

COMBINED EFFECTS OF ULTRAVIOLET RADIATION AND TEMPERATURE ON MORPHOLOGY, PHOTOSYNTHESIS, AND DNA OF *ARTHROSPIRA* (*SPIRULINA*) *PLATENSIS* (CYANOPHYTA)¹

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Natural levels of solar UVR were shown to break and alter the spiral structure of *Arthrospira* (*Spirulina*) *platensis* (Nordst.) Gomont during winter. However, this phenomenon was not observed during summer at temperatures of ~30°C. Since little has been documented on the interactive effects of solar UV radiation (UVR; 280–400 nm) and temperature on cyanobacteria, the morphology, photosynthesis, and DNA damage of *A. platensis* were examined using two radiation treatments (PAR [400–700 nm] and PAB [PAR + UV-A + UV-B: 280–700]), three temperatures (15, 22, and 30°C), and three biomass concentrations (100, 160, and 240 mg dwt [dry weight] · L⁻¹). UVR caused a breakage of the spiral structure at 15°C and 22°C, but not at 30°C. High PAR levels also induced a significant breakage at 15°C and 22°C, but only at low biomass densities, and to lesser extent when compared with the PAB treatment. *A. platensis* was able to alter its spiral structure by increasing helix tightness at the highest temperature tested. The photochemical efficiency was depressed to undetectable levels at 15°C but was relatively high at 30°C even under the treatment with UVR in 8 h. At 30°C, UVR led to 93%–97% less DNA damage when compared with 15°C after 8 h of exposure. UV-absorbing compounds were determined as negligible at all light and temperature

combinations. The possible mechanisms for the temperature-dependent effects of UVR on this organism are discussed in this paper.

Key index words: *Arthrospira platensis*; cyanobacterium; DNA damage; morphology; photosynthesis; temperature; UVR

Abbreviations: CPD, cyclobutane pyrimidine dimers; dwt, dry weight; F'_m , F'_v , maximal and current steady-state chl fluorescence of light-adapted sample; MAA, mycosporine-like amino acids; PAB, PAR + UV-A + UV-B; UV-A, UV-B, UVR, ultraviolet radiation A, ultraviolet radiation B, and ultraviolet radiation, respectively; $\Phi_{PSII} = \Delta F/F'_m$, effective quantum yield of PSII in the light

A. platensis is an economically important filamentous cyanobacterium that is commercially produced as a source of human health food (Ciferri 1983), animal feed (Lu et al. 2002), and cosmetic colorants (Dainippon Ink and Chemicals 1985). Extensive studies on improving its growing condition and cultivation have been carried out (Richmond and Grobbelaar 1986, Vonshak and Guy 1992, Torzillo et al. 1998). However, little has been documented on the effects of UVR (280–400 nm) on this organism (Wu et al. 2005a,b, Helbling et al. 2006). In nature or in

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commercially operated ponds, *A. platensis* cells are exposed to solar radiation and thus are susceptible to UVR. It is known that high levels of UVR can reduce photosynthetic rate (Holm-Hansen et al. 1993, Villafañe et al. 2003), inactivate enzyme activities (Villafañe et al. 2003), and damage DNA (Buma et al. 2001, Häder and Sinha 2005), and low or reduced levels of UVR can enhance carbon fixation (Helbling et al. 2003, Gao et al. 2007) of phytoplankton. It was recently determined that natural levels of solar UVR may also affect cyanobacterial morphology (Wu et al. 2005b). However, the UVR-induced effects might be affected by temperature, since it is a major environmental parameter to be considered in biological production and may rise by 1°C–4°C in the year 2100 as a result of global warming caused by human activities (Meehl et al. 2005). It was observed that photoinhibition of cyanobacteria (Roos and Vincent 1998, Zak and Pakrasi 2000) and repair of UVR-induced DNA damage in a red alga (Pakker et al. 2000) were temperature dependent.

A. platensis usually grows better above 30°C (Vonshak 1997, de Oliveira et al. 1999), although a low-temperature strain has also been reported (Qiao et al. 2001). Ambient levels of temperature usually limit biomass production of *Arthrospira* spp. in open commercial ponds where it fluctuates during the day and between different seasons. The variation in water temperature of an *Arthrospira* culture pond between morning and midday could be up to 20°C in nontropical areas (Vonshak 1997). It is known that temperature affects the ultrastructure (van Eykelenburg 1979), morphology (Mühling et al. 2003), photosynthesis and respiration (Torzillo and Vonshak 1994, Venkataramanaiah et al. 2003), biochemical composition, and growth (Tomaselli et al. 1988, Mühling et al. 2005) of *Arthrospira* sp. Mühling et al. (2003) reported that an increase in temperature from 30°C to 32°C–34°C for 7 d led to reversed spiral orientation (left-handed to right-handed) for three of 10 *Arthrospira* strains tested. The activity of PSII in *A. platensis* decreased at high temperature (35°C), while PSI showed an increase in its activity (Venkataramanaiah et al. 2003). Vonshak (1997) demonstrated that lower temperatures in the morning induced higher photoinhibition under solar radiation, and that heating the culture up to 35°C raised its biomass production.

Most of the studies focused on the independent effect of temperature or UVR on living organisms. Little has been documented on the interactive effects of solar UVR and temperature (Roos and Vincent 1998, van de Poll et al. 2002, Sobrino and Neale 2007), which usually increases or decreases simultaneously along with solar radiation. The aim of this study was to evaluate the interactive effect of temperature and UVR on the morphology, photosynthesis, and DNA damage of *A. platensis*.

