Isolation of Phosphate Solubilizing Bacteria for Production of Biofertilizer

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Introduction

Phosphorus is one of the essential nutrients for plant growth. It is classified as a major nutrient, meaning that it is frequently deficient for crop production and is required by crops in relatively large amounts for optimum growth and reproduction (Gyaneshwar, *et al.*, 2002;). A greater part of soil phosphorus, approximately 95–99%, is present in the form of insoluble phosphates, due to its fixation with Fe and Al especially in acidic and aerobic soil conditions (Vassileva, *et al.*, 1998). To increase the availability of phosphorus to plants, large amounts of fertilizer are used regularly. But after application, a large proportion of fertilizer phosphorus is quickly transformed into insoluble form. Therefore, very little percentage of the applied phosphorus is used, making continuous application necessary (AbdAlla, 1994). The principle mechanism for mineral phosphate solubilization is the production of organic acids (FNCA, 2006). There are some species of bacteria which have the potential to solubilize organic and inorganic phosphorous in soil. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers (Qurban*et al.*, 2012). Application of Phosphate-solubilizing bacteria into the field has been reported to increase crop yield (Toro *et al.*, 1994). The aim of this study was isolation of phosphate-solubilizing bacteria available in natural environments for the production of biofertilizer and investigating the possibility of utilizing sugar industry distillery spent wash as a carrier medium for the biofertilizer.

Methodology

The soil and roots samples of sugarcane and different grasses were collected from Udawalawa, Pelwatta, and Sewanagala areas. The collected samples were carried to the laboratory and kept at 4°C temperature before analyses. Dilution series of collected samples were prepared, and 0.1 ml aliquots were spread on the Pikovskaya's (PVK) media and incubated at 28±2°C inincubator. Bacteria colonies surrounded by clear zones were elected, further purified and maintained on Pikovskaya's (PVK) medium. All the isolates were designated as UWU Priyadarshani 200 (UWUP200) series. Gram stain test was done to observe the morphology of the isolated bacteria. Four bacteria isolates per plate were inoculated on Pikovskaya (pvk) and modified PVK media with Eppawala rock phosphate and Triple Supper Phosphate (TSP).The bacteria that possess the ability to solubilize phosphate form a clear zone around them. The clear zone and colony diameter were measured 2, 4, 6, and 8 days after the incubation of the plates at 30^0C. The results are expressed as solubilization index according to Premono *et al.*, (1996). Where,

$$\text{Phosphate Solubilization Index} = \frac{\text{Total diameter (colony + clear zone)}}{\text{Diameter of colony}}$$

The distillery spent wash samples were collected from distillery unit of Sewanagala sugar factory. The collected samples were carried to the laboratory, analysis to determine the properties of spent wash. The growth ability of PSB in distillery spent wash was studied. PVK medium was prepared replacing distilled water with the distillery spent wash solution and bacteria were cultured on that media plates and incubated at 28±2°C in incubator to study the growth ability of the bacteria on that media. Then the spent wash samples were prepared with five different concentrations (5%, 10%, 15%, 20% and 100%) of solution. Selected bacteria were inoculated into each concentrates. Serially diluted cultures were plated on PVK medium plates, incubated and the numbers of colonies were counted. Twelve (12) bacteria isolates with high phosphate solubilizing ability were selected for this study.

Results and Discussion

Ninety six (96) bacterial isolates were isolated from the collected samples. Forty three isolates were shown to be as Gram-negative and fifty three isolates were Gram-positive. All isolates were able to solubilize Phosphate on PVK media plates.

Table 1. Phosphate solubilizing index of the bacteria isolates

Phosphate Solubilizing Index	Number of Bacteria Isolates
1	05
1-2	68
2-3	21
3-4	01
Higher than 4	01

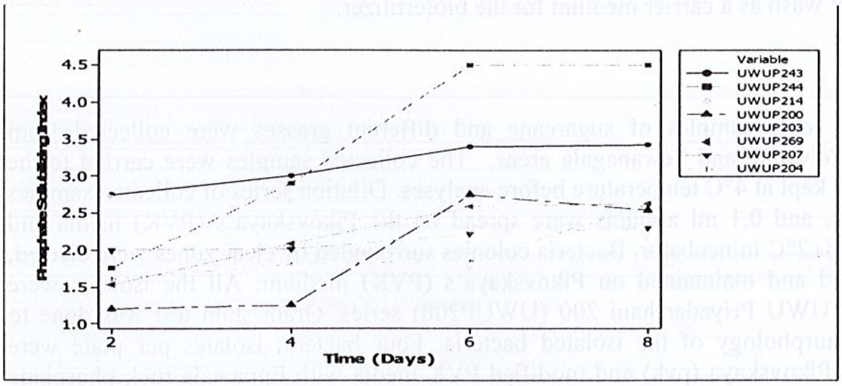

Figure 1: Bacteria isolates with high phosphate solubilizing index

UWUP-244 isolate was recorded significantly higher phosphate-solubilizing index up to 4.5 andUWUP-243, UWUP-214, UWUP-200, UWUP-203, UWUP-269, UWUP-207, and UWUP-204 showed relatively high solubilization index (fig 1). The bacteria isolates UWUP-229, UWUP-270, UWUP-271, UWUP-274 and UWUP-276 showed weak zone of solubilization on pvk plates. Solubilization index of most of the bacteria were increased after 2 and 4 day of incubation, and then the solubilization stopped although the colony was still growing. Therefore, the solubilization index started to decrease after 6 days of incubation.

All isolates were able to solubilize P on PVK media plates, while there was no phosphate solubilization found on media plates with TSP and Eppawala rock phosphate. The phosphate solubilizing bacteria isolates solubilized tricalcium phosphate to a greater extent than rock phosphate (Chakraborty *et al.*, 2010; Chung *et al.*, 2005). These results are in agreement with the findings of Kumar *et al.* (2010) and Parasanna *et al.* (2011).

The spent wash analysis recorded that the pH value of spent wash 4.65, total solids 48.8%, total dissolved solids 37.67%, potassium 0.87 g/L, phosphorous0.56 g/L. Seventy eight (78) bacteria isolates were able to grow on modified PVK medium. But other eighteen (18) bacteria isolates were not grown on those medium plates. All selected isolates (12 isolates) were able to grow in each spent wash solution. The viability of the phosphate solubilizing bacteria in the spent wash solution was measured until one week. Phosphate solubilizing bacteria can survived in all concentration of spent wash solution till one week period. After one week, the 20% spent wash solution supported significantly higher number of viable bacterial cells as compared to that in 5%, 10%, 15% and 100% spent wash solution. Low number of viable bacterial cells was observed in 100% spent wash solution.

Conclusion

Ninety six phosphate solubilizing bacteria were isolated from collected soil and grass root samples. The highest Phosphate solubilizing index (4.5 ± 1.5) is in UWUP-244. Selected (12) phosphate solubilizing bacteria survive seven days in 20% spent wash solution. Therefore the spent wash has potential to utilize as a carrier material for the production of phosphate solubilizing biofertilizer.

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