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Original Research Paper

Phosphate Solubilisation by Four Anoxygenic Phototrophic Bacteria Isolated From Leather Industry Effluents

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ABSTRACT

Four anoxygenic phototrophic bacteria, *Rhodobacterium (Rb.) capsulatus, Rhodopseudomonas (Rps.) acidophila, Rps. rutila* and *Rhodospirillum (Rsp.) rubrum,* isolated from wastewaters, were tested for their ability to solubilize tricalcium and dicalcium phosphate under different conditions. *Rps. rutila* was superior to other bacteria under investigation in solubilisation of tricalcium phosphate. *Rsp. rubrum* was poor in solubilization of tricalcium phosphate. *Rsp. rubrum* and *Rb. capsulatus* could solubilise dicalcium phosphate with almost same efficiency while *Rps. acidophila* was more efficient in solubilization of dicalcium phosphate. Thus, these bacteria can be exploited in solubilization of phosphate.

INTRODUCTION

Phosphorus is one of the most important plant nutrients and also a key element in soil microbiological processes. Soil microorganisms play an important role in solubilisation of bound phosphates which are either in the form of organic or inorganic phosphate compounds and make available to the plants (Singh & Amberger 1990, Seshadri et al. 2004, Sujatha et al. 2004). Various methods to solubilize insoluble phosphate and to increase the phosphate availability by means of phosphate solubilizing bacteria have been tried (Narula et al. 2000). Gothwal et al. (2006) studied the phosphate solubilising capacity of rhizosphere bacterial isolates from economically important desert plants. Influence of pH, organic acids and sugars on phosphate solubilization by phosphate solubilizing microorganisms was studied by Narula et al. (2007).

The participation of phototrophic bacteria such as ***Rhodocyclus*** in phosphorus removal was investigated by Julie et al. (2002). Excessive concentration of phosphorus is the most common cause of eutrophication. Hence, phosphorus removal is of paramount significance in the regulation of eutrophication. However, there is little or no information available on phosphate solubilisation by these bacteria. Hence, solubilization of phosphate by four anoxygenic phototrophic purple non sulphur bacteria was studied and the results have been discussed in this paper.

MATERIALS AND METHODS

The anoxygenic photosynthetic bacteria were isolated by enrichment techniques (Biebl & Pfennig 1981) by adding the effluent water sample into the Biebl and Pfennig's medium. The culture bottles, thus, prepared were incubated anaerobically in light. Identification of isolated bacteria was done by studying the cultural, morphological and biochemical characteristics as suggested by Holt et al. (1994). The capacity of photosynthetic bacteria to solubilize phosphorus was determined by

inoculating 1 mL log phase cultures of four anoxygenic phototrophic bacteria into 15 mL of liquid medium containing 15 mg of phosphorus. The tubes thus inoculated were incubated at 30 ± 2°C under the light intensity of 2000 Lux in screw cap tubes. At the end of 4, 8, 12 and 16 days of incubation the contents of the vials were harvested by subjecting to centrifugation at 10,000 rpm for 10 minutes. The supernatant was collected and the amount of phosphorus was estimated by Subbarao & Bajpai (1965) method. To 2 mL of culture filtrate, 2 mL of ammonium molybdate and 2 mL of amino-naptholsulphonic acid reagent were added and diluted to 10 mL with distilled water. The colour intensity was measured at 660 nm after 5 minutes in a spectrophotometer. The amount of phosphorus was calculated from a standard graph prepared using KH_2PO_4. All the experiments were run in triplicate. Growth was determined by measuring optical density at 660 nm using UV-Vis spectrophotometer. Final pH of the culture supernatant was determined with the help of a pH meter.

RESULTS AND DISCUSSION

Results of the study are given in Table 1. All the bacteria under study could solubilize both tricalcium and dicalcium phosphate. However, degree of phosphate solubilization varied with the incubation period and the organism involved. *Rps. rutila* was more efficient in the solubilization of phosphorus. pH changes in the tricalcium phosphate containing medium were more when compared to dicalcium phosphate medium. *Rb. capsulatus* opted 8th day incubation for maximum solubilization of dicalcium phosphate and 4th day incubation for tricalcium phosphate. Maximum solubilization of both dicalcium and tricalcium phosphate took place on 4th day of incubation by *Rps. acidophila.* Final pH drift was alkaline in *Rb. capsulatus* inoculated medium, whereas it was neutral in the medium growing *Rps. acidophila. Rsp. rubrum* required four days of incubation for maximum solubilization of dicalcium phosphate and tricalcium phosphate. pH of the medium was drifted towards alkaline side. *Rb. capsulatus* and *Rsp. rubrum* could solubilise dicalcium phosphate with almost same efficiency, while *Rsp. rubrum* was comparatively poor in solubilization of tricalcium phosphate. *Rps. rutila* could

Table 1: Phosphate solubilization by anoxygenic phototrophic bacteria.

