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Solubilisation of Uganda low grade Rock Phosphate by *Pseudomonas fluorescence*

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Abstract

Most agricultural soils contain large reserves of phosphorus (P), a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants. In the present study phosphate solubilising activity of Pseudomonas fluorescence against three types of rock phosphate (RP) were studied with respect to different parameters like temperature, incubation period, pulp density and effect of different carbon and nitrogen sources. Results indicated that the lower the concentration of the phosphate in the leaching broth the greater was the dissolution percentage of P_2O_5. A maximum of 44.70, 48.84 and 56.17(mg%) of P_2O_5 solubilisation were obtained after 15 days of incubation at 35ºC from West valley, North valley and South valley rock phosphate, respectively at 0.5 % pulp density. Acidic pH medium was favourable for phosphate solubilisation in all the experiments. Among the carbon sources glucose followed by maltose and sucrose supported the maximum RP solubilization in the presence of 0.5% pulp density as the optimum concentration. Nitrogen in the form of ammonium was very effective in solubilizing rock phosphates by P. fluorescence.

Keywords: *Pseudomonas fluorescence,* phosphate solubilisation, rock phosphate.

Introduction

In the mining of phosphate rock the high grade materials are taken up by fertilizer industries for the manufacture of phosphate fertilizers and a huge quantity of low grade rock phosphates (RP) remain unutilized. As high grade rock phosphate deposits are being depleted day by day low grade rocks containing various impurities will be the alternative source in future. Low grade rock phosphates are not suitable for direct use in agriculture because of their low solubility. Various microorganisms have been reported to solubilise rock phosphates[1,2,3].

In soil, phosphorus (P) is the major nutrient after nitrogen (N) that limits plant growth due to its deficiency[4]. Phosphate fertilizer is the main source of plant available P in agriculture soils, but almost 75 to 90% of added P fertilizer is precipitated due to the formation of complexes by iron, aluminum and calcium present in the soils[5] resulting in phosphorus deficiency[6]. In order to compensate this natural deficiency many expensive phosphate based chemical fertilizer are used in agriculture to improve crop yield [7]. A large portion of phosphate applied to soil as chemical fertilizer is rapidly immobilized after application and becomes unavailable to plants. Current concept in sustainability involves application of less expensive natural resources like rock phosphate which is rich in plant nutrients. Microbial solubilisation of rock phosphate, especially low grade is gaining greater attention in agriculture because of the negative environmental impacts of chemical fertilizers and their increasing costs.

In view of these the present study was undertaken to establish the effect of different temperature, solid concentration and duration on P solubilisation from low grade Uganda rock phosphate by *Pseudomonas fluorescence*.

Material and Methods

Low grade rock phosphate samples from different regions of Uganda (West Valley, North Valley and South Valley) were obtained.

Microorganism: The strain *Pseudomonas fluorescence* was obtained from IMT, Chandigarh and was used for leaching purpose.

Culture medium: In Pikovskaya broth tri-calcium phosphate (TCP) was replaced by different rock phosphate like West valley, North valley and South valley of Uganda. The results on chemical composition of the samples have been presented in table1.

Solubilisation capacity of rock phosphate in liquid culture: P solubilisation activity was carried out in 100 ml PVK broth medium amended with 0.5gm, 1.0 gm, 3.0 gm, 5.0gm, 7.0gm and 10.0gm pulp density of rock phosphate. The pH was

adjusted to 7.0 before sterilization. 10 ml suspension of bacterial culture containing 10^5 cells per ml was added to the broth in triplicate. A control was also kept without inoculation. To study the effect of incubation temperature on P solubilisation the cultures were incubated on rotary shaker at five different temperatures from 25 °C to 45 °C up to 30 days. Cultures were harvested in every 3 days interval by centrifuge in 10,000 rpm for 10 minutes. Water soluble P in the culture filtrate was estimated by chlorostannous reduced molybdophosphoric acid blue method described by Jackson[10]. The pH of the leach liquor was measured by a pH meter.

Results and Discussion

The chemical analysis of rock phosphate samples were given in table-1. *Pseudomonas fluorescence* was capable of solubilising all the three forms of rock phosphate but the solubilisation levels of different rock phosphates varied, i.e 44.70, 48.84 and 56.17% in West valley, North valley and South valley rock phosphate, respectively. Among the different rock phosphate the solubilisation was more in South valley (56.17 mg %). The maximum solubilisation was achieved on 15th day at 35^{0}C at 0.5% pulp density. Hence, the solubilisation of rock phosphate depends on the concentration of nutritive medium, decrease in pH, incubation time and pulp density[10].

