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Optimisation of Parameters for Fermentation Conditions of Phosphate Solubilising Bacteria

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Abstract

The microorganisms play a vital role in fertility of soil and hence favors' large group of plants. In present study we have successfully isolated certain Gram negative bacteria from soil and access their ability in phosphate solubilization. Effects of temperature, pH and different carbon sources, on phosphate solubilization, by these isolates were also determined. High temperature 40 °C, pH 6, Dextrose as carbon source, Yeast as Nitrogen source and Soyacake as waste source were found to be best for phosphate solubilization by most of the isolates. Among all isolates Bacillus sp followed by Pseudomonas sp was found to be the best phosphate solubilizers. Present study highlights the importance of these plants growth promoting bacteria and their uses for agriculture purposes.

Keywords: *Phosphate solubilization, Indigenous Soil, Temperature, pH, Nitrogen Sources and Carbon sources*

1. Introduction

Phosphorus (P) is one of the major plant nutrients limiting plant growth. Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of P fertilizers (Richardson et al., 1994). The concentration of bio-available P in soil is very low reaching the level of 1.0 mg kg 1 soil (Ezawa *et al.*, 2002). Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively and made available for plant growth (Goldstein, 1994; Khiari and Parent, 2002).

PGPB are defined as free living soil, rhizosphere, rhizoplane, endophytic and phylosphere bacteria that are beneficial for plants under certain conditions (Bashan and De-Bashan, 2005). PGPB generally provide natural and harmless means of improving the growth, yield of crops and minimize the use of agrichemicals. PGPR use one or more direct mechanism to improve the plant growth. These mechanisms include improvement of nutrient uptake by phosphate–solubilization, N_2 fixation and phytohormone production like indole -3- acetic acid. Biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzyme, hydrogen cyanide and siderophore or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence, vigor and yield (Antoun *et al.*, 2001)

Phosphate solubilizing microorganisms have established their role under nutrient inequity conditions. (Iguala *et al.*, 2001). Secretion of organic acids and phosphatases to solubilize insoluble phosphate to soluble forms are common in this group (Kim *et al*., 1998). Although several phosphate solubilizing bacteria occur in soil, their numbers are not adequate to compete with other bacteria commonly established in the rhizosphere (Glick *et al*., 1995).

Hence the present study was to investigate the phosphate solubilizing potential of phosphate solubilizing bacterial strain isolated from soil sample.

2. Materials And Methods

2.1 Isolation and Identification of Phosphate Solubilising Bacteria

The soil samples were collected from Soil. Microorganisms mainly the bacterial strains were isolated by serial dilution of soil samples and an aliquot of 100µl from decimal dilutions were considered. The samples were spread onto the Pikovskaya's agar medium and incubated for 24 hours at room temperature for the detection of phosphate solubilising bacteria. The prominent colonies showing a clear zone on the Pikovskaya's agar were selected and purified by repeated culturing on nutrient agar medium. The bacteria were identified and screened by their colony characteristics Staining techniques, microscopic observations and biochemical characteristics and molecular characterization.

2.2 Estimation of Phosphorus

Cultures were harvested after different growth periods in order to record the change in pH and concentration of P released in the medium. After centrifuging at 10,000 rpm for 15 min, the pH of the culture medium was measured with a pH meter equipped with a glass electrode. Dissolved phosphate concentration in the culture filtrate was determined by vanado-molybdate method.

2.3 Effect Of Various Parameters On Efficiency Of Phosphate Solubilization

2.3.1 Effect of pH on efficiency of phosphate solubilisation

Effect of pH on efficiency of phosphate solubilization Optimal media and temperature was used , but the pH of the media was set at pH 6 , pH 7, pH 8, pH 9, pH 10 and pH 11 using NaOH or HCl and growth recorded.

2.3.2 Effect of various Nitrogen sources on efficiency of phosphate solubilisation

Effect of various Nitrogen sources like $(NH_4)_2SO_4$, Urea, Yeast, and Peptone were studied in PVK Broth. The isolates were checked for solubilization activity in PVK broth amended with different phosphorus source. Inoculation was carried out by using pure colony of a bacterial from LB Agar plate. Flask was incubated at 37^{o}C for 4 days

respectively (Fasim *et al*., 2002). Turbidity in media was observed on second in bacterial culture Efficiency of phosphate solubilization was calculated as mentioned above.

