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Influence of Temperature on Growth and Biochemical Composition of *Spirulina platensis* and *S. fusiformis*

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Abstract

The influence of temperature on growth and biochemical constituents of two species of *Spirulina; Spirulina platensis* and *Spirulina fusiformis* was studied. Various temperatures as 20, 25, 30, 32, 35, 37 and 40^{0}C were investigated. Maximum specific growth rate of 0.141 was found at 32^{0}C for *S. platensis* and that of 0.144 was found at 37^{0}C for *S. fusiformis*. Both species showed negligible growth at 20^{0}C and 40^{0}C and after 6–8 days culture collapsed. Maximum protein content was found at 32^{0}C for *S. platensis* and at 30^{0}C for *S. fusiformis* and was 59.0 and 62.3 %, respectively. At 37^{0}C a significant increase in carbohydrates and in lipids were observed for both species; *S. platensis* (29.3 and 10.5%) and *S. fusiformis* (24.3 and 11.0%). Maximum biomass production of 2.4 g l^{-1} and chlorophyll *a* production of 16.6 mg l^{-1} were observed at 32^{0}C for *S. platensis*. Maximum biomass production of 2.3 g l^{-1} and chlorophyll *a* production of 14.2 mg l^{-1} were observed at 37^{0}C for *S. fusiformis.* A net increase in phycocyanin occurred in both species when cultures were grown at the suboptimal temperature of 25^{0}C. From the results the optimal growth temperature for S. *platensis* found at 32^{0}C and that for *S. fusiformis* found at 37^{0}C. It can be concluded from the findings that both species are suitable for culture in the tropics.

Keywords*: Spirulina, optimal growth temperature, pigment, biochemical composition.*

Introduction

Spirulina is a mesophilic algae. Temperature represents one of the major biological limitations for biomass production of S*pirulina*. To enhance yield of biomass it seems important to maintain the culture temperature as close as possible to the optimum. It is an important environmental factor, which affects all metabolic activities. It also affects nutrient availability and uptake as well as other physical properties of cells aqueous environment. The role of chemical environment on the biochemical profile of the *Spirulina* is of prime importance both for laboratory condition and outdoor mass culture. The chemical composition of *Spirulina* reflects its potential as human food, animal feed and as a source of natural products. Protein content of *Spirulina* ranges from 50-70% of dry weight. This variation in protein content of *Spirulina* occurred generally from growth condition and the percentage of ash present.

Commercially important pigments can be extracted from *Spirulina*. The carotenoid pigments including β- carotene are increasingly used in foods; particularly important as the main source of vitamin A. Chemically synthesized carotenoids are available and in widespread use; but there is also a market for "natural" carotenoids produced from algae (Borowizka, 1994). *Spirulina* is a good source of carotenoids. *Spirulina* contains three biliproteins; phycocyanin, allophycocyanin and phycoerythrin. Ciferri (1983) mentioned that phycocyanin is a water-soluble blue pigment. The blue pigment, phycocyanin may be useful in stimulating the immune system in general (Landau, 1992). It is used as a natural pigment for food coloring, drug and cosmetics industries to replace the currently used synthetic pigments that are suspected of being carcinogens.

Vonshakand and Richmond (1988) pointed that high net biomass output may not be achieved in open ponds due primarily to the difficulties in maintaining the optimal temperature throughout the day and throughout the year. Increasing temperature increases photo-synthetic activity (Jensen and Knusten, 1993). Temperature can also affect the biochemical composition of *Spirulina*. Growth and lipids content of *S. platensis* was affected in temperature changes from 25 to 38 ^{0}C (Tedesco and Duerr, 1989). Many *Spirulina* strain differ in their optimal growth temperature as well as their sensitivity to extreme

ranges (Vonshak, 1997). Tomaselli *et al.* (1987) have showed at their laboratory experiment that the maximum biomass yield was obtained when *Spirulina* grown at the optional temperature of 35^{o}C. In this study two *Spirulina* species were grown for the purpose of overall human use and to investigate growth, biomass and pigment properties under different temperature. This work may be of significant importance to select a suitable species for a particular geographical area, which holds temperature close to the optimal.

Materials and Methods

Strain

In the present study two species of *Spirulina* were studied. *S. platensis* was obtained from the collection of Professor Lokman in KUSTEM. *S. fusiformis* was collected from Marugappa Chettair Research Centre (MCRC), Chennai, India. The culture was maintained by repeated transfer to liquid Zarouk medium.

Culture condition

For the experiment thermostatic controlled incubator was used (PROTECH, Model- MB380). Various temperature as 20, 25, 30, 32, 35, 37 and 40^{0}C were investigated. The *Spirulina* was cultivated in 500 ml Erlenmeyer flasks containing 250 ml sterilized Zarouk media. Ten percent (v/v) of the prepared inoculums (0.07 O.D.) were added to the flasks i.e., 25 ml of the inoculums. The flasks were covered perfectly by cotton wool and aluminum foil and sealed with laboratory sealing film. The flasks were vigorously shake manually thrice per day (morning, noon and at night). Growth rate were measured as chlorophyll *a* concentration every 2 days up to 20 days culture period.

