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HPLC analysis of organic acid production by bacterial strains for rock phosphate solubilization

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Fertilizers are widely used to increase crop production and meet the global food demand. Phosphorous is one of the key nutrients in fertilizers that assists plant growth and improve crop yield. Eppawala rock phosphate (ERP) mine is a good source of phosphorous fertilizer. However, water solubility of ERP is substantially low, which makes it only suitable for short term crops. Seed or soil inoculation with Phosphate solubilizing bacteria (PSB) can be used to enhance solubility of ERP and gain higher crop yield. To solubilize insoluble phosphate, PSB biosynthesize and secrete organic acids. Main objective of this study was to analyze organic acid production by PSB. A PSB wild strain named EP11 which is isolated from natural soil and laboratory-improved EP11 mutant strains (mutant 1, mutant 2, and mutant 3) were used in this study. Strains were cultured in PVK broth. Culture supernatants were analyzed by reverse phase HPLC and determined solubilized phosphate levels by phosphate assay. All the experiments were done in triplicates. Results showed that bacterial strains have secreted organic acids to the surrounding medium and solubilized rock phosphate. Amount of solubilized phosphate correlated with the amount of organic acids. Tartaric acid was the dominant organic acid, which contributed for solubilizing rock phosphate. After 72-hour incubation, concentration of tartaric acid produced by the EP11 wild strain was 43.865 ppm, while EP11 mutant 1 produced 110.427 ppm. Relative to the wild strain, EP11 mutant 1 showed 64.30% increase in phosphate solubilization after 5-day incubation period. Pot experiments indicated that wild type and mutant EP11 strains have the ability to survive in natural soil. Plants grown in EP11 mutant 1 and mutant 2-inoculated soil showed a statistically significant increase ($p<0.05$) in growth relative to the controls. Results clearly indicated that high organic acid production contributed for increased phosphate solubilization, leading to higher plant growth and crop yield. Further research is in progress to develop EP11 mutant 1 into a seed inoculum for ERP, with the aim of achieving a higher growth and yield from crop plants.

Keywords: Phosphate solubilizing bacteria, Eppawala rock phosphate, Organic acid biosynthesis, Seed inoculation.