Indian J. Plant Physiol., Vol. 7, No. 1, (N.S.) pp. 52-55 (Jan.-March, 2002)

INFLUENCE OF LIGHT AND TEMPERATURE ON MASS MULTIPLICATION OF *SPIRULINA* STRAINS.

BASIRATH RAOOF AND B.D. KAUSHIK

Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012

Received on 13 July, 2001, Revised on 18 Feb., 2002

SUMMARY

Seven strains of *Spirulina*, five of *S. platensis* and one each of *S. maxima* and *S. lonar* were examined for their tolerance limits to light at optimal and above optimal temperatures under controlled conditions. All the strains showed maximum growth rate and growth (measured as μg chlorophyll ml^{-1}) at 25 Klux light intensity irrespective of temperature optima, although total chlorophyll was less (0.817-0.936 μg ml^{-1}) at 40°C as compared to optimum temperature of 30°C (0.909-1.129 μg ml^{-1}). At 45 Klux light intensity and 30°C temperature, except ARM 727, all other strains survived and exhibited 62-69% growth, whereas at 40°C temperature none of the strains could survive- a phenomenon of photoinhibition was existing. The most ideal temperature for mass multiplication of *spirulina* seems to be 30°C at 25 Klux light intensity in Indian conditions.

Key words : **Light, *Spirulina*, temperature**

INTRODUCTION

Spirulina a filamentous cyanobacterium is commercially cultivated as a source of food, feed, production of fine chemicals and for wastewater treatment (Richmond 1987). Although many *Spirulina* units for commercial production have been set up globally, the annual production, amounts to few hundred tonnes of dry biomass (Venkatarman *et al.* 1995). The major handicap which impedes the large scale culture of this cyanobacterium is the higher cost of production than the conventional protein sources, which results in predominantly lower yields per unit area (Vonshak and Richmond 1985).

Intensive outdoor production of *Spirulina* is limited by daily and seasonal variations in temperature and solar radiation, which leads to marked decrease in productivity (Vonshak *et al.* 1982). The temperature during peak summer months in India attains very high values (40±5°C), whereas in winter months in north India it is as low as 2-5°C. A probable explanation for the limitation of growth on exposure to high light intensity and temperature leading to decrease in photosynthetic activity is the phenomenon of photoinhibition. Loss of photosynthetic activity caused by photon flux density in excess to that required to saturate photosyntheshsis is defined as photoinhibition (Powles 1984). The outdoor cultures are continuously exposed to changes in light intensity and temperature whereby inhibition is not only because of the photon flux density but is also due to temperature associated with it. The present work, therefore, examines the effect of light intensity vis-a-vis temperature on growth of seven strains of *Spirulina*.

MATERIALS AND METHODS

Seven strains of *Spirulina* were obtained from the National Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi. One of the strains (*Spirulina S7*) used was from University of Rajasthan, Jaipur. The detailed sources of these strains are as follows:

Strain	Strain No.	Origin
Spirulina platensis	ARM 727	West Germany
Spirulina platensis	ARM 728	Israel
Spirulina platensis	ARM 729	Vietnam
Spirulina platensis	ARM 730	Mysore, India
Spirulina maxima	ARM 787	Mysore, India
Spirulina lonar	ARM 788	China
Spirulina platensis	S7	Jaipur, India

All the strains of *spirulina* were routinely grown in Zarrouk's medium (Zarrouk 1966) at room temperature 30±1°C in batch cultures at 2500 Lux light intensity. The pH of the growth medium was maintained at 9.0 and the cultures were shaken twice a day manually, till the day of harvest. All the cultures were inoculated with the biomass containing 0.25 μg chlorophyll ml^{-1} at the start of experiment. All the growth experiments were done in Zarrouk's medium at the Institute's Phytotron Facility with precise temperature, light and humidity controls. Illumination in the chambers was provided with cool white fluorescent tubes. The experiment was carried out under three factors completely randomized (Factorial CRD) statistical design. Temperatures at 30°C and 40°C were kept constant in different chambers and light intensity was varied from 2.5-45.0 Klux. All the cultures were harvested after 4 days of growth. Growth was measured as total chlorophyll (μg ml^{-1}) content. Chlorophyll was measured after (McKinney 1941) on DU-64 Beckman Spectrophotometer.

