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Isolation of effective rock phosphate solubilizing bacteria from Sri Lankan soils

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Phosphorus (P) is a multivalent high reactivity element which is never found free in nature. P is essential for plant growth and development and its deficiency can severely limit produc tivity. Plants acquire P as phosphate (PO_4^{-3}) ions, which are highly reactive and precipitate rapidly with cations making it unavailable to the plant and is hence frequently applied to soil as mineral PO_4^{-3} fertilizers. More than 92% of PO_4^{-3} fertilizer consumption of the country is satisfied through imports costing about Rs.1300 million annually. Triple Super PO_4^{-3} is the major PO_4^{-3} fertilizer made by digesting rock PO_4^{-3} with acids, which is a costly treatment. The natural rock phosphate (ERP) deposit in Eppawala contains 60 million metric tons having about 33–40% of PO_4^{-3}. Direct application of ERP is limited to acidic soils due to its low solubility. Rock PO_4^{-3} is one of the basic raw materials for PO_4^{-3} fertilizer production. Certain soil microorganisms are capable of solubilizing insoluble P, making it available to plants. Free-living PO_4^{-3} solubilizing bacteria (PSB) are always present in soils and are often abundant on root surfaces. Seed or soil inoculation with PSB is known to improve PO_4^{-3} utilization efficacy of crop plants. The main objective of the study was to isolate PSB from Sri Lankan soils and to screen them for the efficiency for solubilization of ERP.

Soil samples were collected from various parts of Sri Lanka as bulk rhizosphere soils. Microorganisms extracted into 1% saline solution were initially screened by a plate assay method (Pikovskaya (PVK), 1948) with insoluble $Ca_3(PO_4)_2$ as the sole P source. Based on clear halo production, which is the positive indication for P solubilization, 37 bacterial isolates were selected and further screened on a medium containing partially soluble K_2HPO_4 as the sole P source. Positive clear halo production is indicative of non-suppression of the trait when P is available in the environment. This yielded 30 bacterial isolates and they were coded for convenience. Tertiary screening was conducted to evaluate the ability to solubilize ERP, which contains large amounts of impurities that can hinder P solubilization. All 30 isolates tested, showed the ability to solubilize ERP. The mode of P solubilization was elucidated on a PVK-bromophenol blue medium that changed colour from blue to colourless upon pH reduction. All 30 isolates produced organic acids. The efficiency of solubilizing ERP was measured with respect to the amount of organic acid produced. The centre of PVK-bromothymol blue plates were inoculated with a drop of bacteria suspension having a cell density of 10^7/mL and the diameters of the colourless zones produced were measured and compared using the one-way ANOVA. Acid production was significant at 5 % level with a recorded highest value of 4.26 ± 0.06 cm by WRB1 isolate ($p=0.000$) and suitable candidate for development as an effective P biofertilizer.

Keywords: phosphate, microorganisms, volatilization, screening, ERP