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SCREENING OF INDOLE ACETIC ACID PRODUCTION BY PHOSPHATE SOLUBILIZING BACTERIUM

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Abstract: Microorganisms are integral in the natural phosphorus cycle. Recently, phosphate solubilizing microorganisms (PSMs) have attracted the attention of agriculturists because it provides an ecofriendly and economically sound approach to overcome the P scarcity and its subsequent uptake by plants. This study has been undertaken for the isolation, screening and identification of phosphate solubilizing bacteria (PSB) from rhizosphere soil samples. The strain which showed maximum zone of phosphate solubilization is considered as the most poteintial one and chosen for further studies .The isolated PSB was identified by using morphological and biochemical characteristics up to genus level. The present investigation also dealt with growth study, IAA production. It was also found that, this isolate was able to grow well in pH range between 4.5– 6.8.

Index Terms **- PSB, IAA, Biofertilizers, PSMs, Microorganisms, rhizospere soil.**

1. INTRODUCTION

The maintenance of high level soil phosphorus has been a major challenge to agricultural scientists, ecologists and farm managers because in most of the soils, phosphate is present in unavailable form due to complex formation with Ca^{2+}, Al^{3+}, Fe^{2+} or Mn^{2+} depending on soil pH and organic matter (Kuhad *et al* ., 2011). Because the availability of phosphorus to plants is restricted by various factors, it seems reasonable to study microorganisms that are able to solubilize phosphate from soil and promote its uptake by plants (Jana *et al*., 2001). During the last 10 years knowledge on phosphate solubilizing microorganisms increased significantly (Richardson *et al*, 2001; Rodriguez., 1999). Plant growth promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Glick, 1995), and Phosphate solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant Phosphorus nutrition.

Phosphate Solubilizing Bacteria (PSB) are being used as bio fertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1961). The growth of phosphate-solubilizing bacteria (PSB) often causes soil acidification, playing a key role in phosphorus solubilization (Alla, 1994). Therefore, PSB are considered the important solubilizers of insoluble inorganic phosphate. In turn, plants reimburse PSB with carbohydrates (Goldstein, 1995). Since the beginning of last century, many PSB have been isolated including, for example, those in *Bacillus*, *Pseudomonas*, *Erwinia*, *Agrobacterium*,*Serratia*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Azotobacter*, *Bradyrhizobium*, *Salmonella*, *Alcaligenes*, *Chromobacterium*, *Arthrobacter*, *Streptomyces*, *Thiobacillus*, and *Escherichia* and include some fungi in genus *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium*, and *Sclerotium* (Zhao and Lin, 2001).

Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch, 1985; Franken and Brunner, 1983). Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolites because of the rich supplies of substrates exuded from the roots compared with non rhizospheric soils (Kampert *et al*., 1975; Strzelczyk and Pokojska-Burdziej, 1984). Diverse soil microorganisms including bacteria (Muller *et al*., 1989), fungi (Stein *et al*., 1985) and algae (Finnie and Van, 1985) are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment. Production of IAA by microbial isolate varies greatly among different species and strains and depends on the availability of substrate(s)

Though PSMs have been a subject of research for decades, manipulation of PSMs for making use of increasing fixed P in the soil and improving crop production at the field level has not yet been adequately commercialized. Now a day's research has been focused on isolating different phosphate solubilizing microorganisms (PSM) and using them as biofertilizers as well. Chen *et al*., 2021 isolated seven strains of phosphorus-solubilizing bacteria belong to the genus *Pseudomonas, Pantoea, Enterobacter, Paraburkholderia, Novosphingobium, Ochrobactrum* from the roots, stems, and leaves of Chinese fir and used to study their growth-promoting characteristics, such as their ability to fix nitrogen, produce IAA, and secrete siderophores. The objective of the present study was to isolate and identify phosphate solubilizing bacteria from rhizosphere soil with the ability to effectively solubilize phosphate for plant utilization and screening for the production of phytohormones (IAA).

