

DIFFERENTIAL RESPONSE TO P-STRESS ON THE GROWTH, PHOSPHOMONOESTERASE ACTIVITY AND ORGANIC PHOSPHATE MOBILISATION IN DIAZOTROPHIC CYANOBACTERIA

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The diazotrophic heterocystous cyanobacterial strains of four different taxonomic groups were able to grow under inorganic-P-limited (P-stress) condition with a little cell biomass, which was supported by the stored polyphosphate granules. Nitrate-N could support the growth of all the strains under P-limitation, which acted as an important nutrient, and an able support to overcome P-stress. All the strains under P-limitation synthesised the phosphomonoesterase enzyme and the resultant activity differs among the strains. Almost all the cyanobacteria investigated showed potential to mobilise organic-P substrates such as p-nitrophenyl phosphate (p-NPP), glucose-6-phosphate (G-6-P) and β -glycero phosphate (Gly-P) as evident in the diminishing PMEase activity and growth. Among the strains, *Calothrix brevissima* and *Anabaena flos-aquae* behaved as a novel organism to survive under P-stress and solubilise organic-P substrates.

Key words: combined inorganic-N sources, diazotrophic cyanobacteria, growth, inorganic-P, organic-P mobilisation, P-limitation (P-stress), phosphomonoesterase

INTRODUCTION

Being oxyphotosynthetic, cyanobacteria form a group of procaryotes that are dominant in tropical and sub-tropical regions. They contribute significantly to Carbon and Nitrogen fixation in their habitats. They are an important constituent of many soils, including the surface crusts in semiarid regions and mine-spoilt wastelands. Their presence is prolific in many areas, which are wet or submerged for part of the year, especially rice-fields. The ability to fix dinitrogen is widespread in soil cyanobacteria (Roger and Kulasooriya 1980)

and these N₂-fixing species often constitute more than half of the species present in soils not enriched with nitrogenous fertilisers. Combined inorganic-N is actively assimilated through a series of steps dependent on the availability of N-sources. When combined nitrogen is absent, these organisms differentiate thick-walled cells called "heterocysts" that isolate the N₂-fixing enzyme system (nitrogenase) from the oxygen inactivation by enhanced respiration, and by the barrier of the heterocyst envelop (Wolk *et al.* 1994). Some non-heterocystous species are also able to fix N₂, but the extent of this in the natural system has not been quantitatively estimated (Bergman *et al.* 1997).

Cyanobacteria may not only play a key role in the global cycles of several elements but they are also becoming increasingly important economically in both constructive and harmful ways. Phosphorus is one of the least available essential nutrients in the soil and many of the soils around the world are deficient in inorganic-P (Bielecki 1973). In terrestrial environments a substantial portion of P is sequestered in the form of poorly soluble mineral inorganic-P (usually calcium phosphates) or organic matter (Goldstein 1994).

There is considerable evidence that inorganic-P is more often the limiting nutrient in fresh water and marine ecosystems (Heckey and Kilham 1988, Vaultot *et al.* 1996). Nutrient storage is a valuable attribute to enabling cells to utilise pools of nutrients that are spatially and temporally separated so that growth is maintained during periods of nutrient scarcity. Polyphosphate granules are generally present in exponentially growing cells and may accumulate to high levels in diazotrophic or N-deficient cells. The granules are degraded in P, Fe and C-deficient cells (Tang *et al.* 1995). The phosphorus storage in cyanobacteria may be largely proving them with a competitive advantage (Sommer 1985). In the absence of inorganic-P in natural ecosystem, cyanobacteria can utilise organic-P (Fogg *et al.* 1973). Although the cyanobacterial potential to utilise organic-P cannot be generalised, there are several reports suggesting that they can solubilise phosphomono- and diesters (Ihlenfeldt and Gibson 1975, Whitton and Potts 2000), hydroxyapatite (Cameron and Julian 1988), phytic acids (Whitton *et al.* 1991), glycerophosphates (Sarma and Mehta 1985) and nucleotide phosphates (Hirosawa and Wolk 1979). They perform this function by cleaving the inorganic-P moiety using cell bound surface phosphatases as well as extra cellular phosphatases (Healey 1982). The phosphate depletion of the cells leads to the synthesis of increasing amounts of cellular alkaline phosphatases. The phosphatases of cyanobacteria probably serve a vital role in natural cyanobacterial population. Whitton (1991) while studying the surface phosphomonoesterase activity of cyanobacteria in rice-fields concluded that *Aphanothece* typically grew under P-rich while *Gloeotrichia* under P-limiting condition. Cyanobacteria are often exposed to P-stress

in Indian rice-fields where poor farmers could concentrate only on fertilising their fields with nitrogen.