Rock phosphate solubilisation and pH: A reduction in the pH due to acidic metabolites produced by the bacteria caused phosphate solubilisation. However, no significant relationship could be established between the quantity of phosphate solubilised and reduction in pH. Previous reports of different workers also support our present findings[11]. The maximum reduction in pH to 3.23 was observed on 15th day at 0.5 % pulp density for West valley rock phosphate. The observed pH fluctuation is due to varied rock phosphate solubilisation and the low pH was observed with lowest solid concentration [12] (Figure 4). It was reported that media receiving lowest quantities of rock phosphate remained more acidic than media receiving higher amounts[13]. The maximum rock phosphate solubilisation was achieved at the lowest value of culture pH [14].

Effect on Pulp density: In the present study the maximum solubilisation of 56.17% by *P.fluorescence* was found at 0.5 % of pulp density in South valley rock phosphate. The results indicate that the lower the quantity of rock phosphate the greater the conversion of P_2O_5 in soluble form after leaching. The results presented in (table-2) showed that the highest P solubilisation of 44.70, 48.84 and 56.17 % was found in West valley North valley and South valley respectively and with increasing dosage of rock phosphate, the phosphorous solubilisation decrease. This might be due to the inhibitory effect exerted by Al, Fe and Ca present in rock phosphate on the growth of organism. It appears that the rock phosphate concentration was the main factor determining the level of solubilisation by *P.fluorescence*[15] found that a great part of the acid was neutralized by free carbonates derived from sedimentary rock phosphate. Therefore, high amount of rock phosphate may require more organic acid for their solubilisation and an optimal ratio must be found between the two components.

Effect on culture time: Effect of leaching duration is presented in Figure-2. It was found that the P solubilisation varies with culture time. With increasing incubation period the P solubilisation increases rapidly. The maximum solubilisation was achieved on day 15. After 15 day the P solubilisation decreases. The decrease in solubilisation activity after a particular incubation period might be due to the availability of soluble form of phosphate in the nutritive media that has an inhibiting effect on further phosphate solubilisation [16]. It was reported that the drop in solubilisation after a maximum value might be due to deficiency in nutrients in the culture medium[17].

Effect on temperature: Figure-1 showed that the P solubilisation was dissimilar under different culture temperature. 35°C was the optimum temperature for P solubilisation by *Pseudomonas fluorescence*. However, it can also be seen from fig.-1 that when incubation temperature was increased there was no improvement in leaching. Earlier workers have studied the effect of different temperature on P solubilisation and have reported 25°C as the optimum temperature[18,19]. Some workers have reported 28°C as the optimum temperature[20,21,22,23]. This clearly suggests that different strains adapt to their native environmental temperature so their metabolic activities are linked to the temperature.

Effect of carbon and Nitrogen sources: Phosphate solubilization activity of *Pseudomonas fluorescence* was evaluated in the presence of six carbon and six nitrogen sources, by replacing glucose and $(NH_4)_2SO_4$, respectively of the PVK medium. This strain demonstrated diverse levels of phosphate solubilization activity in the presence of various carbon and nitrogen sources. Production of acids was greatly affected by the nature of carbon sources[24]. Glucose and maltose decreased the pH of the medium to maximum extent and caused highest solubilization of phosphorus, followed by sucrose, xylose and galactose (table-4). The solubilizing ability of a microorganism is related to its organic acid production; however the nature of the acid produced is also important. Nitrogen salts having either ammonium group or nitrate group or both were used as nitrogen source for the study. $(NH_4)_2SO_4$ was found to be best in reducing the medium pH to 3.58, 3.53 and 3.33 and simultaneous solubilization of 44.70, 47.19 and 56,17% of P_2O_5 from West valley, North valley and South valley respectively, out of all the nitrogen sources used (Table 4). It was thus observed that *Pseudomonas fluorescence* was able to utilize $(NH_4)_2SO_4$ most efficiently to decrease the pH of the medium for P solubilization.

A number of bacteria had been reported of being able to solubilize phosphate only in the presence of ammonium as the nitrogen source [25]. The nitrogen source in salt form seems to be important, as it was necessary for increased phosphate

solubilization of rock phosphate[14]. Previous reports on phosphorus solubilizing microorganisms have attributed the differences in phosphate solubilization (when ammonium and nitrate were used) to the use of different mechanisms for the generation of acidity in the culture. Our observation was also similar. However, no significant relationship could be established between the quantity of phosphate solubilized and drop in pH.

