GENETIC IMPROVEMENT OF SOME PHOSPHATE DISSOLVING BACTERIAL STRAINS

By

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ABSTRACT

Hend Fawzy Ahmed Moharam: Genetic improvements of some phosphate dissolving bacterial strains. Unpublished M. Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2012.

Bacillus megaterium is well known for its capacity to solubilize phosphate, has been receiving attention in recent years. The work presented in this thesis examined two aspects of screening and evaluating tricalcium phosphate (Pi) Ca_3 $(PO_4)_2$ solubilizing *Bacillus megaterium*. The objective of this study is to improve phosphate dissolving efficiency through mutagenesis, protoplast fusion and gene manipulation techniques.

In this study, *Bacillus megaterium* strain was evaluated for phosphate solubilization using broth and solid formulations of one medium Botanical Research Institute Phosphate Nutrient medium (NBRIP). The medium contain Ca_3 $(PO_4)_2$ as the only phosphorus (P) source. Three protocols were applied in this study to improve *phosphate* solubilization potentiality the first is mutation induction using UV irradiation, the second; protoplast fusion technique The third; utilization of transformation technique for producing new genetic recombinants. Mutagenesis was more efficient in the improvement of phosphate solubilization potentiality where the mutant strains which obtained after the exposure to UV radiation, recorded the highest phosphate solubilization potentiality, exceeding 2-folds the productivity of its original strain.

Key words:

Bacillus megaterium, E. coli JM107, Bacillus subtillus ,Bacillus polymexa , transformation, induction of mutation, phosphate solubilization, PCR, Gene cloning.

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INTRODUCTION

After N, Phosphorus (P) is the second major plant growth-limiting nutrient despite being abundant in soils in both inorganic and organic forms. **(Gyaneshwar*, et al.,* 2002; Fernandez, *et al*., 2007**). Chemical P fertilizer is the main source of plant available P in the agriculture soils, but almost 75 to 90% of added P fertilizer is precipitated by iron, aluminum and calcium complexes present in the soils **(Gyaneshwar, *et al*., 2002; Turan *et al*., 2006).** In calcareous soils, the pH is ranging between 7.3 and 8.5 depending on the amount of $CaCO_3$ present in the soil **(Lindsay., 1979)**. With high levels of exchangeable Ca, available P ions react with solid phase $CaCO_3$ and precipitate on the surface of these particles to form Ca-P minerals **(Lindsay *et al*., 1989).**

However, many soils throughout the world are P-deficient because the free phosphorus concentration (the form available to plants) even in fertile soil is generally not higher than 10 µM even at pH 6.5 where it is most soluble **(Gyneshwar *et al*., 2002**). To circumvent the problem of P deficiency, chemical fertilizers are added to the soils but cost of chemical phosphatic fertilizers is high **(Goldstein *et al*., 1993).** and has low efficiency (<0.1%) **(Scheffer and Schachtschabel., 1992).** Phosphorus biofertilizers in the form of microorganisms, especially phosphate solubilizing bacteria in rhizosphere, can help in increasing the availability of accumulated phosphates for plant growth by solubilization **(Richarson, 1994; Nautiyal *et al*., 2000).**

Many soil bacteria and fungi have the ability to solubilize phosphate (Pi) minerals and make it available to plants **(Oberson *et al*., 2001; Egamberdiyeva *et al*., 2003).** They are capable of using inorganic and organic forms of P **(Tarafdar and Jung, 1987; Chen *et al.,* 2002).**