Living organisms respond to P-limitation by expressing various genes whose products maintain an appropriate range of phosphate concentrations within each cell. A two component system, that consists of histidine kinase SphS and its cognate response regulator SphR, which regulates the expression of the *phoA* gene for alkaline phosphatase under P-limiting conditions in the cyanobacterium *Synechocystis* sp. PCC6803. The SphS-SphR are two component system in this cyanobacterium is found to be the dominant sensory system that controls gene expression in response to P-limitation (Suzuki *et al.* 2004).

In view of the above, the diazotrophic growth of the cyanobacteria under P-stress and derepression of APase activity was investigated. Whether nitrogen sources in the form of fertilisers may promote some growth of cyanobacteria under P-stress, the experiment was performed using ten diazotrophic cyanobacterial (nine from rice-fields) isolates to observe the impact of combined inorganic-N sources under the laboratory conditions. Further their ability to grow in various organic-P utilising the metabolic functions of PMEase was also looked at in detail.

MATERIALS AND METHODS

Organisms and growth conditions. The heterocystous cyanobacterial strains (Table 1) used in the present investigation, were either obtained from Indian Agricultural Research Institute (IARI), New Delhi or isolated from rice-fields of agricultural farm of the University (BHU). The cyanobacterium *Nostoc muscorum* (*Anabaena* PCC 7120) was obtained through the courtesy of Prof. R. Haselkorn from the University of Chicago. The strains were isolated using standard microbiological techniques (Norris and Ribbons 1968). Their bacteria free clones were raised using germicidal UV lamps. They were identified on the basis of morphological characteristics and cell dimensions (Desikachary 1959, Rippka *et al.* 1979). The strains were routinely cultivated and maintained in BG-11 medium (Rippka *et al.* 1979) under photoautotrophic growth conditions in a culture cabinet at 28 ± 2 °C and illuminated with cool fluorescent tubes of intensity; $25 \mu\text{E m}^{-2} \text{s}^{-1}$ photon flux at the bottom of the culture. The experiments were performed in triplicates and repeated three to four times and the mean data of replicates in a single experiment have been presented in figures.

Table 1
List of cyanobacterial strains and their source of origin

S. No.	Cyanobacterial strains	Taxonomic position		Source of origin
		Desikachary (1959)	Rippka <i>et al.</i> (1979)	
1	<i>Anabaena torulosa</i> (Carm.) Lagerh. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	IARI, New Delhi, India
2	<i>Anabaena flos-aquae</i> (Lyngb.) Breb. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	IARI, New Delhi, India
3	<i>Anabaena variabilis</i> Kütz. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	School of Biotechnology, BHU, India
4	<i>Anabaena spiroides</i> Klebahn	Nostocaceae, Nostocales	Section IV	School of Biotechnology, BHU, India
5	<i>Nostoc muscorum</i> Ag. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	Prof. R. Haselkorn, USA
6	<i>Nostoc calcicola</i> Breb. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	Usar Land Soil, Varanasi, India
7	<i>Nostoc punctiforme</i> (Kütz.) Hariot	Nostocaceae, Nostocales	Section IV	IARI, New Delhi, India
8	<i>Calothrix brevissima</i> G. S. West	Rivulariaceae, Nostocales	Section IV	Agriculture farm, BHU, India
9	<i>Scytonema javanicum</i> (Kütz.) Born. ex Born. et Flah.	Nostocaceae, Nostocales	Section IV	Agriculture farm, BHU, India
10	<i>Hapalosiphon intricatus</i> W. et G. S. West	Stigonemataceae, Stigonematales	Section V	School of Biotechnology, BHU, India

Growth of cyanobacterial strains was measured in terms of absorbance of chlorophyll a (chl a) at 663 nm using spectrophotometer (Spectronic 20, Bausch and Lomb, USA), and protein content of the cell biomass.

Estimation of Chlorophyll a (Chl a). Chl a was extracted in 80% acetone (acetone: water; 80:20, %v/v) by incubating the whole cell overnight at 4 °C. The supernatant obtained after centrifugation (10,000g; 15 min.) was analysed at 663 nm in a colorimeter. Specific growth rate (k , $\log_{10} h^{-1}$) was calculated using the formula of Kratz and Myers (1955).

Estimation of protein. After making a homogeneous suspension with the help of sterilised glass beads (2 mm diameter) on the gravitatory shaker, the protein of the cells was assayed by Folin-phenol reagent (Lowry *et al.* 1959).

Assay of Alkaline Phosphatase (APase) activity. APase (phosphomonoesterase-PMEase) assay was performed using cultures grown in P-supplemented and P-depleted medium depending upon the experimental requirements. Enzyme activity was determined following the method of Ihlenfeldt and Gibson

(1975) using Sigma (USA) reagents and expressed as nmol p-NPP hydrolysed mg^{-1} protein h^{-1} as described by Pandey and Tiwari (2003).

Organic-P. The various organic-P such as p-NPP (p-nitrophenyl phosphate), G-6-P (Glucose-6-phosphate) and Gly-P (β -glycerophosphate) were obtained from Sigma chemicals company (USA) to observe their impact on the growth and APase activity in the cyanobacterial strains. These organic-P were filter (Millipore filters of 0.22 μm pore size; Millipore, INDIA) sterilised and used at 40 mg l^{-1} standard concentration.