Table-1
Chemical composition of different rock phosphate of Uganda (Values are in percentage except LOI)

Rock phosphate	P_2O_5	CaO	MgO	Fe_2O_3	Al_2O_3	SiO_2	LOI
West valley	14.63	22.56	0.56	31.591	23.70	2.06	9.32
North valley	16.70	27.34	0.51	36.44	16.20	2.08	8.64
South valley	19.04	26.89	0.57	32.25	13.31	2.17	8.02

Table-2
Effect of Pulp density on P solubilisation by *P.fluorescence* at 35° C after 15 days of incubation in different rock phosphate

Pulp density (%)	West valley		North valley		South valley	
	P_2O_5 (mg)%	Final pH	P_2O_5 (mg)%	Final pH	P_2O_5 (mg)%	Final pH
0.5	44.70±0.06	3.58±0.02	48.84±0.06	3.43±0.05	56.17±0.06	3.33±0.06
1.0	32.45 ± 0.06	3.93 ±0.02	37.28±0.06	3.73±0.05	43.26±0.06	3.48±0.06
3.0	29.25 ±0.02	4.39 ±0.02	32.44.±0.21	4.10±0.01	39.17±0.28	3.84±0.05
5.0	18.62 ± 0.03	4.53 ±0.06	21.49±0.01	4.29±0.06	25.88±0.55	3.92±0.05
7.0	11.66 ± 0.23	4.75 ±0.03	19.86±0.03	4.84±0.07	21.43±0.69	4.88±0.10
10.0	5.30 ± 0.08	5.83 ±0.07	8.64±0.22	5.34±0.03	14.78±0.10	5.12±0.16

Values are expressed as mean ± standard deviation of three independent readings

Table-3
Effect of incubation period on P solubilisation by *P. fluorescence* in West Valley, North valley and South valley rock phosphate in 0.5% pulp density

Days	West valley		North valley		South valley	
	P_2O_5 (mg %)	Final pH	P_2O_5 (mg %)	Final pH	P_2O_5 (mg %)	Final pH
3	16.12±0.10	4.68±0.02	23.94±0.07	4.71±0.040	25.31±0.04	3.74±0.27
6	21.25±0.02	4.53±0.01	35.68±0.04	4.85±0.040	39.47±0.16	3.84±0.05
9	34.45±0.15	4.45±0.02	40.37±0.05	3.77±0.072	45.26±0.05	3.65±0.04
12	39.46±0.21	3.85±0.04	43.99±0.11	3.69±0.075	51.22±0.11	3.49±0.10
15	44.70±0.06	3.58±0.02	48.84±0.06	3.43±0.055	56.17±0.06	3.33±0.06
18	44.45±0.09	3.63±0.04	47.19±0.11	3.53±0.049	55.11±0.10	3.80±0.07
21	42.36±0.27	3.78±0.05	46.87±0.01	3.50±0.026	54.66±0.10	3.83±0.15
24	41.15±0.11	3.80±0.01	46.40±0.22	3.72±0.11	53.58±0.33	4.07±0.07
27	39.89±0.26	3.86±0.03	44.77±0.33	4.12±0.03	52.62±0.15	4.32±0.18
30	30.16±0.04	3.97±0.03	44.21±0.67	4.37±0.07	52.85±0.64	4.50±0.20

Values are expressed as mean ± standard deviation of three independent readings

Table-4
Effect of different carbon and nitrogen sources on P solubilisation by *P fluorescence* in Uganda rock phosphate at 35° C in 0.5 % pulp density after 15 days of incubation

Carbon source	West valley		North valley		South valley	
	P_2O_5 (mg)%	Final pH	P_2O_5 (mg)%	Final pH	P_2O_5 (mg)%	Final pH
Glucose	44.70±0.06	3.58±0.02	47.19±0.11	3.53±0.049	56.17±0.06	3.33±0.06
Maltose	34.10±0.06	3..76±0.06	35.78±0.04	3.89±0.06	44.78±0.11	3.76±0.12
Mannitol	9.13±0.05	6.63±0.05	8.66±0.06	6.26±0.04	12.67±0.04	2.21±0.06
Xylose	24.28±0.09	5.21±0.09	21.98±0.08	5.21±0.08	31.17±0.02	5.16±0.04
Sucrose	29.67±0.12	4.8±0.12	26.44±0.11	4.64±0.02	38.56±0.06	4.17±0.02
Galactose	12.12±0.14	5.01±0.14	13.18±0.04	5.12±0.11	21.64±0.11	5.11±0.14
Nitrogen source						
Ammonium sulphate	44.70±0.06	3.58±0.02	47.19±0.11	3.53±0.049	56.17±0.06	3.33±0.06
Calcium nitrate	30.81±0.58	5.31±0.08	32.64±0.06	5.89±0.06	38.23±0.16	5.32±0.02
Sodium nitrate	39.02±0.08	4.89±0.02	37.72±0.11	5.13±0.08	42.14±0.14	5.11±0.08
Ammonium nitrate	41.27±0.06	4.35±0.02	44.14±0.14	4.34±0.02	52.15±0.11	4.16±0.06
Potassium nitrate	34.12±0.49	5.95±0.05	35.33±0.16	5.23±0.06	40.68±0.08	5.44±0.08
Urea	7.23±0.12	6.11±0.21	9.87±0.26	6.21±1.41	14.87±0.44	6.08±1.11

