

PHOSPHATE SOLUBILIZATION BY *ALLOCHROMATIUM* SP. GSKRLMBKU-01 ISOLATED
FROM MARINE WATER OF VISAKHAPATNAMKadari Rajya Laxmi*¹, Ramchander Merugu², S.Girisham¹ and S.M.Reddy¹¹Department of Microbiology, Kakatiya University, Warangal, Telangana, India.²Department of Biochemistry, Mahatma Gandhi University, Nalgonda, Telangana, India.

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ABSTRACT: Mineral phosphate solubilization activities by *Allochromatium* sp. GSKRLMBKU-01 on dicalcium and tricalcium phosphate was investigated. The biomass, di- and tricalcium phosphate solubilization increased with the progress of incubation period upto 8th day and decreased with further incremental incubation period. The highest solubility of dicalcium phosphate ($558.0 \pm 9.2 \mu\text{g P/ml}$) and tricalcium phosphate ($568.0 \pm 8.0 \mu\text{g P/ml}$) was recorded on 8th day of incubation period. The maximum optical density of biomass of the bacterium on dicalcium and tricalcium phosphate was 1.389 ± 0.110 and 1.206 ± 0.108 respectively on 8th day of incubation period. A positive correlation coefficient (r) was recorded between growth, dicalcium phosphate ($r=0.965$) and tricalcium phosphate ($r=0.786$) solubilization.

Key words: Anoxygenic phototrophic bacteria, *Allochromatium* sp. GSKRLMBKU-01, Phosphate solubilization, dicalcium phosphate, tricalcium phosphate.

INTRODUCTION

Phosphorus is an essential macronutrient for growth and development of plants and involved in important metabolic pathways like photosynthetic, biological oxidation, nutrients uptake and cell division. The inorganic insoluble phosphorus can be made available by different biogeochemical cycles (Perez *et al.*, 2007). Phosphate solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphorus nutrition. Mostly the soils are supplemented with inorganic phosphate as chemical fertilizers to enhance the crop productivity but excess use of fertilizers deteriorates the soil quality. The negative impact of chemical fertilizers and their increasing costs, the scenario is shifting towards use of biological fertilizers for a more sustainable agriculture. Soil organisms play an important role in solubilization of raw phosphates which are either in the form of organic or inorganic phosphatic compounds which makes availability of phosphorus to the plants (Seshadri *et al.*, 2004 and Sujatha *et al.*, 2004). Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble phosphate to soluble phosphate by releasing low molecular weight organic acids (Goldstein, 1995 and Kim *et al.*, 1997), which chelate readily and ion exchange (Narula *et al.*, 2000 and Whitelaw, 2001). The positive effect of phosphate solubilizers on food and fodder crops has been reported by Dey *et al.* (2004) and Gulati *et al.* (2009). Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganisms has been recognized since 1903 (Khan *et al.*, 2007). Bacteria are more effective in phosphate solubilization among phosphate solubilizing microorganisms (Alam *et al.*, 2002 and Fankem *et al.*, 2006). Among soil bacteria, ectorrhizospheric strains of *Pseudomonas*, *Bacillus* and *Endosymbiotic rhizobia* proved to be effective phosphate solubilizers (Igual *et al.*, 2001). *Arthrobotrys oligospora*, a nematofungus, has the ability to solubilize the phosphate rocks (Dupponnois *et al.*, 2006). Anaerobic treatment of domestic and industrial waste waters releases large amount of phosphorus and nitrogen which are directly responsible for eutrophication (Trepanier *et al.*, 2002).

Compared to chemical precipitation soil microorganisms are reported to be efficient in recycling phosphorus in an ecofriendly manner. Narula *et al.* (2007) have investigated various factors like pH, organic acids and sugars on phosphate solubilization by phosphate solubilizing microorganisms. Though extensive research has been carried out to assess phosphate solubilization activity of heterotrophic bacteria (Subba Rao, 1988, Kucey and Stewart, 1988, Whitelaw, 2001), very limited information is available on phototrophic bacteria (Ramchander *et al.*, 2008). Nagadomi *et al.* (2000) reported removal of phosphate from waste water by using immobilized cells of some phototrophic bacteria. Removal of phosphorus from waste water by using *Rhodocyclus* sp. was reported (Julie *et al.*, 2002). Solubilization of dicalcium and tricalcium phosphate by *Rhodobacter capsulatus* and *Rhodopseudomonas acidophila* was investigated by Ramchander *et al.* (2008). Apart from phosphate solubilization these bacteria also offers several biotechnological applications (Merugu *et al.*, 2012). Eutrophication which is major tropical water bodies due to accumulation of phosphorus is mainly posing a great problem which is needed to study of phosphate solubilization more urgently by anoxygenic phototrophic bacteria may provide some idea about the role of these bacteria in remediation of water pollution and eutrophication. Hence, solubilization of di- and tricalcium phosphate by *Allochroematium* sp. GSKRLMBKU-01 was studied and discussed in this paper.