2.3.2 Effect of various Carbon sources on efficiency of phosphate solubilisation

Effect of various carbon sources like glucose, fructose, maltose, and starch were studied in PVK Broth. The isolates were checked for solubilization activity in PVK broth amended with Tricalcium phosphate. Inoculation was carried out by using pure colony of a bacterial that had been grown, in LB Agar and PVK Agar at 37^{o}C for 4 days respectively (Fasim *et al*., 2002). Efficiency of phosphate solubilization was calculated as mentioned above.

2.3.4 Effect of temperature on efficiency of phosphate solubilization

Media composition to which the bacteria responded best was used as substrate. Bacteria were inoculated and the culture was maintained at 35^{o}C, 40^{o}C, 40^{o}C, 50^{o}C, 55^{o}C and 60^{o}C and growth recorded as described above.Efficiency of phosphate solubilization was calculated.

2.3.5 Effect of Agrobased waste material on efficiency of phosphate solubilization

Agrobased waste material used for the study were Ground nut cake, Coconut cake, Wheat bran and Soya cake. Inoculation was carried out by using pure colony of a bacterial that had been grown, in LB Agar and PVK Agar at 37oC for 4 days respectively (Fasim *et al.*, 2002). Efficiency of phosphate solubilization was calculated as mentioned above.

3. Results And Discussion

3.1 Screening of Ecological Sources and Identification of Phosphate Solubilising Bacteria:

Location of soil sample collection was chosen because of the possibility of occurrence of phosphate solubilizing microbes. Sampling was done at various sites, in order to maintain uniform representation of the micro flora in and around the collection area. For initial growth of micro flora PVK medium was used embedded with tricalcium phosphate as phosphate source.

Preliminary investigation of these cultures in PVK media embedded with tricalcium together with agitation and aeration for 4 day allowed microbial solubilization of P with fall in pH. This was followed by the dilution plating in order to isolate the single colonies. The concentration of 1.5% agar adequately maintained the desired texture of the solid medium, while simultaneously retained enough moisture to promote microbial growth.

Its been reported that organisms capable of doing phosphate solubilization give clear zone around the colony by which it can be concluded that they are phosphate solubilizing microorganisms. Four colonies gave clear zone by which it was confer that they can solubilize phosphate. The bacterial isolates were further characterized by a series of biochemical reaction and identified as *E.coli, Bacillus sp., Pseudomonas sp.,* and *Serratia* sp.

3.2 Phosphate Solubilising activity of Bacteria

3.2.1 Effect of pH on efficiency of phosphate solubilization:

Results show that all the isolates were able to P solubilize in the pH range of 6 to 11. Maximum P solublization and growth was monitored at pH 6. At the same time retardation in growth and P solublization was observed at pH 8 (Figure 1). Acid predation has been reported to be a major mechanism involved in solubilization. pH is the vital factor in solubilization , in most of cases P solublization is the result of organic acid production. There are also other mechanisms such as production of bacterial metabolites and siderophores have also attributes to solubilization(Sadaf Shahab, 2008)

