Available online at www.sciencedirect.com
SciVerse ScienceDirect
Procedia Engineering 46 (2012) 62 – 67
Procedia Engineering
www.elsevier.com/locate/procedia

1st International Symposium on Innovation and Technology in the Phosphate Industry
[SYMPHOS 2011]

Beneficial Microorganisms for the Sustainable Use of Phosphates in Agriculture

Hani Antoun[a]*

Département des Sols et de Génie agroalimentaire & Centre de Recherche en Horticulture,
Université Laval Québec Canada G1V 0A6

Abstract

Phosphorus (P) is vital for plant growth. However, most added soluble P forms insoluble phosphates. Therefore, inorganic P (Pi) accumulates in soils. Soil organic P (Po) is another important reserve (20 to 80% of total soil P). To be available for plant Po must first be mineralized by soil microorganisms. The variability of the results of inoculation trials with P solubilizing microorganisms (PSMs) clearly reflects the complexity of the interactions occurring in the soil-plant-microbes-fauna ecosystem. Important points overlooked in previous studies will be presented and perspectives of the use of PSMs to allow the plant to benefit from soil reserves in Pi and Po will be discussed.

Keywords: bacteria, fungi, rhizosphere, mycorhizosphere, mycorrhizas, phosphate mobilization

---

1. Introduction

Phosphorus (P) is an essential element for life. In fact it is present in adenosine triphosphate (ATP) which transport in cells the energy required for biological reactions. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and phospholipids are also vital P containing molecules in animal and plant cells. After nitrogen, P is the second major element effecting plant growth and yield. And the concentration of P in soil solution is very low, varying from 0.001mg L^{-1} in very poor soils to 1 mg L^{-1} in heavily fertilized soils. Orthophosphate anions (mainly $H_2PO_4^-$ and HPO_4^{2-}) are the form of P taken up by plants from soil solution; however their concentration is very low because they are chemically very active and they react rapidly with cations like calcium in alkaline soils or aluminum and iron in acid soils to form sparingly soluble precipitates, not available to plants. The reactions resulting in the precipitation of soluble P from soil solution are generally referred to as P-fixation or P-retention. It is estimated that because of P-fixation in soil, plants will take up the year of application, only 10 to 15% of the soluble P added as fertilizers or manure [1]. Therefore long term use of P fertilizers and organic amendments, particularly in high P-fixation soils, will cause the accumulation of substantial quantity of P. In general half of total soil P [2] is formed by organic P of which 10 to 50% is formed by inositol phosphates [1].

---

* Corresponding author: Tel.: +418 656 2131; fax: +418 656 3515
E-mail address: hani.antoun.1@ulaval.ca

1877-7058 © 2012 The Authors. Published by Elsevier Ltd. Selection and/or peer-review under responsibility of the Scientific Committee of SYMPHOS 2011
Open access under CC BY-NC-ND license. doi:10.1016/j.proeng.2012.09.446

Growing P demand for food production, the fact that phosphate rock (PR) is a finite non-renewable resource and that high quality PR will be in the long run the first depleted (thus increasing the expenses of future mining and refinement), are examples of good reasons dictating the important need to have a better management of the global P resources, for sustainable food production [3]. Plant growth promoting (PGP) microorganisms, including PGP-rhizobacteria (PGPR), phosphate solubilizing microorganisms (PSMs) and other symbiotic microorganisms like arbuscular mycorrhizas (AM) fungi, may play a major role in developing a sustainable use of P resources. However, the success of the use of these beneficial microorganisms requires an excellent understanding of the complex interactions taking place between the different components of the complex plant-soil-microorganisms ecosystems. It is important to note that it is extremely difficult, if not impossible, to develop efficient PSMs that can totally replace the strong acids used industrially to mobilize P from PR. However, great achievements can be realised with PSMs if they are able to colonize and persist on plant roots, and interact synergistically with AM fungi to create numerous niches in which inorganic P and organic P are mobilized.

