

Short Communications

Salinity and age - induced changes in pigments and biomass production in marine cyanobacterium *Phormidium tenue* (Myxophyceae : Hormogonales)

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The marine cyanobacterium *Phormidium tenue* was studied for biomass, levels of pigments and their photochemical activities as influenced by age of culture and salinity. During the culture period, the trend was similar between the fluorescence excitation of pigments and the production of biomass and that was also similar between the fluorescence emission and the level of pigments such as, phycocyanin and phycoerythrin; but the pattern was not alike between the levels of pigments and the production of biomass. Regarding the salinity-induced responses, the level of pigments, their capacity to transfer energy and biomass production were higher in salinity 40×10^{-3} than in 18 and 100×10^{-3} grown cultures. Thus biomass and pigment characteristics are highly dependant on salinity and age of culture.

Marine cyanobacteria are a group of photo-synthetic organisms adapted to saline stress. Mechanisms of salt adaptation in the marine cyanobacteria were already elucidated in terms of osmoprotective compounds^{1,3} and maintenance of low internal contents of inorganic ions⁴. Saline stress was found to reduce the levels of chlorophyll-*a* in *Microcystis firma* and *Synechocystis* sp.⁵ The pigments especially phycobilins were found efficiently used for photosynthesis at optimal salinity than that at sub- and supra- optimal salinities⁶. These reports reveal that the levels of pigments and their activities in cyanobacteria appear to be sensitive to any stress. As there are only a few such studies⁷, the present work was undertaken to analyse the levels of pigments and their photochemical properties as influenced by salinity and age of culture of cyanobacterium *Phormidium tenue*.

Phormidium tenue (Menegh.) Gomant (Myxophyceae/Hormogonales) was isolated from sediments of the Pichavaram mangroves (lat. $11^{\circ}27'N$; long. $79^{\circ}47'E$) situated along southeast coast of India. *Phormidium tenue* was cultured in 500 ml conical flasks contained with 250 ml of the MN medium⁸ inoculated with 0.06 g of fresh biomass, under sterile conditions at fluorescent light intensity of 160 J.s^{-1} (12:12 h LD cycle ; $29^{\circ}C$) in

MN medium supplemented with urea ($92 \mu\text{g/l}$), potash (4.94 mg/l), superphosphate ($24.7 \mu\text{g/l}$), pH 8 and salinity of 40×10^{-3} , as standardized for *P. tenue*⁹. The culture was harvested every 24 h for 10 days, to study the effect of age-induced responses. The cultures which were grown in salinities of 18, 40 and 100×10^{-3} were harvested after 10 days of inoculation to study the effect of salinity. The harvested cultures were thoroughly washed in distilled water and weighed fresh for their biomass, extracted in ice cold acetone for estimation of chlorophyll-*a* and carotenoid or extracted in phosphate buffer (0.05 M, pH 6.8) for phycobilins (phycocyanin, allophycocyanin and phycoerythrin). The extracts were read in a double beam spectrophotometer (450, 562, 615, 652, 663 nm) (Hitachi 220S, Japan) and the pigments were quantified.^{10, 11}

Fluorescence emitted by acetone extract was estimated in a range from 600-750 nm at a temperature $25 \pm 1^{\circ}C$ with an excitation at 545 nm using a spectrofluorometer (Jasco, Japan). Excitation spectrum was also measured in a range from 500-580 nm with an emission at 685 nm in the same spectrofluorometer. In both the spectral analyses the concentration of pigments in the acetone extracts, was kept constant.

The biomass increased linearly up to 7 days of

culture and declined thereafter (Fig.1). The pigments such as chlorophyll-*a* and carotenoid, showed a similar trend of increment up to 3 days and decreased thereafter. Phycoerythrin and phycocyanin showed a pattern of increase up to 5 days. It is important to note that the levels of chlorophyll-*a* and carotenoids increased only up to 3 days of culture (Fig.1). But, still there was an increasing trend of biomass even after 3 days of culture (Fig.1). There seems no relationship between the levels of photosynthetic pigments and biomass production during the culture period. This is partially in support of a previous report¹² that determination of photosynthetic pigments is only an approximate indication of photosynthetic potential of any species. Hence, we further attempted the photochemical activities of the pigments extracted in acetone for their energy transfer process, as already described by Murthy *et al.*⁷

The acetone extract from *Phormidium tenue* culture was analysed for the emission and excitation spectral activities. Figure 2 shows that the fluorescence emission intensity increased steeply by 74.7% between 24 and 48 h of culture and increased up to 6 days of culture and declined thereafter by 18% between 6 and 7 days of culture. The pattern of emission spectrum (Fig. 2) looks alike to that of chlorophyll increase till days 2-5 (Fig. 1) which also increased up to 2 days of culture. Hence, it is suggested that the energy transfer process during fluorescence emission, might have taken place from phycocyanin and phycoerythrin which are the predominant pigments of *Phormidium tenue* (Table 1). In higher plants, it was opined that the process of energy transfer takes place during emission from chlorophyll pigments and that process during excitation from accessory pigments¹³.