Analytical procedure

Chlorophyll *a* was determined spectrophotometrically (CECIL, Model-7200 Spectrophotometer was used) after absolute methanol extraction utilizing the absorption coefficient factor reported by Vonshak (1997). Dry weight determination was done by 20 ml algal sample of suspension that was filtered through a Whatman GF/C filter of 47 mm diameter. The filter was dried in an oven for overnight at

70°C, 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.

For protein determination, 6 ml 0.5 N NaOH was added to filtered algal samples then sonicate for two minutes and water bath for 20 minutes at 80°C. Then the sample was centrifuged in 100g for 10 minute. The protein content was then measured by Bradford method (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 (1966). The method used for estimating total carotenoids was spectrophotometry (CECIL, Model-7200 Spectrophotometer was used) after extracting the cells in 90% acetone (Vonshak and Borowitzka, 1991). The phycobilliproteins content was estimated after extraction in a phosphate buffer (pH 7) following the procedure of MacColl and Guard-Friar (1987).

Three replicates were taken for each species and for each temperature and the average was calculated.

Results and Discussion

The specific growth rates of *S. platensis* and *S. fusiformis* at different temperature are presented in Figure 1. The best growth of *Spirulina platensis* found was at 32°C and grew well from 30–35°C. *S. fusiformis* showed the best growth at 37°C and grew well in between 32–37°C. At 20°C culture of S. *platensis* collapsed after 8 days having a long lag phase with negligible growth. Similar results happened at 40°C and culture collapsed after 6 days. Culture of *S. fusiformis* collapsed after 5 days at 20°C and after 8 days at 40°C. Mean difference of specific growth in different temperature is significant at the 0.05 levels for both species ($p<0.05$). The results of macromolecular composition and biomass of cultures are reported in Table 1. The results indicated that at highest temperature the protein

content decreased while the carbohydrate and lipid content increased. Maximum protein content of 59.0% was found at 32^0C for *S. platensis*. This value is not statistically different with the protein content found at 30^0C ($p>0.05$) but statistically different with the protein content found at other temperature ($p<0.05$). Maximum protein content of 62.3 % was found at 30^0C for *S. fusiformis*. This value is not statistically different ($p>0.05$) with the protein content found at 25 and 32^0C but statistically different ($p<0.05$) with the protein content found at other temperature. Maximum biomass production of 2.4 g l^{-1} was observed at 32^0C for *S. platensis* and 2.3 g l^{-1} was observed at 37^0C for *S. fusiformis*.

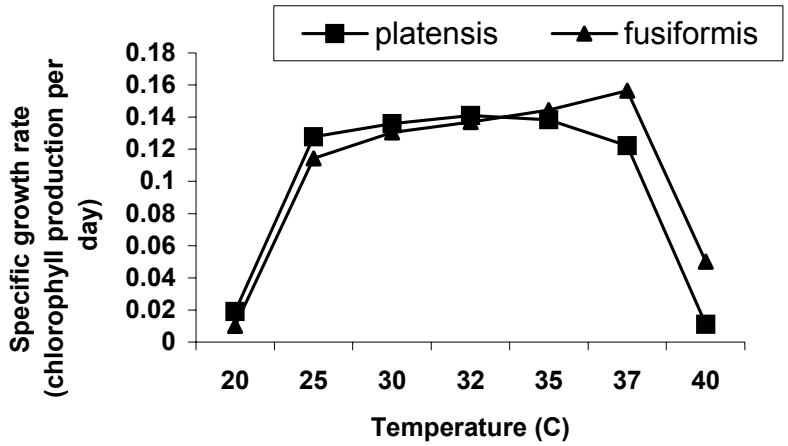

Figure 1- Growth rates of *S. platensis* and *S. fusiformis* at different temperature. The values are means of three replicates.

Many authors reported that by extended growth temperature or by other stress, carbohydrate biosynthesis stimulated with the decreasing of protein (De Oliva *et al.* 1999, Warr *et al.* 1985, Tomaselli *et al.* 1987). The protein, carbohydrate and lipid contents obtained are similar to literature data (Cohen, 1997; Dillon et al. 1995). Trozillo *et al.* (1991) found higher biomass losses in cultures which grown at a low temperature, had a higher carbohydrate content. Skorokhod (1990) studied the influence of temperature on the lipid composition of the blue green algae, *S. platensis* and noticed that the quantity of lipids increased at the temperature 36–38^0C. Tomaselli *et al.* (1993) investigated the physiological and biological responses to

temperature, lipids and salinity in some *Spirulina* species and observed that the degree of saturation in the fatty acid content increased at high temperature and salinity. These findings are in accordance with the results of the present study since lipid content found high at 37^0C. The relative stability of the protein content at elevated growth temperature represents a useful requisite for *Spirulina* biomass production especially in a region where temperature remains high.