RESULTS AND DISCUSSION

All the seven strains of *Spirulina* were grown at varying light intensity (2.5 to 45 Klux) at optimum (30°C) temperature in humidity controlled chambers in Phytotron. Cultures were harvested on the 4th day and growth was determined as μg chlorophyll ml^{-1}. All the strains of *Spirulina* could grow up to 45 Klux light intensity except *Spirulina platensis* ARM 727 from Germany. In general, growth continued to increase with increase in light intensity up to 25 Klux and thereafter, growth reduced gradually with the increase in light intensity. Even at 45 Klux light intensity 62-69% growth compared to growth observed at 25 Klux light intensity (maximum growth) was recorded in all the strains except the strain ARM 727 (Fig. 1A). ARM 727 showed only 32% growth at 40 Klux as compared to growth observed at 25 Klux and a further increase in light intensity (45 Klux) resulted in complete bleaching of cells/filaments (Table 1).

Table 1. Growth (chlorophyll μg ml^{-1}) of *Spirulina* strains (ARM/S numbers) at two different temperatures and varying light intensities (Each value is mean of three replications).

Light intensity (Klux)	*S. platensis* ARM 727		*S. platensis* ARM 728		*S. platensis* ARM 729		*S. platensis* ARM 730		*S. maxima* ARM 787		*S. lonar* ARM 788		*S. platensis* S7	
	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C
2.5	0.612	0.592	0.684	0.656	0.698	0.668	0.745	0.705	0.714	0.682	0.726	0.695	0.729	0.675
5.0	0.697	0.664	0.713	0.692	0.735	0.703	0.762	0.727	0.748	0.715	0.754	0.716	0.768	0.694
10.0	0.741	0.714	0.752	0.715	0.779	0.735	0.802	0.755	0.781	0.725	0.787	0.735	0.802	0.743
15.0	0.809	0.760	0.837	0.782	0.853	0.804	0.872	0.833	0.866	0.823	0.851	0.784	0.849	0.765
20.0	0.878	0.793	0.890	0.802	0.903	0.871	0.956	0.893	0.902	0.853	0.947	0.862	0.936	0.848
25.0	**0.909**	**0.817**	**0.928**	**0.847**	**0.954**	**0.885**	**1.129**	**0.936**	**0.973**	**0.907**	**0.988**	**0.893**	**1.056**	**0.878**
30.0	0.893	0.763	0.893	0.795	0.902	0.845	0.964	0.872	0.911	0.838	0.960	0.853	0.944	0.826
35.0	0.834	0.443	0.820	0.485	0.849	0.496	0.901	0.644	0.862	0.626	0.896	0.606	0.913	0.596
40.0	0.295	0.237	0.698	0.266	0.711	0.262	0.725	0.268	0.705	0.258	0.763	0.280	0.734	0.261
45.0	0	0	0.617	0	0.654	0	0.709	0	0.674	0	0.687	0	0.698	0

C.D at 5%				
	Strain(S)	0.042	Strain x Light	0.132
	Light	0.050	Strain x Temperature	NS
	Temperature	0.022	Light x Temperature	0.071

