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Evaluation of growth, nitrogen fixation and P-solubilizing ability of diazotrophic cyanobacteria under mineral phosphorus sources

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ABSTRACT

Five cyanobacterial strains, viz. *Anabaena variabilis*, *Nostoc muscorum*, *Tolypothrix tenuis, Aulosira fertilissima* and *Westiellopsis prolifica* were utilized extracellular insoluble tricalcium phosphate (TCP) and Mussorie rock phosphate (MRP) when provided as sole P source by replacing the conventional source of P (K_2HPO_4) in BG-11 medium. These strains exhibited a differential response to insoluble forms of phosphorus both in terms of growth and nitrogen fixation. *A. variabilis*, *N. muscorum* and *W. prolifica* showed better growth in presence of TCP while *T. tenuis* and *A. fertilissima* proliferated better in presence of MRP. *N. muscorum* recorded maximum growth in terms of total chlorophyll content (6.21 μg/ml) in presence of TCP (20 mg P/l) followed by *T. tenuis* (6.10 μg/ml) in presence of MRP (20 mg P/l) after 21 days of incubation. Nitrogen fixation measured as acetylene reduction (AR) activity showed significant variation among different strains when grown at varied concentrations of TCP or MRP and time of incubation. *N. muscorum* showed maximum AR activity of 6.17 μmole C_2H_4/mg chl/h in presence of MRP (20 mg P/l) followed by *A. variabilis* which recorded 5.46 μmole C_2H_4/mg chl/h but in presence of TCP (20 mg P/l) after 28 days of incubation. In general, in all the strains tested, AR activity was more in presence of higher concentration of TCP/ MRP than their lower concentrations (10 mg P/l). In terms of P-solubilization, *A. variabilis* was found to be the best, showing maximum amount of available P, i.e. 0.224 μg/ml and TCP (20 mg P/l) was the best utilized source of mineral P compared to MRP.

Key words: AR activity, Available P, Cyanobacteria, Mussorie rock phosphate, Total chlorophyll, Tricalcium phosphate

Phosphorus (P) is second most essential mineral fertilizer for crop production after nitrogen, comprising 0.2% of plant dry weight. Microorganisms solubilize fixed soil phosphates into assimilated form which has greater practical importance. Different groups of phosphate solubilizing microorganisms, particularly bacteria and fungi, have been reported to solubilize inorganic phosphatic compounds (Bardiya and Gaur 1972, Wani *et al.* 2007). Cyanobacteria, besides showing slow release of the fixed and metabolized nitrogen also solubilize mineral phosphorus thereby enabling the crop plants to utilize more nutrients from the soil in presence of algal inoculation. The agronomical potential of BGA has been long recognized and inoculation of BGA in lowland rice is well documented (Venkataraman 1961). Although, reports exist on heterocystous nitrogen fixing cyanobacteria to solubilize mineral P, but not much attention has been paid to this attribute. Some reports have shown that these solubilize insoluble $Ca_3(PO_4)_2$, rock phosphate, hydroxyapatite, $FePO_4$, $AlPO_4$ and ($Ca_5(PO_4)_3OH$) (Roychoudhury and Kaushik 1989, Yandigeri and Pabbi 2005, Yendigeri *et al*. 2010) and some have also been reported to solubilize organic sources of P (Whitton *et al*.1991) in soils, sediments or in pure cultures. In general, these mineral phosphates are biologically not available for nutritional transport and assimilation. With this background, the present investigation was carried out to evaluate the growth, nitrogen fixation and phosphorus solubilization ability of selected cyanobacterial strains when grown with insoluble phosphate sources.

MATERIALS AND METHODS

Two mineral phosphate sources, *viz.* Mussorie rock phosphate (MRP) and tricalcium phosphate (TCP) were taken for the study carried out in 2017. The total P content in MRP and TCP was 18 and 20% respectively. Heterocystous cyanobacterial cultures namely *Anabaena variabilis* (CCC441), *Nostoc muscorum* (CCC442), *Tolypothrix tenuis*(CCC443), *Aulosira fertilissima* (CCC444) and *Westiellopsis prolifica* (CCC474) were obtained from Culture Collection of Cyanobacteria at CCUBGA, ICAR

