ORIGINAL RESEARCH PAPER

Biotechnology

PHOSPHATE IMPACT AND THEIR KINETICS IN BLUE-GREEN ALGA SYNECHOCOCCUS AERUGINOSUS NAGELI

KEY WORDS: *Synechococcus aeruginosus,* Phosphates, growth and kinetics

Dr. Kusuma Bhagya Lakshmi Jyothi

Asst. Professor, Dept. Of Biotechnology (P.G), Dr. Lankapalli Bullayya College, Visakhapatnam

ABSTRACT

Phosphorus is one of the major nutrients required in all phases of metabolism particularly in energy generating and transforming reactions during the development and for the normal growth of microorganisms such as algae which are the primary producers in the food web cycle (Kuhl, 1974). *Synechococcus aeruginosus* collected from the paddy fields of Andhra Pradesh, India. The effect of phosphate sources i.e. K_2HPO_4 and NaH_2PO_4 on growth of the algae and uptake and kinetics of phosphates were also examined for upto 72 hours of life cycle. Uptake of phosphates followed Michaelis-Menton kinetics in *Synechococcus aeruginosus.* Based on the results di-potassium hydrogen orthophosphate (K_2HPO_4) supplemented algal cultures showed better growth than sodium dihydrogen orthophosphate (NaH_2PO_4) at all concentrations.

INTRODUCTION

Phosphorus has been considered as an indispensable macro-element which plays a significant role in multiplication and growth of algal cells and their metabolic processes such as photosynthetic CO_2 fixation, uptake and transport of ions,activation of aminoacids and condensation of polyphosphates which generate energy rich phosphate bond of ATP by incorporating orthophosphate by light reaction in algae, especially cyanobacteria. The phosphorus requirement for optimal algal growth and phosphorous uptake differ from species to species of blue-green algae (Stewart and Alexander, 1971) depending upon the environmental factors besides phosphate availability (Spijkerman and Coesel, 1996).

The photoautotrophic micro-organisms such as algae absorbed phosphate either from inorganic or organic phosphates accumulating on the specific sites which are utilized for the synthesis of ATP under dark conditions till attaining normal quantity in the cells and further absorption depends upon the light. (Batterton and Van Baalen, 1968). The rate of uptake of phosphate depends upon many factors such as temperature, pH and light etc. In *Synechococcus* R-2 PCC 7924, the rate of phosphate uptake in the light was strongly Na^+ and pH dependent. The uptake of phosphate was optimal between pH 7.5 and 9.0 and declined sharply in the acidic range (Healy, 1973) and also fell off at more alkaline conditions (Ritchie et al., 1997). In *Synechococcus* sp. and *Anacystis nidulans*, the metabolic assimilation of phosphate was energy dependent (Ritchei et al., 1997) but was dissimilar in *Gloeotrichia echinulata* (Istvasnovics et al., 1993).

Reichard et al. (1967) and Berman (1970) reported that orthophosphates were hydrolyzed by the dissolved alkaline phosphatase and the released phosphate was utilized as nutrient by algae growing in lakes and oceans. However, most of the cyanobacteria could utilize organic phosphorus for growth if only hydrolyzed by periplasmic phosphatase enzymes (Islam and Whitton, 1992). Some cyanobacteria were also able to use hydroxyapatite and tri-calcium phosphate as source for both calcium and phosphorus as well (Roy Choudhary and Kaushik, 1989).

In Indian rice-fields, bulk quantities of phosphorous fertilizers are required for the growth of improved crops. Therefore the farmers have been employing the chemical phosphate fertilizers like super phosphate (P_2O_5) and rock phosphate (tri-calcium phosphate) etc. in the rice-fields to enhance the crop produce where cyanobacteria are predominantly growing (Tadulingam and Venkatanarayana, 1955). Because of availability of phosphates, growth of blue-green algae in rice-fields and aquatic systems was noted (Baral and Kumar, 1994).

In the present study, the experiments deal with the modifying effects of phosphate on growth and kinetics of phosphates sources of blue-green algae *synechococcus aeruginosus* .

