlorophyll (mg/g fw) of ayapana
C	0.97
T_1	1.31
T_2	1.33
T_3	1.15
T_4	1.31
T_5	1.48
T_6	1.60
T_7	1.70
T_8	1.65
T_9	1.51
T_{10}	1.30
SEM±	0.040
CD at 5%	0.120

T_1 to T_{10}: Treatments for isolates from zone 1 to 10

REFERENCES

[1] Kloepper, J. W and Scroth, M.N. 1978. Plant growth promoting rhizobacteria on radishes. Proceeding of the Fourth International conference on plant pathogenic bacteria. Vol.2. station de pathologie vegetale et phytobacteriologie. INRA. Ed. Angers. *Gilbert-Clarey. Tours* pp. 879-882.

[2] Bryan, H. and Jason, E. 2005. Phosphorous availability with alkaline or calcareous soils. *Western Nutrient Management Conference.* 6:88-93.

[3] Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can. J.Microbiol.*41:533-536.

[4] Hilda Rodríguez. and Reynaldo Fraga. 2007. Phosphate solubilizing bacteria and their role in plant growth promotion in *Arabidopsis thaliana*. Vol. 20. No. 2. 207–217

[5] Rodriguez., Fraga., Gonzalez, T. and Bashan,Y. 2006. Genetics of phosphate solubilisation and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil. J.*287:15-21.

[6] Blazevic, D.S. and Ederer, G.M. 1975. *Principles to diagnostic microbiology.* Weeley and Company. Newyork. pp 13-45.

[7] Eekford, M.O. 1927. Thermophilic bacteria in soils. *Am. J. hygiene.* 7:200-201.

[8] Seeley, H.W. and Vandemark, P.S. 1970. Microbes in action; A laboratory manual of microbiology. D.B. *Taraporevale Sons and Company Limited.* Bombay.pp:86-95.

[9] Seeley, H.W. and Vandemark, P.S. 1970. Microbes in action; A laboratory manual of microbiology. D.B. Tarapogevale *Sons and Company Limited.* Bombay.pp:86-95.

[10] Blazevic, D.S. and Ederer, G.M. 1975. Principles to diagnostic microbiology. *Weeley and Company., Newyork*. pp 13-45.

[11] A.O.A.C. 1980. Official methods of analysis. 13th Edn. Washington D.C. *The association of official analytical chemists.* 61:972-978.

[12] Jackson, M. L. 1973. *Soil Chemical Analysis. Pretice Hall of India, New Delhi.*

[13] J.D Hiscox, Israelstam.1979.A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57: 1332-1334.

[14] T.M. Little, and F. J. Hills. 1978. Agricultural experimentation. *John Wiley and sons. Inc.,* USA.

[15] N.R. Krieg, J.G. Holt. 1984-1989. Bergey's manual of systematic bacteriology. *Williams & Wilkins, Baltimore and London*.

[16] Kim, H. S. and Smithies, O. 1988. Recombinant fragment assay for gene targeting based on the polymerase chain reaction. *Nucl. Acids Res.* 16: 8887-8903.

[17] Rodriguez., Fraga., Gonzalez, T. and Bashan,Y. 2006. Genetics of phosphate solubilisation and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil. J.*287:15-21.

[18] Zopade., S.T. 2001. Sea weeds as a biofertilizer. *J.Sci. Indust.Res.,* 60:378-382.

[19] Rudresh, D.L., Shivaprakash, M.K., Prasad, R.D. 2005. Effect of a combined inoculation of rhizobium, a phosphate solubilizing bacterium and trichoderma spp. on growth, nutrient uptake and yield of chickpea. *Applied soil ecology .* 28(2):139-146.

[20] Basharat Ali Æ Anjum Nasim Sabri Æ Karin Ljung Æ Shahida Hasnain. 2009. Quantification of indole-3-acetic acid from plant associated bacillus spp. and their phytostimulatory effecton vigna radiata (l.). *World journal of microbiology & biotechnology,* 25: 519-526.

[21] Gerestson, T. C., The influence of microorganisms in phosphorous uptake by plant. *Plant Soil*, 1948; **1:** 51-61.

[22] Hesham M. A. EL-Komy. 2005. Coimmobilization of *azospirillum lipoferum* and *bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. *Food technol. biotechnol.* 43 (1): 19–27.