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Strain improvement to enhance phosphate solubilising ability of bacteria by random mutagenesis

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Rock phosphate is a cheap source of phosphorous fertilizer. However, low solubility of rock phosphate makes it agronomically less effective. Combined application of rock phosphate with phosphate solubilizing bacteria (PSB) is an attractive solution to this issue. PSB produce organic acids and lower the pH of the medium which solubilise insoluble phosphates. Phosphate solubilizing abilities of wild strains, however, is considerably low. Qualitative improvement and quantitative enhancement of the phosphate solubilisation ability require genetic improvements of wild strains. Therefore, the aim of the present work is to enhance phosphate solubilizing ability of a wild strain EP11 by mutagenesis. EP11 was subjected to random mutagenesis by exposing to UV (254 nm) at varying time periods, Sodium azide (0.05 mg/L) and Ethidium bromide at varying concentrations. Amount of phosphate solubilised by each strain was determined in the PVK liquid medium supplemented with Eppawala rock phosphate (12.5 g/L) and aliquots were taken for 8 days. Aliquots withdrawn during this period were subjected to the ammonium molybdate assay. Among the mutants produced, the mutant, UV4EP11 generated by UV irradiation showed the highest phosphate solubilizing activity (135%) than the wild strain. Mutant SA5EP11 generated by exposure to sodium azide, exhibited 163% higher activity, whereas the ethidium bromide mutant, EB2EP11 showed 139% phosphate solubilizing activity compared to wild strain. All mutant strains were tested further for three generations to confirm that the mutations are stable and there is no statistically significant difference in the phosphate solubilizing ability between the generations. RP-HPLC analysis of the culture supernatants of the mutant strains UV4EP11, SA5EP11, and EB2EP11 showed that the cultures reached 50 mg/L, 84 mg/L, and 32 mg/L acid concentrations respectively. These values were substantially higher than that produced by the wild strain which was only 22 mg/L. These values correlate well with the amount of phosphate solubilized suggesting that phosphate solubilisation by mutant strains proceeds through acidification of the medium. It can be concluded that, new rock phosphate solubilising mutant strains were successfully created by exposing to mutagens and those mutants showed a qualitative improvement in organic acid production leading to enhancement of rock phosphate solubilisation.

Keywords: Phosphate solubilisation, random mutagenesis, rock phosphate