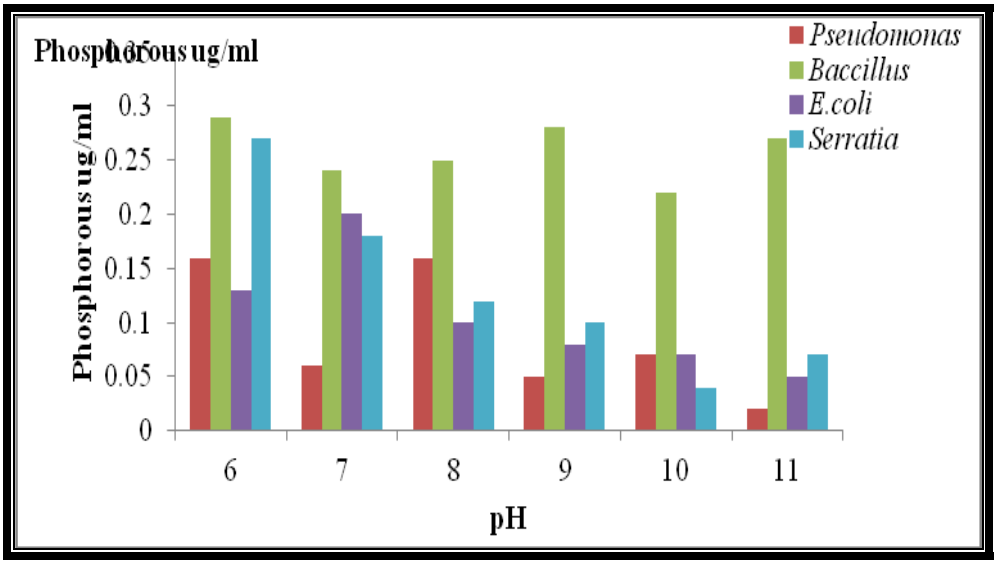

Figure 1: Effect of pH on efficiency of phosphate solubilisation

3.2.2 Effect of Nitrogen Sources on efficiency of phosphate solubilization:

While studying the effect of various nitrogen sources on the phosphate solubilization it was found that Yeast showed maximum P solubilization followed by $(NH_4)_2SO_4$ (Figure 2). In control with no nitrogen source substantial growth was there and drop of pH was there and little solubilization of P which may be due to yeast extract and glucose in medium which was utilized by bacteria as nitrogen source. A number of fungi and bacteria have been reported of being able to solubilized phosphate only in the presence of ammonium as the nitrogen sources (Illmer and schinner, 1992).

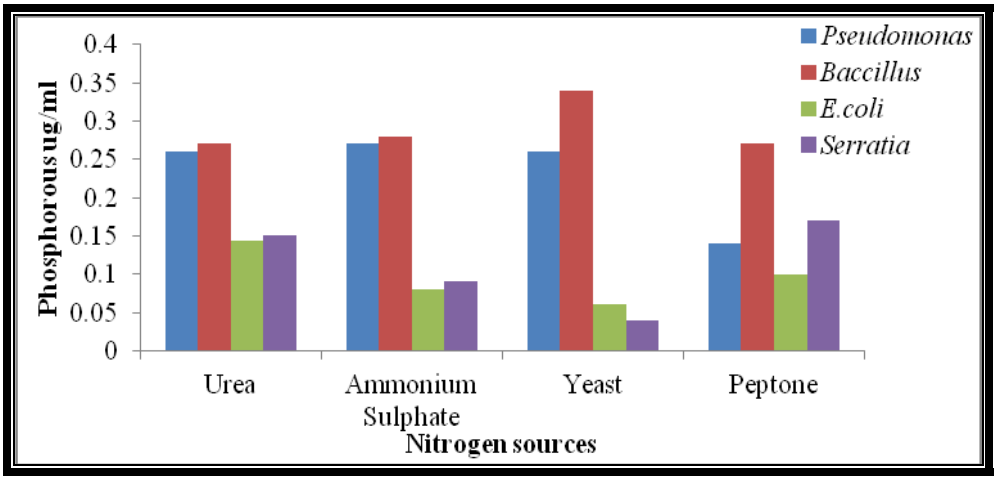

Figure 2: Effect of nitrogen sources on efficiency of phosphate solubilisation

3.2.3 Effect of Carbon sources on efficiency of phosphate solubilization:

When various carbon sources were used to study the P solubilization it was found that PVK with dextrose gave maximum P solubilization with a fall in pH to 4.2 followed by fructose (Figure 3). The solubilization ability of microorganism is related to its organic acid production; however nature of acid produced is also important (Vassileva, 1998). Fasim *et al*. 2002 have reported bacterial isolates which solubilize P only in presence of glucose and while co solubilization in presence of gluconate, galactose and fructose.