2. Important plant root ecological niches

The germinating seed and the soil surrounding it are known as the spermosphere [4]. This ecological niche supports a more intense microbial activity because of seed carbon exudation. This is the first habitat that will be used, though for a short period of time, by any developed PSM introduced by seed inoculation. Therefore, the behavior of PSM in the spermosphere will significantly influence future plant growth and health. Unfortunately, the spermosphere is not well studied and future investigations should establish if spermosphere competent PSMs are also rhizosphere or mycorhizosphere competent, and how introduced PSMs affect microbial communities in this habitat. When plants get older, the growing roots are surrounded by strongly adhering soil particles. In this root-soil interaction there is four distinct ecological niches: the root surface (mainly plant material) is known as the rhizoplane. Some microorganisms can colonize the interior of the roots (endosphere) and are considered endophytes. The rhizosphere is the soil under the influence of roots. Root exudates are energy rich photosynthates supporting important microbial activities. Therefore the number of microorganisms in the rhizoplane and the rhizosphere are several times more important than their number in the bulk soil, which is the soil distant from the roots. Since most agricultural crops are mycorrhizal (form a symbiotic association with mycorrhizas), the concept of the mycorrhizosphere replaced that of rhizosphere [5] to include the zone under the influence of plant roots and the symbiotic fungi, and it includes the hyphosphere which is the zone surrounding mycelia. Efficient PSM should therefore be able to colonize the mycorrhizosphere and have beneficial interactions with plant roots and with mycorrhizas, to support high yields in sustainable agriculture systems while using less input in fertilizers and pesticides or in organic agriculture by using direct application of PR or other sources of Po.

3. Naturally occurring phosphate solubilizing microorganisms

Chabot et al. [6] reported that PSM present in four bulk soils from the province of Quebec, with contrasting available P, ranged from 2.8 to 3 $x10^6$ cfu g^{-1} (colony forming unit per g of dry soil), and they represented from 26 to 46% of the total soil microflora. To determine if there is any potential correlation between soil physicochemical properties and the incidence of PSM, Bouchard [7] studied 20 different soils used for potato production. PSM represented from 4 to 30% of the total soil microflora and their number ranged from 2 to 30 x 10^7 cfu g^{-1} of bulk soil. The number of PSM found in soil will greatly depend on the sparingly soluble P source used, which is often a di or tri-calcium phosphate. In fact, out of 287 PSM isolated from Quebec soils on $CaHPO_4$, 53% and 67 % were able mobilize P from $AlPO_4$ and $FePO_4$, respective [7]. Although soils contain an important number of PSMs, these microorganisms mobilize enough P to cover their own needs, but not in excess to supply the plant [8]. It is important also to note that many free living microorganisms are not necessarily adapted to life in the mycorrhizosphere.

4. What are phosphate solubilizing microorganisms?

PSM are bacteria and fungi mobilizing inorganic P by the production of organic acids or the release of protons, and if they produce phosphatases they are able to mineralize organic P. Many genera and species of fungi have been described as PSMs [9]. Chabot et al. [10] obtained an efficient PSM isolate of *Rhizopus* sp. from a soil in Québec, and Reyes et al. [11] extensively studied an isolate of *Penicillium rugulosum* isolated from a pasture soil formed on top of an unexploited apatite PR mine in Venezuela. Babana and Antoun [12] described two fungi solubilizing the Tilemsi PR isolated from Malian soils and belonging to the genera *Aspergillus awamori* and *Penicillium chrysogenum*. To our knowledge, the only phosphate inoculum presently sold commercially on a large scale, is JumpStart®, developed in Western Canada with a strain of *Penicillium bilaii*, and now sold by Novozyme [13].

