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Salt Stress Culture of Blue-green Algae *Spirulinafusiformis*

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Abstract: A Study was conducted on the salt stress culture of blue green algae *S. fusiformis.* The blue green algae *S. fusiformis* was grown at the different salinity of sea water as 0.2, 0.5, 1.0, 1.2, 1.5, 3.0, 5.0, 7.0, 14.0, 15.0, 16.0, 17.0 and 18.0 ppt which were enriched with Zarouk medium. A new steady state was established after carry out an initial lag phase. The observed growth rate was slower and inversely related with concentration of salt stress i.e. the more was salinity, the slower was growth rate during the experiment. Instead of growth, a decrease in biomass was also observed in high salinity. The specific growth rate at all salt stress culture was lower than that of control (0 ppt). The result showed that the protein and carbohydrate content were varied from 37.3 to 56.1 % and 16.8 to 31.4 %, respectively. At 1.0 and 1.2 ppt a marked increase in lipids of 19.6 and 15.6% were observed. Highest carotenoid content of 3.53 mg^{-1} g dry weight was found at 16 ppt, which is significantly ($P<0.05$) higher compared to control. Phycocyanin was high at 1 ppt (93 mg^{-1} g dry weight), which is significantly lower than that of control. The result showed that the production of lipids and carotenes in salt stress culture which would be 1-1.2 ppt and 15-16, ppt respectively in laboratory culture conditions.

Key words: *Spirulina*, Salinity, pigments, growth, biochemical composition

Introduction

Cyanobacteria inhabit environments that vary drastically in their saline levels. In the last 15 years many studies were published on the response of cyanobacteria to different environments. It has been suggested that exposure to high salinity is accompanied by a higher demand for energy by the stressed cells (Blumwald and Tel-or, 1982). Salt stress modifies photosynthesis and respiratory activities of *Spirulina* (Vonshak and Richmond 1981) and make variations in the protein synthesis pattern (Hagemann *et al*., 1991). *Spirulina* is commercially produced as a nutrient source in health food, feed and pharmaceutical industries especially in developing countries. The response of *Spirulina* to salinity varies widely, depending on several physiological mechanisms including the accumulation of inorganic and organic osmoregulators (Mackay *et al*., 1984; Reed *et al*., 1986) It was indicated that *S. platensis* adapted to salinity stress by increasing carbohydrate metabolism in cells (Warr *et al*., 1985; Vonshak *et al*., 1988; Martel *et al*., 1992). The objective of this study was to observe the effect of salt stress on *Spirulina fusiformis* by measuring the changes in growth, pigments and biochemical composition.

Materials and Methods

The strain *Spirulina fusiformis* was collected from Marugappa Chettair Research Centre (MCRC), Chennai, India. The culture was maintained by repeated transfer to liquid Zarouk medium. This experiment was done in Zarouk media with different salt stress. Seawater encompasses 1, 3, 5, 7 and 16 ppt salinity were used for the culture of the 1st stage of experiment. Then 0.2, 0.5, 1.2, 1.5 ppt salinity and 14, 15, 17, 18 ppt salinity were used to get the exact change of lipid and carotenoid, respectively. All the experimental seawater comprises of different salinity were then enriched with Zarouk media and autoclaved at 121^{0}C for 15 min accordingly. Three replicates were taken for each experiment and the average was calculated.

Analytical procedure: Chlorophyll-a was determined by using spectrophotometer after absolute methanol extraction utilizing the absorption coefficient factor as described by Vonshak (1997a). Dry weight determination was done by using 20 ml algal sample of suspension which was filtered through a Whatman GF/C filter of 47 mm diameter. The filter was dried in an oven for overnight at 70^{0}C, then put in desiccators for 20 min for cooling and weighed. The specific growth rate was measured by using the formula:

$$M = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

Where M = Growth Rate
x_1 and x_2 are biomass concentration at time interval t_1 and t_2

Protein determination: Six ml 0.5 N NaOH was added to filtered algal samples then sonicate for 2 min and 80°C water bath for 20 min. Then the sample was centrifuged at 3000 rpm for 10 min. The protein content was then measured by Bradford method (Bradford, 1976) using BSA as protein standard. Carbohydrate was determined based on method of Kochart (1978) using glucose as a standard. Total Lipid determination was done based on the method of Bligh and Dyer (1959) as modified by Kates and Volcani (1996). The method used for estimating the total carotenoids was spectrophotometry after extracting the cells in 90% acetone. The phycobilliproteins content was estimated after the extraction in a phosphate buffer (pH 7) following the procedure of MacColl and Guard-Friar (1987).