MATERIALS AND METHODS

All the chemicals used in the present investigations were purchased from Sigma Aldrich (Mumbai, India) and Hi Media company (Mumbai, India). Samples for isolation of anoxygenic phototrophic bacteria were collected from marine coastal region at Visakhapatnam. The anoxygenic phototrophic bacterium *Allochroematium* sp. strain GSKRLMBKU-01 was isolated by enrichment technique (Biebl and Pfennig, 1981) by inoculating the marine sample into the 15 ml medium containing screw capped tubes. Strict anaerobic conditions are maintained and incubated under 2000 lux light. The cultures thus obtained by enrichment technique were streaked on the solid enriched medium repeatedly by paired petriplate method and flushed with nitrogen gas to maintain the anaerobic condition. The colonies were picked up and inoculated into liquid medium. The bacterium thus isolated was identified as *Allochroematium* sp. with the help of Bergey's Manual of Systematic Bacteriology (1994). The morphologically identified bacterium was further confirmed by precise molecular identification by 16S rRNA sequencing analysis. Sequence thus obtained was submitted in Centre for Biotechnology Information (Gene Bank Accession number HF677171.1).

Solubilization phosphorus was determined by inoculating 1ml of fresh culture of *Allochroematium* sp. GSKRLMBKU-01 into screw capped tubes containing 15 ml of liquid medium supplemented with 15 mg of dicalcium and tricalcium phosphate individually and incubated under the light intensity of 2000 lux at $30 \pm 2^\circ\text{C}$ for 12 days. The phosphate solubilization activity of these bacteria was estimated at end of 4, 6, 8, 10 and, 12 days. At the end of incubation period cultures were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and the amount of phosphorus was estimated by the method suggested by Subbarao and Bajpai (1965). To 2 ml of supernatant, 2 ml of ammonium molybdate and 2 ml of amino-naphtho-sulphonic acid reagent (ANSA) were added and diluted to 10 ml with distilled water and incubated at room temperature for 5 minutes. The blue colour intensity thus developed was measured at 660 nm by UV spectrophotometer and the amount of phosphorus was calculated by a standard curve prepared using KH_2PO_4 . Growth of bacterium was determined by turbidity method by measuring optical density at 660 nm. pH of the culture supernatant was determined by Elico pH meter. The results obtained were statistically analyzed. The data obtained were subjected to analysis of variance (ANOVA) using GraphPad Prism.InStat Version 6 (GraphPad Software, Inc.) to test the significance of treatment at $P \leq 0.05$.

RESULTS AND DISCUSSION:

Table 1 and Figure (1&2) reveals that the ability of *Allochroematium* sp. GSKRLMBKU-01 to solubilize both di- and tricalcium phosphate varied with the incubation periods. *Allochroematium* sp. GSKRLMBKU-01 accomplished maximum optical density of biomass on 8th day of incubation period (1.389 ± 0.110) and (1.206 ± 0.108) during dicalcium and tricalcium phosphate solubilization respectively. Solubilization of both the phosphates was recorded maximum on the 8th day of incubation period. Dicalcium phosphate solubilization activity was observed from 4th day to 8th day which ranged between 255.0 ± 5.0 to 558.0 ± 9.2 $\mu\text{g P/ml}$. Similarly the solubilization of tricalcium phosphate solubilizing activity ranged from 305.0 ± 5.0 to 568.0 ± 8.0 $\mu\text{g P/ml}$. Such rate variation in phosphate solubilization was also reported (Gaur, 1990, Goenadi *et al.*, 2000 and Ramchander *et al.*, 2008). The reason for this may be attributed to the fact that when the rate of phosphorus uptake is higher than that of solubilization, a decrease in phosphorus concentration in the media was recorded. On the otherhand, when the uptake rate decreased, the level of phosphorus in the medium increased (Rodroguéz and Faga, 1999).