MATERIALS AND METHODS

A unicellular *synechococcus aeruginosus* was isolated from rice-fields of Krishna District in Andhra Pradesh. The general experimental culture methods were employed in this study. During this study, to observe the effect of different concentrations (0.1, 1.0, 1.5, 2.0 mg per ml) of phosphates i.e. di-potassium hydrogen orthophosphate (K_2HPO_4) and sodium dihydrogen orthophosphate (NaH_2PO_4) on the growth of *synechococcus aeruginosus*, short-term experiments were conducted by inoculating the algae grown in the phosphate deficient media and starvation was observed by the reduction of chlorophyll-a and other pigments in algae. The growth of the algae was recorded by measuring the cell number per ml with Neubauer's Haemocytometer and also chlorophyll-a and proteins at "0" hour, after 24 and 48 hours by MacLachlan and Zalik (1963) and Lowry et al. (1951) methods respectively. Moreover, in this study, the uptake of phosphates was recorded in the experimental cultures and phosphate was estimated by the method of ammonium molybdate and aminonapthosulphonic acid described by Fiske and Subba Row (1925).

In these experiments, phosphate supplementation was inoculated with a fixed number of phosphate starved algal cells (0.2 ml containing 405 x 10^5 per ml) of *synechococcus aeruginosus*. The control and experimental cultures [(basal medium (BM); BM-PO_4; BM+ NaH_2PO_4/K_2HPO_4] were kept under white fluorescent light (600 lux) at room temperature 28 ± 2^{0}C. Among the experimental cultures, the uptake of phosphate was measured and estimated in control and various phosphate supplemented cultures evidenced as the difference between phosphate quantity before and after inoculation of growth periods i.e. after 72 hours in *Synechococcus aeruginosus*. The S/V values indicate the rate of uptake of phosphate chemical per hour by the algae and the Km (Michaelis constant) values give clues to the affinity between substrate and the activity of enzymes involved in permeability and uptake of phosphate and the changes in reaction rate.

RESULTS

To observe the effect of phosphate sources on growth and their kinetics in *Synechococcus aeruginosus*, different concentrations (0.10, 1.0, 1.5, 2.0 mg per ml) of di-potassium hydrogen orthophosphate (K_2HPO_4) and sodium dihydrogen orthophosphate (NaH_2PO_4) supplemented cultures besides control were inoculated with the vegetative cells of *Synechococcus aeruginosus* grown in phosphate depleted basal medium (BM-PO_4) and the growth measured in terms of chlorophyll-a, proteins, cell number was lower in control (BM-PO_4) when compared to basal medium cultures (BM) and phosphate supplemented cultures which indicates that different concentrations of phosphate sources caused significant influence on growth. When the effect of two phosphate sources on growth in respect of

chlorophyll-a, protein content and cell number were compared di-potassium hydrogen orthophosphate (K_2HPO_4) supplemented algal cultures appeared to be better than sodium dihydrogen orthophosphate (NaH_2PO_4) at all concentrations. The supplemented concentrations (0.1 and 1.0 mg per ml) of di-potassium hydrogen orthophosphate (K_2HPO_4) caused a significant enhancement in the levels of chlorophyll-a, protein and cell number, while at higher concentrations (1.5 and 2.0 mg per ml), a decrease in the above contents (chlorophyll-a, protein and cell number) was observed but in sodium dihydrogen orthophosphate (NaH_2PO_4) cultures, the amount of chlorophyll-a, proteins and cell number increased with increasing concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml)(Table 1). The uptake of phosphate capacity of *Synechococcus aeruginosus* was greater in di-potassium hydrogen orthophosphate (K_2HPO_4) than sodium dihydrogen orthophosphate (NaH_2PO_4) depending upon the increased concentration of phosphate.

The S/V values indicating the rate of uptake of phosphate in *Synechococcus aeruginosus* at 0.1, 1.0, 1.5 and 2.0 mg per ml concentration of di-potassium hydrogen orthophosphate (K_2HPO_4) and sodium dihydrogen orthophosphate (NaH_2PO_4) respectively which were varied with the concentrations of phosphate sources and their uptake rate. 0.27 and 0.25 mM were the Km values of sodium dihydrogen orthophosphate (NaH_2PO_4) and di-potassium hydrogen orthophosphate (K_2HPO_4) cultures respectively.