MATERIALS AND METHODS

Organism and culture conditions. *A. platensis* strain 439 (strain synonyms: UTEX LB 2340 that was originally isolated from Natron Lake, Chad) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences (Wuhan, Hubei). A single trichome was obtained by micropipetting and grown in sterile Zarrouk's medium (Zarrouk 1966) for the clonal culture. The culture was grown with aeration of filtered (0.22 µm) ambient air (0.1 L · min⁻¹) in an illuminated chamber at 20°C with a 12:12 light:dark (L:D) period, receiving a PAR irradiance of 50 µmol photons · m⁻² · s⁻¹ (supply by cool-white fluorescent lights) before being used for the experiments.

Simulated solar radiation and experimental conditions. Experiments were carried out under a solar simulator (Sol 1200W; Dr. Hönle, Martinsried, Germany). The output of irradiance of the simulator was measured using a broadband filter radiometer (ELDONET; Real Time Computer Inc., Möhrendorf, Germany) that has three channels, UV-B (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm), respectively. Quartz tubes (Pacific Quartz Products Co. Ltd., Lianyungang, China) with the samples placed under the simulator received 344.0, 76.0, and 2.6 W · m⁻² of PAR (400–700 nm), UV-A (315–400 nm), and UV-B (280–315 nm), respectively. The biologically weighted UV-B irradiance was 0.26 W · m⁻² (Setlow 1974, normalized at 300 nm), estimated on the basis of the irradiance reaching the cells in the quartz tubes covered with a 295 nm cut-off filter (Fig. 1). The spectrum of the solar simulator was obtained from Prof. Donat-P. Häder, and that of local solar radiation was calculated using the STARsci model (Ruggaber et al. 1994). The ratio of PAR:UV-A:UV-B on a sunny day in summer at the study site is 100.0:15.7:0.52, while that for the simulator is 100.0:22.1:0.76.

Subsamples of *A. platensis* were collected during exponential growth phase, resuspended in Zarrouk's fresh medium, and dispensed in 340 mL UV-transparent quartz tubes (inner diameter, 5.4 cm; length, 16 cm). The cultures were aerated with filtered (0.22 µm) ambient air at a rate of 0.1 L · min⁻¹. Two different radiation treatments were implemented: (i) samples receiving full radiation (280–700 nm, PAB treatment), quartz tubes covered with an Ultraphan 295 film (UV OpaK, Digefra, Munich, Germany); (ii) samples receiving only PAR (400–700 nm, PAR treatment), quartz tubes covered with

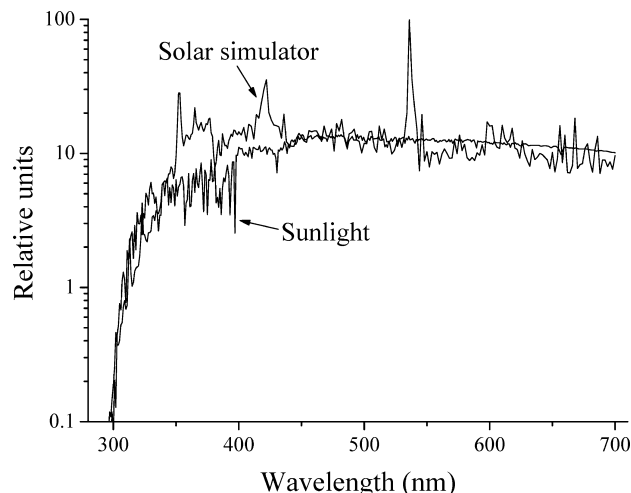


FIG. 1. Spectrum of the simulator solar radiation reaching the cells in the quartz tubes covered with a 295 nm cut-off filter, in contrast to that of local solar radiation.

Ultraplan film 395. For each radiation treatment, three levels of *A. platensis* biomass density were used: 100, 160, 240 mg dwt · L⁻¹, representing the low, medium, and high cell concentrations, respectively.

Temperature control and treatments. Quartz tubes with the cells were placed in a water bath, and water was circulated after being cooled with a refrigerating circulator (Cole-Parmer, Chicago, IL, USA). Three different temperatures (15, 22, and 30°C) were set up and controlled to assess the combined impacts with UVR and temperature on *A. platensis*. The cells were preacclimated to the experimental temperatures for 3 d in an incubator under the same light conditions used to maintain the cultures before the experiments. The temperature levels used in our experiments reflect those usually experienced by *A. platensis* cells in cultured ponds from early morning to noon in subtropical areas (Vonshak 1997).

Morphological observation. While the cells were exposed to different radiation and temperature treatments, subsamples were taken at time 0, 4, and 8 h after the exposure, and morphological changes were examined by using an inverted microscope (IX51; Olympus, Tokyo, Japan). Digital images were recorded using a Canon digital camera (S50; Canon, Tokyo, Japan) and analyzed. At least five fields were observed, and 30–40 filaments were examined. The spiral length (L), helix pitch (P), and angle (A) were measured at the same time, but for the helix pitch and angle, only the trichomes that had at least two helices were measured.