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- Indian Agricultural Research Institute, New Delhi, India and maintained and grown in N-free BG-11 medium (Stanier *et al*. 1971) at 28± 2°C with 16/8 h L/D cycles at 2500–3000 Lux light intensity. The experimental flasks were supplemented with alternate P sources, i.e. MRP or TCP at varying concentrations (equivalent to 10 and 20 mg P/l) by replacing K_2HPO_4 in the conventional BG-11 medium and inoculated with 2% inoculum of actively growing and P-starved, 7 days old cultures of BGA. Potassium chloride of equivalent amount (34.2 mg/l) was added in the medium to maintain the availability of potassium. Conventional BG-11 medium with K_2HPO_4 as source of P was maintained as control. The treatments were replicated three times. Growth as total chlorophyll content, nitrogen fixation and P-solubilization were measured periodically at 7 days interval up to 28 days. Total chlorophyll in cyanobacterial strains was estimated by the Mackinney method (1941) and nitrogenase activity was estimated by measuring the Acetylene Reduction (AR) activity as described by Hardy *et al.* (1973). The available 'P' in cell free supernatant at desired intervals i.e. 7, 14, 21 and 28 days was determined by vanadomolybdi-phosphoric yellow complex method (Watanabe and Olsen 1965).

RESULTS AND DISCUSSION

In the present study it was found that the five strains namely *A. variabilis*, *N. muscorum*, *T. tenuis, A. fertilissima* and *W. prolifica* were able to exhibit growth and solubilize phosphorus (P) when grown in presence of MRP or TCP as sole source of P. Initially, after 7 and 14 days of incubation, all the strains showed good growth in presence of K_2HPO_4 (control) followed by TCP and MRP. Among the concentrations of MRP and TCP used, maximum growth (measured in terms of total chlorophyll) was exhibited at 20 mg TCP/MRP than 10 mg TCP/MRP. All the strains showed maximum total chlorophyll content after 14 days of incubation when K_2HPO_4 was used as P-source. But when MRP/TCP was used, all the strains tested showed gradual increase in total chlorophyll content as the days of incubation progressed and showed maximum total chlorophyll after 21 days of incubation. When compared between insoluble P-sources, i.e. TCP and MRP, *W. prolifica, N. muscorum*

Table 1 Time course effect of different phosphate sources on total chlorophyll* content in cyanobacteria

Treatment	Days	K_2HPO_4	TCP (mg P/L)		MRP (mg P/L)		Mean (S)
(Strain)			10	20	10	20	
A. variabilis	7	2.34	0.59	0.73	0.15	0.47	2.302
	14	6.05	1.28	2.14	0.88	1.01	
	21	4.16	2.55	5.14	2.34	3.45	
	28	3.76	1.53	3.50	2.08	1.92	
N. muscorum	7	2.51	0.92	1.91	0.68	0.19	3.214
	14	6.44	2.93	4.93	1.97	2.64	
	21	4.70	4.71	6.21	2.48	4.52	
	28	3.94	3.69	4.22	1.97	2.74	
W. prolifica	7	1.83	0.84	1.22	0.91	1.03	3.412
	14	6.14	2.81	3.59	2.85	3.22	
	21	4.16	3.83	5.28	4.38	5.01	
	28	3.91	3.58	5.02	4.12	4.50	
A. fertilissima	7	1.97	0.85	0.99	0.8	1.26	3.028
	14	5.95	2.80	3.89	2.18	4.03	
	21	5.01	3.82	4.59	2.43	4.91	
	28	4.68	2.80	3.13	1.67	2.80	
T. tenuis	7	2.77	0.98	1.82	1.02	1.41	3.620
	14	6.56	2.74	4.48	3.20	5.20	
	21	5.79	4.76	5.46	5.04	6.10	
	28	2.73	2.41	3.07	2.89	3.96	
	S	*T×S*	*T×D*	*S×D*	*T×S×D*		
SEM±	0.002	0.006	0.005	0.005	0.011		
CD (P=0.05)	0.007	0.015	0.014	0.014	0.031		

*Values are µg chlorophyll/ml.