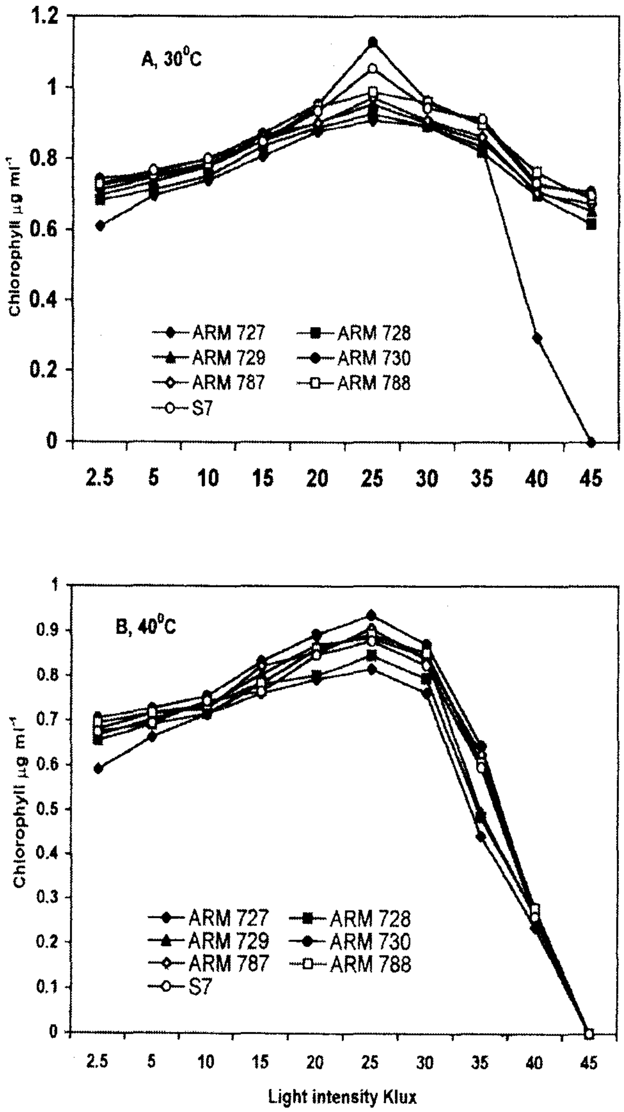

Fig. 1. (A-B). Growth of *Spirulina* sp. strains (chlorophyll µg ml^{-1}) at different light intensities at (A) optimum temperatures, 30°C, (B) elevated temperature, 40°C

In the second set of experiment temperature was maintained at 40°C in humidity controlled chambers and light intensity was varied. At elevated (40°C) temperature also all the seven strains of *Spirulina* showed increased growth in terms of chlorophyll accumulation with the increase in light intensity from 2.5 to 25 Klux. Beyond 25 Klux light intensity a gradual decrease in growth (up to 35 Klux) was observed. At 40 Klux light intensity only 28-31% growth was observed as compared to maximum growth recorded at 25 Klux light intensity. None of the cultures could survive at 45 Klux light intensity and 40°C temperature in this set of experiment (Fig. 1B). These studies clearly indicated that the best growth rate (µg chlorophyll ml^{-1} day^{-1}) observed was at 25 Klux light intensity and at 30°C temperature.

Growth rate of each strain of *Spirulina platensis* calculated at three different light intensities is presented in Table 2. The results showed that the highest growth rate (GR) of 0.219 µg ml^{-1}chlorophyll d^{-1} was attained at 30°C in *S. platensis* ARM 730 at 25 Klux light intensity and closely followed by *S. platensis* S-7. With the remaining strains GR varied from 0.164-0.184 µg ml^{-1} chlorophyll d^{-1}. At elevated temperature (40°C), the GR of *Spirulina* strains was less than that observed at 30°C but the trend was same as observed at 30°C. The interesting observation was made with *S. platensis* ARM 730 whose GR though low (0.171 µg ml^{-1} chlorophyll d^{-1}) at 40°C but still higher than the *Spirulina sp.* ARM 727 and ARM 728 (0.164-0.169 µg ml^{-1} chlorophyll d^{-1}) at ideal temperature of 30°C (Table 2).

The estimation of chlorophyll or proteins as indirect measure of growth is practised in filamentous cyanobacteria, as the biomass in small volumes invariably results in erratic measurements. In the present investigation chlorophyll estimation was taken as a measure of growth,

Table 2. Growth rate (chlorophyll µg ml^{-1} day^{-1}) of *Spirulina* strains at three different light intensities (Each value is a mean of three replications).