Fluorescence measurements. To estimate the impact of UVR on photosynthesis of *A. platensis* during the exposure period, the effective quantum yield was measured at time 0, 30 min, 1 h, and thereafter every hour during the experiment (8 h) using a water-PAM (pulse amplitude modulated) fluorometer (PAM-control, Walz, Effeltrich, Germany). The parameters were determined within 1 min after collection. The effective quantum yield of PSII (Φ_{PSII}) was calculated as follows:

$$\Phi_{\text{PSII}} = (F'_m - F'_t)/F'_m = \Delta F/F'_m \quad (1)$$

where F'_t is the current steady-state fluorescence, and F'_m is the maximal fluorescence of light-adapted cells determined at a saturating pulse of 5,600 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in 0.8 s. The recovery of Φ_{PSII} in the photoinhibited samples was followed at 30 min, 1 h, and thereafter every hour for 5 h (15°C and 22°C) or 6 h (30°C). The samples were maintained at 5 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of PAR (supplied by a cool-white fluorescent light) and the same temperature as during the previous exposure in the incubator (LRG-250-G; Zhujiang, Guangdong, China) during the recovery period.

DNA damage. Damage to the DNA molecule was evaluated via the formation and accumulation of cyclobutane pyrimidine dimers (CPD), after the samples were exposed for 4 and 8 h to different radiation treatments. Aliquots of *A. platensis* culture (30 mL) were collected on 0.2 μm CN-CA membrane filters (Xinya, Shanghai, China) and immediately frozen (liquid nitrogen -180°C) until analysis. DNA was extracted according to Helbling et al. (2001), which is a method modified from Doyle and Doyle (1990). Cells on the filters were resuspended in a solution (25 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl, pH 8.0) and then broken by sonication (CPX600; Cole-Parmer, Chicago, IL, USA) on ice. Thereafter, prewarmed (60°C) cetyltrimethylammonium bromide (CTAB) extraction buffer (3% [w/v] CTAB, [Amresco, Solon, OH, USA] 1.4 M NaCl, 0.5% (v/v) β -mercaptoethanol, 25 mM EDTA, 100 mM Tris-HCl, pH 8.0) was added and incubated at 60°C for 30 min. A 1:1 proportion of chloroform-isoamyl alcohol (24:1 vol/vol) was added to precipitate proteins. After centrifugation (12,000g for 15 min at 4°C; 5804R, Eppendorf, Germany), 20 $\mu\text{g} \cdot \text{mL}^{-1}$ RNase (Sigma-Aldrich, St. Louis, MO, USA) was added to the upper phase and incubated at 37°C for 30 min to remove

RNA. A 1:1 proportion of chloroform-isoamyl alcohol (24:1 vol/vol) was added and centrifuged (12,000g for 15 min at 4°C). The supernatant was transferred to sterile microcentrifuge tubes, and DNA was precipitated at -20°C for 2 h in 2/3 volumes of isopropanol. Following another centrifugation (12,000g for 20 min at 4°C), the supernatant was removed, and the pellet was washed once with 80% ice-cold ethanol. Finally, the DNA pellet was dried and resuspended in TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and maintained at -20°C until analyzed within 24 h. The purity and concentration of DNA were determined by using a DNA/protein analyzer (DU 530; Beckman Coulter, Fullerton, CA, USA).

The amount of CPD was determined via ELISA according to Armstrong et al. (2002) by employing a primary antibody H3 (Sigma-Aldrich), which mainly binds to the thymine dimer (T<>T). Since T<>T accounts for most of the CPD caused by UV-B (Friedberg et al. 1995, Buma et al. 2001), the term CPD was used in the following sections for clarity. A 96-well flat-bottomed microplate (Canada JET Biochemicals Int'l. Toronto, ON, Canada) was precoated with 0.04% protamine sulfate (Bio Basic Inc., Mississauga, ON, Canada) for 2 h. Samples of 100 ng heat-denatured DNA were added to each well and incubated for 20 h or until the plate was dry. After 30 min blocking with BSA (Bio Basic Inc.) followed by four times washing (PBS-T), 100 μL primary antibody H3 (monoclonal antithymine dimer antibody; Sigma-Aldrich) was added and then incubated for 30 min. After four times washing steps (PBS-T), a secondary antibody IgG-HRP (goat antimouse IgG, conjugated with horseradish peroxidase) was added and incubated for another 30 min. After washing four more times with PBS-T and twice with citrate-phosphate buffer (pH 5.0), 100 μL *O*-phenylene diamine (Amresco, Solon, OH, USA), a hydrogen peroxide in citrate-phosphate buffer (pH 5.0) was added and incubated for ~45 min in the dark. The reaction was stopped with sulfuric acid (2 mol · L⁻¹), and the absorbance was read at 490 nm with a microplate reader (ELx800; BioTek, Winooski, VT, USA). All steps were carried out at 37°C. At the same time, a serial of standard DNA with known amounts of CPD were analyzed to calculate the CPD in the tested samples.

Statistical analysis. The microscopic measurements were randomly performed for at least 30–40 filaments at each time and treatment. One-way analysis of variance (ANOVA) and *t*-test were used to establish differences among treatments. A confidence level of 95% was used in all analyses.