2. MATERIALS AND METHODS

For the present study rhizospheric soil samples of Yam were collected from the local markets in and around Chromepet in sterilized polythene bags and stored in the refrigerator at 4°C. The soils samples were air dried and used for the isolation and identification of PSB.

2.1 Isolation of Phosphate Solubilizing Bacteria

One gram of soil from each sample was taken and isolation of PSB was carried out by serial dilution and pour plate method on petri plates containing Pikovskaya's (PKV) medium, supplemented with tricalcium phosphate as insoluble inorganic phosphate source, and incubated at 37 ± 2° C for 5days. Colonies showing clear zone of phosphate solubilization were counted as PSB (Gyaneshwar *et al*., 1999). To detect the PSB, isolated strains were streaked onto PKV agar medium. After three days of incubation at room temperature, strains that produced clear halo zone around the colonies were considered as positive. These colonies were selected, purified, sub-cultured and stored on the slants of PKV agar for further studies.

2.2 Identification of the microorganism

The isolated bacterium was identified by the following routine microbiological and biochemical tests using the key provided by Bergy's Manual of Determinative Bacteriology (Holt *et al*., 1994; Krieg and Garrity, 2001).

2.3 Growth study

The isolated bacterium was inoculated in flasks containing 50 ml of PKV broth in a rotary shaker at 120 rpm for 5 days at 37 ± 2° C. The culture was harvested at every 12 h intervals and the absorbance of the culture was read at 660 nm using Elico (SL 159) UV – VIS Spectrophotometer.

2.4 Phosphorus solubilization, pH and titrable acidity

The isolated bacterium was evaluated for the ability to solubilize Tricalcium phosphate in PKV medium qualitatively and quantitatively. Qualitatively the plates were incubated at 37 ± 2° C for 5 days and observed regularly for solubilization zone. The Phosphate Solubilization Efficiency (PSE) was identified by measuring the total halo zone of the colony and the colony diameter (Premono *et al*., 1996).

$$PSE = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

To quantify the soluble P and organic acids present in culture media, 100 ml of Pikovskaya's broth containing a single colony of PSB was incubated in a 250 ml Erlenmeyer flask at 37 ± 2° C for 5 days in a shaker at 180 rpm. After every 24 h of growth, 20 ml of each culture was harvested and centrifuged at 10000 rpm for 10 min to obtain cell-free supernatants. The supernatant was analyzed for inorganic P (Phosphorus) content by the method of Olsen *et al.* (1954). To 1ml of culture, 10 ml of distilled water was added and shaken well. Then 5 ml of freshly prepared ascorbic acid and ammonium molybdate solution were added and made up to 25 ml. The Absorbance was measured at 882 nm after half an hour. The available phosphorous (P) was determined using spectrophotometer at 882nm after half an hour and calibrated with standard KH_2PO_4 curve.

A change in the PH of the medium due to the growth of PSB was measured with a pH meter. In order to study the titrable acidity, 5 ml of supernatant was added with a few drops of phenolphthalein indicator and titrated against 0.01N NaOH. The titrable acidity was expressed as ml of 0.01N NaOH consumed per 5 ml of culture filtrate (Ponmurugan and Gopi, 2006).

2.5 IN VITRO SCREENING OF PSB FOR IAA PRODUCTION

The PSB isolate was inoculated in PKV broth containing L-Tryptophan (without L-tryptophan or supplemented with 500 µg/ml of L-tryptophan) as substrate for the production of IAA. The cultures were incubated at 37 ± 2° C on an orbital shaker with gentle agitation (100 rpm). After every 12 h of incubation, the bacterial cultures were centrifuged at 10,000 rpm for 10 min. To the supernatant (2 ml), 2 drops of Orthophosphoric acid was added and incubated at room temperature for 10 min, followed by addition of 4 ml of Salkowski reagent (50 ml, of 35% sulphuric acid, 1 ml of 0.5 M $FeCl_3$). Development of pink colour indicates the positive result for IAA production and no color change indicates the negative result for IAA production. The IAA produced by the strain was measured spectrophotometrically at 530nm. Optical density values were compared with a standard curve prepared from known concentrations of IAA and quantification was hence done.

