Effects of solar ultraviolet radiation on the photochemical efficiency, photosynthetic pigments and biomass production of *Spirulina platensis* *

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Abstract Effects of solar ultraviolet radiation (UVR) on *Spirulina platensis* were studied by investigating its photochemical efficiency, photosynthetic pigments and biomass production while exposed to full spectrum solar radiation or depleted of UVR for understanding how and to what extent UVR influences its photosynthetic physiology and production. It was found that UVR brought about an extra inhibition of photochemical efficiency by 26%—30%. The greatest inhibition of photochemical efficiency in *S. platensis* was observed at noontime, and then recovered to some extent in late afternoon no matter which treatment they were exposed to. The contents of chlorophyll *a*, phycocyanin and carotenoids increased during initial stage of the exposure, but decreased with elongated exposure. UVR decreased the biomass yield by about 6%. It indicated that filtering out UVR of solar radiation would raise the productivity of *S. platensis*, which is an important factor that should be considered in the production.

Keywords: ultraviolet radiation, *Spirulina platensis*, photochemical efficiency, biomass production, pigments.

The cyanobacterium, *Spirulina platensis*, is an important source of protein with its annual production of about 2000 tons[1,2], and it has additional commercial potentials in β-carotene production[3], H_2 generation[4] and wastwater treatments[5,6]. Although *S. platensis* has been harvested on a relative large-scale with the production of 9—28 g dry mass per m^2 per day, the study on optimizing its growing conditions is still being performed[7—9]. It has been known that high levels of irradiance or solar radiation usually result in photoinhibition and reduce the productivity of *S. platensis*[10—12]; depletion of stratospheric ozone can enhance the levels of ultraviolet radiation (UVR) reaching the earth's surface, and excessive UVR will affect photosynthetic performance and damage DNA in algae[13—15]. It is also found that *S. platensis* cells in outdoor cultures are susceptible to photoinhibition and their photochemical efficiency declines in response to increasing sunlight[11]. However, how solar UVR affects photochemical process in *S. platensis* remains unknown. To answer this question, we investigated the effects of solar UVR on the photochemical efficiency, photosynthetic pigments and biomass production of *S. platensis* to understand if and how UVR influences its physiology and production.

1 Material and methods

1.1 Algae culture and UVR treatment

Spirulina platensis was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences, where it was clonally maintained, and was cultured in Zarrouk's medium at 25 ℃ under fluorescent light of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (12∶12 LD) with continued aeration.

UVR exposure experiments were carried out on December 11, 2002 in the campus of Shantou University (116.6° E, 23.3° N). Daily changes of the photosynthetically active radiation (PAR: 400—700 nm) in solar irradiance was monitored by a PAR sensor (SKP-200, Skye Instruments Ltd, UK). The UVA (315—400 nm) and UVB (280—315 nm) radiations were calculated based on their ratios to PAR using a DAYLIGHT model and the information on ozone concentrations (245 DU on 11 Dec., 2002) supplied by NASA (http://toms.gsfc.nasa.gov/ozone). The *Spirulina* cultures were harvested, resuspended

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in the fresh medium, and then dispensed to a final concentration of $OD_{560\,nm}$ 0.3 in six 500 mL UV-penetrable plexiglas containers, which transmit 90% of the irradiance of $\lambda > 280$ nm. A combination of two radiation treatments was set up: (1) 3 cultures received full solar radiation (PAR + UVR) and the plexiglas containers uncovered; (2) 3 cultures received only PAR and the plexiglas containers covered with Ultraphan film 395, a product of Digefra, Germany, with total UV radiation cutting off. The transmission spectra of these filter foils and plexiglas have been published elsewhere[16,17]. The plexiglas containers were placed under the sunlight from 08:30 to 17:30, and during this period the temperature was controlled at 23±1 ℃ by running water.