RESULTS

Morphological changes. Cells of *A. platensis* strain 439 were arranged in a spiral-shaped filament, with a mean length of about 470 μm and 4.7 spirals per filament (Fig. 2, A–C). At 15°C, the filaments were completely (100 mg dwt · L⁻¹) or severely (160, 240 mg dwt · L⁻¹) broken when exposed to both PAR and PAB treatment for 8 h (Fig. 2). At 22°C, the culture of 100 and 160 mg dwt · L⁻¹ biomass density became pellucid (Fig. 2, B4 and B6), and that of the highest biomass (240 mg dwt · L⁻¹) showed less breakage (Fig. 2B2) when exposed to PAB. Much less (100 mg dwt · L⁻¹) or no breakage (160 and 240 mg dwt · L⁻¹) was seen when UVR was filtered out (Fig. 2, B1, B3, and B5). At 30°C, no changes in spiral morphology were observed even under the PAB treatment for 8 h (Fig. 2, A2, A4, and A6) at any biomass level, and the filaments became longer ($P < 0.05$) when UVR was filtered out (Fig. 2, A1–A3; Fig. 3).

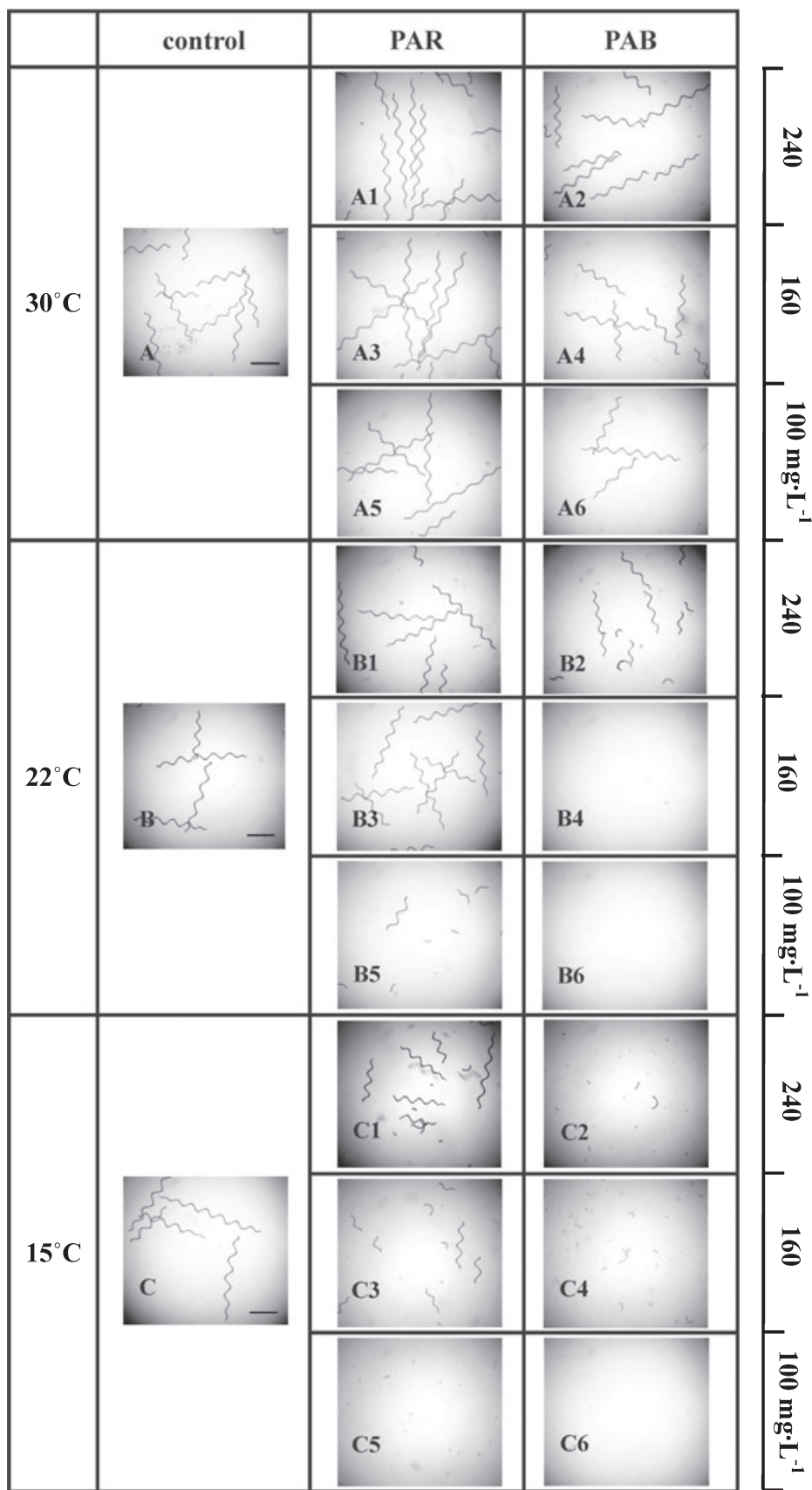


FIG. 2. Spiral structures of *Arthrospira platensis* after 8 h exposure to the solar simulator at 15, 22, and 30°C and different initial biomass densities. The scale bars represents 200 μm . PAB, PAR + UV-A + UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B.