Organism	Soluble phosphate (µg/mL)						
	Incubation Period (in days)	Growth (O.D.) at 660 nm	Final pH	Dicalcium (P)	Growth (O.D.) at 660 nm	Final pH	Tricalcium (P)
Rb. capsulatus	4	0.865	7.2	421	0.912	7.5	400
	8	1.152	7.6	453	1.110	8.0	391
	12	0.715	8.0	360	0.862	8.4	378
	16	0.612	8.2	320	0.612	8.6	305
Rps. acidophila	4	0.752	6.2	460	0.812	6.0	410
	8	0.968	6.5	420	0.985	6.5	395
	12	0.624	6.7	371	0.715	7.0	361
	16	0.521	6.9	330	0.523	7.2	280
Rsp. rubrum	4	0.855	7.0	443	0.796	7.4	368
	8	0.824	7.4	410	1.015	7.8	354
	12	0.949	7.8	336	1.250	8.2	300
	16	0.596	8.0	320	0.896	8.5	286
Rps. rutila	4	1.033	7.0	402	0.910	7.5	452
	8	1.168	7.4	420	0.985	7.8	362
	12	0.985	7.6	345	1.256	8.0	315
	16	0.812	8.0	330	0.872	8.4	250

solubilize more tricalcium phosphate than dicalcium phosphate on the 4th day of incubation. Phosphate solubilization decreased considerably under alkaline conditions. Present investigations are in agreement with Sujatha et al. (2004) who also reported decrease in phosphate solubilization under alkaline conditions. The decrease in soluble phosphorus at later incubation may be either due to decreased solubilization activity and increased phosphate absorption. In general, dicalcium phosphate was solubilized more readily than tricalcium phosphate.

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REFERENCES

Beibl, H. and Pfenning, N. 1981. In: The Prokaryotes (Eds.) M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel, Spriger-Verlag, Berlin.

Gothwal, R.K., Nigam, V.K., Mohan, M.K., Samal, D. and Ghosh, P. 2006. Phosphate solubization by rhizospheric isolates from economically important desert plants. Indian J. Microbiol., 46: 355-361.

Holt John, G., Krieg Noel, R., Sneath Peter, Staley, H., James, T. and Willimas Stanley, T. 1994. Bergeys Manual of Determinative Bacteriology, Fifth edition, Willimas and Wilkins Publishers.

Julie, L., Zilles, Jordan Peccia, Myeong-Woon Kim, Chun-Hsiung Hung, and Daniel R. Noguera. 2002. Involvement of *Rhodocyclus* related organisms in phosphorus removal in full-scale wastewater treatment plants. Appl. Environ. Microbiol., 68: 2763-2769.

Kapoor, K.K., Mishra, M.M. and Kukreja, Kamalesh 1989. Phosphate solubilization by soil microorganisms. Indian J. Microbiol., 29: 119-127.

Narula, N., Kumar, Vivek, Behl, Deubel, R.K., Gransee, A. and Merrbach, W. 2000. Effect of phosphate solubilizing *A.chrooccum* on N,P, K uptake in P-responsive genotypes grown under green house consitions. J. Plant Nutr. Soil. Sci., 163: 39-98.

Narula, N., Deubel, A., Gransee, A. and Merbach, W. 2007. Phosphate solubilizing microorganism: Mechanism of solubilization as influenced by pH, type of organic acid and sugars. Asian Jr. of Microbiol. Biotech. Env. Sc., 9: 397-404.

Seshadri, S., Ignacimuthu, S. and Lakshminarasimhan, C. 2004. Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. Chemical Engineering Communications, 191: 1043-1052.

Subbarao and Bajpai 1965. Fungi on the surface of legume root nodules and phosphate solubilization. Experientia, 21: 386-387.

Sujatha, E., Girisham, S. and Reddy, S.M. 2004. Phosphate solubilization by thermophilic microorganisms. Indian J. Microbiol., 44: 101-104.

Singh, C.P. and Amaberger, A. 1990. Humic substances in straw compost with rock phosphate. Biol. Waste, 31: 165-174.