Inorganic-N. Nitrate was used as KNO_3 (10 mM) and ammonium as NH_4Cl (1 mM). Both were obtained from BDH (BDH Qualigenes, INDIA) and were steam sterilised in an autoclave.

Statistical analysis. The data were analysed statistically using analysis of variance (Two Way ANOVA) as described by Underwood (1997).

Enzyme nomenclature. Alkaline phosphatases (ALPs; orthophosphoric-monoester phosphohydrolase-1 PMEase; EC 3.1.3.1) are a group of membrane-bound phosphomonoesterases (Sergeant and Stinson 1979).

RESULTS

The cyanobacterial strains mentioned in Table 1 differentiate heterocyst and belong to four taxonomic groups. The pattern of heterocyst differentiation differs among them as it is terminal in *C. brevissima*, apical in *H. intricatus* and intercalary in the rest of the strains. The cyanobacterium *S. javanicum* generally shows false branching while the *H. intricatus* is characterised by heterotrichous pattern of morphogenetical differentiation, i.e. prostrate and erect systems.

Growth characteristics. Before coming to the major objectives that how the APases are derepressed under P-stress and scavenge inorganic-P moiety from the organic-P of low as well as higher molecular weight compounds, it was desired to study the growth characteristic of selected rice-field cyanobacterial strains in P-sufficient, P-deficient, diazotrophic conditions and in the presence of combined inorganic-N. A general visual growth of the strains in broth culture was not uniform as evident in *C. brevissima* that exclusively grew by sticking on the walls of glassware while *S. javanicum* and *H. intricatus* form clumps.

Growth under P-rich and P-stress condition. A comparison of growth characteristics of ten cyanobacterial strains examined, revealed that the species of *Anabaena* and *Nostoc* required about 2 days of lag period for the initiation of growth in P-rich condition, while the lag period in *C. brevissima*, *S. javanicum* and *H. intricatus* was extended to 3 days. The presence of phosphate (40 mg l^{-1}) in the growth medium supported luxuriant growth of all the strains examined. The optimal growth yield in the terms of chl *a* under this condition was in *N.*