3. RESULTS AND DISCUSSION

3.1 Isolation and screening of phosphate solubilizing bacteria

Phosphorus is one of the essential macro minerals for the growth and development of plant (Schachtman *et al*., 1998). It is a major component in ATP, the molecule that provides energy to the plant for such processes as photosynthesis, protein synthesis, nutrient translocation, nutrient uptake and respiration (Deepak and Kirti, 2011). But after application, a considerable amount of them are rapidly transferred into less available forms by forming complexes with Fe, Ca and Al cations before roots have a chance to absorb it (Alam *et al*., 2002). Under such conditions PSB play fundamental role in biogeochemical phosphorous cycling in natural and agricultural ecosystem. Extensive use of chemicals as fertilizer improves the plant health and productivity but disturbed the ecological balance of soil and resulted in nutrient depletion. This has necessitated the search for alternate source of this element. The use of PSB in agriculture practice is not only offset high cost of manufacturing phosphate fertilizers but also make availability of insoluble P fertilizer.

The PSB strain, isolated from the rhizospere soil samples of Yam collected from the local market, Pallavaram, near Chennai produced clear zone around the colonies when incubated in Pikovaskaya's medium. The phosphate solubilizing strain exhibited halo zones due to the presence of phosphate enzyme (Fig. 1) on PKV medium. The PKV medium is used in the present study because it acts as specific isolation medium for phosphate solubilizing microorganisms, because the microorganisms grow and utilize calcium phosphate and produce clear zones. The colony which produced maximum phosphate solubilization is picked up, purified by repeated streaking (Fig. 2) and preserved for further studies.

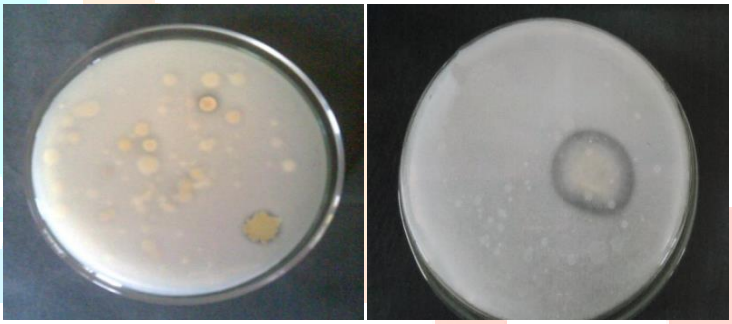

Fig.1 Isolation of PSB

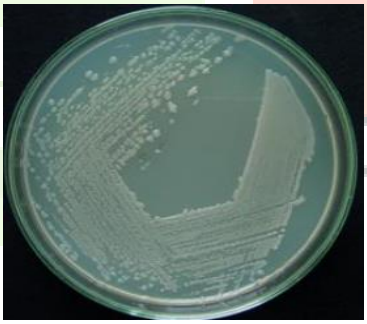

Fig.2 Purification by streak plate method

3.2 Identification of the microorganism

Over decades bacteria have been characterized and identified according to a few phenotypic characters such as morphology, pigmentation, reaction to dyes, the presence or absence of spores, nutritional requirements ability to produce acids from sugars etc. These simplified characterization methods are still the basis of classification. Besides the enzymatic and metabolic activities, fattyacid composition profiles have been proposed as method for taxonomic identification, in which fattyacids are methylated etherified and then analyzed by gas chromatography (Emmanuel *et al*., 2000). In the present study based on the biochemical tests, the Phosphate solubilizing bacterium was identified up to generic level. The results of various biochemical tests for the isolated strain are summarized in (Table1). Based on biochemical analysis the isolated strain PSB strain was identified as *Micrococcus* sp. by using the key provided by Bergy's Manual of Determinative Bacteriology. Similarly, Alexander (1977) reviewed that phosphate solubilizing bacteria include members of *Pseudomonas*, *Micrococcus*, *Bacillus* and *Flavobacterium*.

