Screening of Efficient Phosphate Solubilizing Microorganisms from Rhizosphere of Some Export Agricultural Crops

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Introduction

Nutrient deficiency, chemical fertilizer application and soil deterioration is a well-known cyclic occurrence associated with agriculture in which successive cycles lead to more and more deterioration of soil. With the advancement of technology, sustainable and environmentally friendly solutions are sought to address such problems. Development of biofertilizers to supplement soil with nutrients eases the demand on chemical fertilizers and its negative impacts on the environment. Phosphorus (P) is one of the major plant nutrients. Its deficiency limits the plant growth and development in almost all agricultural soils. Due its high reactivity, applied P readily combines with soil minerals forming insoluble phosphates, lowering its availability to plants. As such, development of an economical and environmentally friendly alternative for successful soil P management is a major concern in crop production and phosphate solubilizing microorganisms (PSM) attracted attention in this regard (Rodriguez and Fraga, 1999).

Export agricultural crops (EAC) are important contributors to the Gross National Product. Although Eppawala rock phosphate (ERP) is applied to EACs concerning the perennial nature of the plant, it has been shown that phosphate availability of ERP for EACs can be increased using PSMs (Mala *et al*, 2010). Hence, the main objective of this study is to develop a phosphate biofertilizer for EACs using suitable phosphate solubilizing microorganisms.

Methodology

Screening Phosphate Solubilizing Microorganisms (PSMs)

Soil samples were collected from rhizospheres of wild and cultivated species of three EACs: Pepper, Cocoa and Cinnamon and screened on Pikovskaya (PVK) medium (Pradhan and Sukla, 2005) containing tri calcium phosphate as the sole P source. PSMs were isolated based on the clear halo production on the medium. Isolated organisms were subjected to a secondary screening on a modified PVK medium containing a partially soluble phosphate to identify the persistent expression of the character. Isolates that showed a positive response were selected for further experimentation and coded for easy reference.

Evaluation of the efficiency of phosphate solubilization

All selected isolates from secondary screening were evaluated for the efficiency of P solubilization using two parameters: quantity of P solubilized at a given time and amount of organic acid secreted as denoted by a decrease in the medium pH. Isolates were assessed in PVK and a modified PVK medium containing ERP as the P source. For fungi, the respective broth media were inoculated with approximately 1×10^8 fungal spores/mL from a respective fungal isolate and incubated at room temperature for 24 and 48 h under 100 rpm constant oscillation. Three replicates were maintained. After incubation, media

were filtered to remove any suspended materials and 10 mL of the filtrate were used to measure pH values and another 20 mL was taken to measure solubilized P content using Murphy and Riley method (Murphy and Riley, 1962). Efficiencies of the bacterial isolates were measured in the same way, except that the inoculum density was adjusted to 1 x 10^8 cfu/mL and the incubation time was increased up to 72 hours.

Statistical Analysis

The experimental data were subjected to one-way ANOVA tests, correlation analysis and multiple mean comparison tests using Minitab 15 statistical software package.

Results and Discussion

Isolation of PSM

From the primary screening, 12 fungi and 41 bacteria were isolated. All the fungal isolates showed the persistence of the trait in the presence of partially soluble P while only 26 bacteria out of 41 were successful.

Evaluation of the efficiency of phosphate solubilization

The amount of phosphates solubilized in both PVK and ERP modified PVK liquid media by fungal and bacterial isolates showed significant variability at 5% significance level (p=0.000). Almost all the isolates exhibited significant increment in soluble P content compared to control, showing effective conversion of insoluble P forms in to soluble forms. The highest P solubilization values of 145.79(±9.09) mg P/L in PVK and 16.15(±9.00) mg P/L in ERP-PVK media were recorded by the fungal isolate MPsRF1. Among bacterial isolates, the highest P solubilization of 222.33(±28.39) mg P/L in PVK medium was recorded by the isolate MPlRB3 while isolate MPlRB2 gave the highest P solubility (68.23(±5.90) mg P/L) in ERP-PVK medium.

Significant pH reductions (p=0.000) were observed in all treatments in comparison to the control. Highest organic acid productions in PVK medium were exhibited by the fungal isolate TClRF1 and the bacterial isolate MPcRB1 with 3.6403(±0.9865) and 3.251695 (±0.152753) pH values, respectively. The highest organic acid producers in ERP-PVK medium were fungal isolates MTcRF1 and MPsRF1, recording a pH of 4.1691(±0.0577) and the bacterial isolates MCvRB1, TUcBB1, TCvRB1, TCvgRB1 and TCvjBB1 (pH 3.94 (±0.058)). This indicates the production of organic acids by the microorganisms to acidulate the medium for P solubilization. Yet, the correlation analysis revealed an absence of a correlation between the decrease in pH and the amount of solubilized phosphates by fungi in both media (r=0.320 (p=0.311) and r=0.534 (p= 0.074) in PVK and ERP-PVK media, respectively) indicating the existence of other mechanisms in fungi to solubilize P other than acid production. In contrast, bacterial isolates showed a significant correlation of r = 0.714 (p=0.000) in PVK medium and r=0.602 (p=0.001) in ERP-PVK medium. This indicates that organic acid production is a major factor contributing to solubilization of phosphates. Accordingly, fungal isolate MPsRF1and bacterial isolates MPlRB2 and MPlRB3, the best solubilizers of phosphates were selected for further studies.

Conclusion

Fungal isolate MPsRF1 and bacterial isolates MPlRB2 and MPlRB3 are effective in phosphate solubilzation and hence are the best candidates for the production of a phosphate biofertlizer. A strong relationship between organic acid production and phosphate solubilization was observed in bacterial isolates whereas in fungal isolates such indication was not found.

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