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Buffering Reduces Phosphate Solubilizing Ability of Selected Strains of Bacteria

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Abstract: Phosphate solubilizing bacteria (PSB) possessing the ability to solubilize insoluble inorganic phosphates were isolated from rhizosphere soil. Eighty-one potential PSBs thus obtained were quantitatively screened for phosphate solubilization. Of these, four bacteria found to be efficient phosphate solubilizers, were selected for further evaluation and found that they solubilize tricalcium phosphate in buffered as well as non-buffered media. The efficiency of phosphate solubilization was decreased in buffered media compared to non-buffered media. The buffering capacity of the medium reduced the effectiveness of PSBs in releasing P from tricalcium phosphates.

Key words: Tricalcium phosphate · Rhizosphere · phosphate solubilization

INTRODUCTION

Phosphorus exists in nature in a variety of organic and inorganic forms, primarily in either insoluble or very poorly soluble inorganic forms. Soluble forms of P fertilizers applied to the soil are easily precipitated as insoluble forms [1]. Phosphate solubilizing microorganisms solubilize insoluble P by producing various organic acids. Plants take up this available P [2]. However, P solubilization ability of microorganisms in soil may be different from that found under laboratory conditions [3]. Most PSMs have been isolated using non-buffered conditions [4]. The buffering capacity of soils could limit solubilization soil phosphates by microorganisms as it has been shown that solubilization Ca-P complexes are mediated mainly by lowering the pH of the medium [5]. Hence the present study was undertaken to evaluate the effect of buffers in bacterial phosphate solubilization.

Rhizosphere soil samples of 29 crop plants were collected from different parts of Kerala during the monsoon season of 2004. Pikovaskaya's medium incorporated with tricalcium phosphate used for the enrichment of phosphate solubilizing bacteria. The samples were serially diluted, plated on the medium and incubated at 30± 2°C for 4 days [6]. The colonies showing solubilization were picked up and purified by streaking on the surface of soil extract agar medium. The purified colonies were preserved on Pikovaskaya's agar slants. Pikovaskaya's broth medium containing tricalcium phosphate as P source was inoculated by selected PSBs (0.1 mL of 1 OD inoculum added to 50 mL media in triplicate) for 14 days and the soluble P released in the medium was estimated by using vandomolybdophosphoric acid method [7]. Un inoculated flasks were used as control.

To evaluate the effect of buffering on phosphate solubilizing activity the PSBs were grown on agar medium containing 100 mM glucose, 25 µM $MgSO_4$, 10 mM NH_4Cl and the following micronutrients at concentrations mentioned in the brackets (mg/L): $FeSO_4$ $7H_2O$ (3.5); $ZnSO_4$ $7H_2O$ (0.16); $CuSO_4 5H_2O$ (0.08); H_3BO_3 (0.5); $CaCl_2$ $2H_2O$ (0.03); $MnSO_4$ $4H_2O$ (0.4). The phosphate source was 5 g of tricalcium phosphate. The pH of the medium was adjusted to 7. Sterilized tricalcium phosphate was added to media before pouring. For buffering the media 100 mM Tris HCl, pH 7 was used. Methyl red indicator dye was used at 0.01% in plates. The effect of buffering on phosphate solubilization was recorded by measuring the diameter of red zone around the colony after different time intervals. Quantitative estimation of soluble P in buffered and non-buffered broth media were also determined by standard protocols[7].

A total of 81 PSBs obtained were screened for phosphate solubilizing activity and maximum potential showing 18 isolates were double screened for phosphate solubilizing ability (Table1). Of these 4 bacteria (PSB 12, PSB 73, PSB 67 and PSB 58) showed maximum phosphate solubilizing potential selected for the study. The organisms were identified by biochemical reactions as

