academicJournals

Vol. 8(18), pp. 1872-1876, 16 May, 2013
DOI: 10.5897/AJAR10.995
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Halophilic phosphobacteria for raising vigorous growth improvement in Rice (*Oryza sativa*)

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Accepted 18 January, 2011

Phosphorus is one of the major essential macronutrients limiting plant growth owing to its low bioavailability in soils. Fertilizer phosphorus tends to be fixed soon after application and becomes mostly unavailable, low recovery by crops and a considerable phosphorus accumulation in soils. Several processes of the phosphorus cycle in soils remain obscure, despite large research efforts devoted to increasing the phosphorus availability to plants. The biofertilizer effect of phosphate solublizing bacteria on growth, yield and nutrient uptake of *Oryza sativa* was studied in a field experiment. Positive effect on plant growth, nutrient uptake, grain yield and yield components in *O. sativa* plants was recorded in the treatment inoculation of phosphate solubilizing bacteria (PSB). The PSB inoculated with *O. sativa,* increased significantly the average root length by 32.95%, average shoot length by 11.00%, numbers of roots by 38.46%, shoot biomass by 90.48%, root biomass by 3.77% as compared to control. The pigments increased the level of total chlorophyll by 68.70%, chlorophyll-a by 53.50%, chlorophyll-b by 80.47% and carotenoids by 45.83%, as compared to control and they also increase the level of carbohydrate by 33.33%, protein by 41.21% and amino acids by 30.23% as compared to control. Thus PSB is beneficial in raising vigorous of *O. sativa* under nursery and field conditions.

Key words: Biofertilizer, growth improvement, phosphate solubilising bacteria, *Oryza sativa,* root biomass.

INTRODUCTION

Phosphorus as an essential mineral nutrient for plant growth and development is the world's second highest chemical input in agriculture. Soluble P is often the limiting mineral nutrient for biomass production in agricultural ecosystems as well (Hameeda et al., 2006). Plants utilize fewer amounts of phosphate fertilizers that are applied and the rest is rapidly converted into insoluble complexes in the soil. So this phenomenon encourages farmers to frequent application of phosphate fertilizers. Modern agriculture is severely modifying and polluting the natural environment, due to the widespread application of chemical fertilizers, herbicides and pesticides. Therefore, thinking about valid alternative for chemical fertilizers is too necessary. Phosphate solubilizing bacteria (PSB) are used as biofertilizer since 1950's (Kudashev, 1956; Krasilinikov, 1957). These microorganisms secrete different types of organic acids e.g., carboxylic acid (Deubel and Merbach, 2005) thus lowering the pH in the rhizosphere (He and Zhu, 1988) and consequently dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils.

The role of phosphate solublizing microorganism in their abilities to reduce the pH of the surroundings by the production of organic acids (Sperber, 1958; Kim et al., 1998; Chen et al., 2006), production of acid and alkaline phosphatises (Rodríguez and fraga, 1999) and to H^+

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protonation (Illmer and Schinner, 1995). These organic acids can either dissolve phosphates as a result of anion exchange or can chelate Ca, Fe or Al ions associated with the phosphates (Gyaneshwar et al., 2002).

Various kinds of bacteria (Rodríguez and Fraga, 1999; Harris et al., 2006; Perez et al., 2007) have been isolated and characterized for their ability to solublize unavailable reduced phosphorus to available forms. Such transformations increase phosphorus availability and promote plant growth (Rodríguez and Fraga, 1999; Rudresh et al., 2005; Harries et al., 2006). The most abundant phosphates in the semiarid Argentinean pampas are bound to Calcium (Buschiazzo et al., 1994). The continuous agricultural during more than 20 years caused considerable decreases in the soluble phosphorous and in similar degree in the total inorganic phosphorus (Urioste et al., 1996).

Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizers could help increase the availability of accumulated phosphate (by solubilization), efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc., through production of plant growth promoting substances (Kucey et al., 1989). Trials with PSB indicated yield increases in rice (Tiwari et al., 1989), maize (Pal, 1999) and other cereals (Afzal et al*.,* 2005; Ozturk et al., 2003). Hence, the present study aims to evaluate which extent a phosphate solubilizing bacteria strain has the ability of *Oryza sativa* plants fertilized with different phosphatase solubilizing bacteria and to determine the effect of inoculation with a phosphate solubilizing bacterial strain on the growth and yield of *O. sativa.*

MATERIALS AND METHODS

Isolation and identification of PSB

All the samples were subjected for Pikovkya's medium (glucose: 10g; tricalcium phosphate: 5 g; NH_4SO: 0.5 g; $MgSO_4.7H_2O$: 0.1 g; KCl: 0.2 g; $MnSO_4$: trace; $FeSO_4$: trace; yeast extract: 0.5 g; Agar: 15.0 g; aged seawater: 500 ml; distilled water: 500ml; pH 7.2 ± 0.2; autoclaved at 15lbs for 15 min). The plates were incubated at 28 ± 2°C for 7 days. Morphologically different phosphor-bacterial species were identified by repeated streaking and identified by Bergey's Manual (Holt et al., 1994).

Preparation of bacterial inoculums

Identified phosphobacterial species of *Bacillus subtilis*, *Escherichia coli*, *Arthrobacter ilicis*, *Micrococcus roseus*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Micrococcus luteus* were isolated from mangrove environments and were incoculated separately into 100ml of Pikocsky's broth and were cultured at 28 ± 1°C for 5 days in a shaker. The culture was centrifuged at 12, 000 rpm for 15 minutes. The pellet were suspended in phosphate buffer ($NaH_2Po_4.2H_2O$-32.2g, Na_2HPO4-28.39g in 100ml sterile distilled water) and washed repeatedly with the buffer and were resuspended in the same buffer solution.

Phosphobacteria induced growth on *Oryza sativa*

To study the effect of phosphobacteria on the growth of coastal rice crop, an experiment was conducted. Certified seeds of paddy (IR36) were procured from Department of Agriculture, Thirupathisaram, Kanyakumari district. The seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and the seeds were soaked for 1 hr in liquid cell suspension (10^8 cells ml^{-1}) separately and spread on to the soil moistured with sterile distilled water. 5 replicates of 100 seeds of paddy were maintained for each bacterial species. The seeds without bacterial treatment served as control.

After 20 days of treatment, the plant growth characteristics *viz.,* average root length, average shoot length, number of roots, root and shoot biomass were analyzed. The pigments such us chlorophyll, chlorophyll-a, chlorophyll-b and carotenoides (Hiscox and Israelstan, 1979) which were extracted in 80% ice cold acetone from leaves, were measured by following respectivey the methods of Arnon (1949) and Reddy (1977). The biochemical constituents viz., carbohydrate (Dubois et al., 1956), aminoacid (Moore and Stein, 1948) and protein (Lowry et al., 1951).

RESULTS

The inoculation of different phosphobacterial species of PSB on the growth parameters of *Oryza sativa* reveals that, the *Micrococcus roseus* enhanced the root length by 32.95%, the *Bacillus megaterium* and *Enterobacter aerogenes* enhanced the shoot length by 38.46%, the *Escherichia coli, Arthrobacter ilicis, Micrococcus roseus, Bacillus cereus* and *Enterobacter aerogenes* enhanced the root biomass 3.77% and *Arthrobacter ilicis*, *Escherichia coli* enhanced the average shoot length and shoot biomass was increased by 11.00% and 90.48% respectively (Table 1).

The content of total chlorophyll, chlorophyll-a and chlorophyll-b in *O. sativa* seedlings were found to be increased by 68.70, 53.50 and 80.47%, respectively by the inoculation of the bacterial species of *Enterobacter aerogenes*, the content of carotenoids was increased by 45.83% by the inoculation of *Micrococcus luteus*. The content of carbohydrate was increased by 33.33% by *B. cereus* and the content of protein was increased maximum by 41.21% by the inoculation of *Bacillus megaterium*, the amino acid content was increased by 30.23% by *B. subtilis* over control (Table 2).

