PHOSPHATASE ACTIVITY OF NON-HAIR FORMING CYANOBACTERIUM *RIVULARIA* AND ITS ROLE IN PHOSPHORUS DYNAMICS IN DEEPWATER RICE-FIELDS

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Abstract. Phosphatase activity of *Rivularia* strains II and I isolated from a deepwater rice field was studied. When grown under conditions of P-limitation the strains did not form hair and showed great variations in induction time and pattern of enzyme activity in relation to growth. Phosphomonoesterase activity (PMEase) was induced earlier in the *Rivularia* strain I which was maintained in high phosphate concentration in the medium before the study. *Rivularia* strain II on the other hand showed induction of PMEase activity after 3-4 days and this strain was maintained in low phosphorus concentrations in the medium before the study. The comparison of K_m and V_{max} values early in the growth phase showed that affinity of both PMEase (phosphomonester) and PDEase (phosphodiester) for the substrate was higher in early growth phase rather than in later stages suggesting that there is presence of more than one phosphatase enzymes in these organisms. The observations from the above study have important implications in rice fields where constant fluctuations in nutrients can be observed – especially by phosphorus in its most available form phosphate. Equal enzyme activity in both light and dark for the two strains (non hair-forming) implies that phosphatase activity may not be affected by changes in light regimes in rice fields which do occur due to the plant growth. This can in turn contribute significantly to maintain the growth of cyanobacteria for prolonged periods even under unfavorable conditions of phosphorus depletions. These strains of *Rivularia* can therefore biotechnologically exploited as bio-fertilizers (phosphatic fertilizer) when there is phosphorus deficiency. During the unfavorable conditions of phosphorus deficiency, phosphatase activity may play a significant role in providing phosphate for its own growth and for the rice plants.

Key words. *PMEase, PDEase, K_m, V_{max}.*

Introduction

Phosphatases are enzymes, which promote the degradation of complex phosphorus compounds into orthophosphate and an organic moiety, are thus believed to have an essential function in the nutrient dynamics of most of the ecological niches [11]. Microorganisms with phosphatase activity are able to hydrolyze phosphate from a variety of organic phosphorus compounds [2, 3]. Many cyanobacteria are therefore particularly amenable to studies on phosphatase activity, because they can be studied both as field samples and as laboratory isolates. Phosphatase activity has been found in all major groups and numerous species of algae and is found widespread among P-limited cyanobacteria though not universally [9, 12, 17, 18]. Rice fields constitute a very interesting habitat for the study of phosphatase activity as they are dominated by cyanobacteria and contribute significantly to maintain soil fertility [1, 5, 15]. There is evidence that cyanobacteria belonging to Rivulariaceae occur in environments where organic phosphate is an important source of phosphate [10]. Cyanobacteria with the

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ability to form hairs occur in environments where organic phosphates are especially important [16]. As the strains showed the tapering characteristics of *Rivularia* under conditions of P-limitations and these strains studied did not form hairs, it seemed important to determine how it differed from hair forming strains.

The aim of the present investigation was therefore to study the changes in (PMEase and PDEase) activity and induction during the different stages of growth of the two *Rivularia* strains II and I isolated from the rice fields of Uttar Pradesh and West Bengal (India). In particular variations in K_m and V_{max} values of substrate concentration curves using the Michaelis–Menten kinetics denoting the substrate affinity relationships at early and later stages of growth of cyanobacteria was studied. Another attempt has been the comparison between the strain II and I subjected to high and low concentrations of phosphate before the study respectively.

Materials and methods

Isolation and purification of algal samples

Rivularia strain I was isolated from the rice fields of Uttar Pradesh, India, identified by taxonomic keys [6, 7, 14] purified and maintained in culture collections of Laboratory of Algal Biotechnology, Department of Bioscience Barkatullah University, Bhopal, India, where it had been subjected to high phosphate concentration and stored in liquid nitrogen, while strain II was isolated from the rice fields of West Bengal (India) and was subjected to low phosphate concentration before the experimentation. The cyanobacterial filaments were microscopically examined, which revealed these strains did not form hairs in culture and they contained scytonemin pigment in abundance.