Table. 1 Biochemical characteristics of the isolated strain

Test	PSB 1
Gram stain	-
Shape	coccus
Motility	-
Catalase	+
Oxidase	+
Urease	-
MR	-
VP	+
Citrate	-
Indole	-
Gelatine	+
Nitrate	-
H_2S	-
Glucose	+
Lactose	+
Sucrose	+

3.3 Growth study

In the present study it was found that the isolated PSB**,** M*icrococcus* showed a maximum growth at 84 h. It was also observed that after 84 h it shows a gradual decrease in growth (Fig .1).

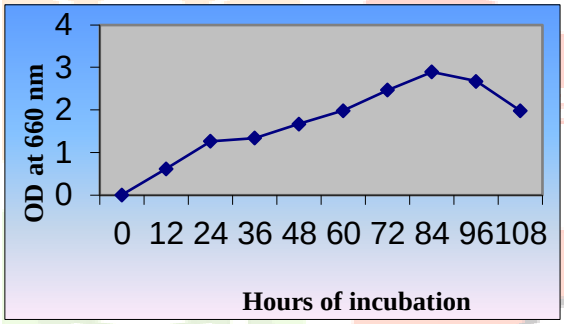

Fig.3 Growth of PSB

3.4. Screening of phosphorus solubilization, pH and titrable acidity

Phosphate solubilizing activity of the isolated bacterium *Micrococcus* sp. is analyzed by qualitative as well as quantitative methods. It showed a Phosphate Solubilization Efficiency of 2.73 on agar plates containing PKV medium after 5 days of incubation.

Quantitative estimation of phosphate solubilization is presented in Table 2. The bacterium solubilized P in an efficiently increasing manner with reference to incubation time. After 96 h of incubation, the soluble P content reached a maximum of 15 µg/ml, after which the solubilization declined slightly.

The beneficial microorganisms can solubilize bound phosphate in soil and bring into solution making it available for plant uptake. Pikovskaya (1948) was the pioneer in isolating an organism capable of actively solubilizing tricalcium phosphate which is termed as 'Bacterium P' from soil and phosphorite. The transformation of insoluble phosphate into soluble form is performed by soil microorganisms which play a key role in soil P dynamics and subsequent availability of phosphate to plants (Richardson, 1994). Phosphate Solubilizing Microorganisms (PSMs) especially Phosphate Solubilizing Bacteria (PSB) enhance the solubilization of insoluble phosphorous compounds through the release of organic acids and phosphatase enzyme which is present in a wide variety of soil microorganisms. Species such as *Pseudomonas, Mycobacterium, Micrococcus and Flavobacterium among bacteria and Penicillium, Sclerotium, Aspergillus* and many other fungi are active in the conversion. During the conversion process, a part of phosphorous is assimilated by microorganisms, but the amount made soluble and released is in excess to the requirement of the microorganisms. The excess amount thus released is made available to plants. During this conversion process, organic acids play an important role. Equally important are nitric acid and sulphuric acid. As a result, these organic and inorganic acids convert calcium phosphate to di or monobasic phosphates and then easily made available to plant phosphates (Khan *et al*., 2007; Chen *et al*., 2006 and Kang *et al*., 2002).

Table.2 *In vitro* screening of phosphate solubilization efficiency, pH and Titrable acidityof PSB strain

Incubation time (h)	P Solubility (**µg/ml**)	pH*	Tirable acidity**
24	5.05	6.8	1.9
48	7.08	6.4	2.1
72	13.1	5.8	2.7
96	15.0	4.5	3.4
120	14.7	4.7	3.8

*Initial pH was 6.8, ** Titrable acidity expressed as ml of 0.01 N NaOH consumed per 5.0 ml of culture filtrate. Titrable acidity of control (uninoculated broth) was 1.8.