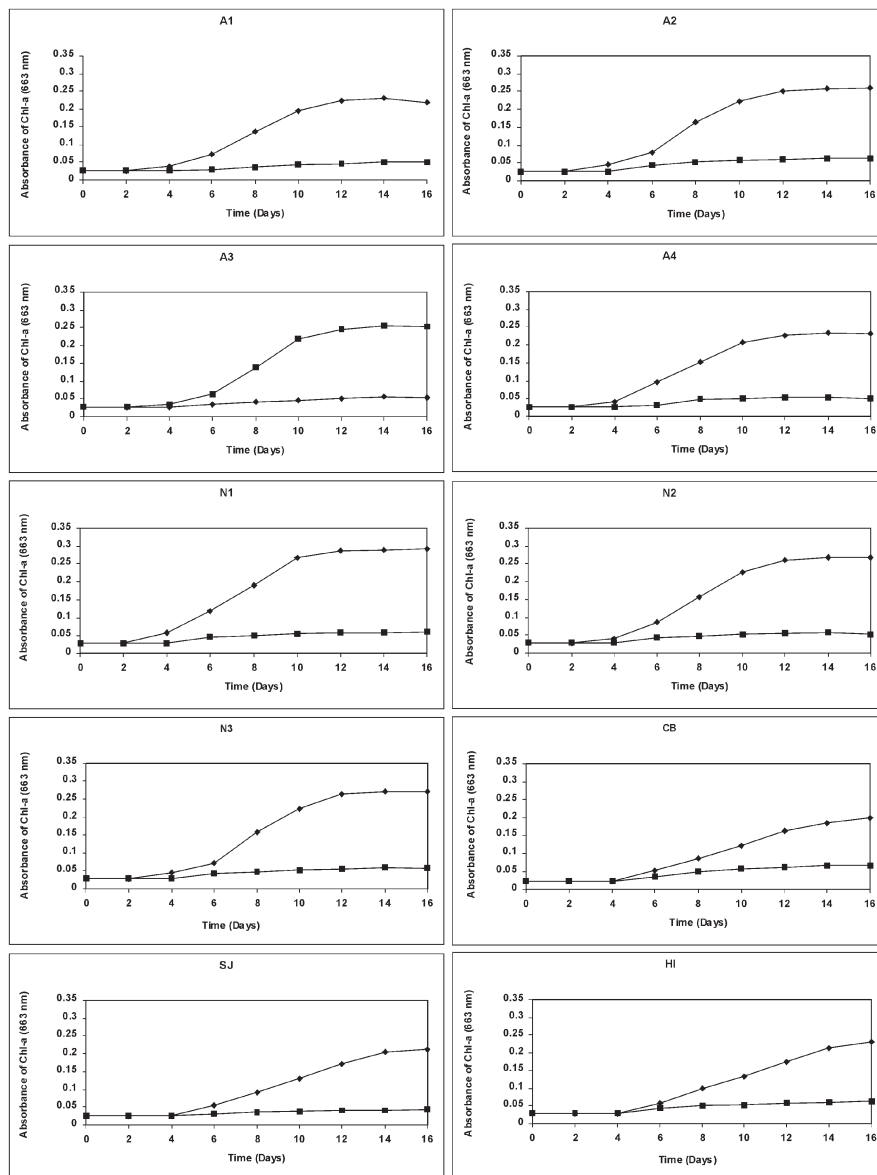


Fig. 1. A comparative growth characteristics of cyanobacterial strains in the P-sufficient (40 mg l^{-1} ; $\blacklozenge$) and P-deficient (P-stress; $\blacksquare$) condition. A time course differential growth pattern was illustrated under the diazotrophic condition. Two-way ANOVA ($F_{9,179}$ cyanobacterial growth against P-sufficient/P-deficient condition = 81.34; $p < 0.001$) showed a significant effect of inorganic-P on growth (A1 = *Anabaena torulosa*; A2 = *A. flos-aquae*; A3 = *A. variabilis*; A4 = *A. spiroides*; N1 = *Nostoc muscorum*; N2 = *N. calcicola*; N3 = *N. punctiformis*; CB = *Calothrix brevissima*; SJ = *Scytonema javanicum*; HI = *Hapalosiphon intricatus*)

muscorum while the minimum growth was observed in *C. brevissima* probably due to its glass-sticking nature. The species *Nostoc* and *Anabaena* reached to stationary phase after 12 days. The other three taxonomically different strains grew slowly and their exponential phase was extended (Fig. 1).

In contrast, there was little growth under P-stress (P-deficient) condition in all the strains compared to P-rich condition. In general the growth yield was nearly 20% under the P-stress and it was difficult to assess exponential phase due to inadequate growth. The maximum growth under P-stress was observed in *C. brevissima* compared to P-sufficient, which might speculate the role of APase under P-stress (Fig. 1).

Growth under the influence of combined inorganic-N sources. Since the growth of all the cyanobacterial strains was inadequate under the P-stress, it was interesting to observe whether the supplementation of combined inorganic-N sources could support some growth under this adverse condition. Growth media prepared with NO_3^- (10 mM) or NH_4^+ (1 mM) were buffered with HEPES buffer (2.5 mM) to maintain the sustainability of the medium at pH 8.0.

Nitrate-N invariably supported maximum growth of all the cyanobacterial strains under P-rich conditions compared to diazotrophic growth or NH_4^+ supplementation. Optimum growth among the strains was observed in *N. muscorum* while minimum being in *C. brevissima*. Ammonium-N, the most reduced and easily available N-source although supported the growth of all the strains but it was always less than that of the diazotrophic ones and was far less than the NO_3^- supplemented growth (Fig. 2A).

In P-stress conditions, growth was invariably much less than P-rich, however, when supplemented with nitrate-N, showed enhanced growth. The growth in the presence of ammonium-N was either analogous to or less than the growth facilitated by N_2 -fixing conditions. Thus the maximum growth of cyanobacterial strains was obtained in the medium supplemented with both NO_3^- and PO_4^{3-} . On the other hand, under P-stress condition, the nitrate-N supported growth was found to offset the adverse scenarios created by P-deficiency (Fig. 2B).

PMEase induction under P-stress. In the natural fresh water ecosystem, cyanobacteria often face P-stress which induces the derepression of APase synthesis. An induction and development of cellular PMEase activity in four taxonomically different species of cyanobacteria with time-course incubation was determined and the results of PMEase activity in ten cyanobacterial strains under P-sufficient and P-stress were presented in Figure 3. The cells grown in P-sufficient in exponential phase were used as the inoculum, showing a little PMEase activity.

Each strain required "switch-on" time for the induction of PMEase activity which differed invariably among the strains. The minimum time of 15 h