and *A. variabilis* showed maximum total chlorophyll content in presence of TCP whereas *T. tenuis* and *A. fertilissima* recorded maximum total chlorophyll content in presence of MRP (Table 1). Among all the strains, *N. muscorum* showed maximum total chlorophyll content, i.e. 6.21 µg/ml followed by *T. Tenuis* (6.10 µg/ml) in presence of TCP and MRP respectively after 21 days of incubation. It was also observed that all the cyanobacterial strains showed maximum total chlorophyll content in presence of higher concentration of TCP or MRP, i.e. 20 mg P than its lower concentration (10 mg P). Variations were observed with regards to the ability of these strains to solubilize P at different concentration of TCP and MRP. Available 'P' content in cultural filtrates showed that most of the total 'P' was in the available form when K_2HPO_4 was used as a source of 'P' and maximum amount of available 'P', i.e. 3.74 µg/ml was found in the culture filtrate of *A. variabilis* when K_2HPO_4 was used as a source of 'P' followed by *W. prolifica* which showed 3.71 µg/ml after 7 days of incubation. The amount of available 'P' in the culture filtrates of the organisms was very less when 'P' was supplied as an insoluble source (MRP or TCP) and it was observed that the availability was concentration dependent (10 mg/20 mg 'P'). Available 'P' followed a definite pattern of initial increase with incubation time and then decrease irrespective of its concentration. In the present study, when different strains of BGA were used for solubilizing TCP/MRP, different quantities of available P were found in the culture filtrates. The observations are similar to the one reported by Roychoudhury and Kaushik (1989). This indicated that the organisms were continuously solubilizing the insoluble phosphate, using some amount of it for their own growth and metabolism, and the remaining was left in the medium. The maximum availability of P was shown at 21 days of incubation which gradually decreased with further increase in time of incubation in all the strains when TCP was used as P source except *W. prolifica* and *T. tenuis*. These two strains showed maximum availability of P in presence of MRP. Among all the strains, *A. variabilis* and *W. prolifica* had better ability to solubilize P than *N. muscorum*, *T. tenuis* and *A. fertilissima*. Maximum amount of available 'P', i.e. 0.224 $\mu g.ml^{-1}$ was found in the culture filtrates of *A. variabilis* followed by *W. prolifica* and *N.*

Table 2 Time course effect of different phosphate sources on available phosphorus* in cell free supernatant of cyanobacteria

Treatment	Days	K_2HPO_4	TCP (mg P/L)		MRP (mg P/L)		
(Strain)			10	20	10	20	Mean (S)
A. variabilis	7	3.74	0.045	0.083	0.042	0.061	0.598
	14	2.870	0.094	0.171	0.057	0.083	
	21	2.320	0.121	0.224	0.084	0.102	
	28	1.56	0.077	0.113	0.102	0.054	
N. muscorum	7	3.48	0.036	0.059	0.057	0.084	0.553
	14	2.640	0.054	0.087	0.079	0.127	
	21	2.181	0.063	0.107	0.102	0.196	
	28	1.34	0.055	0.069	0.096	0.152	
W. prolifica	7	3.71	0.064	0.091	0.065	0.087	0.602
	14	2.810	0.093	0.209	0.114	0.182	
	21	2.260	0.116	0.185	0.094	0.120	
	28	1.470	0.082	0.117	0.081	0.093	
A. fertilissima	7	3.39	0.031	0.069	0.036	0.054	0.508
	14	2.580	0.040	0.087	0.045	0.073	
	21	2.013	0.081	0.133	0.079	0.114	
	28	1.102	0.044	0.072	0.061	0.057	
T. tenuis	7	3.41	0.061	0.084	0.067	0.088	0.548
	14	2.620	0.083	0.141	0.105	0.169	
	21	2.153	0.075	0.122	0.093	0.132	
	28	1.311	0.034	0.097	0.035	0.088	
	S	*T×S*	*T×D*	*S×D*	*T×S×D*		
SEM±	0.002	0.005	0.004	0.004	0.010		
CD (P=0.05)	0.006	0.013	0.012	0.012	0.027		

*Values are µg available P/ml.

muscorum which showed 0.209 and 0.196 µg/ml respectively (Table 2). It was also found that amount of available 'P' was less at lower concentration (10 mg P/l) than at higher concentration (20 mg P/l) irrespective of MRP or TCP. The results also showed that TCP was solubilized more effectively by most of the BGA strains, which resulted in more available 'P' throughout the experimental phase as well as at the end of incubation. In this study, it was observed that not all insoluble phosphate got converted into soluble form and a major portion still remained insoluble. This may be because certain portions of insoluble phosphates remain bound and are not solubilized by microorganisms (Yandigeri *et al*. 2011).