DISCUSSION

Phosphorous deficiency is the major constraint on crop production, as reported by many researchers. Use of phosphate solubilising bacteria has been reported promising in reducing phosphate fixation and increasing the phosphorous availability from soluble and insoluble phosphatic fertilizers. Beneficial effect of inoculation of phosphate solubilizers on the uptake of nutrients and on the yield of crops has been reported by many workers. Gerretson (1948) was the first to demonstrate that plants take up more phosphate from insoluble phosphatic fertilizers in the presence of microorganisms with these

Table 1. Effect of PSB on the average root length, shoot length, number of roots, shoot biomass and root biomass of *Oryza sativa* seedlings as percentage increase of decrease over control.

PSB treated	Average root length	Average shoot length	Number of roots	Shoot biomass	Root biomass
Bacillus subtilis	8.70 (5.75)	17.31 (5.41)	11.00 (27.27)	0.161(2.48)	0.157(2.55)
Escherichia coli	10.02 (18.16)	18.20 (9.78)	6.00 (-33.33)	1.650 (90.48)	0.159 (3.77)
Arthrobacter ilicis	10.64 (22.93)	18.45 (11.00)	6.00 (-33.33)	1.600 (90.19)	0.159 (3.77)
Micrococcus roseus	12.23 (32.95)	18.18 (9.68)	7.00 (-14.29)	0.159 (1.26)	0.159 (3.77)
Bacillus cereus	10.69 (23.29)	17.32 (5.20)	11.00 (27.27)	0.168 (6.55)	0.159 (3.77)
Bacillus megaterium	8.90 (7.89)	16.86 (2.61)	13.00 (38.46)	0.167 (5.99)	0.158 (3.16)
Pseudomonas aeruginosa	10.52 (22.05)	17.07 (3.81)	9.00 (11.11)	0.159 (1.26)	0.156 (1.92)
Enterobacter aerogenes	11.31 (27.50)	18.39 (10.71)	13.00 (38.46)	0.174 (9.77)	0.159 (3.77)
Micrococcus luteus	9.18 (10.68)	16.53 (0.67)	9.00 (11.11)	0.159 (1.26)	0.156 (1.92)
Control	8.20 (0.00)	16.42 (0.00)	8.00 (0.00)	0.157 (0.00)	0.153 (0.00)

Values are parentheses are percent increase over control.

Table 2. Effect of PSB on the chlorophyll-a, chlorophyll-b, carotenoid, carbohydrate, protein and aminoacids of *Oryza sativa* seedlings as percentage increase of decrease over.

PSB treated	Total content of chlorophyll	Content of chlorophyll-a	Content of chlorophyll-b	Carotenoids	Carbohydrate	Protein	Amino acids
Bacillus subtilis	0.198 (27.27)	0.087 (-6.90)	0.109 (54.13)	0.09 (-44.44)	0.091 (25.27)	0.160 (39.38)	0.043 (30.23)
Escherichia coli	0.181 (20.44)	0.113 (17.70)	0.067 (25.37)	0.14 (7.14)	0.079 (13.92)	0.119 (18.49)	0.039 (23.08)
Arthrobacter ilicis	0.193 (25.39)	0.072 (-29.17)	0.119 (57.98)	0.03 (-333.33)	0.075 (9.33)	0.112 (13.39)	0.037 (18.92)
Micrococcus roseus	0.167 (13.77)	0.103 (9.71)	0.060 (16.67)	0.02 (-550.00)	0.073 (6.85)	0.129 (24.81)	0.036 (16.67)
Bacillus cereus	0.190 (24.21)	0.096 (3.13)	0.094 (46.81)	0.13 (0.00)	0.102 (33.33)	0.161 (39.75)	0.038 (21.05)
Bacillus megaterium	0.296 (51.35)	0.106 (12.26)	0.187 (73.26)	0.16 (18.75)	0.094 (27.66)	0.165 (41.21)	0.041 (26.83)
Pseudomonas aeruginosa	0.241 (40.25)	0.108 (13.89)	0.130 (61.54)	0.08 (-62.50)	0.076 (10.53)	0.101 (3.96)	0.034 (11.76
Enterobacter aerogenes	0.460 (68.70)	0.200 (53.50)	0.256 (80.47)	0.21 (38.39)	0.086(20.93)	0.160 (39.38)	0.036 (16.67)
Micrococcus luteus	0.349 (58.74)	0.193 (51.81)	0.153 (67.32)	0.24 (45.83)	0.098 (30.61)	0.132 (26.52)	0.034 (11.76)
Control	0.144 (0.00)	0.093 (0.00)	0.050 (0.00)	0.13 (0.00)	0.068 (0.00)	0.097 (0.00)	0.030 (0.00)