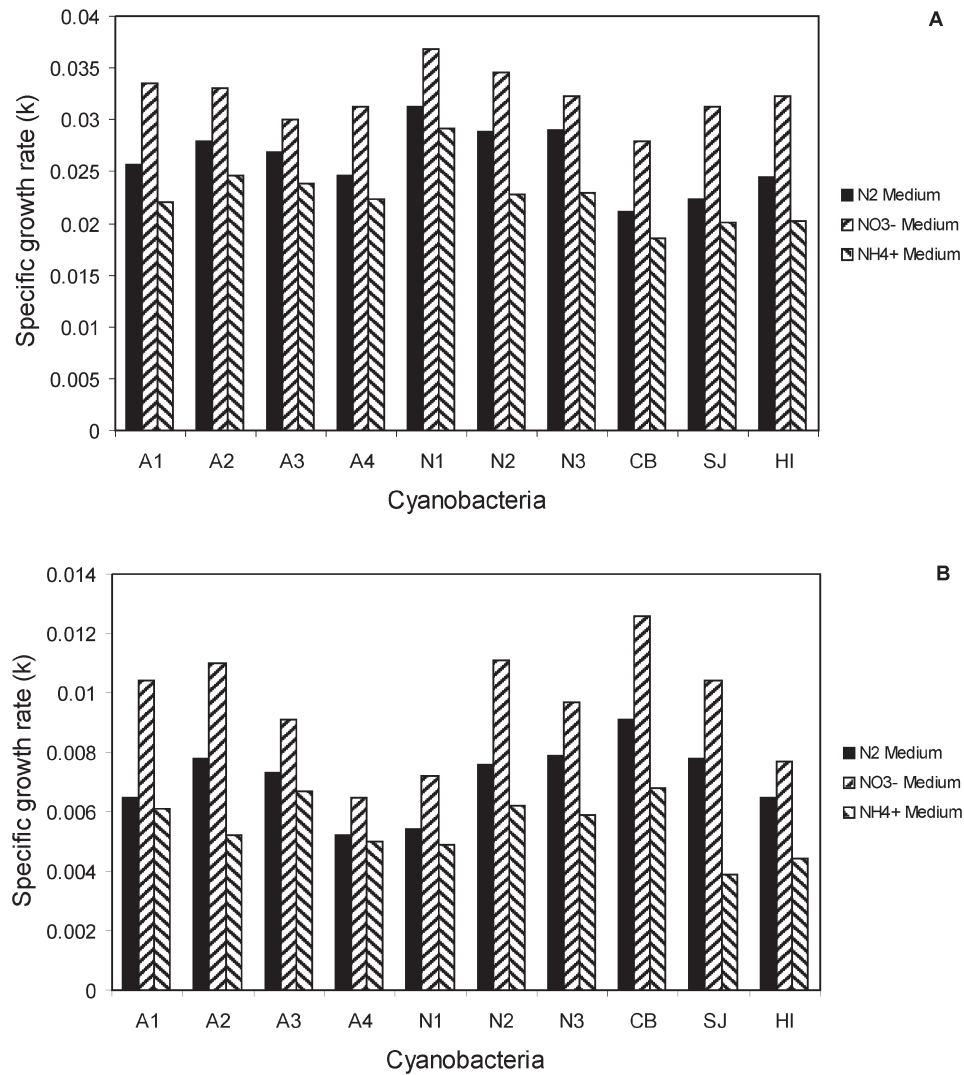


Fig. 2. Effect of combined inorganic-N sources (KNO_3 , 10 mM; NH_4Cl 1 mM) on the relative growth rate of cyanobacterial sample group against P-sufficient (Fig. 2A) and P-deficient (Fig. 2B) while keeping the diazotrophic growth condition as the control parameter (P-rich cells were used as inoculum). Symbols as indicated in blocks. Two-way ANOVA ($F_{9,29}$ cyanobacterial growth in various N-sources against P-sufficient and P-deficient = 149.61/47.34; $p < 0.001$) demonstrated a significant role of combined inorganic-N sources under P-stress (A1 = *Anabaena torulosa*; A2 = *A. flos-aquae*; A3 = *A. variabilis*; A4 = *A. spiroides*; N1 = *Nostoc muscorum*; N2 = *N. calcicola*; N3 = *N. punctiformis*; CB = *Calothrix brevissima*; SJ = *Scytonema javanicum*; HI = *Hapalosiphon intricatus*)