Bacteria include Gram positive as well as Gram negative isolates [14] and the number of new PSM species is growing. Interestingly, Antoun et al. [15] observed that 54% of the 266 species of rhizobia obtained from the culture collections of different laboratories were able to solubilize dicalcium phosphates. Out of 446 strains of rhizobia isolated from legume fields in different parts of Iran, 44% were able to solubilize tricalcium phosphate and 76% mineralized inositol hexaphosphate [16]. Therefore, if properly selected efficient strains of rhizobia compatible with AM fungi, might benefit their plant symbiotic legumes partners, by supplying nitrogen through fixation of atmospheric N_2, and by improving plant P nutrition by mobilization of soil inorganic and organic P. Also when studying the effect of mixed inocula containing PSM and rhizobia on legumes P-uptake, the ability of rhizobia to solubilize organic and inorganic P should be appraised. Rhizobia have also an excellent potential to be used as PGPR and PSM with non-legume plants [10].

Phosphate solubilizing bacteria belong to the PGPR [17]. PGPR exert their beneficial effect on plant growth by different mechanisms of action, having a direct effect like the production of phytohormones and cytokinins or by improving plant nutrition, or by indirect mechanisms which improve plant health, like biological control of pathogens or the induction of systemic resistance in plants which can be compared to the improvement of the immune system in animals. Some PGPR can stimulate the symbiosis between plants and AM fungi.

5. How is phosphate mobilized by phosphate solubilizing microorganisms?

Organic acid production is the main mechanism by which PSM mobilizes P from sparingly soluble phosphates [18]. Solubilization of phosphates may result from the drop in pH or from cations chelation by organic acids. Mineral phosphate solubilization (MPS) is greatly affected by the carbon [11] and nitrogen [19] sources available to PSM. When glucose is used as the carbon source the Venezuelan strain IR-94MF1 of *Penicillium rugulosum* produced gluconic acid in the presence of hydroxyapatite as sole P source [11]. However sucrose was the carbon source producing the highest P mobilization. In the presence of sucrose, the gluconic acid was responsible for the solubilization of a Florida apatite and a Monte Fresco PR from Venezuela, while citric acid was very active in solubilizing a Utah variscite, and both organic acids were involved in the solubilization of a the Venezuelan Navay PR [20]. To elucidate the mechanisms by which *Penicillium rugulosum* IR-94MF1 mobilized different sources of P, in addition to the wild type MPS^+ phenotype, two UV induced mutants were obtained, MPS^- with an altered and MPS^{++} with an amplified phenotype [11]. The MPS^- did not produce any organic acid, but it supported good biomass production when a Florida apatite and Navay PR from Venezuela were used as sole P source, and it did not acidify the liquid culture medium used [20]. It was suggested that a H^+ pump mechanisms might be involved in P-solubilization by MPS^-. This is in agreement with an earlier report indicating that P-mobilization is not always caused by the release of organic acids, but the release of protons accompanying respiration and NH_4^+ assimilation, might also play and important role [21].

It is important to note that mineral P solubilization (MPS) phenotype might disappear when some PSMs are isolated on laboratory agar media. In this case these bacteria are solubilizing P with gluconic acid produced by the enzyme glucose dehydrogenase (GDH). This enzyme requires the cofactor pyrroloquinoline quinone (PQQ), which is not produced by many soil bacteria. Babana and Antoun [22] retained 44 bacteria and 18 fungi isolated from Malian soils for their high ability to solubilize Tilemsi PR. After several subcultures in solid and liquid media only 6 bacteria and 2 fungi retained their P-solubilizing phenotype.

6. Root colonization and interaction with mycorrhizas

A good PSM should be able to colonize plant root and persist in the mycorrhizosphere to supply plants with available P. Chabot et al. [23] marked P-solubilizing PGPR with *lux* genes for bioluminescence and they noted that their population averaged log 3.0 to 4.1 cfu/g of fresh root of lettuce and maize four weeks after seeding in non-sterile Promix substrate (Premier Tech, Rivière-du-Loup). They also observed that a potential PGPR *Serratia* strain was not detected on lettuce roots and was absent from maize roots two weeks after planting. By using Lux^+ transconjugants mutants of a PSM strain R1 of *Rhizobium leguminosarum* bv. *phaseoli* altered in their MPS phenotype Chabot et al. [24] found that mutants altered in P-solubilization colonized maize roots at lower degree than strain R1 Lux^+ which solubilized significantly more hydroxyapatite. They also noted that maize root colonization by the PSM was not affected by soil P-fertilization.