Cyanobacterial strains tested also showed nitrogen fixation activity in presence of insoluble phosphates as was shown in normal BG-11 medium with soluble P source (K_2HPO_4). There was, however, different response for nitrogen fixation, measured as Acetylene Reduction (AR) activity in presence of TCP and MRP. Initially, all the strains showed maximum AR activity after 14 days of incubation which further decreased as the days of incubation increased when K_2HPO_4 was provided as a 'P' source. It was reported that the nitrogenase activity reaches its peak and then decreases quickly as the culture becomes old (Yendigeri *et al.* 2010). This may be due to the presence of readily available P (K_2HPO_4) from the beginning, which was quickly utilized to show normal nitrogenase activity. It was found that nitrogenase enzyme activity gradually increased and formed a plateau to maintain enhanced activity for relatively longer period of incubation when insoluble 'P' source is utilized. These strains thus, showed increase in AR activity as days of incubation progressed irrespective of the 'P' source (TCP or MRP) provided in medium. *N. muscorum* and *W. prolifica* showed maximum AR activity in presence of 20 mg MRP whereas *A. variabilis, T. tenuis* and *A. fertilissima* showed maximum AR activity in presence of 20 mg TCP after 28 days of incubation. This may be due to the fact that in presence of insoluble phosphate source (MRP and TCP), the 'P' was slowly made available to the organisms and based on their growth required a longer

Table 3 Time course effect of different phosphate sources on acetylene reduction (AR) activity*of cyanobacteria

Treatment (Strain)	Days	K_2HPO_4	TCP (mg P/L) 10	TCP (mg P/L) 20	MRP (mg P/L) 10	MRP (mg P/L) 20	Mean
A. variabilis	7	2.17	0.73	1.04	0.80	0.98	2.876
	14	5.73	2.72	2.96	1.54	2.35	
	21	3.82	3.07	3.58	2.08	3.95	
	28	1.40	4.13	5.91	3.12	5.46	
N. muscorum	7	2.14	0.71	0.83	0.94	1.12	2.830
	14	6.02	1.47	1.65	1.52	2.09	
	21	4.46	2.61	2.86	2.13	3.98	
	28	2.86	3.95	4.64	4.45	6.17	
W. prolifica	7	1.93	0.63	0.94	0.74	1.01	2.486
	14	4.98	1.22	2.08	1.37	2.42	
	21	2.25	2.37	2.92	3.13	3.81	
	28	0.96	3.63	4.54	3.94	4.87	
A. fertilissima	7	1.82	0.81	0.93	0.52	0.60	2.090
	14	4.73	1.11	1.24	0.90	1.00	
	21	2.91	2.52	2.98	1.89	2.03	
	28	0.92	4.24	4.45	2.73	3.46	
T. tenuis	7	2.02	0.72	0.78	0.98	0.81	2.194
	14	4.92	1.11	1.06	1.15	1.28	
	21	2.32	2.08	3.01	2.24	2.96	
	28	1.10	2.99	4.81	3.15	4.42	
	S	*T×S*	*T×D*	*S×D*	*T×S×D*		
SEM±	0.006	0.013	0.012	0.012	0.026		
CD (P = 0.05)	0.016	0.036	0.032	0.032	0.072		

*Values are µmole C_2H_4/mg chlorophyll/h.