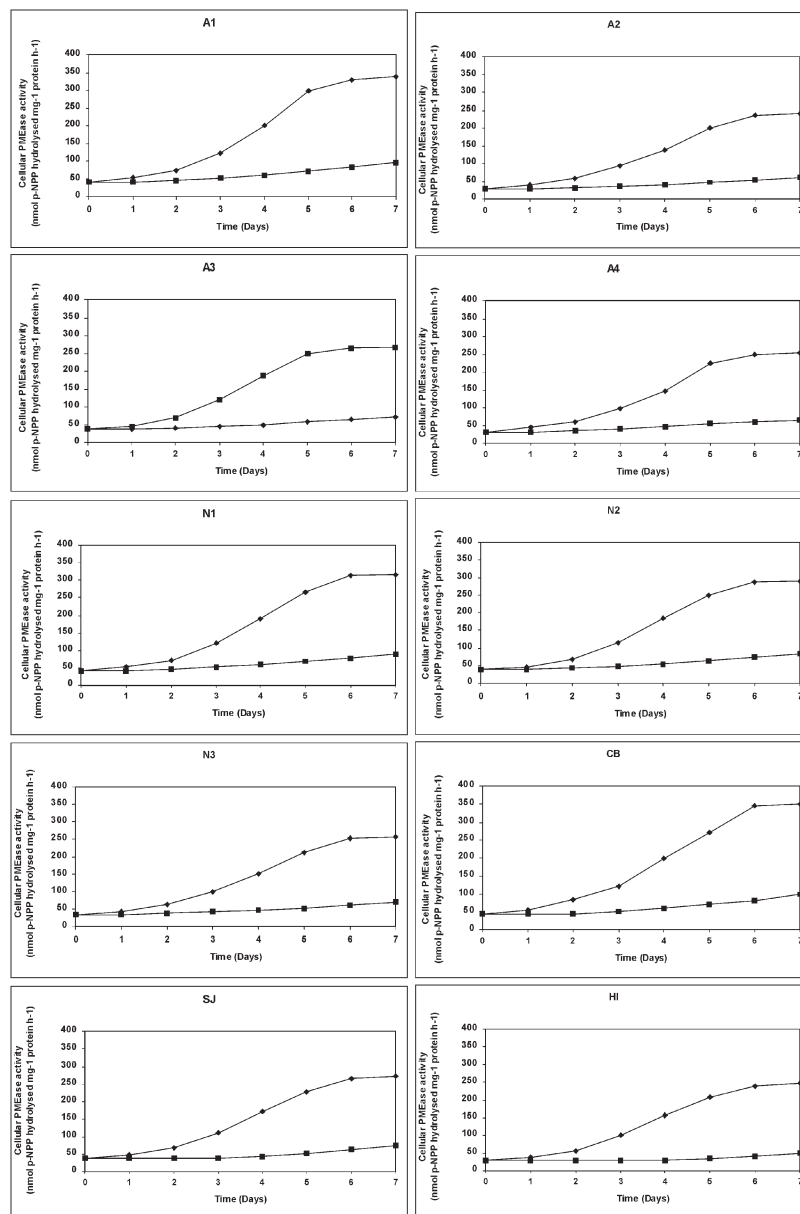


Fig. 3. Influence of presence (40 mg l^{-1} ; ♦) and absence (■) of inorganic-P on the derepression of PMEase activity in cyanobacterial strains. Two-way ANOVA ($F_{9,159}$ cyanobacterial PMEase against P-sufficient/P-deficient condition = 77.53; $p < 0.001$) showed a substantial effect of inorganic-P on PMEase induction (A1 = *Anabaena torulosa*; A2 = *A. flos-aquae*; A3 = *A. variabilis*; A4 = *A. spiroides*; N1 = *Nostoc muscorum*; N2 = *N. calcicola*; N3 = *N. punctiformis*; CB = *Calothrix brevissima*; SJ = *Scytonema javanicum*; HI = *Hapalosiphon intricatus*)

was taken up by *N. muscorum* to express its measurable activity which was followed by *C. brevissima* with 16 h. The maximum "switch-on" time required in *H. intricatus* was 26 h. Even the other species of *Nostoc* and *Anabaena* belonging to the same taxonomic group showed marked differences in their induction time for cellular PMEase.

Once synthesis of PMEase started, as evident in their activity, a gradual increase in the enzyme activity was observed in the cells incubated under P-stress condition. All the four species of *Anabaena* and the three species of *Nostoc* (Fig. 3) investigated showed a sharp increase in their enzyme activity up to the 5th and 6th day of incubation, respectively and further incubation resulted in the stabilisation of enzyme activity. A similar pattern of PMEase induction appeared in *Calothrix*, *Scytonema* and *Hapalosiphon* spp. In general, there was seven to eightfold increase in PMEase activity recorded in cyanobacterial strains compared to its initial level except in *N. muscorum* and *C. brevissima*, where the increase was nine to tenfold. Almost all the strains behaved similarly with respect to the induction in PMEase activity under the P-stress.

In contrast, P impetus could not support any increase in enzyme (PMEase) activity in all the strains up to 5th day but a slight increase in the enzyme activity was observed afterwards, which could be attributed to the depletion in phosphate level of the medium, though to a lesser extent. Among the cyanobacterial strains, *C. brevissima* expressed the highest recorded level of enzyme activity (Fig. 3). The results establish the role of inorganic-P as a feedback inhibitor for the PMEase activity.

Organic-P solubilisation. Cyanobacteria are very often exposed to P-limiting condition in rice-field and natural environment, which may pose a severe problem to the biomass production and their survival. At the same time the natural ecosystems are rich in organic-P and insoluble-P contents. In order to assess the mobilisation of organic and insoluble-P by the cyanobacterial strains, in terms of growth (Fig. 4A) and enzyme activity (Fig. 4B), an experiment was performed in which the strains were grown in p-NPP, G-6-P and Gly-P at 40 mg l⁻¹ concentration.