The decrease in soluble phosphorus may also be due to decreased solubilization activity of microorganisms or increased phosphorus absorption (Ramchander *et al.*, 2008). Reduction in release of soluble phosphorus during later phase of the incubation might be due to the depletion of nutrients in the culture medium, in particular, carbon source needed for the production of organic acids (Kang *et al.*, 2006, Chaiarn, 2009 & 2011). However, Narsian *et al.* (1994) feels that availability of soluble phosphorus in the culture medium might also have an inhibitory effect on further phosphate solubilization. Excretory ions of some toxic products may also responsible for such decline in phosphate solubilization (Narsian *et al.*, 1994). The pH of the growth medium changed during the process of solubilization by *Allochroamatium* sp. from its initial value of 8 to 6. A similar change in the pH of the growth medium was noticed by many workers (Vora and Shelat, 1996; Sujatha *et al.*, 2004). The inverse relationship between pH and soluble phosphorus concentration was suggested that decrease in pH of the medium could facilitate the inorganic phosphate solubilization (Yasmin and Bano, 2011; Yu *et al.*, 2011). Ramchander *et al.* (2008) reported an increase in the pH of the medium was associated within phosphate solubilization by *Rba. capsulatus* and *Rps. acidophila*. Pandey *et al.* (2006) reported the high concentration of tricalcium solubilization (247 $\mu\text{g P/ml}$) with decrease in the pH of the medium during the growth of *Pseudomonas putida* B0. The elevation of pH of the medium on prolonged incubation was also noticed which could be either due to the death and lysis of microorganisms (Illmer and Scinner, 1992) or due to the consumption of organic acids by the organism (Dave and Patel, 1999). A positive correlation was observed between the biomass, phosphate solubilization and final pH of the medium. However, many workers failed to find such correlation between phosphate solubilization and decrease in pH (Narsian and Patel, 2000). Statistically analysis of di- and tricalcium phosphate solubilization by *Allochroamatium* sp. at different incubation period was carried out and their minimum, maximum, mean and standard deviation were depicted in Table 3. The mean growth 1.103 and dicalcium phosphate solubilization 395.3 $\mu\text{g P/ml}$ was recorded by this bacterium. Similarly the mean growth 0.991 and tricalcium phosphate solubilization 448.5 $\mu\text{g P/ml}$ was recorded. The positive correlation coefficient was observed between biomass, dicalcium phosphate (0.965), and tricalcium phosphate (0.786) solubilization.

Table 1 Quantitative assay of phosphate solubilization of dicalcium and tricalcium phosphate by *Allochroamatium* sp. GSKRLMBKU-01 at different incubation periods

Dicalcium phosphate	Growth *	Final pH	Phosphate ($\mu\text{g P/ml}$)
4	0.8 \pm 0.04	7.7 \pm 0.1	255 \pm 5.0
6	1.1 \pm 0.10	7.2 \pm 0.2	427 \pm 7.5
8	1.4 \pm 0.11	6.7 \pm 0.2	558 \pm 9.2
10	1.2 \pm 1.00	6.4 \pm 0.1	431 \pm 6.6
12	1.0 \pm 0.12	6.0 \pm 0.2	305 \pm 5.0
Tricalcium phosphate			
4	1.0 \pm 0.90	7.5 \pm 0.1	305 \pm 5.0
6	1.4 \pm 1.17	7.2 \pm 0.3	446 \pm 7.1
8	1.7 \pm 1.47	6.6 \pm 0.2	568 \pm 8.0
10	1.5 \pm 1.27	6.3 \pm 0.2	508 \pm 7.5
12	1.2 \pm 0.97	6.0 \pm 0.1	415 \pm 6.0

* Expressed in units OD units at 660 nm

The experimented was conducted in triplicates and the results are expressed in mean and standard deviations are statistically significant at ≤ 0.05 level by using SPSS software tool.

Table 2 Statistical analysis of phosphate solubilization by *Allochroamatium* sp. GSKRLMBKU-01

	Growth *	Dicalcium P($\mu\text{g/ml}$)	Growth *	Tricalcium P($\mu\text{g/ml}$)
Minimum	0.783	255.0	0.729	305.0
Maximum	1.389	558.0	1.206	568.0
Mean	1.103	395.3	0.991	448.5
Std. Deviation	0.234	118.9	0.196	99.32
Std. Error	0.104	53.17	0.087	44.42
One sample t test				
95% CI of discrepancy	0.8126 to 1.394	247.7 to 542.9	0.7477 to 1.234	325.2 to 571.8
t, df	t=10.54 df=4	t=7.434 df=4	t=11.31 df=4	t=10.10 df=4
Correlation coefficient (r)	0.965	-	0.786	-