period of time to show gradually higher activity which is another reason for maintenance of nitrogen fixation under these conditions (Natesan and Shanmugasundaram 1989, Degerholm *et al*. 2006). Among all the strains, *N. muscorum* showed maximum AR activity, i.e. 6.17 µmole C_2 H_4 /mg chl/ h followed by *A. Variabilis,* i.e. 5.46 µmole C_2 H_4 /mg chl/ h (Table 3). In general, it was also found that most of the strains showed more AR activity in presence of higher concentration of TCP/MRP than that in presence of lower concentration. Overall, these cyanobacterial strains showed best mean AR activity at TCP and MRP (20 mg P/l) which was more than the positive control (K_2HPO_4) irrespective of the days of incubation. This study has clearly shown that the preference of insoluble phosphate source to solubilize P is a strain dependent character and the cyanobacteria could maintain nitrogenase activity for a longer duration in presence of insoluble phosphate as compared to soluble phosphate source. No doubt, cyanobacteria are considered to be an efficient sink for N and continuously causing N-transformations, there are reports available which show that cyanobacteria also have ability to solubilize P along with fixing atmospheric nitrogen (Natesan and Shanmugasundaram 1989, Yandegeri *et al.* 2011). Therefore, it indicates that there is highly significant correlation between P-solubilization/availability and nitrogen fixation activity during incubation (Yendigeri *et al.* 2010). The results of current study are in agreement with the above reports and a clear correlation between available P and AR activity was observed (Fig 1). As the availability of phosphorus increased, the nitrogenase enzyme activity also increased. Stewart and Alexander (1971) found that uptake of phosphorus results in a rapid stimulation in acetylene reduction by *Anabaena cylindrica*, *A. flos-aquae*, *Anabaenopsis circularis* and *Chlorogloea fritschii*, with a response being obtained to less than 5 µg/L of phosphorus. The rapid response observed in the rate of acetylene reduction when phosphorus is supplied suggests that the response may be via a stimulation of the formation of ATP, which is required for nitrogenase activity in blue-green algae (Smith and Evans, 1970, Haystead *et al*. 1970). On the other hand, the available phosphorus and growth measured in terms of total chlorophyll content are not linearly correlated and showed a curvilinear relationship (Fig.2). It is observed that initially, there is no effect on chlorophyll content by the availability of phosphorus but on further incubation, the chlorophyll content decreases irrespective of increase in phosphorus availability. It is because, available phosphorus is utilized for formation of ATP for nitrogenase activity and thus does not affect growth. This is similar to the observation reported by Bbalali *et al*. (2013) where they observed no significant correlation between chlorophyll *a* and logarithm chlorophyll *a* with dissolved phosphorus (P>0.05). They could not find any relationship between chlorophyll *a* and phosphorus since the phosphorus-chlorophyll relationship most probably is outcome of the dependence of algal growth rates on phosphorus availability. Hoyer *et al*. (2002) suggested that phosphorus accounts for more variance in chlorophyll than in nitrogen in nearshore coastal Florida waters and fresh water. Meeuwig *et al.* (1998) also illustrated that the relation between chlorophyll and total nitrogen is marginally stronger than that between chlorophyll and total phosphorus. These organisms also have ability to synthesize phosphatases to mobilize organic P fraction of the soil and making it available to plants besides dissolution of inorganic P. Thus, due to their ability to grow well and fix atmospheric nitrogen in the absence of combined nitrogen and soluble phosphates, these heterocystous cyanobacteria especially *A. variabilis* may be used for the preparation of microbial inoculant or all these cyanobacterial strains tested i.e. *N. muscorum*, *T. tenuis, A. fertilissima* and *W. prolifica* can be developed as a consortium inoculant which will contribute both for nitrogen and phosphorus. It would be more beneficial in developing countries like India with limited P resources.

Conclusion

These cyanobacterial strains showed best mean AR activity in presence of TCP and MRP (20 mg P/l) more than the positive control (K_2HPO_4) irrespective of the days of incubation and *A. variabilis* was best in terms of P-solubilization whereas *N. muscorum* was best in nitrogen fixation. TCP (20 mg P/l) was found to be the best source of insoluble P rather than MRP or K_2HPO_4. This study has

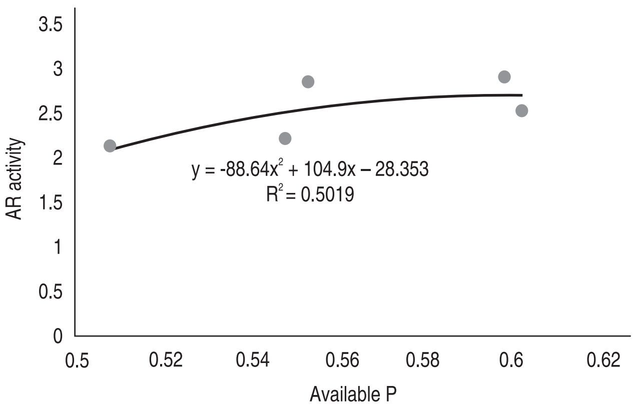

Fig 1 Correlation between available P and AR activity as observed in different cyanobacteria.