Table 1: Kinetics and effect of phosphate sources on growth of *Synechococcus aerugonisus*

Concentration (mg/ml)	No. of cells for 72 hrs x 10^5	Chlorophyll-a (mg/g)	Proteins (µg/100 ml fw)	Phosphate uptake rate / hr (mM x 10^{-5})	S/ V (mM)	Km Value (mM)
B.M – PO_4 (Control)	11.0	0.044	31.43			
B.M + PO_4	21.0	0.165	45.48			
NaH_2PO_4						
0.1	18.8	0.080	28.18	50.15	0.0127	
1.0	19.0	0.116	36.82	300.12	0.0213	0.27
1.5	19.2	0.130	38.14	320.10	0.0300	
2.0	20.4	0.145	43.06	440.45	0.0290	
K2HPO4						
0.1	20.8	0.142	40.32	188.25	0.0030	
1.0	22.0	0.200	44.54	261.42	0.0219	0.25
1.5	19.8	0.135	39.14	315.13	0.0273	
2.0	19.2	0.128	37.88	481.45	0.0238	

NaH2PO4 = sodium dihydrogen orthophosphate; K2HPO4 = di-potassium hydrogen orthophosphate; f.w. = fresh weight; mM = Milli molar; S/V = Rate of uptake of phosphate; Km= Michaelis constant

DISCUSSION

The algal biomass production mostly depends upon phosphate absorption and uptake, which is controlled by various physical factors and limitation of the phosphate in the surrounding media (Gerloff and Skoog, 1954; Alkholy, 1956). Algal growth rates plotted versus phosphate concentrations from batch cultures showed a linear relationship at very low concentrations and virtual independence at concentrations above 10 mg phosphate per litre (Thomas and Dodson, 1968).

Singh (1975), Anand and Karuppusamy (1987) and Anand (1990) studied the interaction of several species of cyanobacteria with superphosphate (10 to 500 µg per ml concentration) in laboratory cultures and observed that up to 100 µg per ml concentration phosphorus stimulated the growth and nitrogen fixation of blue-green algae whereas higher concentrations of phosphorus decreased photosynthetic O_2 evolution, CO_2 fixation, nitrogenase activity and glutamine synthetase activity of all the organisms.

The results in the present study on the effects of different concentrations of phosphates on growth, phosphate uptake, rate of uptake of phosphate and changes in Km values in the cultures of *Synechococcus aeruginosus* were recorded. The growth of algae and the uptake of phosphate sources by algae varied depending upon the type of phosphate source, physical factors, chemical interactions and was also dependent upon the physiological state of the algal cells (Kuhl, 1974; Murali et al, 2009).

In short-term experiments, phosphorus – starved cells of *Synechococcus aeruginosus* were inoculated and grown in a phosphate deficit basal medium (BM-PO_4) supplemented with ;different concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of sodium dihydrogen orthophosphate (NaH_2PO_4) and di-potassium hydrogen orthophosphate (K_2HPO_4) individually as well as in basal medium (BM) and control (BM-PO_4). The growth measured in terms of chlorophyll-a and protein and also cell number of *Synechococcus aeruginosus* was lower in phosphate deficient basal medium (BM-PO_4) than in phosphate supplemented cultures (Tables 1), that indicated the significant involvement and role of phosphate in the growth of these blue-green algae as reported by Batterton and Van Baalen (1968) and in green algae (Ketchum et al., 1958; Kuhl, 1962, 1974).

It was evident that, lower concentrations (0.1 mg per ml) of both phosphate sources i.e. sodium dihydrogen orthophosphate ($N_aH_2PO_4$) and di-potassium hydrogen orthophosphate (K_2HPO_4) augmented the growth either in terms of chlorophyll-a, proteins and cell number in *Synechococcus aeruginosus* whereas at higher doses, biomass was decreased slightly than control cultures (BM-PO_4), however at all concentrations of sodium dihydrogen orthophosphate ($N_aH_2PO_4$) and di-potassium hydrogen orthophosphate (K_2HPO_4) supplemented cultures could enhanced the growth than phosphate depleted basal medium cultures (BM-PO_4). Similarly the growth enhancement was observed and reported in blue-green algae (Singh, 1975; Anand and Karuppusamy, 1987 and Anand, 1990), thereby stating the requirement and role of phosphate in promoting the growth of these algae.