Before titration After titration

Fig.4 Estimation of titrable aciditty

In the present study, the isolated PSB that produced halo zone around the colony in PVK medium was able to produce organic acids in broth culture (Fig. 4). Similar observations were also found by Chen *et al*., (2006) and Ponmurugan and Gopi (2012). Studies related to the production of organic acids have shown that citric and oxalic acids were two major organic acids produced by PSB (Alam *et al*., 2002). The pH of the broth was found to decline in each case due to bacterial activity, lowering of pH coincided with phosphate solubilizing activity. It was also observed that during the same period of growth there was a reduction in the pH and an increase in titrable acidity. The pH was lowered from control 7.0 to 4.45 at 96 h of incubation.

***In vitro* screening for IAA production**

The isolated bacterial strain was screened for its ability to produce plant growth regulator, IAA. The phosphates solubilizing bacterial strain have produced pink coloration when qualitative assay was done, which indicated the production of IAA in their culture filtrates (Fig.5). From quantification experiment, varying levels of IAA production were recorded at different time intervals (Fig .6). The range of IAA production in the present study was 2.0 – 8.7 µg/ml.

Our findings of IAA production by PSB isolate, *Micrococcus* is in agreement with those of other researchers. A test for production of the auxin IAA was positive for the isolated strain, suggesting a potential mechanism whereby this bacterium may regulate plant growth. This interpretation is in line with the well- known characteristic of certain phytohormones (e.g. Auxin, ethylene). Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by other rhizobacteria.

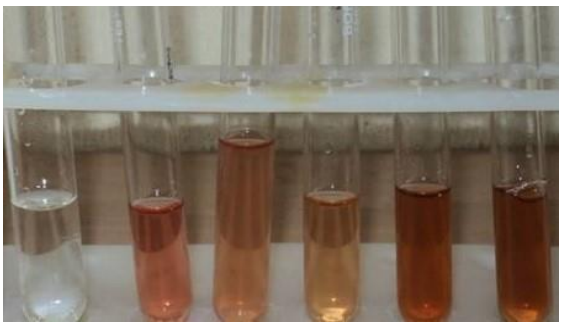

Fig. 5 Qualitative analysis of IAA

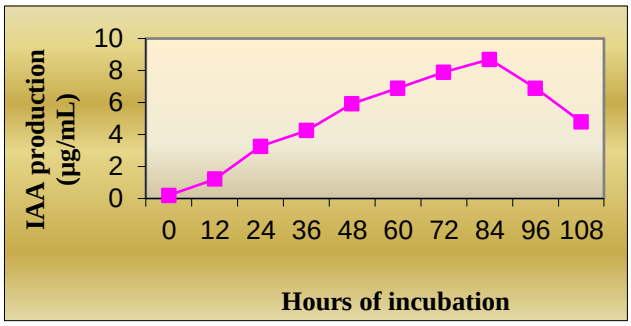

Fig. 6. Quantitative analysis of IAA

Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolites because of the rich supplies of substrates exuded from the roots compared with non-rhizospheric soils (Ahmad *et al*., 2005). The production of auxins by bacteria may offer a chance to use these bacteria as bio fertilizers to improve the growth and yield of agricultural crops. Phosphate solubilizing bacteria isolated from the rhizosphere soils are capable of dissolving phosphate (Barea *et al*., 1978).

The phosphate solubilizing bacterial cultures release a maximum quantity of IAA in the presence of tryptophan in a culture medium. Production of IAA varies greatly among different crops and is also influenced by culture conditions, growth stage, and availability of substrate (Vijila, 2000). In the present study IAA production by Phosphate solubilizing bacteria was investigated and found that the isolated strain had the ability to produce IAA.