*Expressed in units OD units at 660 nm

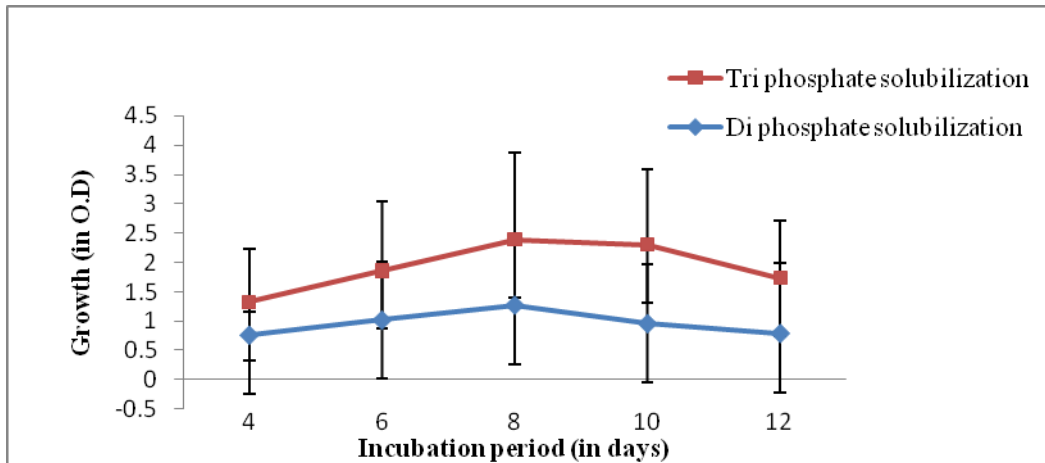


Figure-1: Growth of *Allochroamatium* sp. GSKRLMBKU-01 during dicalcium and tricalcium phosphate solubilization

Mean error bars in the graph represent the mean \pm standard error from the triplicate samples that were tested and the results are statistically significant at ≤ 0.05 level.

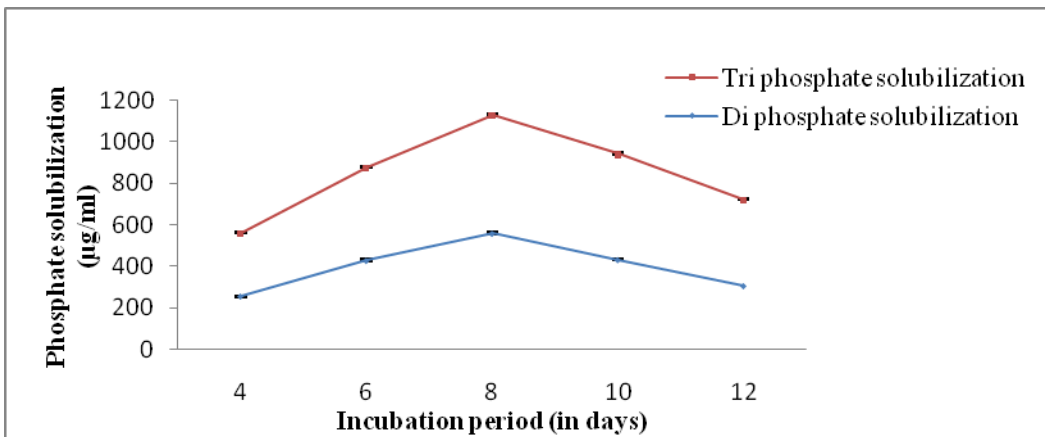


Figure-2: Phosphate solubilization of dicalcium and tricalcium phosphate by *Allochroamatium* sp. GSKRLMBKU-01

Mean error bars in the graph represent the mean \pm standard error from the triplicate samples that were tested and the results are statistically significant at ≤ 0.05 level.

CONCLUSION

The insoluble inorganic phosphates present in soil and different waste waters become available by the activity of microflora by secreting organic acids and acid phosphatase. Although, high buffering capacity of soil reduces the effectiveness of phosphate solubilizing bacteria in releasing phosphorus from bound phosphates, enhancing microbial activity through phosphate solubilizing inoculants may promote considerably in plant phosphorus uptake. Anoxygenic phototrophic bacteria are efficient in removal of phosphorus from wastes and solubilization of different inorganic phosphates. Present investigations revealed that phosphate solubilization capability of *Allochroamatium* sp. strain GSKRLMBKU-01 was found to be efficient. However, further in depth studies for optimizing the nutritional conditions are desired for more phosphate solubilization and also its exploitation in bioinoculation capabilities.

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