1.2 Measurements of photochemical efficiency, pigments and biomass production

The photochemical efficiency of *S. platensis* was determined with a Plant Efficiency Analyser (Hansatech Instrument Ltd., UK). The ratios of variable to maximal chlorophyll *a* fluorescence (Fv/Fm) of dark-adapted samples were employed to represent the photochemical efficiency of photosystem II (PSII) or the maximal quantum yield of the photosynthetic apparatus[18]. Cell density of the cultures was measured as dry mass density by filtering 30 mL of the cultures on a pre-dried filter, drying in an oven at 80 ℃ for 24 h, weighing on an electronic balance and substracting the known weight of the dried filter. The biomass yield was estimated from the changes in the biomass density. The concentrations of chlorophyll *a* (Chl *a*) and carotenoids (Car) were determined according to Parsons and Strickland[19] by extracting in 100% methanal (> 12 h), centrifuging at 3000 g for 10 min, and then measuring the absorbances of the supernatant with a spectrophotometer (Shimadzu UV-1206, Japan). Phycocyanin (PC) was extracted by repeated freezing and thawing at 4 ℃ in 0.1 mol/L phosphate buffer containing 0.2 mol/L NaCl, centrifuging at 5000 g for 10 min, and measuring the absorbances of the supernatant spectrophotometrically, and its concentration was determined according to Siegelman et al.[20]

2 Results

Daily changes of solar irradiance are shown in Fig. 1.

The photochemical efficiency (Fv/Fm) of *S. platensis* decreased significantly ($p < 0.01$, *t*-test) after exposed to either solar radiation (with UVR) or depleted of UVR (PAR) (Fig. 2). Compared with the initial Fv/Fm values at 08:30, UVR treatment resulted in much lower Fv/Fm values, indicating a strong inhibitory effect. After exposed to UVR, the Fv/Fm decreased by 46% at 11:00 and 59% at 13:30, while without UVR exposure, Fv/Fm decreased only by 20% at 11:00 and 29% at 13:30 when compared with the measures at 08:30. UVR brought about extra inhibition of the photochemical efficiency at noontime by 26%—30%. The photochemical efficiency showed a recovery in late afternoon despite of the radiation.

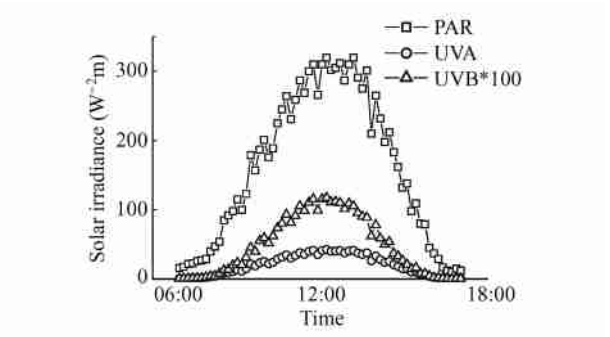

Fig. 1. Daily changes in the photosynthetically active radiation (PAR) and ultraviolet radiation (UVA and UVB) on December 11, 2002, in Shantou, China. UVB irradiance is magnified 100 times.

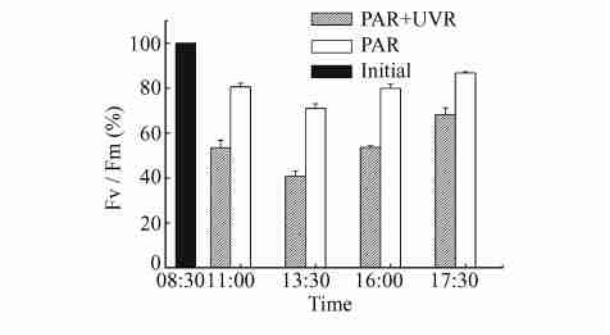

Fig. 2. Changes in the photochemical efficiency (Fv/Fm) of *Spirulina platensis* cultures under full spectrum solar radiation (PAR+UVR) and solar radiation deprived of total UVR (PAR). The absolute value determined at 08:30 was 0.57±0.02 and was set as initial for comparison. Data are the means ± SD of triplicate cultures.