The inorganic-P (40 mg l⁻¹ standard concentration) supported a luxuriant growth in all the cyanobacterial strains which served as a yardstick (100%) on which the relative growth of the strains in different organic-P were assessed. The growth under P-stress was about 20–25% in the strains which indicated the polyphosphate granules supported growth in all the strains. p-NPP, a substrate used for the measurement of PMEase activity was able to support some growth in the strains which was although less than the inorganic-P supported one but was always better than the growth observed in P-stress. The relative growth under this condition was maximum in *A. flos-aquae* and *C. brevissima*

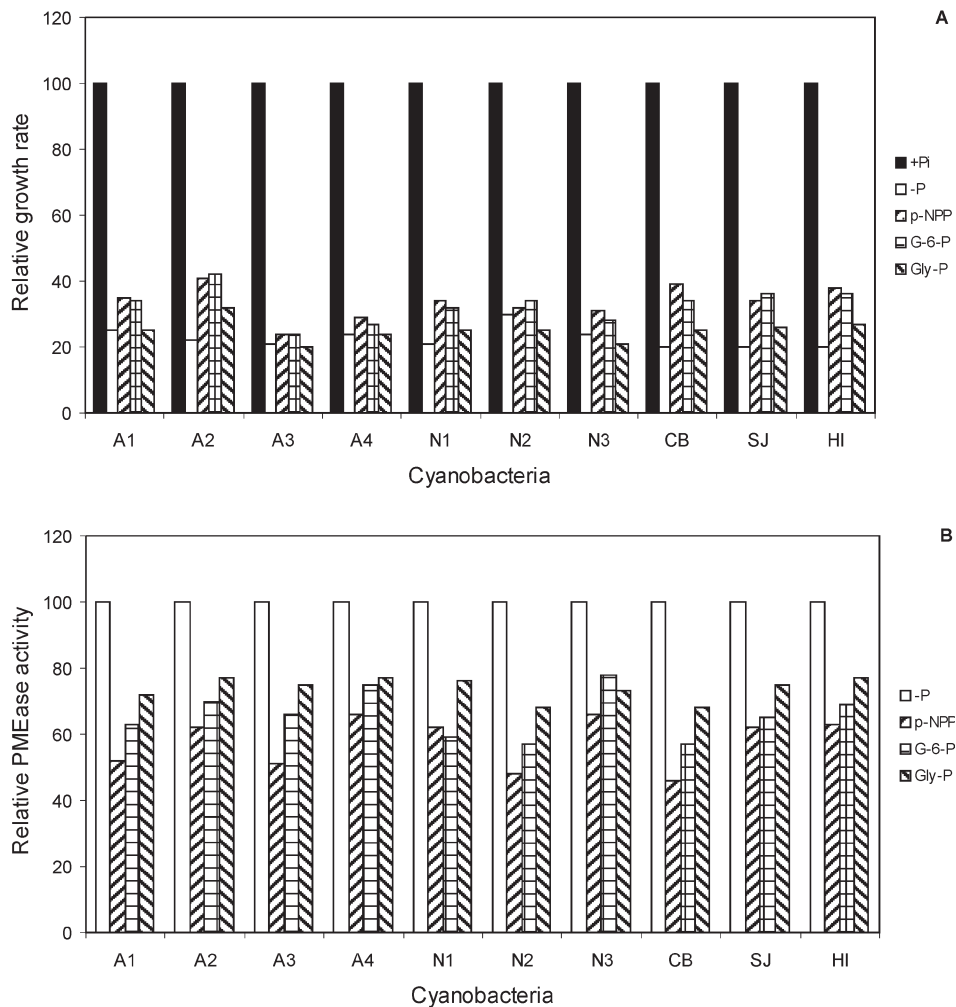


Fig. 4. Organic-P mobilisation by the cyanobacterial strains in terms of growth (Fig. 4A) and PMEase activity (Fig. 4B). Growth and PMEase activity are measured against P-sufficient and P-deficient conditions, respectively, in terms of their mutual relativity, while keeping the P-sufficient and P-deficient conditions as control parameters. Symbols as indicated in blocks. Two-way ANOVA for Fig. 4A ($F_{9,49}$ cyanobacterial growth against various P-sources = 1034.9; $p < 0.001$) and Fig. 4B ($F_{9,39}$ cyanobacterial PMEase against various P-sources = 188.96; $p < 0.001$) showed a considerable effect of organic-P in offsetting P-stress conditions (A1 = *Anabaena torulosa*; A2 = *A. flos-aquae*; A3 = *A. variabilis*; A4 = *A. spiroides*; N1 = *Nostoc muscorum*; N2 = *N. calcicola*; N3 = *N. punctiformis*; CB = *Calothrix brevissima*; SJ = *Scytonema javanicum*; HI = *Hapalosiphon intricatus*)

(ca 40%). The *A. variabilis* with a relative growth rate of 24% was slightly better than the P-stress supported growth (ca 21%). Like p-NPP, G-6-P also supported some growth in all the strains, which was either analogous to p-NPP supported growth or slightly better than that. Among the strains *A. flos-aquae* was found to be the most efficient in mobilising this organic-P whereas the response of *A. variabilis* was the worst in the above respect. The cyanobacterial endeavour to mobilise Gly-P was not up to the mark as the increase in the relative growth rate compared to the P-stress, was dismal (Fig. 4A).