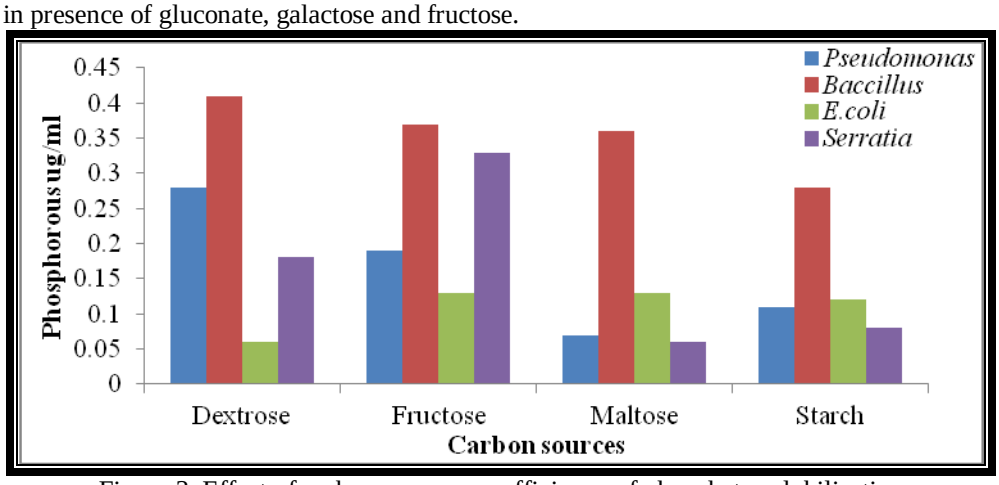

Figure 3: Effect of carbon sources on efficiency of phosphate solubilisation

3.2.4 Effect of Temperature on efficiency of phosphate solubilization:

For all the isolates 40° C temperature is the optimum temperature for the growth and phosphate solubilization. P solublization was also seen at 35°C and it was found that after 40°C there was growth retardation and fall in P solublization (Figure 4). Different temperature have been reported by earlier workers for solubilization , most of them have found 25oC to 28oC to be optimum temperature (Sayer and Gadd, 1998). There are reports that have shown P solublization at 45oC and some at 10oC. This shows that bacteria adapt to their indigenous environment so their metabolic activites are linked to the temperature of the environment. (SadafShahab, 2008,Varsha NHH (2002).

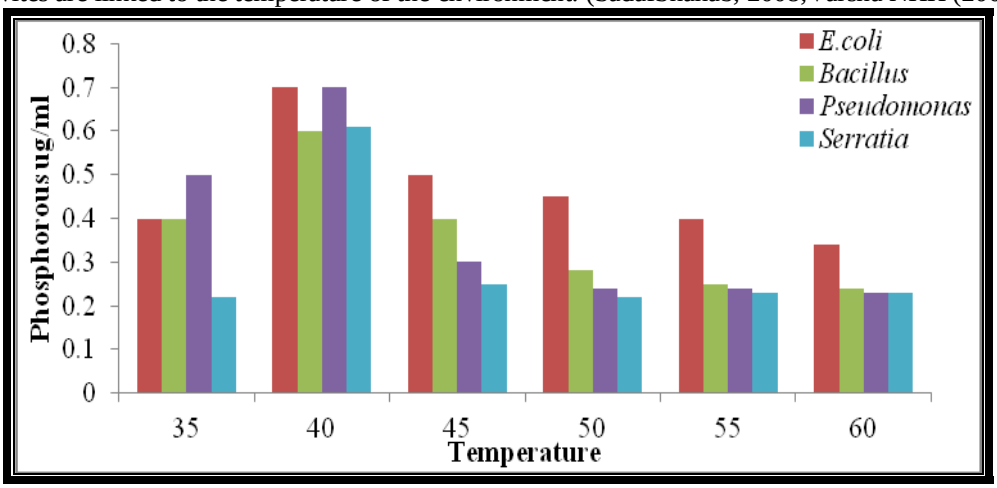

Figure 4: Effect of temperatures on efficiency of phosphate solubilisation

3.2.5 Effect of Agrobased Waste on efficiency of phosphate solubilization:

Effect of various agrobased waste sources like Groundnut cake, Soyacake, Coconut cake and Wheat bran showed that Soya cake is the best agrobased waste. Next was coconut cake which showed medium P solubilization. (Figure 5).