IV. CONCLUSION

The PSB strain obtained in this study may be more effective and attractive as phosphate solubilizer. It requires further in depth studies based on the plant growth promoting activities of this isolate under pot culture as well as field conditions before they are recommended as bio fertilizers. The findings of the present investigation high lightened that IAA producing bacteria from local soil could be easily isolated and may be exploited after strain improvement for local use. It is concluded from the present study that PSB showed variation in their biochemical characteristics and is an efficient strain on the basis of screening and production of IAA. Further research should be continued with such efficient PSB isolates. These may be used for inoculum production and their inoculation effect on the plant growth.

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REFERENCES

[1] Abd-Alla, M. H. 1994. Phosphatases and the utilization of organic phosphorus by *Rhizobium leguminosarum* biovarviceae. *Letters in Applied Microbiology*, 18(5) 5: 294–296.
[2] Ahmad, F., Ahmad, I. and Khan, M.S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turk. J. Biol*, 29: 29-34.
[3] Alam, S., Khalil, S., Ayub,N. and Rashid, M., 2002, *Invitro* solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize. *Int. J. Agric. Biol*., 4: 454-458.
[4] Alexander, M. 1977. Introduction to soil microbiology. New York, John Wiley.
[5] Barea, J.M., Ocampo,.J. A., Azcon, R., Olivares, J. and Montoya, E. 1978. Effect of ecological factor on the establishment of *Azotobacter* in the rhizosphere. *Ecological Bulletin (Stockholm)*, 26:325-330.
[6] Chen, Y. P., Rekha, P. D., Arunshen, A. B., Lai, W. A. and Young, C. C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl Soil Ecol:* 34:33-41.
[7] Chen, J., Zhao, G. and Wei, Y. 2021. Isolation and screening of multifunctional phosphate solubilizing bacteria and its growth-promoting effect on Chinese fir seedlings. *Sci Rep* 11, 9081. https://doi.org/10.1038/s41598-021-88635-4
[8] Emmanuel Bossis., Philippe Lemanceau., Xavier Latour and Louis Gardan. 2000. The taxonomy of Pseudomo nas fluorescens and *Pseudomonas putida*: Current status and need for revision. Agronomie, 20, 51-63.
[9] Franken Berger, W. T., Brunner. W.1983. Methods of detection of auxin indole acetic acid in soil by high performance liquid Chromatography. *Soil Soc. Am. J.*47:237-241.
[10] Finnie, J. F. and Van Staden, J. 1985. Effect of seed weed concentrate and applied hormones on in vitro cultured tomato roots. *J. Plant Physiol*.120: 215-222.
[11] Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol*. 41: 109-117.
[12] Goldstein, A. H. 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria. *Biological Agriculture and Horticulture*, 12 (2): 185–193.
[13] Gyaneshwar P, Parekh LJ, Archana G, Poole PS, Collins MD, Hutson RA, Kumar GN(1999).Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphatesolubilizationbyEnterobacterasburiae.*FEMSMicrobiolLett.* 171:223–229.
[14] Holt, J.G., Krieg, N.R., Sneath, P.H.A. and Staley, J.T.1994. Bergy's Manual of Determinative Bacteriology. 9th Ed., Williams and Willkins Company, Ballamore, Md, USA,pp.255-273.
[15] Jana, B. B., Chakraborty, P., Biswas, J. K. and Gangly, S. 2001. Biogeochemical cycling bacteria as indices of pond fertilization: importance of CNP ratios of input fertilizers. *J Appl. Microbiol.* 90:733–740.
[16] Kampert, M., Strzelczyk, E. and Pokojska, A. 1975. Production of auxins by bacteria isolated from pine roots (*Pinus syIvestris* L.) *Acta Microbiol. Poll*.7: 135-143.
[17] Khan, M. S., Zaidi, A. and Wani, P. A. 2007. The role of phosphate-solubilizing microorganisms in the sustainable agriculture-a review. Agron Sustainable Dev: 27:29-43.
[18] Kang, S. C., Hat, C. G., Lee, T. G.and Maheshwari, D. K. 2002. Solubilization of inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102. *Curr Sci*., 82:439-42.
[19] Krasilnikov, M.1961. On the role of soil bacteria in plant nutrition. *J Gen Appl Microbiol.*, 7:128–44.
[20] Krieg, N.R. and Garrity, G.M. 2001. On using the manual, *In*:Garrity G.M.(Ed.), Bergy's Manual Trust. Springer-Verlag, New-York, pp.15-19.
[21] Kudashev, I. S. 1956. The effect of *phosphobacterin* on the yield and protein content in grains of Autumm wheat, maize and soybean. *Doki. Akad. Skh. Nauk*. 8:20-23.
[22] Kuhad,R. K., Singh, S. and Lata, Singh, A. 2011. Phosphate-Solubilizing Microorganisms. In: Singh A. *et al*., editors. Bio augmentation, Bio stimulation and Biocontrol. *SoilBiology* 28, Springer-Verlag Berlin Heidelberg.
[23] Lynch, J. M. (1985). Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. *In: Soil*