The results of the experiment conducted with three organic-P substances; p-NPP, G-6-P and Gly-P, to analyse the PMEase response to them under P-sufficient and P-stress conditions, have been presented in Figure 4B. No measurable enzyme (PMEase) activity in strains was detected in the P-sufficient condition after 3rd day of incubation, which indicated the complete repression of enzyme synthesis in almost all the strains. Under P-stress, the enzyme activity was recorded at its peak on the 6th day of incubation and values were benchmarked to compare relative enzyme activity. In general, an inverse correlation between PMEase activity and the growth was observed. A lower level of enzyme activity was evident in p-NPP followed by G-6-P, while a higher level of enzyme activity in Gly-P in all the strains indicated inorganic-P deficient (P-stress) status of the incubation medium. Among the strains studied, the *A. flos-aquae* was found to be the most efficient in mobilisation of the organic-P though the enzyme activity was at its lowest inorganic-P in *C. brevisissima*.

DISCUSSION

Cyanobacteria under the nutrient limitation evolved certain defence measures to counteract nutrient deficiency. N₂-fixing cyanobacteria synthesise nitrogenase enzyme during N-deprivation to fix atmospheric N₂ to fetch the nitrogen demand. Likewise under P-starvation they synthesise several classes of APases which function at different pH utilising various kinds of substrates to overcome the P-deficiency in the natural environment.

Under P-limiting conditions, cellular phosphorous concentration declines while the phosphorous uptake potential increases. As a consequence, a pulse of phosphorous delivered to P-limited cells results in substantial formation of polyphosphate reserves (the polyphosphate “overplus” phenomenon) with cellular phosphorous level able to exceed those occurring under steady-state maximum growth conditions (Allen 1984, Riegman and Mur 1984).

P-limitation in filamentous heterocystous cyanobacteria results into the formation of akinetes, which acts as a perennating structure (Pandey and

Kashyap 1987). Motility in many heterocystous forms like *Nostoc* and *Calothrix* is restricted to hormogonia, which are formed under P-limited condition (Whitton 1992). Although no data have been presented on akinete differentiation in cyanobacterial strains but some of them differentiated into akinete under P-stress in present investigation.

Most cyanobacteria do not have to access either organic-P or poorly soluble mineral inorganic-P forms of phosphate which must be converted to soluble inorganic-P in the first place or a low molecular weight organic-P. During the P-deprivation they need to increase their capacity to harness inorganic-P and scavenge phosphorous from any available source, which may require the induction of several genes. Scanlan *et al.* (1997) reported that the Ptr-A protein is synthesised in cyanobacterium *Synechococcus* during the P-starvation and the protein is homologous to the periplasmic phosphate binding protein of *Escherichia coli*. The present study with cyanobacterial strains further supports the above view, as all of them are able to utilise organic-P such as p-NPP, G-6-P and Gly-P efficiently. The utilisation of G-6-P by the cyanobacteria *Oscillatoria* BA010 is well evident in a high level of initial APase activity only to witness its complete inhibition at the later stage (Marco and Orus 1988). Sarma and Mehta (1985) observed the utilisation of Gly-P by the cyanobacteria *Anabaena torulosa*, however the mobilisation of this phosphate was strain specific.

Frequently N:P ratio is used to access the occurrence and diversity of cyanobacteria but the nutrient ratio alone is not a reliable indicator as the critical limiting level of total inorganic-N required for the induction of N₂-fixation is irrespective of N:P ratio (Horne and Commins 1987). They concluded that total inorganic-N should be below 50–100 mg m⁻³ to be able to set off nitrogenase activity. In several North American lakes, nitrogen is just as frequently acted as a limiting factor as phosphorus (Elser *et al.* 1990). The general response to P-stress is an apparent lowering of the cyanobacterial bio-mass, however the extent and nature of that response is dependent upon the internal P-load (polyphosphate granules) and the magnitude of the external load (inorganic-P in the medium).

The above can presumably state that the source of inorganic-N available to the cyanobacteria does influence their cell biomass. The non-N₂-fixing cyanobacteria are favoured by ammonim-N and the diazotrophic strains behave better under N-deficient condition (Oliver and Gant 2000). This is also evident in present investigation that the diazotrophic growth is always better than the cell biomass obtained in NH₄⁺-supplemented medium.

To overcome P-stress condition, almost all the cyanobacterial strains synthesise APase, however their metabolic function is restricted due to non-availability of suitable substrate. The cell death under this condition can be prevented to some extent by the addition of nitrate to the medium. A similar re-

sult has been reported by Pandey and Kashyap (1986) to observe that the addition of ammonium-N to the *Synechococcus* and nitrate-N to the *Nostoc muscorum* has reversed the toxicity of herbicide Machete to a certain degree. These studies imply that probably the energy limitation due to P-stress is responsible for the inhibition in several metabolic functions.

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