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Table 1: Secondary screening of selected phosphate solubilizing isolates

phosphate solubilizing bacteria (PSB)	pH	amount of phosphorus solubilized mg/100 ml	solubilization zone diameter in mm.Z	spot diameter in mm. C	phosphate solubilizaton efficiency(SE%)=Z-C/C×100
PSB 12	3.75	72.9±0.7	35	6	483.33
PSB 13	3.26	50.9±0.5	27	4	575.00
PSB 20	4.46	43.2±0.6	28	5	460.00
PSB 21	5.29	40.2±0.8	22	4	450.00
PSB 22	3.61	49.2±1.5	19	6	216.66
PSB 23	3.93	40.2±1.1	17	7	142.85
PSB 30	3.69	45.1±1.3	15	5	200.00
PSB 31	4.67	22.1±1.2	20	8	150.00
PSB 38	4.04	42.1±0.9	16	8	100.00
PSB 40	5.85	23.9±1.1	*	*	*
PSB 47	4.23	32.9±1.0	12	6	100.00
PSB 55	4.15	37.4±1.6	18	8	125.00
PSB 56	5.01	11.3±1.3	*	*	*
PSB 58	3.56	59.0±0.7	18	5	260.00
PSB 66	3.27	55.7±2.0	20	5	300.00
PSB 67	3.77	63.8±1.4	19	3	533.00
PSB 73	3.22	68.8±1.3	21	5	320.55
PSB 75	4.24	43.0±1.1	18	6	200.00

* indicates no zone formation results are expressed as mean ± SD of three independent reading

Table 2: Effect of buffering on selected strains of phosphate solubilizing bacteria

P-Source	Buffer	Bacteria	Zone of discolouration diameter in mm*72 hr	pH	Amount of P solubilized mg/100 ml*
$Ca_3PO_4^{2-}$	-	PSB 12	54±3	3.05	77.5±0.8
	+	PSB 12	40±5	3.32	66.6±1.2
	-	PSB 73	52±5	3.65	61.6±2.7
	+	PSB 73	50±1	3.91	31.5±1.5
	-	PSB 67	67±4	4.00	40.5±0.8
	+	PSB 67	50±1	4.08	22.3±2.4
	-	PSB 58	67±4	2.99	88.8±0.1
	+	PSB 58	51±2	3.75	63.2±1.5

* results are expressed as mean ± SD of three independent readings - without buffer + with buffer

mentioned in the Bergey's Manual of systematic bacteriology and fatty acid methyl ester (FAME) analysis (Sherlock version 4.0 B, MIDI aerobe method saved on chemStation version 4.02) as follows, *Acetobacter liquefaciens* (PSB12), *Acetobacter* sp. (PSB67), *Pseudomonas gladioli* (PSB 73) and PSB 58 (unidentified strain).

P solubilizations of selected PSBs in buffered medium are presented in Table 2. PSB 67 showed a significant difference in zone of discolouration (17 mm) followed by PSB 58 and PSB 12. But the PSB 73 showed only a 2 mm difference in zone of discoluration. PSB 58 (non buffered) showed maximum P solubilization and least by PSB67 in buffered media.

Our results showed that phosphate solubilizing organisms were able to reduce the pH of the medium in presence of supplemented buffers. The results cope up with the earlier observations [8,9], where the plant growth was limited by availability of P despite the abundance of PSBs in the rhizosphere due to buffering. The reduction P solubility has been shown to be due to high buffering capacity of soils and reduced or loss of P solubilizing efficiency of bacteria under buffered media conditions. It is also evident that buffering has a more pronounced effect on the growth of bacteria using rock phosphate as a P source [10]. Presumably, in this case, drop in pH in the absence of buffer is rapid and PSBs were not severally affected by buffering. The PSMs are known to solubilize Ca-P complexes mainly by lowering the pH of the media by secreting organic acids and studies with mineral phosphates have shown that the nature of the organic acids was more important than the amount [11]. Taken together, the results presented indicate that buffering capacity of the medium reduced the effectiveness of the

PSBs in releasing P from tricalcium phosphates. In conclusion the effectiveness of the PSBs isolated earlier should be determined in the buffered media conditions and screening of PSBs using a buffered media may lead to the selection of more effective PSBs.

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