Values are parentheses are percent increase over control.

ideas in view present investigation was undertaken to see the effect of phosphate solubilising microorganisms on growth and yield of rice crops. Katznelson and Bose (1959) found that rhizosphere bacteria have greater metabolic activity and suggested that they might contribute significantly to the phosphate economy of the plant. Laboratory studies reviewed by Kucey et al. (1989) have shown that the microbial solubilising of soil phosphate in liquid medium studies has often been due to the excretion of organic acids as a result of which a decrease in pH was affected. A few reports have indicated the phosphate solubilising activity of some nitrogen fixers (Mahesh kumar et al., 1999 and Seshadri et al., 2000).

The maximum and significantly high shoot length was observed in *Bacillus* sp. with phosphate solubilising bacteria. The inoculation of PSB has also increased the shoot growth. The control plants have recorded the lowest shoot length. Similarly PSB was observed on block pepper (Kandiannan et al., 2000) and tomato (Kim et al., 1998). The inoculation of PSB recorded significantly high root length over control. The control plants produced less root mass.

These results suggest that, treatment with PSB is beneficial as a general increase in growth and length as compared to control, was observed in all cases. Enhancement of growth in *O. sativa* seedlings might be due to treatment with phosphate solubilizing bacteria so as to enable to release the available phosphorous to the plants (Ponmurugan and Gopi, 2006).

The bacterial species that facilitate phosphate solubilisation by inoculation with mangroves are not well characterized, although some of the organisms involved in the inoculation processes have been identified (Ravikumar et al., 2002a, b; 2004). It was previously observed that mangrove seedlings usually grow better after inoculation with the diazotrophic filamentous cyanobacteria (Palaniselvam, 1998), *Azospirillum* and *Azotobactor* (Ravikumar et al., 2004). Based on this observation, it was reasoned that mangrove seedlings might also benefit by being inoculated with plant growth promoting bacteria (Bashan and Holguin, 1998). PGPBs have been reported to stimulate regeneration of temperate forests (Toledo et al., 1995; Chanway and Holl, 1992 and Li et al., 1992). Phosphobacterial species are well known PGPBs that facilitate the growth of terrestrial plant species (Bashan and Holguin, 1997a; Bashan and Holguin, 1997b). But there are only few reports describing the inoculation of halophilic phosphobacteria on to plants. Hence, the present study has been carried out to find out the effect of 9 halophilic phosphobacteria on the growth of *O. sativa.* It reveals that all the 9 phosphobacterial species. A total 9 phosphobacterial species enhanced the growth and physiology of *O. sativa.*

In the present study, halophilic phosphobacteria had positive effects on the growth characteristics, biochemical constitutions and pigments of *O. sativa*. This promontory effect may be attributed to ability of the PSB and making it available to the growing coastal crops. In this present study, all of the 9 bacterial species of PSB also synthesizing the phytohormone, which are required for better growth and pigment production of *O. sativa* (Ravikumar, 1995) and observed the halophilic bacterial species of phosphobacteria enhanced the maximum number of plant growth parameters in *O. sativa.*

In conclusion, organic fertilizer and chemical fertilizers could increase the phosphate level available in soil. Present investigation was therefore carried out on phosphate solubilising bacteria and their biofertilizer effect on coastal rice crops. This result revealed many interesting and important facts about the halophilic PSB, as well as the role of P-availability, for crop production and agricultural sustainability.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Alagappa University for providing required facilities and also to Ministry of Environment and Forest, New Delhi for financial assistance.

REFERENCES

Afzal A, Ashraf M, Asad SA, Farooq M (2005). Effect of phosphate solubilizing microorganisms on phosphorus uptake, yield and yield traits of wheat (*Triticum aestivum* L.) in rainfed area. Int. J Agric. Biol. 7:207-9.