Phosphorous–deficient blue-green algal cells can assimilate phosphorus immediately in excess of their requirement both in light and dark and incorporate and store it in high energy polyphosphate granules such as ATP molecules (Batterton and Van Baalen, 1968; Volk and Phinney, 1968; Stewart and Alexander, 1971; Fogg et al., 1973). Therefore the luxuriant growth observed in the blue-green algae in the phosphate supplemented cultures was significantly higher than in phosphate -deficient cultures as evidenced from the growth and survival experiments of *Synechococcus aeruginosus.*

CONCLUSION

Blue-green algae, generally prefers to utilize lower concentrations of phosphate fertilizers for optimum growth (Anand and Karuppusamy, 1987). The present study reveals that the phosphate uptake capacity of *Synechococcus aeruginosus* was greater in K_2HPO_4 than NaH_2PO_4 as well as lower concentrations (0.1 mg per ml) of both phosphate sources i.e. NaH_2PO_4 and K_2HPO_4 augmented the growth in *Synechococcus aeruginosus* whereas at higher doses, biomass was decreased slightly than that of control cultures (BM-PO_4). The present study suggests that the introduction of blue-greens as biofertilizers in rice-fields in combination with lower concentrations of phosphate fertilizers was found to increase grain yield of rice and also soil fertility.

REFERENCES

1. Anand, N and Karuppusamy, A. (1987). Growth of nitrogen fixing and non-nitrogen fixing blue-green algae in presence of some common fertilizers. Phykos 26: 22-26.
2. Baral, S.R. and Kumar, H.D. (1994). Nitrogen fixation rates in Paddy soils of Kathamandu valley. In: Recent advances in Phycology. (Ed) Kashypa, A.K. and Kumar H.D. Rastogi and Company, Meerut, India. Pp. 191-193.
3. Batterton, J.C. and Van Baalen, C. (1968). Phosphorus deficiency and phosphate uptake in the blue-green alga Anacystis nidulans. Can. J. Microbiol. 14: 341-8.
4. Gerloff, G.C. and Skoog, G. (1954). Cell contents of nitrogen and phosphorus as a measure of their availability for growth of Microcystis aeruginosa. Ecology 35: 348-353.
5. Healy, F.P. (1973). Characteristics of phosphorus deficiency in Anabaena. J. Phycol. 9: 383-394.
6. Islam, M.R. and Whitton, B.A. (1992). Phosphorus content and phosphatase activity of the deep water rice-field cyanobacterium (blue-green alga) Calothrix D 764. Microbiol. 69: 7-16.
7. Istvanovics, V; Pettersson, K; Rodrigo, M.A.; Pierson, D; Padisak, J. and Colom, W. (1993). Gloeotrichia echinulata, a colonial cyanobacterium with a unique phosphorus uptake and life strategy. J.Plankton Res. 5: 531-552.
8. Kuhl, A. (1962a). Inorganic phosphorus uptake and metabolism. In "Physiology and Biochemistry of Algae". (Ed.) Lewin, R.A. Academic Press, New York. Pp. 211-29.
9. Murali, R, Subramanian, V.V, Sumathi, p. and Sivasubramanian, V. 2009. Studies on kinetics of phosphate uptake by blue-green algae. J.Algal Biomass Utln., 1 (1): 41-60.
10. Reichardt, W., Overbeack, J. and Steubing, 1. (1967). Free dissolved enzymes in lake waters. Nature 216: 1345-47.
11. Ritchie, R.J; Trautman, D.A. and Larkum, A.W.D. (1997). Phosphate uptake in the cyanobncterium Synechococcus R-Z PCC 7942. Plant cell Physiol. 31: 1232-1241.
12. Singh, P.K. (1975). Fertilizers tolerance of blue-green algae and their effect on heterocyst differentiation. Phykos. 14: 81-88.
13. Spikerman, E and Coesel, P.F.M. (1996) Phosphorus uptake and growth kinetics of two planktonic desmid species. Europ. J. Phycol. 31: 53-60.
14. Stewart, W.D.P. and Alexander, G. (1971). Phosphorus availability and nitrogenase activity in aquatic blkue-green algae. Fresh water Biol. 1: 389-404.
15. Thomas, W.H. and Dodson, A.N. (1968). Effect of phosphate concentration on cell division rates and yield of a tropical oceanic diatom. Biol. Bull. 134: 199-208.