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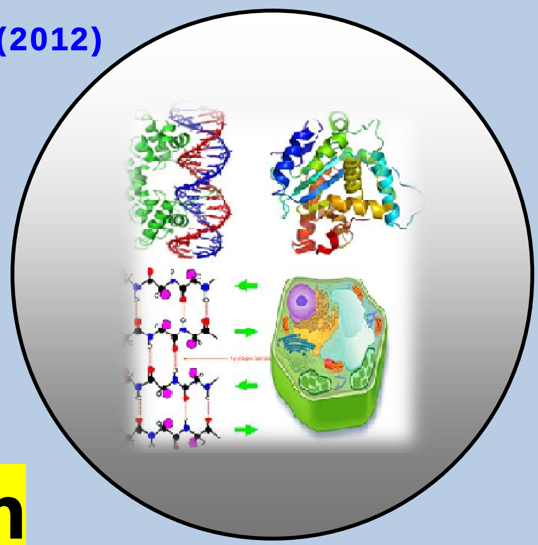
ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)

J. Biol. Chem. Research
Volume 31 (1) 2014 Pages No. 589-594



Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 1: 589-594 (2014)
(An International Journal of Life Sciences and Chemistry
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ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)


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RESEARCH PAPER

Received: 09/05/2013 Revised: 07/04/2014 Accepted: 10/04/2014

Production of Biological Phosphorus Fertilizer and Investigation of its Revenue from Medicinal Plants in Esfahan

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ABSTRACT

Phosphate solubilizing bacteria (PSB) are known to convert the insoluble forms of phosphate to soluble one and make them available for plant uptake. Phosphorus as an important mineral nutrient is essential for growth and metabolism in the plants. Phosphorus fertilizers are insoluble in calcareous soils and therefore could not be absorbed by the plants. Therefore, in most areas of Iran which soils are calcareous, phosphorus fertilizer efficiency is low. The results of this experiment showed that the bacterial growth which were considered at different salinities, pH, and temperatures. The bacterium, PS1 could tolerate the temperature up to 40^{0}C, and that will be able to continue its growth at the pH ranges of 5 to10. Other results showed that the bacteria had the hazy tolerance at saltiness of 2.5%. We conclude that the bacterium PS1 could tolerate wide ranges of severe environmental stress and most often there is a potential for PS1 to be used as a good biological fertilizer in the most agricultural soils of Iran. At the final stage of the processes we measure the yield of some plants with ornamental, pharmaceutical and herbal properties when coped with the bacteria. All the results showed that the yield of the plant was significant when we used it with the inoculated bacteria.

Keywords: Phosphorus, Biological Fertilizer, Salinity, Environmental Stress and Solubilizing Bacteria.

INTRODUCTION

Phosphorus (P) is one of the major essential macro nutrients for plants and is applied to soils in the form of phosphatic fertilizers. However, a large portion of soluble inorganic phosphates applied to the soil as a chemical fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Although P is abundant in soils in both inorganic and organic forms, it is a major limiting factor for plant growth as it is in an unavailable form for root uptake. Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants (Rengel and Marschner 2005). Organic matter is also an important reservoir of immobilized P that accounts for 20–80% of P in soils (Richardson 1994). Only 0.1% of the total P exists in a soluble form available for plant uptake (Zhou et al. 1992) because of its fixation into an unavailable form due to P fixation. The term P fixation is used to describe reactions that remove available phosphate from the soil solution into the soil solid phase (Barber 1995). Knowing the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PGPB is advantageous in the sustainable agricultural practices. Soil micro-organisms act as sink and source of phosphorus (P) and mediate the key processes in the soil P cycle, e.g., P mineralization and immobilization (Obersonand, Jones., 2005). Micro-organisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle. In particular, soil micro-organisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Hilda and Fraga, 1999). In calcareous soils, phosphorus fertilizers are very low in efficiency of phosphorous. The noticeable characteristics of soil organism's especially micro-organisms and over use of chemical fertilizers make the producing biological fertilizers, one of the most important issues to study. The realization of all these potential problems associated with chemical P fertilizers together with the enormous cost involved in their manufacture, has led to the search for environmental compatible and economically feasible alternative strategies for improving crop production in low or P-deficient soils (Zaidi et al. 2009). The use of microbial inoculants (biofertilisers) possesing P-solubilizing activities in agricultural soils is considered as an environmental-friendly alternative to further applications of chemical based P fertilizers. These biofertilisers can be used as fertilization with seed, soil or compost. They can also be used for grass, trees and perennial ornamental plants as spray, soluble powders or granule fertilizers. The new common microbial fertilizers offered commercially as phosphate, are phosphate solvent micro-organisms.

MATERIAL AND METHODS

To separate bacteria from the soil, the soil around corn root was diluted by distilled water and cultured in solid culture (sperber). The light parts showed bacteria which is called ps1. To evaluate solvency of mineral derivatives in ps1- bacteria is as follows, after preparing sperber culture including insoluble 3-phosphated calcic bacterium, it was cultured linearly.

