Study of the effects of rock phosphate application with phosphate solublizing bacteria on P availability for corn

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Abstract
Rock phosphate is the source of chemical phosphorus fertilizers but its solubility is very low and one of the solutions for increasing its efficiency is application of phosphate solubilizing microorganisms. A greenhouse experiment was performed with *Bacillus subtilis* and *Pseudomonas putida*, five levels of rock phosphate (0, 25, 50, 75 and 100 percent of the difference to an optimum level of 16 ppm), in three soils with different amounts of available P (Low, Medium and High) in four replications. Their effects on single plant dry matter of corn and plant P uptake was determined using standard analytical methods. Results showed that *pseudomonas*, with maximum fertilizer treatments and soil with medium P had highest dry matter. Treatments without bacteria had highest P uptake, one of the reasons could be excretion of deleterious materials by bacteria. Fertilizer levels were not significantly different from each other. Perhaps it is because of the small differences between selected levels. In addition, applied rock phosphate had low solubility in these soils (pH= 7.5-8), even under the effects of phosphate solubilizing bacteria.

Key Words
Rock phosphate, bacillus, pseudomonas, phosphate solubility.

Introduction
Currently, about 800 millions of the world population suffers from malnutrition because of limitation of suiTable food (FNCA Biofertilizer Project Group 2006). Some limitations in land use for agriculture could be overcome with application of suiTable irrigation methods and use of fertilizers on nutrient deficient land. Phosphorus is necessary for all forms of life because of its genetic role in RNA and energy transformation by ATP. It is a factor for shorter growth period especially in cereals. Phosphorus deficiency effects metabolic processes such as transforming sugar to starch, and causes the production of anthocyanin. Phosphate fertilizers soon after spreading in the land, transform to low soluble or insoluble forms. Only 15 to 20% of phosphate fertilizer is available and a small portion of it will be taken up by plants. Roots can deplete or concentrate mineral phosphorus in the rhizosphere, and so change phosphorus availability (Havlin *et al.* 2004). Plants excrete organic acids such as citric, oxalic and tartaric acid to increase phosphorus solubility and availability in rhizosphere (Marschner *et al.* 1990).

Rock phosphates are the raw materials for production of phosphate fertilizers. Increasing of rock phosphate solubility rhizosphere of some plant species has been observed in alkaline soils. Application of rock phosphates of Iranian mines is not common, because of its low availability given the fact that most soils are alkaline, high pH, under drought stress, high bicarbonate in irrigation water and low organic matter. Yet researches have shown that rock phosphate application as a phosphate fertilizer along with the activity of soil microorganisms can be effective (Kang *et al.* 2002). Most soil microorganisms such as bacteria, fungi and actinomycetes have the ability to change insoluble phosphates to soluble forms. Bacillus and Pseudomonas are important genera of phosphate solubilizing bacteria. Phosphate solubilizing microorganisms can attain some of their nutritional needs directly from minerals, so temporarily transform them to available forms (Taalab and Badr 2007). Soil fertility and phosphorus efficiency depend on biological, chemical and physical complex interactions and processes in soil. The purpose of this experiment was to investigate the effect of phosphate solubilizing bacteria at different rates of rock phosphate in soils with different levels of available phosphorus, on corn yield and phosphorus uptake.

Materials and methods
A greenhouse experiment with randomized complete block design in factorial form and three replications of each treatment was conducted. Factors included three levels of bacteria treatments (control, *Bacillus subtilis, and Pseudomonas putida*), three soils with different levels of available phosphorus(0-5, 5-10,10-15 mg/kg)

and five rates of rock phosphate application based on P requirement of each soil to reach an optimum of 16 mg/kg (including 0, 25, 50, 75 and 100 percent of the differences). Five kg pots were prepared with each soil and have fertilizers of nitrogen and potassium applied based on soil test. The phosphorus fertilizers were also applied for each treatment. Five corn seeds (DC370) were planted in each pot and thinned to three after the second week. Inoculation was done over the seeds after planting with phosphate solubilizing bacteria at a population of 1×10^8 cell/g soil. Above ground plant material was harvested after 75 days and dry matter yield per single plant and amount of plant phosphorus (Murphy and Riley 1962) were determined. Phosphorus uptake was also calculated. Results were analyzed by Gen-Stat software.

Results and discussion

The results indicated that medium P soil had highest dry matter and phosphorus uptake for all treatments ($P<0.01$). (Results not shown). The mean of the 3 soil P ranges of the single plant D.M. showed the difference between fertilizer level treatments and that there was an increasing trend, although only the difference between 75% and 100% with control was statistically significant (Table 1). This could be due to the small difference between treatments in the lower range and low solubility of the fertilizer source. P. putida treatment had highest D.M. of single plant and was significantly different from control and B. subtilis treatments. P uptake of control treatment was highest compared to both bacteria treatments (Table 2) which may be due to decrease in the number of bacteria after inoculation as the competition between bacteria and plants for nutrient uptake. Changes in the available phosphorus content of all these soils between the start and the end of the experiment were much higher than the fertilizer inputs. This could indicate a other soil characteristics influenceing P solubility of the residual phosphorus of the soil, which perhaps will be more effective if inoculation with higher pH resistance strains of bacteria is practised in these soils (FNCA Biofertilizer Project Group 2006).

Table 1. Mean comparison of the interaction between fertilizer rate and treatment of bacteria on dry matter of single plant (g).

Bacteria / Fertilizer (%)	control	Bacillus subtilis	Pseudomonas putida	Mean total
0	15.98^{c}	17.21^{c}	18.91^{bc}	17.37^{c}
25	18.79^{c}	19.9^{bc}	20.47^{bc}	19.72^{b}
50	20.19^{bc}	18.74^{c}	19.34^{bc}	19.42^{b}
75	20.69^{bc}	21.65^{b}	22.45^{ab}	21.6^{a}
100	20.85^{bc}	20.31^{bc}	24.79^{a}	21.99^{a}
Mean total	19.3^{b}	19.56^{b}	21.19^{a}	

Table 2. Mean comparison of the interaction between fertilizer rate and treatment of bacteria on plant phosphorus uptake (mg/pot).

Bacteria / Fertilizer (%)	control	Bacillus subtilis	Pseudomonas putida	Mean total
0	64.33^{ab}	62.26^{ab}	60.03^{ab}	62.2^{a}
25	66.53^{a}	68.5^{a}	53.73^{b}	62.92^{a}
50	68.7^{a}	47.56^{b}	59.9^{ab}	58.72^{a}
75	69.33^{a}	54.7^{b}	65.93^{a}	63.32^{a}
100	64.46^{ab}	64.2^{ab}	62.3^{ab}	63.65^{a}
Mean total	66.67^{a}	59.44^{b}	60.38^{b}	

Conclusion

In conclusion, the results of this experiment indicated that solubility of rock phosphate used was very low and could not impose a significant effect on P availability on the relatively high pH soils used in this study. At the same time, the enhanced microbial activities due to bacterial inoculation or plant root exudates have had a positive effect on changes in availability of phosphorus in soil. Although, the trend and relative effectiveness of microorganisms in the soil are very complicated and unpredicTable. It is suggested that for further studies, variation in plant, bacteria population and species, and the soil type be considered.

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