Contents of Chl *a*, Car and PC increased significantly ($p < 0.01$, *t*-test) after *S. platensis* being exposed to the solar radiation for 2.5 h. UVR treatment raised the contents of Chl *a* and PC by up to 30%, and that of Car by up to 43%. The contents of Chl *a* and PC decreased with a further solar exposure in the afternoon, while Car sustained a high concentration throughout the period (Fig. 3).

The yield of biomass production during the daytime was higher with solar radiation deprived of UVR than that with UVR. It increased by about 6.6% without UVR and only 0.3% with UVR. UVR reduced the biomass yield by 6%.

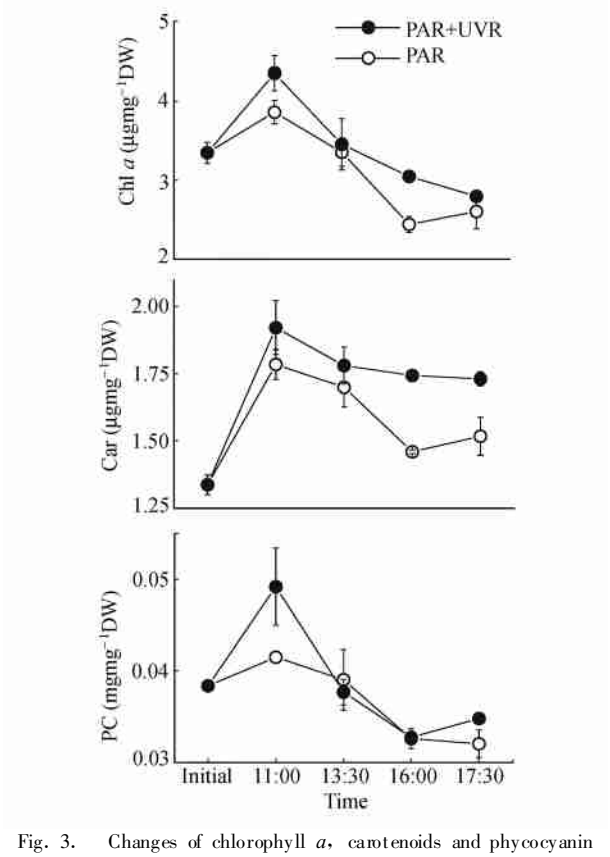

Fig. 3. Changes of chlorophyll *a*, carotenoids and phycocyanin contents of *Spirulina platensis* cultures under full spectrum solar radiation (PAR+UVR) and solar radiation deprived of total UV (PAR). Data are the means ±SD of triplicate cultures.

3 Discussion

Photoinhibition caused by PAR in *S. platensis* has been well investigated in laboratories, and even in outdoor cultures under high solar radiation, causing a decrease of 15%—55% in photochemical efficiency and a loss as high as 30% of the potential production rate[8—11]. Whether solar UVR results in the photoinhibition in *S. platensis* has not been evaluated and documented. The present work demonstrated that solar UVR negatively affects the photochemical efficiency and biomass production of *S. platensis*, and that the photosynthetic inhibition was observed either under the solar radiation depleted of UVR or with it. UVR brought about extra inhibition by 26%—30% in the photochemical efficiency, and about 6% in the biomass production of this cyanobacterium. The observations made in December were similar to that in October (Table 1). We also observed that the greatest inhibition happened at noontime, and in late afternoon there was a recovery despite what treatments *S. platensis* received. The down-regulation of photosynthetic activity in response to light stress is a common physiological strategy as a protective regulatory mechanism to dissipate excess excitation energy, thereby lowering the quantum yield[21]. However, a slow recovery of photochemical efficiency with UVR exposure indicated that the photosynthetic apparatus of *S. platensis* could have suffered chronic photoinhibition caused by UVR, characterized by protein damage[22] and a slower (hours to days) restoration. Rajagopal et al. have reported that exposure of *S. platensis* to a moderate intensity of UVR resulted in decreased levels of D1 and D2 proteins[23]. *De novo* synthesis of the D1 and D2 reaction center subunits, a key step in the repair process[24], needs more time than the other protective mechanisms. Thus the recovery of UVR-induced photoinhibition of *S. platensis* in the present study could consequently become slower compared with that induced by PAR alone.