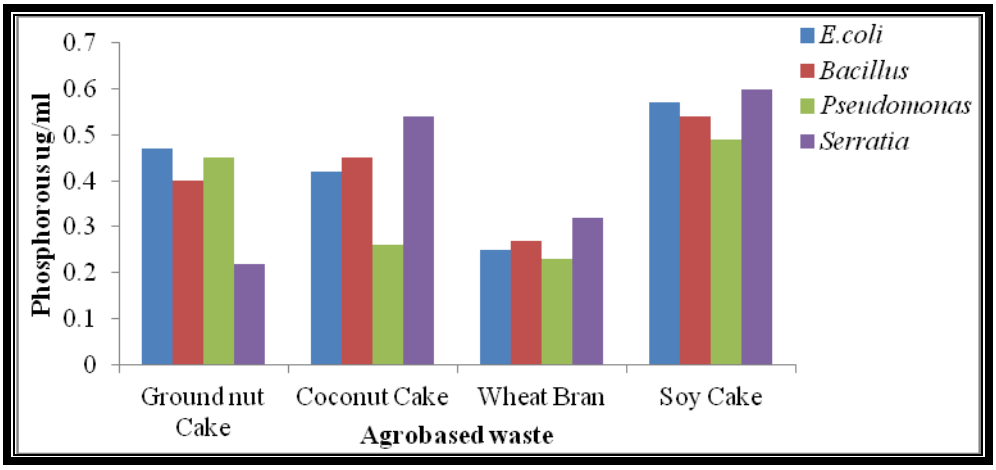

Figure 5: Effect of nitrogen sources on efficiency of phosphate solubilisation

A bacteria may be called as PGPRB if it has the ability to solubilize the phosphate and make them available to plant for their growth, if they can stimulate plant growth, if they have aggressive colonization around root, if they can control Phytopathogens (Vessey 2003) or at least have any two of these criteria.

Phosphate solubilizing microorganisms (PSM) which can solubilize phosphates and can demonstrate phosphate solubilizing ability (Perrez *et al*.,2007). The common feature of gram negative bacteria is mineral phosphate solubilization which has already been reported (Khan *et al*., 1995). The present results are in agreement with (Mello et al., 2011), as reported that 90 % isolates of rhizosphere were gram negative.

Temperature, pH, carbon and nitrogen sources are different parameters and their effects were observes on phosphate solubilizing ability of bacteria. The isolates were grown at 37^0C which is the best suited temperature for growth of mesophilic bacteria (Fasim *et al*., 2002). For optimum temperature there were differ the views of different researchers according to Shahab and Ahmed 2008 25^0C is the optimum temperature for phosphate solubilization. Kang (2002) reported that in few studies 28^oC was found as optimum temperature for phosphate solubilization. The solubilization can occurred at extreme temperatures i.e. 45^0C and 15-4^oC respectively. According to these studies, the bacteria can adapt themselves and show their activities according to conditions.

Conditions like type of organisms, pH., nitrogen source in media and carbon play an important role in solubilization of phosphates (Nahas 1996). For best solubilization pH of strain should be 5-7 and there is no growth below 5 and above 8 pH. These results match with the results of Farhat et al., 2009.

To check their effect on phosphate solubilizing ability different sources of carbon were added to minimal medium. The results of Narsian and patel (2000) are also in agreement with the above results according to which A. aculeatus was able to show phosphate solubilization in the presence of different carbon sources in order of >glucose >fructose >mannitol>xylose >aranbinose >maltose >sorbitol>sucrose >glycerol >galactose>Lactose. These results are same as that of Nautiyal, (2000). *Serratia marcesance* CTM 50650 can use different carbon sources with highest solubilization in presence of Glucose (Farhat et al., 2009).

$(NH_4)_2SO_4$, Urea, Yeast and Peptone are four different nitrogen sources were used to determined the effect of nitrogen sources on phosphate solubilizing ability of isolates. All the isolates showed a big halo zone in the presence of Yeast followed by Ammonium sulphate as a source of nitrogen. According to several studies ammonia is a better source of Nitrogen as compared to nitrate (Illmer et al., 1995).

4. Conclusion

In the present research work we able to isolates such bacteria which can promote the growth of plants from indigenous soil samples. These bacteria were quite effective in phosphate solubilization and effect of various factors on growth were also studied, however, further research is needed to explore other potentials of these isolates and to find out genetic stability of these properties.

5. References

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