Culture conditions

Cultures were grown at 30 °C and 100 μmol photon m^{-2} s^{-1} PAR continuous light regime using cool florescent source. The media used was CHU 10 (CHU 10-D) medium as given by [4] and slightly modified by [8] with P reduced to 1 mg^{-1}, EDTA as chelator and pH buffered to 7.6 with HEPES [12]. Materials for inoculations for phosphatase activity and growth were sub cultured twice at 4 days interval, centrifuged inoculated in fresh medium to give 10 mg^{-1} dry weight. Three replicates were used and the flasks were pooled at the time of harvesting to provide sufficient material for measurements of dry weight. The organisms were dried at 105 °C and the yield was recorded as dry weight.

Phosphatase assay

Cyanobacterial cells were harvested every day till induction and there after every two days during growth follow till 20 days for the two strains to monitor changes in phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity. Cellular material was obtained by centrifugation at 5000 g for 10 minutes.

PMEase activity was assayed routinely using colorimetric method using para-nitro-phenyl-phosphate (p-NPP) and PDEase activity using bis-para-nitro-phenyl-phosphate (bis-p-NPP). The assay was studied by the method given by [19]. As the two strains II and I were subjected to high and low phosphate concentration before the study, cyanobacterial cells were transferred to P-minus medium before the phosphatase assay for two weeks to deplete the cells of P. Assays were carried out in a P free version of the medium and buffered with 100 μM glycine (final concentration) to give a pH of 9. For each replicate, a bottle was filled with definite amount of cold sterile medium and

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algal pellets obtained after centrifugation were added to each bottle excluding the control set. Assays were conducted in a water bath with gentle shaking at 30 °C and light flux (100 μmol photon m^{-2} s^{-1} PAR). Dark conditions were also tested and were obtained by wrapping the bottle twice with aluminum foil. Practical details were given in [13, 19]. Substrate was added to the bottles and bottles were tightly capped and run for 30 min. After the assays supernatant was passed through a GF/C filter (Whattman) and the activity ended using the 10% v/v correct base/acid terminator. For the spectrophotometeric analysis, the absorbance of p-NP and bis p-NP was measured by recording the optical density in a Systronic spectrophotometer Model-169 at 405 nm (nanometers). The results are expressed in terms of product (p-NP, bis-p-NP) formed g^{-1} d wt h^{-1} (p-NP = para-nitro-phenol, bis-p-NP = bis-para-nitro-phenol). The filter containing algal material was dried overnight at 105 °C. The mass of the filter, biomass was recorded and biomass of the algae was determined after correction for the mass of the filter. The yield was expressed in terms of dry weight mg l^{-1}.

Results

Fig. 1 shows the induction of PMEase activity using p-NPP was detectable on day 2, while PDEase activity on the other hand was induced on the 2nd day with negligible amount in strain I.

Induction of PMEase activity was detectable on the 4th day in strain II whereas PDEase activity on the 2nd day (*Fig. 2*).

The PMEase and PDEase activity of strain I increased in the initial stages of growth reaching a maximum on days 10 and 8, respectively, and decreased during the later stages of growth. In strain II both monoesterase and diesterase activity increased with the growth of the alga while decreased during the later stages of growth i.e. around day 28, while in strain I the PMEase and the PDEase activity was induced on day 2 of growth and declined slightly around day 20. In general in the two strains phosphomonoesterase activity was more than phosphodiesterase activity. *Fig. 3* shows the yield in terms of dry weight of strain II and I, during the initial growth of the organism PMEase and PDEase activity was found to be maximum while the activity tends to decrease at the later stages of growth.

Table 1 shows the V_{max} and K_m value of the two strains using p-NPP as monoesterase substrate and bis-p-NPP as a diesterase substrate on days 5 and 15 of growth.