Table 1. Relative inhibition (%) of the photochemical efficiency by UVR

Time	Relative inhibition by UVR (%)	
	27 Oct.	17 Dec.
11:00	30.7±3.3	33.6±1.5
13:30	43.8±5.9	42.5±3.6
16:00	33.8±1.7	32.9±1.4
17:30	22.1±3.3	21.5±3.3

The total solar radiation is about 8% higher on 27 October than 17 December, 2002. The relative inhibition was calculated as $[(P_{PAR}-P_{UV})/P_{PAR}\times 100]$, where P_{UV} and P_{PAR} represent the values obtained in the cultures exposed to full spectrum solar radiation or those to that depleted of UVR. Data are the means ±SD of triplicate cultures

When the laboratory-maintained *S. platensis* was switched to outdoor conditions, contents of Chl *a*, Car and PC were raised after the exposure to sunlight. This could be attributed to a reaction that characterizes the irritative stage of stress, being indicative of a protecting dynamic photoinhibition. Such a responsive phenomenon was also observed in *Chondrus crispus*[25]. UVR exposure gave rise to higher contents of the pigments, implying that it might have enhanced their synthesis in *S. platensis*. The contents of Car in *S. platensis* sustained high values during the solar exposure probably due to their protective functions: interacting with triplet state chlorophyll, quenching singlet oxygen, dissipating the excitation energy as heat and providing a nondestructive energy-dissipating mechanism[26].

Responses of laboratory-maintained *Spirulina* strains to outdoor UVR exposure might be different from that of outdoor cultured *Spirulina* strains or those freshly isolated from nature, because the strains that had been grown in an environment largely free of UVR for years might have lost their tolerance to UVR, which may lead to greater extent of damage (Whitton, personal communication). Mühling et al. reported that helix orientation of *Spirulina* strains could be reversed due to genetic drift and by environmental factors, temperature upshift and mechanical stress[27]. Genetic drift might also happen in terms of changes in levels of solar UVR even for the strains outdoor cultured or newly isolated from nature because depletion of stratospheric ozone due to industrial activities is enhancing the UVR reaching earth surface. Nevertheless, responses of *Spirulina* strains of different backgrounds (laboratory-stock, outdoor culture, nature-isolated) to UVR should be compared to gain further information on its relationship with changing UVR levels.

The present study conclusively indicated that solar irradiance, when depleted of UVR, generated a higher productivity of *S. platensis*. In view of raising production efficiency, filtering out UVR of the solar radiation by screening UV-opaque films would raise the productivity of *S. platensis* by more than 6%, and should be considered if cost of the practice pays off.

References

1 Vonshak A. and Tomaselli L. *Arthrospira* (*Spirulina*): Systematics and ecophysiology. In: The Ecology of Cyanobacteria: the Diversity in Time and Space. The Netherlands: Kluwer Academic Publishers, 2000. 505—522.

2 Hu H. J. Biology and Biotechnology of *Spirulina* (in Chinese). 1st ed. Beijing: Science Press, 2003. 1—8.

3 Gireesh T., Jayadeep A., Rajasekharan K. N. et al. Production of deuterated β-carotene by metabolic labeling of *Spirulina platensis*. Biotechnol. Lett., 2001, 23: 447—449.

4 Tomonou Y. and Amao Y. Photoinduced H_2 production with Mg chlorophyll-a from *Spirulina* and colloidal platinum by visible light. Biotechnol. Lett., 2002, 24: 775—778.

5 Olguín E. J., Galicia S., Mercado G. et al. Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastwater recycling process under tropical conditions. J. Appl. Phycol., 2003, 15: 249—257.

6 Siew-Moi P. Agro-industrial wastewater reclamation in Peninsular Malaysia. Arch. Hydrobiol., 1987, 28: 77.

7 Cogne G., Lasseur C., Comet J. F. et al. Growth monitoring of a photosynthetic microorganism (*Spirulina platensis*) by pressure measurement. Biotechnol. Lett., 2001, 23: 1309—1314.