The spot lights seen around the bacteria showed that the solubility of mineral phosphate. To evaluate the solubility of organic phosphate in ps1 bacteria: In order to evaluate the phosphates activity in the mentioned bacteria, the cultured bacteria in sperber culture was prepared including 50 mg/ml, 5-bruma, 4-choloro , 3-Indolil phosphate (BCIP). Appearing the blue color and its darkness is considered a criterion for phosphate activity. Scattering and purification of ps1 bacteria: The bacterium produced in the light parts of sperber culture were placed in a new culture after taken up by a disinfected loop to be propagated. After propagation, the bacteria collected by disinfected loop and solved in distilled water in large Erlenmeyer flasks were placed on shaker to be propagated. Investigating the quality of sp1 bacteria growth in different salt concentrations, pH and temperatures: At first, the solid sperber culture was prepared with different pH's of (5,6,7,8,9 and 10), and then NaCl concentrations of (0, 1 and 2.5) were made, and then the propagated bacteria cultured linearly and transferred to different temperatures of (25,30,35 and 40) centigrades.Yield measurement of some inoculated plants with ps1 bacteria: The seeds of Rosmarinus officinalis, officinales, Achillea millefolium, Salvia and Hyssopos officinalis were planted in pots in the greenhouse. The results showed that the flat leaf measurement of the mentioned plants have increased in comparison to the control group.

RESULTS

Table 1, showed that the ps1 bacteria in all chosen ranges of pH, NaCl and temperatures has a good activity. It means that it could tolerate the temperature up to 400C and wide range of pH's. It grows in more than 2.5% salt concentration; this suggests that it was resistant to salinity. Considering the above data, we can conclude that the ps1 bacteria can tolerate the unsuitable environmental conditions. Knowing that the high salinity and most alkali soils of Iran, the use of this biological fertilizer is recommended.

Table1. Investigate PS_1 bacteria growth in levels of different temperatures, salt concentrations and pH's.

Treatments	pH	NaCl (%)	Temp (C^o)
	5 6 7 8 9 1 0	0 1 2.5	25 30 35 40
PS_1Bacteria	- +++++	+ + +	+ + + +

Figure 1. Yield comparison of Zufa plant after bacterial inoculation.

DISCUSSION

Biological fertilizers are essential for improving growth in the plants especially in calcareous soils. Phosphorus fertilizers are insoluble in calcareous soils and therefore, could not be absorbed by the plants. In this study we used inoculated micro-organisms as microbial fertilizers which were extracted from rhizosphere to increase growth rate and widened flat leaf. H. Rodríguez and R. Fraga(1999) showed that the use of phosphate solubilizing bacteria as inoculant simultaneously increases P uptake by the plant and the crop yield. Micro-organisms play an important role to improve P use efficiency through use of specific inoculants. In this experiment, after inoculation with PS_1 bacteria in some medicinal plants an increase in leaflets can be perceived. It is also shown that the bacterium PS_1 could tolerate wide ranges of severe environmental stress, and so often a potential to be used as a good biological fertilizer in the most agricultural soils of Iran. A. E. Richardson and R. J. Simpson (2011) mentioned that opportunities for enhancing microbial mediated P availability in soils appear feasible and might be achieved by management of existing populations of micro-organisms to optimize their capacity to mobilize P or through the use of specific microbial inoculants. They also reported that a wide range of micro-organisms are able to solubilizing inorganic P. Their capacity to solubilize P is generally associated with the ability in culture to acidify growth media and release organic anions with other substances (Khan et al., 2007). In many cases, inoculation of plants with P-solubilizing micro-organisms in controlled experiments resulted in improved growth and P nutrition, especially under glasshouse conditions and in fewer cases in the field (e.g. for review, see Kucey et. al (1989), Rodrı´guez and Fraga, (1999), White law (2000), Gyane shwar et. al (2002), Jakobsen et. al (2005), Khan et. al(2007), Harvey et. al (2009), Zaidi et. al (2009), yosefi, Galavi, et. al (2011). Researches on maize shown that the use of pseudomonas inoculants bacteria resulted in improving seed function, increasing stem length and stem weight (Zahir *et al*., 1998). Micro-organisms may have capacity to directly solubilize P to meet their own requirements. For example, various commercial products (primarily based on bacterial isolates that reportedly solubilize P are widely promoted as plant growth promoting bacteria. Stimulation of root growth or greater elongation of root hairs (Vessey and Heisinger, 2001)

ACKNOWLEDGEMENTS

The project was funded by the Department of Biotechnology and Advanced Sciences & Technology, University of Esfahan in Iran, and Agricultural Biotechnology Research Institute Central of Isfahan (ABRICI). The authors would like to thank Dr. mandana behbahani for critical review of this manuscript.

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