The excitation spectrum of acetone extract from *Phormidium tenue* (Fig. 2) shows that fluorescence increased by 141% between 24 and 48 h of culture. The level of fluorescence remained rather constant up to 6 days of culture and declined by 40.3 % between 6 and 7 days of culture. The trend of excitation spectrum (Fig.2) appears akin to that of biomass production (Fig.1), suggesting the possible involvement of excitation process of pigments in the production of biomass.

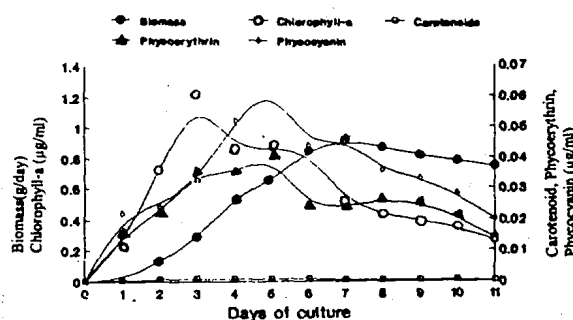


Fig. 1—Biomass production (g/day) and levels of chlorophyll-*a* (µg/ml), carotenoids (µg/ml), phycoerythrin and phycocyanin (µg/ml) in *Phormidium tenue* with least square significance (LSD) values at 5% level are 0.07, 0.09, 0.94, 0.08 and 0.08 respectively

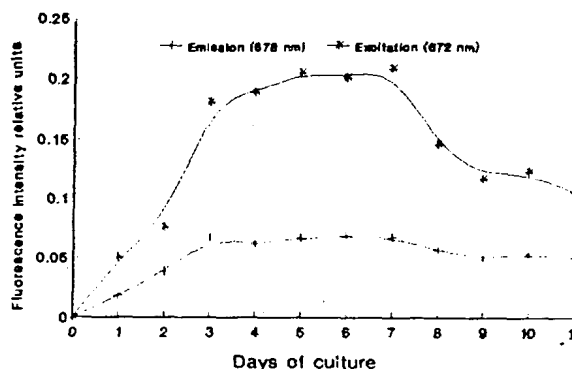


Fig. 2—Emission (678 nm) and excitation (672 nm) for acetone extract from *Phormidium tenue* with least square significance (LSD) values at 5% level are 0.05 and 0.08 respectively

Growth of *Phormidium tenue* was measured in terms of biomass and pigment contents as influenced by salinities of 18, 40 and 100×10^{-3} (Table 1). The cyanobacterial response to salinity varied with external salt concentration, as reported earlier^{2,3}. The salinity of 40×10^{-3} induced higher biomass production which was lower by 15.8 and 29% at salinities 18 and 100×10^{-3} respectively. Also the culture grown in 40×10^{-3} exhibited higher levels of chlorophyll, phycocyanin and phycoerythrin by 35, 26 and 41% respectively. But, the value of carotenoid was found high by 62% in 100×10^{-3} (Table 1).

To understand the influence of salinity on photochemical activities of pigments, fluorescence spectra (emission and excitation) were studied (Table 1). The fluorescence emission was maximum at 673 nm and this might be from

Table 1—Influence of salinity on biomass production, levels of pigments, emission (673 nm) and excitation (672.5 nm) spectra for acetone extract from *Phormidium tenue* grown in MN medium for 11 days under laboratory conditions

Salinity ($\times 10^{-3}$)	Pigments ($\mu\text{g ml}^{-1}$)				Biomass (g)	Fluorescence intensity (673 nm)	
	Chloro- phyll- <i>a</i>	Carote- noids	Phycoery- thrin	Phyco- cyanin		Emission spectrum	Excitation spectrum
						(Relative units)	
18	0.176	0.315	10.35	22.00	0.76	0.19	0.70
40	0.238	0.409	13.00	31.10	0.90	0.35	0.82
100	0.146	0.512	10.04	27.80	0.63	0.24	0.57
LSD 5%	0.101	0.090	2.70	9.00	0.13	0.08	0.07

Least Significant Difference at 5% level

allophycocyanin⁷. The fluorescence emission was nearly 2 fold higher in 40×10^{-3} than that in 18×10^{-3} grown cultures (Table 1). The fluorescence excitation was noticed with two peaks, one was minor at 614 nm and another one was major at 673 nm. The excitation peak at 614 nm may be from phycocyanin, while the peak at 673 nm may be due to allophycocyanin as already reported in *Spirulina platensis*⁷. The fluorescence excitation was nearly 40% more in salinity 40×10^{-3} than that in 100×10^{-3} grown *Phormidium tenue* (Table 1). This clearly indicates that extreme salinity caused drastic changes in the pigment systems which are responsible for energy transfer process in the cyanobacterial species.

Salinity-induced changes on fluorescence excitation and biomass production were alike. For instance, these two parameters fell in the same decreasing order in different salinities of 40, 18 and 100×10^{-3} (Table 1). This trend was also evident with the age-induced responses (Figs. 1,2). Salinity-induced alterations in fluorescence emission and level of phycocyanin were similar (Table 1). These parameters fell in the same decreasing order in different salinities of 18, 40 and 100×10^{-3} . This association was also found with the age influenced responses.

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