8 Xia J. R. and Gao K. Studies on photoinhibition of *Spirulina maxima* grown in high CO_2 concentration. Acta Hydrobiologica Sinica (in Chinese), 2002, 26(1): 14—18.

9 Torzillo G., Accolla P., Pinzani E. et al. On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (cyanobacteria). J. Phycol., 1998, 34: 504—510.

10 Vonshak A., Guy R., Poplawsky R. et al. Photoinhibition and its recovery in two strains of the cyanobacterium *Spirulina platensis*. Plant Cell Physiol., 1988, 29: 721—726.

11 Vonshak A. and Guy R. Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors. Plant Cell Environ., 1992, 15: 613—616.

12 Jensen S. and Knutsen G. Influence of light and temperature on photoinhibition of phtotosynthesis in *Spirulina platensis*. J. Appl. Phycol., 1993, 5: 495—504.

13 Helbling E. W., Villafañe V. E., Buma A. G. J. et al. DNA damage and photosynthetic inhibition induced by solar UVR in tropical phytoplankton (Lake Titicaca, Bolivia). Eur. J. Phycol., 2001, 36: 157—166.

14 Ravanat J. L., Douki T. and Cadet J. Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol. (B): Biol., 2001, 63: 88—102.

15 Figueroa F. L., Escassi L. and Pérez-Rodríguez E. et al. Effects of short-term irradiation on photoinhibition and accumulation of mycosporine-like acids in sun and shade species of the red algal genus *Porphyra*. J. Photochem. Photobiol. (B): Biol., 2003, 69: 21—30.

16 Figueroa F. L., Salles S. and Aguilera J. et al. Effects of solar radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticta*. Mar. Ecol. Prog. Ser., 1997, 151: 81—90.

17 Underwood G. J. G., Nilsson C., Sundbäck K. et al. Short-term effects of UVB radiation on chlorophyll fluorescence, biomass, pigments and carbohydrate reactions in a benthic diatom mat. J. Phycol., 1999, 35: 656—666.

18 Krause G. H. and Weis E. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant mol. Biol., 1991, 42: 313—349.

19 Parsons T. R. and Strickland J. D. H. Discussion of spectrophotometric determination of marine plant pigments, with revised equation for ascertaining chlorophylls and carotenoids. J. Mar. Res., 1963, 21: 155—163.

20 Siegelman H. W., Chapman D. J. and Cole W. J. The bile pigments of plants. Biochem. Soc. Symp., 1968, 28: 107—120.

21 Osmond C. B. and Chow W. S. Ecology of photosynthesis in the sun and shade: summary and prognostications. J. Phycol., 1988, 15: 1—9.

22 Campbell D., Eriksson M. J., Öquist G. et al. The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction-center D1 proteins. Proc. Natl. Acad. Sci. USA, 1998, 95: 364—369.

23 Rajagopal S., Murthy S. D. S. and Mohanty P. Effect of ultraviolet-B radiation on intact cells of the cyanobacterium *Spirulina platensis*: characterization of the alterations in the thylakoid membranes. J. Photochem. Photobiol. (B): Biol., 2000, 54: 61—66.

24 Sass L., Spetea C., Máté Z. et al. Repair of UV-B induced damage of photosynthesis II via *de novo* synthesis of the D1 and D2 reaction center subunits in *Synechocystis* sp. PCC 6803. Photosyn. Res., 1997, 54: 55—62.

25 Yakovleva I. M. and Titlyanov E. A. Effect of high visible and UV irradiance on subtidal *Chondrus crispus*: stress, photoinhibition and protective mechanisms. Aquat. Bot., 2001, 71: 47—61.

26 Powles S. B. Photoinhibition of photosynthesis induced by visible light. Ann. Rev. Plant Physiol., 1984, 35: 15—44.

27 Mühling M., Harris N., Belay A. et al. Reversal of helix orientation in the cyanobacterium *Arthrospira*. J. Phycol., 2003, 39: 360—367.