Results and Discussions

The growth rate of *Spirulina fusiformis* significantly inhibited in salt stress compared to control (0 ppt). An initial lag phase was observed before a new steady state growth was established. After adaptation new growth rate was slower and inversely correlated with concentration of salt stress i.e. the more was the salinity, the slower was the growth rate. High concentration of saline condition reduces the specific growth rate (Vonshak, 1997b). This is due to the inhibition of photosynthetic and respiratory system after exposed to high salt concentration (Ehrenfeld and Cousin, 1984). Not only growth, a decrease in biomass was also observed in the high salinity (Table 1). It has been suggested that the inhibition of photosynthesis arising from rapid entry of sodium, might be the result of the detachment of phycobillisomes from the thylakoid membranes (Blumwald *et al*., 1984) Protein content varies from 38.0 to 58.2 % (Table 2). Carbohydrate content varies from 18.5 to 36.4 and lipid content varies from 7.5 to 19.6 %. At 1.0 ppt a marked increase in lipids of 19.6 was observed which were significantly higher compared to control. Stress cells have a lower protein synthesis capacity increasing carbohydrate and lipid metabolism (Table 3). Infact while the stimulation of carbohydrate biosynthesis by stress condition is well known (Warr *et al*., 1985; Tomaselli *et al*., 1987); the increase in lipid biosynthesis has been seldom documented. Vonshak *et al*. (1996) reported that the cultivation with higher saline concentration had lower chlorophyll and protein contents. Chlorophyll-a content was high at 1 ppt, which is not significantly different from control. At 16 ppt highest carotenoid content of 3.53 mg^{-1}g dry weight was found which is significantly higher than the control. Phycocyanin was high at 1ppt (93 mg^{-1} g dry weight), which is significantly lower than that of control.

Table 1: Dry biomass and Specific growth of *S.fusiformis* grown in different salinity using Zarouk media

Salinity	Biomass (g l^{-1})	Sp. growth
0.0	2.3	0.121
0.2	2.1	0.117
0.5	2.1	0.115
1.0	1.8	0.107
1.2	1.7	0.106
1.5	1.5	0.104
3.0	1.2	0.092
5.0	0.9	0.084
7.0	0.4	0.069
14.0	0.3	0.065
15.0	0.3	0.053
16.0	0.3	0.039
17.0	0.2	0.040
18.0	0.2	0.037

Table 2: Biochemical constituents of *S.fusiformis* grown in different salinity using Zarouk media

Salinity (ppt)	Protein (%DW)	Carbohydrate (%DW)	Lipids (%DW)
0.0	61.5	21.5	8.8
0.2	58.2	27.5	7.5
0.5	57.3	22.1	8.8
1.0	57.1	28.5	19.6
1.2	53.8	20.1	9.2
1.5	52.7	18.5	15.6
3.0	46.1	25.4	12.2
5.0	43.3	34.0	13.9
7.0	38.9	36.4	13.0
16.0	38.0	41.4	15.2

Note: Biochemical constituents of 14, 15, 17 and 18 ppt were not done,
* %DW – Amount in 100 gm dry *Spirulina* powder

Table 3: Pigment content of *S. fusiformis* grown in different salinity using Zarouk media

Salinity (ppt)	Chlorophyll-a (mg l^{-1})	Carotenoids (mg g^{-1} DW)	Phycocyanin` (mg g^{-1} DW)
0	12.5	2.80	132
1	12.47	1.86	93
3	9.5	1.70	74
5	8.25	1.57	74
7	6.24	1.40	60
14	4.8	2.96	58
15	3.9	3.34	46
16	3.6	3.45	44
17	3.1	3.02	42
18	2.9	2.53	35

Note: Pigment contents of 0.2, 0.5, 1.2 and 1.5 was not done, mg g^{-1}
DW – Milligram pigment in one gm dry *Spirulina* powder

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