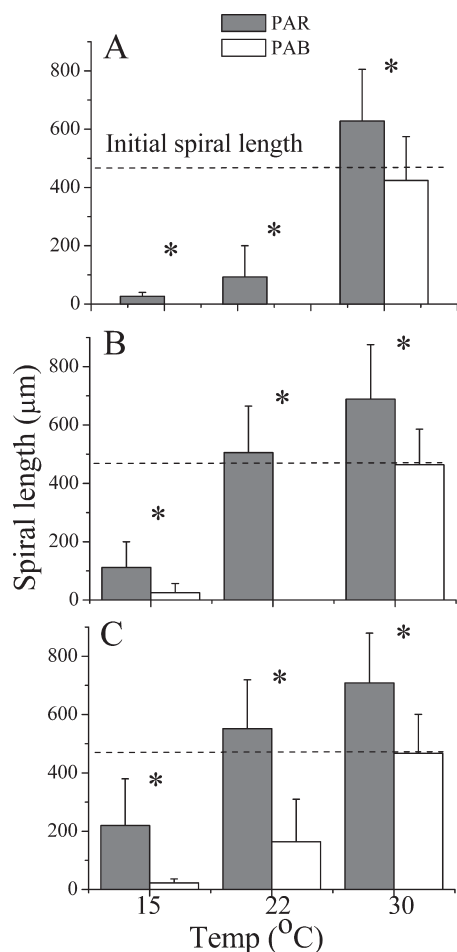


FIG. 3. Spiral length of *Arthrospira platensis* after exposed to PAR and PAB radiation treatments at 15, 22, and 30°C. (A–C) The inoculated biomass densities of 100, 160, and 240 mg dwt · L⁻¹, respectively. The dashed line shows the initial spiral length, 475 μm. Asterisks indicate significant differences between PAR and PAB treatments. Data are the means ± SD for 30–40 filaments in at least five fields. PAB, PAR + UV-A + UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B; dwt, dry weight.

The mean trichomes length (Fig. 3), helix pitch, and helix angle (Table 1) were estimated based on digital images. At all of the biomass levels, the smallest trichomes were associated with the lowest temperature (15°C), while the largest were associated with the highest temperature (30°C). The helix pitch and angle of *A. platensis* filaments in the culture of 240 mg dwt · L⁻¹ biomass density became significantly ($P < 0.05$) enlarged under PAR and significantly ($P < 0.05$) decreased under PAB treatment (Table 1), reflecting that the presence of UVR lead to compressed spirals.

Photosynthetic inhibition and recovery. The effective quantum yield of *A. platensis* decreased significantly ($P < 0.01$) in all radiation and temperature treatments. It was higher under PAR than under PAB treatment, indicating an additional inhibition by UVR in the PAB treatment. The inhibition due to both PAR and UVR decreased, while the tempera-

TABLE 1. Helix pitch and angle of *Arthrospira platensis* after 8 h exposure to PAR and PAB radiation treatment at 15, 22, and 30°C in a high biomass culture (240 mg dwt · L⁻¹).

	Control	PAR	PAR+UV-A+UV-B
Helix pitch (μm)			
30°C	118.9 ± 1.3 ^a	136.0 ± 2.5 ^b	115.0 ± 1.3 ^c
22°C	118.5 ± 3.0 ^a	122.2 ± 3.5 ^a	119.5 ± 1.3 ^a
15°C	115.4 ± 1.1 ^a	118.7 ± 3.1 ^a	– (pellucid)
Angle (degree)			
30°C	100.3 ± 0.3 ^a	114.8 ± 1.4 ^b	100.5 ± 1.1 ^a
22°C	102.5 ± 1.62 ^a	106.2 ± 2.4 ^a	102.2 ± 1.8 ^a
15°C	101.3 ± 0.7 ^a	101.7 ± 3.7 ^a	– (pellucid)

Data are the means ± SD for 30–40 filaments in at least five fields. Control shows the value at time zero.

Different superscript letters indicate significant difference at 95% confidence level among radiation treatment. PAB, PAR+UV-A+UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B; dwt, dry weight.

ture increased from 15°C to 30°C (Fig. 4, A–C). Increased biomass density, which leads to higher self-shading, decreased the photoinhibition at all levels of temperature tested. The recovery of the quantum yield, after 0.5 and 1 h exposures, was followed for 5 h (15°C and 22°C) or 6 h (30°C; Fig. 5). Samples that were exposed for 0.5 h recovered to >80% of the control (initial value) at all levels of temperature and biomass (Fig. 5, A, C, and E). One hour exposure significantly suppressed and delayed such recovery at the low levels of temperature and biomass (Fig. 5, D and F). The recovery was achieved faster at high temperature and biomass density and in the samples exposed to PAR alone when compared with other temperature and radiation treatments. Presence of UVR during the pre-exposure markedly hindered the recovery of the yield. At 15°C, samples preexposed to the PAB treatment at the low biomass level recovered to only 8.5% of the original yield, while it reached 61% for the PAR pretreatment (Fig. 5F). At 22°C, samples in the PAB treatment and low biomass recovered to only 30% of the initial value, while at medium and high biomass, the yield recovered to >71% of the control value (Fig. 5D). At the highest temperature tested (30°C), the samples recovered to >80% of the control value (Fig. 5B). In general, samples exposed to 30°C had less inhibition and a faster recovery than samples incubated at 15°C or 22°C. A similar pattern, but not as marked as with temperature, was observed when considering the biomass density, with the inhibition being less and recovery faster at 240 mg dwt · L⁻¹ when compared with the 100 mg dwt · L⁻¹ cultures.