To study root colonization by strain IR-94MF1 of *Penicillium rugulosum* a transformant w-T3, resistant to hygromicin B was produced by Reyes et al. [25]. In a non-sterile soil microcosm assay, colonization of the rhizosphere of 5-week-old maize plants by w-T3 was significantly affected by the source of P used. The lowest rate of colonization was observed in the non-fertilized control or when ordinary superphosphate was added, while a tenfold increase in colonization was observed when the Navay PR from Venezuela was used. Interestingly, plants inoculated with w-T3 and fertilized with Navay PR contained significantly more P in their shoots, confirming the importance of a good root colonization by PSM, to properly supply P to the plant. Root colonization by PSM should not be studied under axenic conditions, since PSM should compete with other indigenous soil microbes for colonizing sites on plant roots.

Mycorrhizas and in particular AM fungi are ubiquitous and they form symbiotic association with most cultivated crop plants. Under natural conditions spores and hyphae of mycorrhizas are exposed to bacteria, some are loosely attached and some are endophytes [26]. The mycorrhiza helper bacteria can operate either by helping the establishment of the symbiosis or they can help the functioning of the symbiosis [27]. In selecting PSMs their compatibility with AM fungi should not be overlooked, because these interactions can have a beneficial effect on plant growth [28] and on P solubilization [29].

7. Plant response to inoculation with phosphate solubilizing microorganisms

In 1947 in the former U.S.S.R., phosphobacterin, a soil inoculum, was produced by adsorbing spores of *Bacillus megaterium* var. *phosphaticum* on Kaolinite. This Gram positive bacteria is a PSM mobilizing poorly soluble sources of organic and inorganic P [30]. For many years phosphobacterin field inoculation trials were performed with different crop plants and legumes, in different soils and the results obtained varied from no response to 70% yield increase, with an average of 10%. The best plant response to phosphobacterin was observed in neutral or alkaline soils rich in organic acids. The outcome of inoculation of maize with PGPR is significantly influenced by the nature of the soil used [31], and this can be attributed in part to the variability of the indigenous microflora and microfauna which interact with the introduced PGPR. In fact soil fauna plays an important role in the soil-plant-microbes interactions because grazing of bacteria by a wide diversity of protozoa affects plant growth by modifying bacterial community structures and by keeping them young and active, or by acting as concentrators of phytohormones produced by bacteria, or by releasing nitrogen to the plant (for a review see Antoun and Prévost [32]). Mycorrhizas are also an important factor influencing the interaction between plant and PGPR, and they can influence the microbial communities structure in the roots since a mycorrhizal plant have root exudates different than non-mycorrhizal plants [28, 32]. Unfortunately many studies still overlook today the mycorrhizal effect and several reports presumably on the rhizosphere might be indeed the results of the study of the mycorrhizosphere. Very simple method can be used to determine if a plant is mycorrhizal or not and to evaluate the importance of root colonization by these symbiotic fungi [33].

Because of the low percentage of the P-fertilizers available to plants the year of application, high rates of soluble P-fertilizers are used. With field cultivated forage and grain maize, it is possible to reduce the rate of soluble P-fertilizer added by 50% without reducing yield, if plants are inoculated with appropriate PSMs [10, 34]. This suggests that it might be advantageous to add PSMs directly to soluble P-fertilizers to increase the availability of P the year of application.