Organic Matter and Biol. Activity. Eds. Vaughan D and Malcom RE. Martinus.
[24] Muller, M. Deigele, C., Ziegler, H. 1989. Hormonal interactions in the rhizospheres of maize (Zea mays L.) and their effect on plant development. *Z Pflanzenernahar. Bodenkd* 152: 247-254.
[25] Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate *U.S. D.A. Circular, U.S. Govt. Printing Office, Washington D.C*. pp: 939.
[26] Pikovskaya, R. I. 1948. Mobilization of phosphates in soil in connection with the vital activities of some microbial species. *Mikrobiologiya*, 17: 362-370.
[27] Ponmurugan, P. and Gopi, C. 2006. Distribution Pattern and Screening of Phosphate solubilizing bacteria isolated from different food and forage crops. *J. Agron*., 5(4): 600-604.
[28] Ponmurugan, P. and Gopi, C. 2006. In vitro production of growth regulators and phosphate activity by phosphate solubilizing bacteria. *Afr. J. biotechnol*., 5: 384-350.
[29] Premono- Edi, Moawad, M.A and Vleck, P.L.G. 1996. Effect of phosphate solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indonesian Journal of Crop Sciences, 11: 13-23.
[30] Richardson, A. E. 1994. Soil microorganisms and phosphorus availability. In: Soil Biota, Management in sustainable farming systems. Pankhurst CE, Doube BM, Grupta VVSR, Grace PR. Eds. CSIRO, Melbourne, Australia. p. 50-62
[31] Richardson, A. E., Hadobas, P. A., Hayes, J. E. O., Hara, C. P., and Simpson, R. J. 2001. Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil microorganisms. *Plant Soil*. 229:47–56.
[32] Rodrıguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv*. 17:319–339.
[33] Schachtman, D. P., Reid, R. J., and Ayling, S. M. 1998. Phosphate uptake by plants from soil to cell. *Pl. Physiol*. 116: 447- 453.
[34] Stein, A., Fortin, J. A. and Vallee, G.1985. Enhanced rooting of Picea mariana cuttings by ectomycorrhizal fungi. *Can. J. Bot*. 68: 492-498.
[35] Strzelczyk, E., Pokojska-Burdziej, A. 1984. Production of auxins and gibberellin like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorhizosphere of pine (Pinus syIvestris L.). *Plant Soil.*, 81: 185 -194.
[36] Tabatabai, M. A., Bremner, J. M., Deepak, V. and Kirti, S. 2011, Nutritional value of rice and their importance. Indian Farmer's Digest. ISSN 0537- 1589.
[37] Vijila, K. 2000. Estimation of IAA production in nitrogen fixing microorganisms. In: *practical manual-microbial interaction in soil*. Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India, pp: 38-39.