A general observation was that for monoesterase, the affinity of phosphatase enzymes for two substrates was higher on days 5 than on day 15. A study to see whether dark condition had any effect on phosphatase activity reveal that the activity in light and dark was the same when assayed on days 8 and 16 with both p-NPP and bis-p-NPP (*Table 2)*.

Discussion

There were three distinct phases with respect to phosphatase activities during growth of Rivularia in batch culture at optimum light flux. During the first phase (to day 4) there were no cell bound or extracellular activity, this coincides with the period when trichomes of sufficient length appear capable of forming hormogonia.

The second phase (days 5–15) is one of rapidly increasing phosphatase activities, which coincides with the gradual loss of ability to form hormogonia. The third phase

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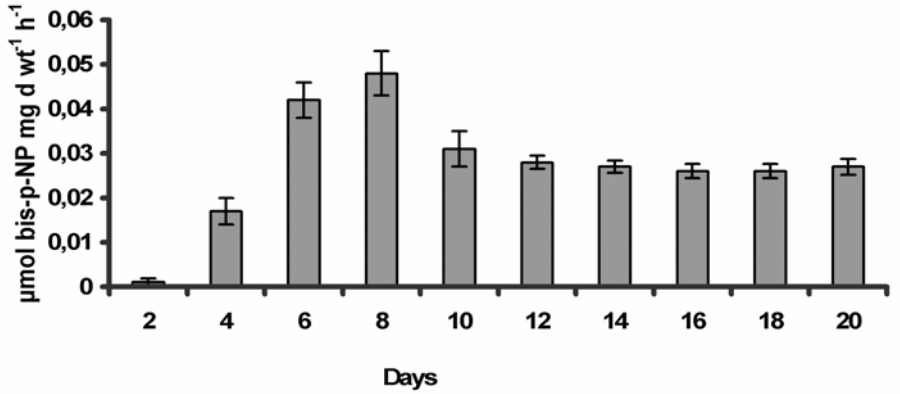

Figure 1. *Phosphomonoesterase (µmol p-NP mg d wt^{-1} h^{-1}) and phophodiesterase (µmol bis-p-NP mg d wt^{-1} h^{-1}) activity in Rivularia strain I.*

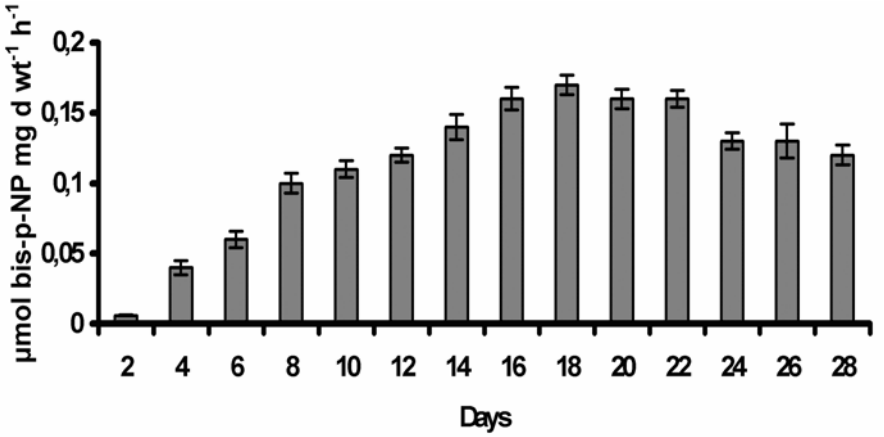

Figure 2. *Phosphomonoesterase (µmol p-NP mg d wt^{-1} h^{-1}) and PDEase (µmol bis-p-NP mg d wt^{-1} h^{-1}) activity in Rivularia strain II over the growth period.*