8. The plant effect

When P is scarce in soil solution, plants use different strategies to acquire P, and many like the release of organic acids or the production of phosphatases are common in both plants and microorganisms. Richardson et al. [35] reviewed the different plant and microbial strategies that can lead to the improvement phosphorus efficiency in agriculture, and a new model involving root Ca-uptake controlling P nutrition under P-deficient conditions was recently described [36]. Root hairs play a very important role in P uptake by plants, and selecting for root hair length and density often improves P acquisition. Interestingly, inoculation of pea with *Penicillium bilaii* the P solubilizing fungus of the inoculum JumpStart® increases by 22% the proportion of roots containing root hairs and by 33% the mean root-hair length [37]. Noh Medina [38] isolated a strain of *Bacillus benzoevorans,* with PGP activity on tomato, that increased root-hair density and root-hair length by 24 and 70% respectively and these effects were associated to the production of the plant hormone indole-acetic acid and the cytokinin isopentenyl adenosine.

9. Concluding remarks

There is an abundant literature showing positive, neutral or negative effects on plant growth and yield, in response to inoculation with PSMs. This illustrates the complexity of all the interactions taking place in soil between plants and their symbiotic mycorrhizal fungi partners, and the different microbial communities associated with AM fungi or plant roots, and fauna particularly protozoa and nematodes grazing on microorganisms. To overcome the problem of variability of the results and to develop effective microbial inocula for the improvement of plant growth and P-nutrition, the following points may be considered.

- Inocula containing a mixture of microorganisms should be developed. Indeed plant growth promoting microorganisms have multifaceted beneficial effects [39] and they can complement each other. For example, Becker Underwood produces a new BioStacked® inoculant for soybean containing the plant symbiotic nitrogen fixing partner

Bradyrhizobium japonicum mixed with a unique patented *Bacillus subtilis* acting as a Nodulating Trigger® resulting in greater nodule biomass, faster canopy and ultimately higher yields [40]. *Bacillus subtilis* strain MBI600 is used commercially as a biofungicide in Premier Tech products like Pro-Mix BX Biofungicide™* [41]

- Mycorrhizas are ubiquitous and most agricultural crop plants are mycorrhizal, and some soil bacteria can grow at the expenses of AM fungi [42]. Developing PSMs that can form syntropic associations with these symbiotic fungi, mixed with rhizosphere competent PSM might secure a better colonization of the roots and an inoculation success in soils with diverse microbial communities. Industrial production of the obligatory symbiotic AM-fungi is not a problem anymore, in fact Premier Tech produces commercially the AM fungus *Glomus intraradices*, used in their products like PRO-MIX MYCORRHIZAE™ [43].
- Biochar the product of transformation of organic materials by pyrolysis has several attributes associated with beneficial effects on plant growth and on soil biota [44]. Future PSMs development and inoculation trials should evaluate the benefits of using biochar as a carrier.
- For a sustainable use of P, in conventional agriculture direct addition of PSMs to soluble P fertilizers should be considered, and mixing PSM with PR or other organic P fertilizers should be appraised as an avenue to improve PR reactivity and P availability to plants.

Improving P acquisition by plants and elaborating sustainable strategies for the use of P, are problems that will be solved only by using global approaches and by collaboration of scientists from different fields. However, PSMs will be playing a major role in solving the P problem. Although there is a debate going on the report indicating that a bacterium can use arsenic instead of P [45], more research in this area will probably lead to the discovery of new mechanisms used by the bacterium to grow in an environment where there is a dearth of P.

Acknowledgements

I would like to thank all my present and past graduate students and collaborators, for their passionate discussions and their excellent work and findings reported in this paper The continuous and generous financial supports from NSERC, FQRNT and the MAPAQ are greatly appreciated.

References

[1] Brady, N.C.,Weil, R.R. The nature and properties of soils.14th ed. Pearson/Prentice Hall, 2008.

[2] Richardson, A.E. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant physiol.2001,**28,** 897-906

[3] Global phosphorus research initiative. 2008. , consulted online April 15, 2012.