Arnon DI (1949). Copper enzymes in isolated chloroplasts.Polyphenol oxidase in *Beta vulgaris.* Plant physiol. 24:1-15.

Bashan Y, Holguin G (1997a). *Azospirillum* plant relationships: environmental and physiological advances (1990 – 1996). Can. J. Microbiol. 43:103-121.

Bashan Y, Holguin G (1997b). Short and medium – term avenues for *Azospirillum* inoculation. In: Plant growth – promoting Rhizobacteria. Present status and Future prospects. Ogoshi, A Kobayashi K Homma Y Kodama F Kondo N and Akino S (Eds) Hokkaido University, Sapporo. Japan pp. 130-149.

Bashan Y, Holguin G (1998). Proposal for the division of plant growth promoting rhizobacteria into two classifications: biocontrol – PGPB (plant growth – promoting bacteria) and PGPB. Soil Boil. Biochem. 30:225-1228.

Buschiazzo D, Hevia K, Urioste A, Hepper E (1994). Phosphate forms and sorption in virgin and cultivated soils of the semiarid Argentineans Pampas. In: XV Int. Congress Soil Sci. pp. 97-98.

Chanway CP, Holl HB (1992). Influence of soil biota on Douglas – fir (*Pseudotsuga menziessii*) seedling growth: The role of rhizosphere bacteria. Can. J. Biol. 70:1025-1031.

Chen YP, Rekha PD, Arun AB, Shen, Lai WA, Young CC (2006). Phosphate solubilising bacteria from subtropical soil and their tricalcium phosphate solubilising abilities. App. Soil Ecol. 34:33-41.

Deubel A, Merbach W (2005). Influence of microorganisms on phosphorus bioavailability in soils. In: Buscot, F. and A. Varma (eds.), Microorganisms in Soils: Roles in Genesis and Functions. pp. 177-191.

Dubois M, Gills KA, Hamilton JK, Reser PA, Smith F (1956). Calorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Gerretson FC (1948). The influence of microorganisms on the phosphate intake by the plant. Plant Soil 1:51-81.

Gyaneshwar P, Kumar GN, Parkekh LJ, Poole PS (2002). Role of soil microorganism in improving P nutrition of plants. Plants Soil 245:83-93.51-81.

Hameeda B, Harmi G, Rupela OP, wani SP, Reddy G (2006). Growth promotion of maize by phosphate solubilising bacteria isolated from composts and macrofauna. Microbiol. 59:23-147.

Harris JN, New PM, Martin PM (2006). Laboratory tests can predict beneficial affects of phosphate solubillizing bacteria on plants. Soil Biol. Biochem. 38:1521-1526.

He ZL, Zhu J (1988). Microbial utilization and transformation of phosphate adsorbed by variable charged minerals. Soil Biol. Biochem. 30:917-923.

Hiscox JD, Israielstan GF (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.

Holt JG, Krieg NR, Sheath PHA, Stanley JT, Williams ST (1994). Bergey's manual of determinative bacteriology, 9^{th} Edn. (Wilkins and Wilkins, Baltimore, Maryland. USA), P. 787.

