# Optimizing environmental parameters affecting *in-vitro* rock phosphate solubilization by native Phosphate Solubilizing Microorganisms

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Direct application of Rock phosphate (RP), a cheap and environmental friendly source of Phosphorous (P) fertilizer, is limited for perennials due to its low solubility. Phosphate solubilizing microorganisms (PSMs) can improve the solubility of RP making it feasible to apply for annual crops. Efficiency of solubilization by microorganisms largely depends on the environmental parameters. Hence attempts were made to ascertain the effect of incubation time, aeration, carbon (C) and nitrogen (N) concentrations on *in-vitro* RP solubilization by native *Aspergillus* sp. and *Burkholderia* sp., inoculated alone and in combination with each other into a RP containing medium. The optimized medium can be used as the basal medium for the production of a liquid P fertilizer for the application into annual cropping systems.

To determine the optimum incubation time, 1 ml of each culture (1 x $10^7$ spores $ml^{-1}$ of *Aspergillus* sp., 1 x $10^8$ cfu $ml^{-1}$ *Burkholderia* sp.) was inoculated separately and in combination, to 50 ml of Pikovskaya medium modified by replacing $Ca_3(PO_4)_2$ with Eppawala rock phosphate (ERP) quantitatively (7.2 g $l^{-1}$) (PVK-ERP). Samples were incubated at room temperature, at 100 rpm oscillation and as still cultures, for different time periods: 24 h, 48 h, 72 h, 96 h and 120 h. Three replicates were maintained and 1 ml of sterile distilled water added PVK-ERP medium served as the control. Soluble P concentrations were determined by using the Murphy and Riley method. After identifying the appropriate conditions from the above experiments, the same procedure was carried out with varying N and C concentrations and the data were statistically analyzed using SAS 9.0 version, at 5% level of significance.

It was revealed that P solubilization was significantly high in aerated samples ($p \leq 0.05$). The optimum incubation time for *Aspergillus* sp., *Burkholderia* sp. and their combination were 72, 96 and 48 hours, respectively. Statistical analysis (one way ANOVA) indicated that P solubilization by each species and the combination of species under different N and C levels were significantly different ($p \leq 0.05$). It was noted that the highest ERP solubilization by *Aspergillus* sp. (170.37 ± 3.97 $mgl^{-1}$) was in $N_1$ $C_1$ simulation ($N_{ammonium\ sulphate}$ = 0.5 $gl^{-1}$, $C_{glucose}$ = 10.0 $gl^{-1}$); *Burkholderia* sp. (126.15 ± 5.13 $mgl^{-1}$) was in $N_5$ $C_1$ simulation ($N_{ammonium\ sulphate}$ = 3.0 $gl^{-1}$, $C_{glucose}$ = 12.0 $gl^{-1}$) and the combination of *Aspergillus* sp. and *Burkholderia* sp. (118.17 ± 2.72 $mgl^{-1}$) was in $N_1$ $C_5$ simulation ($N_{ammonium\ sulphate}$ = 0.5 $gl^{-1}$, $C_{glucose}$ = 50.0 $gl^{-1}$). Among the PSMs tested, efficiency of ERP solubilization was highest in *Aspergillus* sp. and thus it is feasible to utilize *Aspergillus* sp. in liquid P fertilizer production.

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