[4] Nelson, E.B. Microbial dynamics and interactions in the spermosphere. Annu. Rev. Phytopathol. 2004,**42**,271-309.

[5] Johansson, J.F., Paul, L.R., Finlay, R.D. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microb. Ecol. 2004,**48**, 1-13.

[6] Chabot, R., Antoun, H., Cescas, M.P. Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique. Can. J. Microbiol. 1993,**39**,941-947.

[7] Bouchard, D. Influence des propriétés physico-chimiques et biochimiques du sol sur la présence des microorganismes dissolvant le phosphore inorganique. Mémoire de maitrise, Université Laval, 2002, 109 pages.

[8] Richardson, A.E., Barea, J-M.,McNeill, A.M.,Prigent-Combaret, C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promoting by microorganisms. Plant & Soil 2009, 321,**305**-339.

[9] Whitelaw, M.A. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv. Agron. 2000,**69**,99-151.

[10]Chabot, R., Antoun, H. Cescas, M.P. Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar. *Phaseoli*, Plant & Soil 1996,**184**,311-321.

[11] Reyes, I., Bernier, L., Simard, R.R., Tanguay, P., Antoun, H. Characteristics of phosphate solubilisation by an isolate of a tropical *Penicillium rugulosum* and two UV-induced mutants. FEMS Microb. Ecol. **28,** 291-295.,

[12] Babana, A.H., Antoun, H. Effect of Tilemsi phosphate-rock solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali. Plant & Soil 2006, **287**,51-58.

[13] What is JumpStart®. http://www.bioag.novozymes.com/en/products/canada/biofertility/JumpStart/Pages/default.aspx consulted April 16, 2012.

[14] Rodriguez, H., Fraga, R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 1999,**17**, 319-339.

[15] Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R. Lalande, R. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). Plant & Soil 2004,**204**,57-67.

[16] Alikhani, H.A., Saleh-Rastin, N., Antoun, H. Phosphate solubilisation of rhizobia native to Iranian soils. Plant & Soil 2006, **287**, 35-41.

[17] Antoun, H., Kloepper, J. Plant growth promoting rhizobacteria (PGPR). In: Brenner,S., Miller, J.,editors. Encyclopedia of genetics, b Academic Press , 2001, 1477-1480.

[18] Khan, M.S., Zaidi, A., Wani, P.A. Role of phosphate-solubilizing microorganisms in sustainable agriculture- A review. Agron.Sustain.Dév. 2007,**27**,29-43.

[19] Reyes, I, Bernier, L., Simard, R.R. , Antoun, H. Effect of nitrogen source on the solubilisation of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiology Ecology 1999,**28**, 281-290.

[20] Reyes, I. Baziramakenga, R., Bernier, L. Antoun, H. Solubilization of phosphate rocks and minerals by a wild-type strain and two UV-induced mutants of *Penicillium rugulosum*. Soil Biol. Biochem. 2001, **33**, 1741-1747.

[21]Illmer, P., Schinner, F. Solubilization of inorganic calcium phosphates- Solubilization mechanisms. Soil Biol.Biochem.1995,257-263.

[22] Babana, A.H., Antoun, H. Biological system for improving the availability of Tilemsi phosphate rock for wheat (Triticum aestivum L.) cultivated in Mali. Nutrient Cycling Agrosyst. 2006**, 76**,285-295.

[23] Chabot, R., Antoun, H., Kloepper, J.W. , Beauchamp, C.J. Root colonization of maize and lettuce by bioluminescent Rhizobium leguminosarum biovar phaseoli. Appl. Environ. Microbiol. 1996, **62**, 2767-2772.

[24] Chabot, R., Beauchamp, C.J., Kloepper, J.W., Antoun, H. Effect of phosphorus on root colonization and growth promotion of maize by bioluminescent mutants of phosphate solubilizing *Rhizobium leguminosarum* biovar *phaseoli.*Soil Biol. Biochem. 1998,**30**,1615-1618.