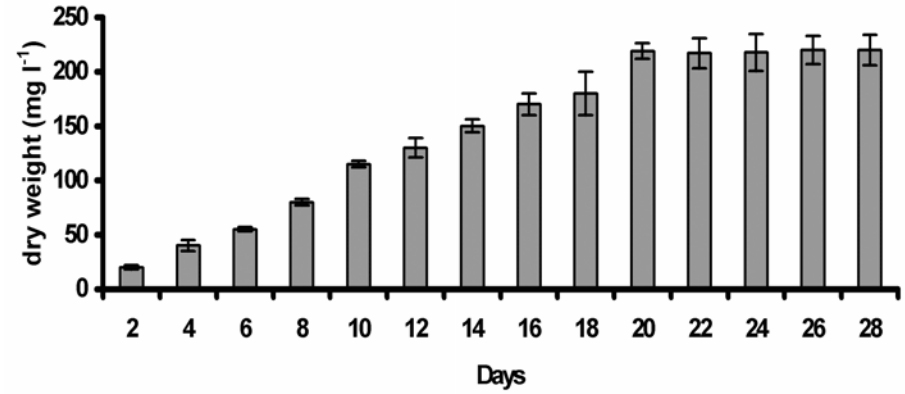

Figure 3*. Graph showing yield in terms of dry weight (mg l^{-1}) in Rivularia strains I and II.*

(days 15–26) is one where activity (PMEase and PDEase) decreases this could be due to the fact that phosphate was removed from the medium within the first few days and stored by the trichomes which had already formed, hormogonia released subsequently were exposed to an environment low in phosphate.

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Table 1. *V_{max} (μ mol product m g^{-1} h^{-1}) / K_m (μM) values of Rivularia strain II and I on days 5 and 15 (μmol product mg^{-1} h^{-1} / μM).*

day	substrate	cyanobacterial strains	
		I	II
day 5	p-NPP	0.21 / 11.21	0.13 / 12.98
	bis-p-NPP	0.019 / 10.76	0.08 / 37.86
day 15	p-NPP	0.17 / 74.26	0.23 / 56.88
	bis-p-NPP	0.16 / 26.01	0.33 / 78.02

Table 2*. Effect of light and dark conditions on PMEase and PDEase (μ mol product mg d wt^{-1} h^{-1}) activity of Rivularia strains on days 8 and 16.*

strains	conditions	p-NPP		bis-p-NPP	
		day 8	day 16	day 8	day 16
I	light	0.101±0.005	0.078±0.006	0.045±0.004	0.025±0.005
	dark	0.100±0.004	0.079±0.019	0.045±0.004	0.025±0.004
II	light	0.118±0.009	0.109±0.019	0.107±0.010	0.104±0.010
	dark	0.119±0.02	0.108±0.018	0.108±0.011	0.124±0.012

The difference in V_{max} and K_m values on days 5 and 15 suggests the presence of more than one phosphatase enzymes whether monoesterase or diesterase enzymes in these organisms. It appears that either one enzyme is induced at a later stage than the first one or that both were induced at the same time, but the activity of one was very low at the initial stages of growth and the activity reaches maximum in the later stages of growth when the activity of the first one was nominal. This could account for the different K_m and V_{max} values.

The K_m values of the three strains at day 15 showed less affinity for the substrate. Equal enzymes activity in light and dark suggests that although there are distinct light regimes in rice fields, fluctuations and decreases in light intensity may not affect phosphatase activity in cyanobacteria.

Due to heterogenity of cyanobacteria filaments a rice-field population might be able to form phosphatases in some filaments leading to hydrolysis of organic phosphorus, which in turn would support the growth and development of other filaments. However when the ambient phosphate is entirely organic, cyanobacteria would depend entirely on phosphatases whatever the external concentrations may be.

Unlike some Rivulariaceae strains the two strains studied lack the ability to form hairs. Both strains I and II (non hair-forming) are similar in that the formation of phosphatase activity coincides with the loss of ability to form hormogonia probably these non hair forming strains have an added advantage over the hair forming strain in not wasting cells as a result of hair lyses every time a P-limited trichome encounters a P-rich environment.

Thus phosphatase activity plays a significant role in rice fields by providing phosphorus for the growth of rice plants under unfavorable conditions of P-depletion and under such conditions cyanobacteria may be able to hydrolyze phosphates to support their growth for prolonged periods.

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