Illmer P, Schinner F (1995). Solubilising of inorganic calcium

phosphates solubilisation mechanisms. Soil Biol. Biochem. 27:257-263.
Kandiannan K, Sivaraman K, Annadaraj M, Krishnamurthy KS (2000). Growth and utrient content of black pepper (*Piper nigrum* L.) cuttings as influenced by inoculation with biofertilizers. J. Speices Aromat. Crops. 9(2):145-147.
Katznelson H, Bose B (1959). Metabolic activity of phosphate dissolving ability of bacterial isolates from wheat root, rhizosphere and non-rhizosphere soil. Can. J. Microbiol. 5:79-85.
Kim KY, Jordan D, Mc Donald GA (1998). Effect of phosphate solubilizing bacteria and Vesicular – Arbuscular Mycorrhiza on tomato growth and soil microbial activity. Biol. Fertil. Soil 26:79-87.
Krasilinikov NA (1957). On the role of soil micro-organism in plant nutrition. Microbiologiya 26:659-672.
Kucey RMN, Janzen HH, Leggett ME (1989). Microbially mediated increases in plant-available phosphorus. Ad Agron. 42:199-228.
Kudashev IS (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.
Li CY, Masssicote HB, Moor LVH (1992). Nitrogen – fixing *Bacillus* sp. associated with Douglas-fir tuberculated ectomycorrhizae. Plant Soil 140:35-40.
Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193:265-275.
Mahesh kumar KS, Krishnaraj PU, Algawadi AR (1999). Mineral phosphate solubilising activity of *Azotobacter diazotraphicus*: A bacterium associated with sugarcane. Curr. Sci. 76:874-875.
Moore S, Stein WH (1948). Photometric method for use in the chromatography amino acids. J. Biol. Chem. 176:367-388.
Ozturk A, Caglar O, Sahin F (2003). Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutr. Soil Sci. 166:1-5.
Pal S (1999). Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 213:221-230.
Palaniselvam V (1998). Epiphytic cyanobacteria of mangrove: Ecological, physiological and biochemical studies and their utility as biofertilizer and shrimp feed. Ph.D. thesis, Annamalai University. India. P. 141.
Perez E, Sulbaran M, Ball MM, Yarzabal LA (2007). Isolation and characterization of mineral phosphate solubilizing bacteria naturally colonizing a limonitic crust in the South-eastern Venezuelan region. Soil Boil. Biochem. 39:2905-2914.
Ponmurugan P, Gopi C (2006). *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr. J. Biotechnol. 5(4):348-350.
Ravikumar S (1995). Nitrogen-fixing azotobacters from the mangrove habitat and their utility as biofertilizer. Ph.D. thesis, Annamalai University. India. P. 120.
Ravikumar S, Kathiresan K, Thadedus Maria Ignatiammal S, Babuselvam M, Shanthy S (2004). Nitrogen fixing azotobacters from mangrove habitat and their utility as marine biofertilizers. J. Exp. Mar. Biol. Ecol. 312(1):5-17.
Ravikumar S, Ramanathan G, Babuselvam M, Prakash S (2002a). Quantification of halophilic phosphobateria from Pichavaram mangroves (South east coast) and their potential application to crop culture. Proc Nat Sem Creeks, Estuaries and mangroves. Pollution and conservation, 28th to 30th November 2002, Thane, Quadios, G (Ed), Thane, India. Vidya-Prasarak Mandal-S-B-N-Bandodkar Coll. Sci. 301-303.
Ravikumar S, Ramanathan G, Suba N, Jeyaseeli L, Sukumaran M (2002b). Quantification of halophilic *Azospirillum* from mangroves. Ind. J. Mar. Sci. 31(2):157-160.
Reddy SM (1977). Interaction of chloroplast withinhibitor: induction of chlorosis by diuron during prolonged illumination in vitro. Plant Phyl. 59:724-732.
Rodríguez H, Farag R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17:319-339.
Rudresh DL, Sivaprakash MK, Prasad RD (2005). Effect of combined application of *Rhizobium,* phosphate solubilising bacterium and *Trichoderma* sp. on growth, nutrient uptake and yield of chickpea (*Cicer arietinum* L). Appl. Soil Ecol. 28:139-146.
Seshadri S, Muthukumarasamy R, Lakshminarasimhan C, Ingacimuthu S (2000). Solubilisation of inorganic phosphates by *Azospirillum halopraeferens.* Curr. Sci. 79(5):565-567.
Sperber JI (1958). Solution of apatite by soil microorganisms producing organic acids. Aust. J. Agric. Res. 9:782-787.
Tiwari VN, Lehri LK, Pathak AN (1989). Effect of inoculating crops with phospho-microbes. Exp. Agric. 25:47-50.
Toledo G, Bashan Y, Soeldner A (1995). *In vitro* colonization and increase in nitrogen-fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Can. J. Microbiol. 11:1012-1020.
Urioste AM, AA Bono, DE Buschiazzo, Hevia GG, Hepper EN (1996). Fracciones de fósforo en suelos agrícolas y pastoriles de la Región Semiárida Pampeana Central (Argentina). Ciencia del Suelo 14(2): 92-95.