[25] Reyes, I, Bernier, L, Antoun, H. Rock phosphate solubilisation and colonization of maize rhizosphere by wild and genetically modified strains of Penicillium rugulosum. Microb. Ecol.2002,**44**,39-48.

[26] Bonfante, P., Anca, I-A. Plants, mycorrhizal fungi and bacteria: A network of interactions. Annu. Rev. Microbiol.2009, **63,** 363-383.

[27] Frey-Klett, P., Garbaye, J., Tarkka, M. The mycorrhiza helper bacteria revisited. New phytologist 2007,**176**,22-36.

[28] Artursson, V., Finlay, R.D., Jansson, J.K. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. 2006**, 8**, 1-10.

[29]Villegas, J., Fortin, J.A. Phosphorus solubilisation and pH changes as a results of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH_4^+ as nitrogen source. Can.J.Bot.2001,**79**,865-870.

[30] Smith, J.H., Allison, F.E., Soulides, D.A. Evaluation of Phosphobacterin as a soil inoculant.Soil Sci. Soc. Am. Proc. 1961, 25,109-111.

[31]Lalande, R.,Bissonnette, N., Coutlée, D., Antoun, H. Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. Plant & Soil 1989,**115**,7-11.

[32] Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. editor, PGPR: Biocontrol and Biofertlization. Springer 2005, chapter 1,1-38.

[33] Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Environ. Microbiology 1998, **64**, 5004-5007.

[34] Yazdani, M., Bahmanyar, M.A., Pirdashti, H. Esmaili M.A.. Effect of phosphate solubilisation microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (Zea mays L.). World Acad. Sci. Eng. Technol. 2009, **49**, 90-92.

[35] Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., Simpson, R.J. Plantr and microbial strategies to improve the phosphorus efficiency of agriculture. Plant & Soil 2011, **349**,121-156.

[36] Devau, N., Le Cadre, E., Hinsinger, P., Gérard, F. A mechanistic model for understanding root-induced chemical changes controlling phosphorus availability. Annals of Botany 2010, **105,** 1183-1197.

[37] Gulden, R.H. , Vessey, J.K. Penicillium bilaii inoculation increases root-hair production in filed pea. Can. J. Plant Sci.2000,**80**. 801-804.

[38] Noh Medina, J.A. Rhizobacteria promoting the growth of plants infected with viruses. Laval University, Ph.D. thesis. 2007, 135 Pages.

[39] Avis, T.J., Gravel, V., Antoun, H., Tweddell, R.J. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol. Biochem. 2008, **40**, 1733-1740.

[40] Biostacked inoculants technical bulletin –Soybean. http://www.histicknt.com/pdf/tech-bulletin.pdf, consulted on line April 25th, 2012.

[41] PRO_MIX BX BIOFUNGICIDE^{TM*}http://www.pthorticulture.com/en/pro-mix-bx-biofungicide-biostimulant-growing-medium/ , consulted online April 25th, 2012.

[42]Lecomte, J., St-Arnaud, M., Hijri, M. Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. FEMS Microbiol. Lett. 2011, **317**, 43-51.

[43] PRO-MIX MYCORRHIZAE^{TM}, http://www.pthorticulture.com/en/pro-mix-mycorrhizae-growing-medium/, consulted on line April 25th, 2012.

[44] Lehmann, J.,Rillig,M.C., Thies,J., Masiello,C.A.,Hockaday, W.C., Crowley,D. Biochar effects on soil biota-A review. Soil Biol. Biochem. 2011,**43**, 1812-1836.

[45] Wolfe-Simon, F., Blum,J.S., Kulp, T.R., Gordon, G.K., Hoeft,S.E., Pett-Ridge,J.,Stolz,J.F., Webb,S.M., Weber, P.K. Davies, P.C.W., Anbar, A.D., Oremland,R.S. A bacterium that can grow by using arsenic instead of phosphorus. Science, 2011, 332, 1163-1166.