DNA damage. Cyclobutane pyrimidine dimers were not detected under the PAR treatment in the absence of UVR. For the PAB treatments, the amount of accumulated CPD was lower at high temperature within the range tested (Fig. 6), indicating decreased DNA damage at increased temperature. Such temperature-dependent DNA damage was

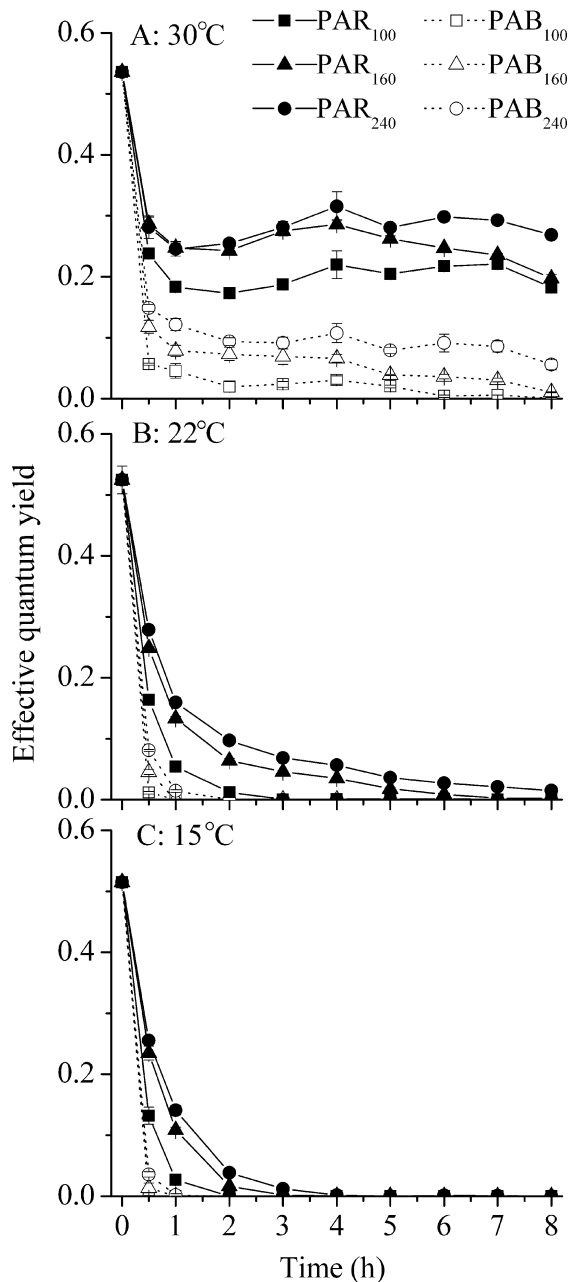


FIG. 4. The effective quantum yield ($\Delta F/F_m'$) of *Arthrospira platensis* during the 8 h exposure to PAR (solid lines) and PAB (dotted lines) radiation treatments at 30°C (A), 22°C (B), and 15°C (C) and at 100, 160, and 240 mg dwt · L⁻¹, respectively. Data are the means \pm SD for at least four measurements. PAB, PAR + UV-A + UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B; dwt, dry weight.

more severe in the longer (8 h) than in the shorter (4 h) exposures. High biomass density resulted in less DNA damage regardless of the temperature, reflecting that self-shading played a photoprotective role during the exposures. The maximal DNA damage was seen in 100 mg dwt · L⁻¹ biomass culture at 15°C and 22°C with approximately 450 CPD · 10⁶ nucleotides⁻¹ after 8 h exposure in the PAB

treatment; in all other cases, CPD amounts were <200 CPD · 10⁶ nucleotides⁻¹ (Fig. 6B). The minimal CPD accumulations were observed at 30°C at 160 and 240 mg dwt · L⁻¹ biomass densities, which was only \sim 2.5% of the maximal damage (100 mg dwt · L⁻¹, 15°C and 22°C).

DISCUSSION

The spiral structure of *A. platensis* was previously observed to be broken and altered by natural levels of solar UVR during the winter season when the temperature ranged from 18°C to 20°C (Wu et al. 2005b). This study clearly showed that such breakage was temperature dependent, and that the morphological changes were coupled with physiological and molecular ones at low temperatures.

Many cyanobacteria are capable of synthesizing UV-absorbing compounds, such as mycosporine-like amino acids (MAA) and scytonemin, to screen off UVR before it reaches intracellular components (Garcia-Pichel and Castenholz 1993). *A. platensis* strains D-0083 and 439 were determined to possess negligible amounts of UV-absorbing compounds even under solar radiation (Wu et al. 2005b). In this study, the amounts of UV-absorbing compounds at different temperatures were also negligible and were not induced during the short-term exposures to simulated solar radiation (data not shown). *A. platensis* strains of tighter helical structure could tolerate higher light intensity compared with those with looser spirals (Jeeji Bai and Seshadri 1980). In addition, loose or straight spirals could be transformed to tight coiled shapes when shifted to high light conditions (Fox 1996). Wu et al. (2005b), working with two different strains of *A. platensis*, suggested that decreasing helix pitch in the presence of UV-B could be an effective protective mechanism. Increased self-shading due to tightened spirals was also observed in this study at higher temperatures in the presence of UV-B. Such morphological changes from loosened to tightened helix appear to be associated with the protective strategy of this organism to counteract solar UVR. The photochemical efficiency of *Arthrospira* species grown in outdoor photobioreactors was highly reduced at midday at low levels of biomass density (Torzillo et al. 1996). In this study, at any given temperature, spiral breakage, photosynthetic inhibition, and DNA damage were lower at high biomass densities compared with low biomass density, reflecting a protective effect with increasing self-shading.