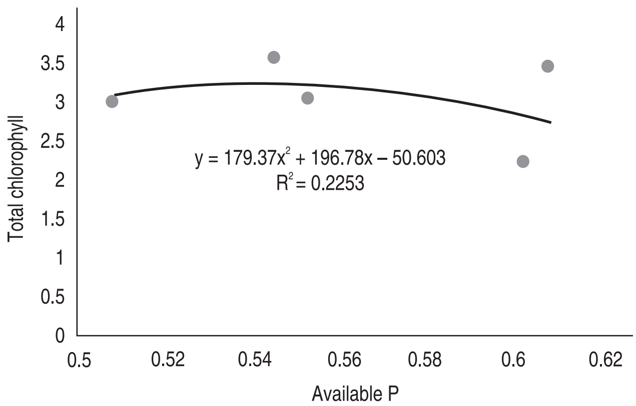

Fig 2 Correlation between available P and total chlorophyll (growth) as observed in different cyanobacteria.

clearly shown that the preference of insoluble phosphate source to solubilize P is a strain dependent character and the cyanobacteria could maintain nitrogenase activity for a longer duration in presence of insoluble phosphate as compared to soluble phosphate source. Further, these organisms also have the ability to synthesize phosphatases to mobilize organic P fraction of the soil and making it available to plants besides, dissolution of inorganic P.

REFERENCES

Bardiya M C and Gaur A C.1972. Rock phosphate dissolution by bacteria. *Indian Journal of Microbiology* **12**: 269–71.

Bbalali S, Hoseini S A, Ghorbani R and Kordi H. 2013. Relationships between nutrients and chlorophyll *a* concentration in the International Alma Gol Wetland, Iran. *Journal of Aquaculture and Research Development* **4**(3): 68–75.

Degerholm J, Gundersen K, Bergman B and Söderbäck E. 2006. Phosphorus-limited growth dynamics in two Baltic Sea cyanobacteria, *Nodularia sp*. and *Aphanizomenon sp*. *FEMS Microbiology Ecology* **58**(3): 323–32.

Hardy R, Burns R C and Holsten R D. 1973. Application of acetylene ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry* **5**(1): 47–81.

Haystead A, Robinson R and Stewart W D P. 1970. Nitrogenase activity in extracts of heterocystous and non-heterocystous blue-green algae. *Arch. Mikrobiol.* **74**(3): 235–43.

Hoyer M V, Frazer T K, Notestein S K and Canfield D E. 2002. Nutrient, chlorophyll, and water clarity relationships in Florida's nearshore coastal waters with comparisons to freshwater lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59**(6): 1024–31.

Khan M S, Zaidi A and Wani P A. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agronomy for Sustainable Development* **27**(1): 29–43.

Mackinney G. 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* **140**(2): 315–22.

Meeuwig J J, Rasmussen J B and Peters R H.1998. Turbid waters and clarifying mussels: their moderation of empirical chknutrient relations in estuaries in Prince Edward Island, Canada. *Marine Ecology Progress Series* **171**(1998): 139–50.

Natesan R and Shanmugasundaram S. 1989. Extracellular phosphate solubilization by the cyanobacterium *Anabaena* ARM 310. *Journal of Bioscience* **14**: 203–8.

Roychoudhury P and Kaushik B D. 1989. Solubilization of Mussorie rock phosphate by cyanobacteria. *Current Science* **58**(10): 569–70.

Smith R C and Evans M C W. 1970. Soluble nitrogenase from vegetative cells of the blue-green alga *Anabaena cylindrical. Nature* **225**(5239): 1253–4.

Stanier R Y, Kunisawa R, Mandel M and Cohen-Bazier G. 1971. Purification and properties of unicellular blue green algae (Order Chroococcales). *Bacteriological Reviews* **35**(2): 171–205.

Stewart W D P and Alexander G. 1971. Phosphorus availability and nitrogenase activity in aquatic blue-green algae. *Freshwater Biology* **1**(4): 389–404.

Venkataraman G S. 1961. The role of blue-green algae in agriculture. *Science Culture* **27**: 9–13.

Watanabe F S and Olsen S R. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Science Society of America Journal* **29**(6): 677–8.

Whitton B A, Grainger S L J, Hawley G R W and Simon J W. 1991. Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. *Microbial Ecology* **21**(1): 85–98.

Yandigeri M S and Pabbi S. 2005. Response of diazotrophic cyanobacteria to alternative sources of phosphorus. *Indian Journal of Microbiology* **45(2**): 131.

Yandigeri M S, Yadav A K, Meena K K and Pabbi S. 2010. Effect of mineral phosphate on growth and nitrogen fixation of diazotrophic cyanobacteria *Anabaena variabilis* and *Westiellopsis prolifica*. *Antonie van Leeuwenhoek* **97**(3): 297–306.

Yandigeri M S, Meena K K, Srinivasan R and Pabbi S. 2011. Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. *Indian Journal of Microbiology* **51**(1): 48–53.