Values are expressed as mean ± standard deviation of three independent readings

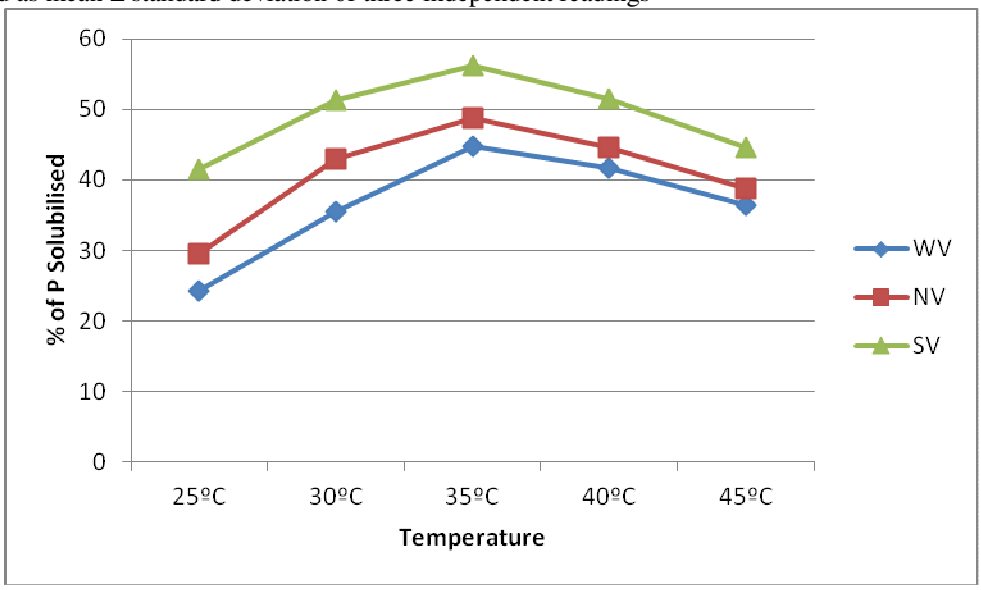

Figure-1
Effect of temperature on P solubilisation by *P.fluorescence* in different Rock Phosphate

Conclusion

Present investigation indicates that all the process parameters have significant influence on the solubilisation of P from different rock phosphate used. A maximum of 56.17% of P as P_2O_5 could be solubilised from South valley rock phosphate after 15 days of incubation at 35°C with 0.5% pulp density. The use of *P.fluorescence* strain as a phosphate solubiliser has promising potential. The addition of low grade Uganda rock phosphate along with *P. fluorescence* can help to enhance the phosphorous mobilization and availability in P deficient soil for plant growth in lieu of chemical fertilizers.

References

1. Chunqiao Xiao, Ruan Chi, Xiao Pan, Feng Liu and Jiawi He, Rock phosphate solubilisation by four yeast strains, *Annals of Microbiology*, DOI 10.1007/s 13213-012-0458/z **(2012)**

2. Prasanna Aadarsh, Deepa V., Murthy Balakrishnan P, Deccaraman M., Sridhar R., Dandapani P, Insoluble phosphate solubilisation by Bacterial strains isolated from Rice rhizosphere soil from Southern India**,** *Int. J. of Soil Sc.,* **6(2)** 134-141 **(2011)**

3. Raval A.A and Desai P.B., Rhizobacteria from Rhizosphere of Sunflower (Helianthus annus. L.) and their effect on plant growth, *Research J. of Recent Sciences,* **1(6)** 58-61 **(2012)**

4. Fernandez L.A., Zalba P., Gomez M.A., Sagordoy M.A., Phosphate solubilisation activity of bacterial strains in soil and their effect on soybean growth under green house conditions, *Biol. Fertil. Soils* **43**, 805-809 **(2007)**