It is well known that UVR induces damage, such as pigment bleaching, protein degradation, enzyme inactivity, and reduction of DNA, in many organisms. However, some organisms have developed repairing strategies against it, such as de novo synthesis of protein (e.g., D1 and D2 protein) (Sass et al. 1997), DNA repair (Britt 1995, Häder and Sinha 2005), and oxygen radical scavenging (Mittler

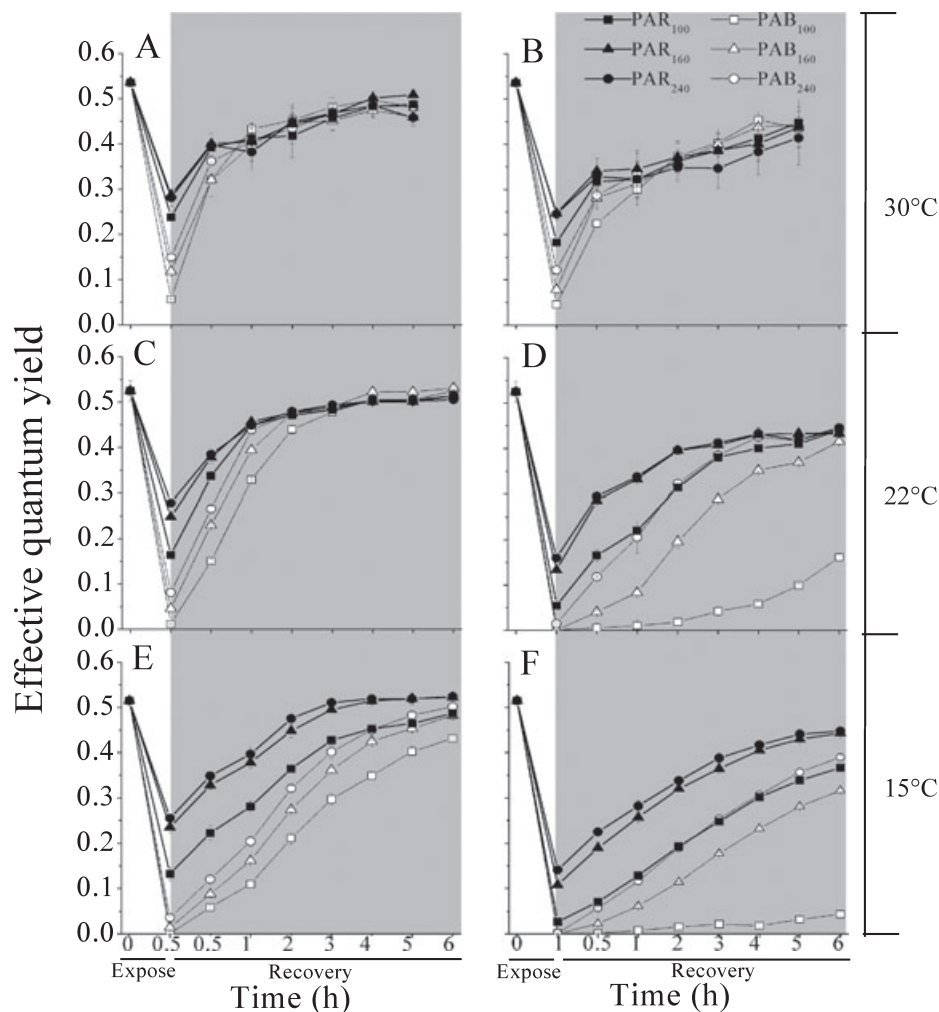


FIG. 5. Recovery in dim light ($\sim 5 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) of the effective quantum yield after 0.5 (A, C, E) and 1 h (B, D, F) exposures to PAR (solid symbols) and PAB (open symbols) radiation treatments at 30°C (A, B), 22°C (C, D), and 15°C (E, F) and at 100, 160, and 240 $\text{mg dwt} \cdot \text{L}^{-1}$, respectively. Data are the means \pm SD for at least four measurements. PAB, PAR + UV-A + UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B; dwt, dry weight.

and Tel-Or 1991, Middleton and Teramura 1993). Net damage in response to UVR represents the balance between total damage induced by UVR and repair via various repair pathways. The photochemical damage process was shown to be independent of temperature (Q_{10} of 1), while the repair mechanisms were temperature dependent ($Q_{10} \neq 1$; Pakker et al. 2000, Li et al. 2002). The de novo synthesis of D1, D2 protein involves a number of biochemical processes, which are thought to be temperature dependent (Gong and Nilsen 1989, Roos and Vincent 1998). Van de Poll et al. (2002) showed less UVR-induced damage to PSII (F_v/F_m) at 12°C and 18°C than at 6°C in some red algae. In this study, low morning temperature together with high light intensity could induce photoinhibitory stress to *Arthrospira* sp., and heating of the culture up to 35°C reversed the inhibition and led to almost four times higher daily production (Vonshak et al. 1994, Vonshak 1997). Repair of UV-B-induced DNA damage relies on temperature in the red alga *Palmaria palmata* (Pakker et al. 2000), Archaeobacterium *Halobacterium cutirubrum* (Eker et al. 1991), and higher plants (Pang and Hays 1991, Takeuchi et al.