Table 1- Biomass yield and biochemical composition of two *Spirulina* species grown at different temperatures. The values are means of three replicates.

Temp (^{0}C)	Protein (% DW)		Carbohydrate (% DW)		Lipid (% DW)		Biomass (g/l)	
	S. plat.	*S. fus.*	*S. plat.*	*S. fus.*	*S. plat.*	*S. fus.*	*S. plat.*	*S. fus.*
25	57.0	60.8	24.2	18.7	8.4	7.9	1.6	1.2
30	58.2	62.3	24.9	19.3	7.0	8.2	2.2	1.5
32	59.0	61.4	23.5	17.8	8.1	10.2	2.4	1.7
35	57.3	58.2	26.7	22.5	9.2	9.8	2.2	1.9
37	53.6	56.5	29.3	24.3	10.5	11.0	1.4	2.3

Note that estimation of 20 and 40^{0}C were not done.

A net increase in phycocyanin occurred in both species when cultures were grown at the suboptimal temperature of 25^0C, which was 133 mg g^{-1} dry weight for both *S. platensis* and *S. fusiformis*. Chlorophyll *a* of 16.6 mg l^{-1} was found for *S. platensis* at 32^0C and 14.2 mg l^{-1} for *S. fusiformis* at 37^0C. Carotenoids were high at 32^0C for both species (Table 2).

The product, phycocyanin is an odorless, non-toxic blue powder with a slight sweetness but when dissolved in water is brilliant with a faint reddish fluorescence. It is used as a natural pigment for food colouring, drug and cosmetics industries to replace the currently used synthetic pigments that are suspected of being carcinogenic. Dainippon Ink and Chemicals, Inc. Company extracts phycocyanin and 1 to 2 tons per year are sold as a food colouring in Japan. It is also used as a colouring agent in candy, ice cream, dairy products and soft drinks. Chlorophyll *a* is a primary photosynthetic pigment in algae and cyanobacteria and is an indicator of the biomass production in the

culture. *Spirulina* is one of the largest sources of chlorophyll in nature. In Brazil, the chlorophyll used as natural green colorant is obtained from spinach, which contains approximately 0.06 mg g^{-1}, whereas *Spirulina* contains more than 1 mg g^{-1} of chlorophyll. The carotenoid pigments including β-carotene are increasingly used in foods; particularly important as the main source of vitamin A. Chemically synthesized carotenoids are available and in widespread use; but there is also a market for "natural" carotenoids produced from algae (Borowizka, 1994). In 1982, the monograph Diet, Nutrition and Cancer published by US national research Council concluded that epidemiological evidence was sufficient to suggest that foods rich in carotene or vitamin A were associated with reduced rate of cancer (Henrikson, 1989).

Table 2- Pigment contents of two *Spirulina* species grown at different temperatures. The values are means of three replicates.

Temp (^{0}C)	Chlorophyll-a (mg/l)		Carotenoids (mg/g DW)		Phycocyanin (mg/g DW)		Allophycocyanin (mg/g DW)		Phycoerythriin (mg/g DW)	
	S. plat.	*S. fus.*	*S. plat.*	*S. fus.*	*S. plat.*	*S. fus.*	*S. plat.*	*S. fus.*	*S. plat.*	*S. fus.*
25	11.4	7.4	1.25	1.84	133	133	62	56	11.7	9.8
30	14.1	11.8	1.32	2.65	132	125	58	54	9.5	8.7
32	16.6	12.4	1.55	2.80	130	130	45	49	10.6	11.0
35	15.6	13.2	1.10	2.74	118	114	31	40	6.7	7.9
37	10.6	14.2	1.06	1.72	97	99	24	22	5.8	6.3

Note that Pigment estimation of 20 and 40ºC were not done.

From the results the optimal growth temperature for *S. platensis* found at 32^{0}C and that for *S. fusiformis* found at 35^{0}C. Different *Spirulina* strain may differ in their optimal growth temperature as well as their sensitivity to high ranges (Vonshak, 1997). He reported that in his lab one *Spirulina* strain marked DA has a relatively low temperature optimum of 30-32^{0}C while one marked EY-5 grew well at temperature up to 40-42^{0}C. Richmond (1992) in his lab experiment found that the optimal temperature for growth of different *Spirulina* strain was between 35-37^{0}C with 40^{0}C definitely injurious. These results compare favorably with data obtained in the present study. It can be concluded from the findings that both species is suitable for culture in the tropics but relatively high and stable protein content of

S. fusiformis at temperature between 25–37^{0}C indicates its more usefulness.

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