Light intensity (Klux)	*S.platensis* ARM 727		*S.platensis* ARM 728		*S. platensis* ARM 729		*S. platensis* ARM 730		*S. maxima* ARM 787		*S.lonar* ARM 788		*S.platensis* S7	
	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C
2.5	0.090	0.085	0.108	0.100	0.112	0.104	0.123	0.113	0.116	0.108	0.119	0.111	0.119	0.106
25.0	**0.164**	**0.141**	**0.169**	**0.149**	**0.176**	**0.158**	**0.219**	**0.171**	**0.180**	**0.164**	**0.184**	**0.160**	**0.201**	**0.157**
35.0	0.146	0.045	0.142	0.080	0.149	0.061	0.162	0.098	0.114	0.094	0.154	0.089	0.165	0.086

as chlorophyll is directly responsible for harvesting light used for generation of assimilatory power essential for CO_2 fixation. It is generally accepted that photoinhibition of photosynthesis is due to the absorption of photon flux density in excess of what is utilised in photosynthesis or dessipated in an orderly fashion (Osmond 1981). It is thus expected that cells will be better able to handle excess energy when grown at optimum temperature for photosynthesis. In the present study, *Spirulina* cultures exposed to high light intensity (45 Klux) and high temperature (40°C) exhibited photoinhibition (Fig. 1A and 1B). Further, it was also shown that all *Spirulina sp.* except ARM 727 were able to survive at 45 Klux but at low temperature (30°C). Similar photoinhibition of *Spirulina platensis* M2 strain was reported by Torzillo and Vonshak (1994) and suggests that cultures when grown at optimum temperature are in as position to handle excess light energy possibly due to its better utilization in photosynthesis and active repair mechanism. A complete bleaching of *Spirulina platensis* ARM 727 at 30°C temperature and at 45 Klux light intensity shows that this particular strain does not tolerate high light intensity stress. Vonshak *et al.* (1988) also demonstrated that *S. platensis* strain Sp-RB was more sensitive to high photon flux densities than strain Sp-G due to a difference in turnover rate of specific protein D1 which is a part of pigment system II.

The study indicated that the exposure of the cells to high light intensity and high temperature results in photoinhibition of *Spirulina strains*. Since such conditions are common during summer months in northern India, hence these findings have direct implication for outdoor mass multiplication of *Spirulina* in these areas. A possible way to overcome inhibitory effects of high light intensity would be through the erection of temporary sheds with locally available material e.g. wheat or rice straw which would cut off at least 25-30% of light intensity and maintain temperature in out door production units. This system would perhaps create conditions near optimal for maximum biomass production of *Spirulina* i.e. about 25 Klux light intensity and 30°C temperature.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. K.V. Prabhu for providing necessary Phytotron Facility to undertake this work. One of us (BR) is thankful to the Indian Agricultural Research Institute for financial support.

REFERENCES

Mac Kinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **140**: 315-322.

Osmond, C.B. (1981). Photorespiration and photoinhibition. Some implications for the energetic of photosynthesis. *Biochim. Biophys. Acta* **639**: 77-98.

Powles, S.B. (1984). Photoinhibition of photosynthesis induced by visible light. *Ann. Rev. Plant Physiol.* **35**: 15-44.

Richmond, A. (1987). *Spirulina*. In: M. Borowitzka and L Borowitzka (eds.), Microalgal Biotechnology, pp. 85-121. Cambridge Univ. Press, Cambridge.

Torzillo, G. and Vonshak, A. (1994). Effect of light and temperature on the photosynthetic activity of the cyanobacterium *Spirulina platensis*. *Biomass and Bioenergy* **6**: 399-403.

Venkatraman, L.V., Bhagyalakshmi, N., Ravishankar, G.A. (1995). Commercial production of micro and macro algae, problems and potentials. *Indian J. Microbiol.* **35**: 1-99.

Vonshak, A. and Richmond, A (1985). Problems in developing the biotechnology of algal biomass production. *Plant and Soil* **89**: 129-135.

Vonshak, A., Abeliovich, A., Boussiba, S., Arad, S. and Richmond, A. (1982). Production of *Spirulina* biomass: Effects of environmental factors and population density. *Biomass* **2**: 175-185.

Vonshak, A., Guy, R., Poplawsky and Ohad, I. (1988). Photoinhibition and its recovery in two strains of the cyanobacterium *Spirulina platensis*. *Plant Cell Physiol.* **29**: 721-726.

Zarrouk, C. (1966). Contribution a c' etude d' une cyanophyceae. Influence de divers facuteurs physigques et chiniques sur la croissance et la photosynthese de *Spirulina maxima*. Thesis, Univ. Paris, France.