5. Turan M.N., Ataoglu and Sahin F., Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture, *J. Sustainable Agri.,* **28**, 99-108 **(2006)**

6. Arcand M.M. and Schneider K.D., Plant and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: A review, *Ann. Acad. Bras. Cienc*., **78**, 791–807 **(2006)**

7. Gyaneshwar P., Kumar G.N., Parekh L.J. and Poole P.S., Role of soil microorganisms in improving P nutrition of plants, *Plant Soil*, **245**, 83–93 **(2002)**

8. Sharma Sonam, Kumar Bijay and Tripathy Ram Babu, Isolation of phosphate solubilising microorganism from soil, *J. Microbiol. Biotech Re*s, **1(2),** 90-95 **(2011)**

9. Jackson M.L., Soil chemical analysis, Prentice-Hall, New Delhi, India **(1967)**

10. Zhu Fengling, Qu Lingyun, Hong Xuguang and Sun Xiuqin, *Evidence based Complementary and alternative medicine* **11,** 1-6 **(2011)**

11. Reddy M.S., Kumar S., Babita K. and Reddy M.S., Biosolubilization of poorly soluble rock phosphate by *Aspergillus tubingensis* and *Aspergillus niger*, *Bioresource Technology,* **84**, 187-189 **(2002)**

12. Gaur A.C. and Sacher S., Effect of rock phosphate and glucose concentration on phosphate solubilisation by *Aspergillus awamori*, Curr Sci., **49**, 553-554 **(1984)**

13. Dave A. and Patel H.H., Phosphorous uptake by *P. fluorescence* and effect of external phosphate on microbial solubilisation, *Asian J. of Microbiol. Biotech. Env. Sc*., **10(1),** 1-4 **(2008)**

14. Ivanova R., Bozinova D. and Nedialkova K., Rock phosphate solubilisation by soil bacteria, *Jr. of the Univ. of chemical Technol. and Metallurgy*, **41(3),** 297-302 **(2006)**

15. Kpomblekou A.K. and Tabatabai M., AEffect of organic acids on release of phosphorous from phosphate rocks*, Soil Sci.,* **158**, 442-444 **(1994)**

16. Narsian V., Thakker J. and Putei H.H., Mineral phosphate solubilisation by *Aspergillus aculeatus*, *Ind. J. Exp. Biol.,* **33**, 91-93 (**1995**).

17. Gaind S. and Gaur A.C., Influence of temperature on the efficiency of phosphate solubilising microorganisms, *Ind. J. Micro boil,* **30**, 305-310 **(1990)**

18. Sayer J.A. and Gadd G.M., Solubilization and precipitation of metals by fungi, *Minerol. Soc. Bull*., 3-5 **(1998)**

19. Gharieb M.M., Sayer J.A. and Gadd G.M., Solubilization of natural gypsum ($CaSO_4.2H_2O$) and the formation of calcium oxalate by *Aspergillus niger* and *serpula himantioidies, Mycol. Res,* **102**, 825-830 **(1997)**

20. Kim K.Y., Jordan D. and Kirshanan H.B., *Rahnella aquatilis*, a bacterium isolated from soyabean rhizosphere, can solubilize hydroxyapatite, *FEMS Micobiol. Lett,* **153**, 273-277 **(1997)**

21. Seshadre S., Muthukumarasamy R, Lakshminarasimhan C and Ignaacimuthu S., Solubilization of inorganic phosphates by *Azospirillum halopraeferans*, *Curr. Sci.,* **79(5),** 565-567 **(2002)**

22. Kang S.C., Ha G.C., Lee T.G. and Maheshwari D.K., Solubilization of insoluble inorganic phosphates by a soil inhabiting fungus sp. Ps 102, *Curr. Sci*., **79(5),** 439-442 **(2002)**

23. Rosado A.S., De Azevedo F.S., Da Croz, D.W. Van Elas and J.D. Seldin L., Phenotypic and genetic diversity of *Paeni bacillus azatofeixans* strains isolated from the rhizosphere soil of different grasses, *J. Appl. Microbiol*., **84**, 216-226 **(1998)**

24. Gilberto O. Mends, Carla S. Dias, Ivo R. Silva, Jose Ivo Ribeiro Junior, Olnto l. Pereira and Mauricio D. Costa, Fungal Rock Phosphate solubilisation using sugar cane baggase, *World J. of Microbiology and Biotechnology*, **12(1)** 43-50 **(2013)**

25. Varsha N.H.H., *Aspergillus aculeatus* as a rock phosphate solubilizer, *Soil Biol. Biochem,* **32**, 559-565 **(2002)**