1996, Li et al. 2002). The results of our study suggest that the damage and repair balance may also have shifted toward damaged cells at low temperature. As a result, severe spiral breakage, photosynthetic inhibition, and DNA damage were seen at the lowest temperature tested in our experiments.

High PAR can also induce the degradation of D1 protein (Friso et al. 1994, Jansen et al. 1999) and the generation of reactive oxygen species (Foyer et al. 1994, Franklin and Forster 1997), and it can also reduce the photochemical efficiency of *A. platensis* (Vonshak and Guy 1992, Vonshak et al. 1994, Torzillo et al. 1996, Lu and Vonshak 1999, Helbling et al. 2006). In our present study, high PAR resulted in a considerable part of the photoinhibition. However, no DNA damage was observed under PAR irradiance at any temperature and cell density. The formation of pyrimidine dimers and other photochemical products are thought to be produced only in the presence of UV-B or wavelengths shorter than 280 nm (Setlow 1974, Quaitte et al. 1992); however, the latter wavelengths do not reach the Earth's surface. The solar simulator has a higher ratio of UVR to PAR than natural sunlight, especially in the wave-

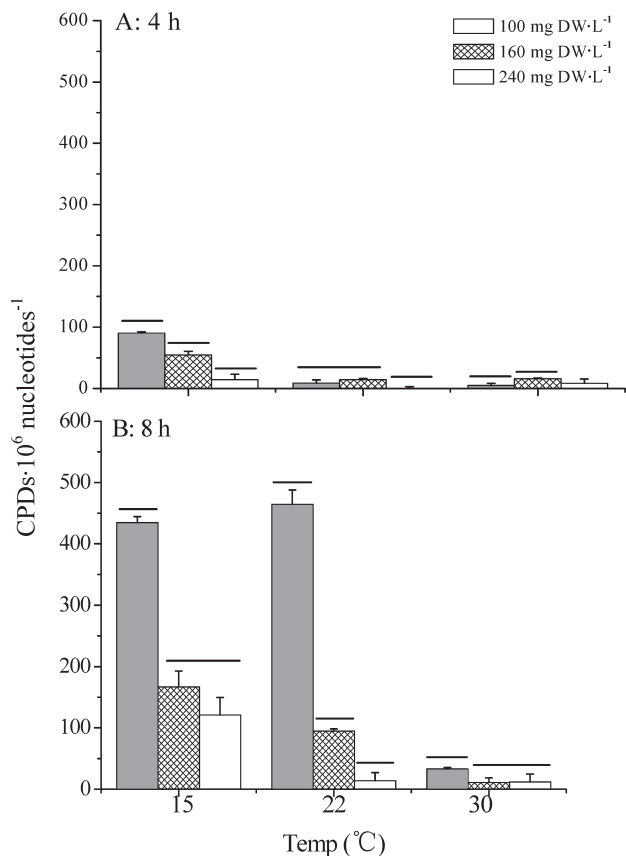


FIG. 6. DNA damage measured as the number of CPD · 10⁶ nucleotides⁻¹ of *Arthrospira platensis* after 4 (A) and 8 h (B) exposures to PAB at 15, 22, and 30°C and at 100, 160, and 240 mg dwt · L⁻¹, respectively. The vertical lines on top of the bars represent SD ($n = 3$ or 4); different horizontal lines (at different levels) indicate significant difference among treatments. PAB, PAR + UV-A + UV-B; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B; dwt, dry weight; CPD, cyclobutane pyrimidine dimer.

lengths <305 nm, which would result in a greater formation of photochemical product such as CPD and 6-4PPs (pyrimidine [6–4] pyrimidinone photo-products) when compared with incident solar UVR. However, such an additional portion of UVR may reflect the significance of increasing UV-B radiation due to depleted stratospheric ozone or when an organism moves latitudinally toward the equator. In addition, changes in the UVR/PAR ratio are normally encountered at different depths in the water column, with the highest ratio at the surface due to the differential attenuation by water and particles in it. Enhanced UV-B radiation would lead to more DNA damage in *A. platensis* and probably other cyanobacteria.

The PAR alone treatment did not result in any DNA damage but led to a significant breakage of the spirals at 15°C and 22°C. This finding indicates that morphological changes might be, to some extent, independent of the DNA damage. The breakage of the spiral structures might be due to degradation of membrane proteins that are essential for maintaining

the spirals. Degradation of proteins might happen when the cells experience high levels of PAR at low temperature at which their synthesis is relatively slow. Under the condition of high light and low temperature, the imbalance between the energetic transfer and carbon fixation processes will result in excessive energy that can cause photoinhibition and damage to proteins (Huner et al. 1998), which consequently lead to the alteration or breakage of the spirals.

In conclusion, the results presented here indicate that solar UVR not only harms photosynthesis, but also affects the morphology and the DNA of the cyanobacterium *A. platensis*, with these impacts being dependent on temperature. To raise production of *A. platensis*, both the temperature and culture biomass density in commercial ponds should be increased during the morning and on winter days to reduce the negative effects caused by UVR. On the other hand, increasing UV-B radiation due to ozone depletion might have negative effects on cyanobacteria; however, the increasing global temperature due to human activities might counteract some of the negative effects of UV-B for some cyanobacteria.

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