Vol. 71, No. 9

Effects of Solar UV Radiation on Morphology and Photosynthesis of Filamentous Cyanobacterium *Arthrospira platensis*

Hongyan Wu,[1] Kunshan Gao,[1,2]* Virginia E. Villafañe,[3] Teruo Watanabe,[4] and E. Walter Helbling[3]

Marine Biology Institute, Shantou University, Shantou, Guangdong 515063, China[1]; Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China[2]; Estación de Fotobiología Playa Unión & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rifleros 227, Playa Unión (9103) Rawson, Chubut, Argentina[3]; and Hainan DIC Microalgae Co., Ltd., Haikou International Commercial Centre 38, Da Tong Road, Haikou, Hainan 570102, China[4]

To study the impact of solar UV radiation (UVR) (280 to 400 nm) on the filamentous cyanobacterium *Arthrospira* (*Spirulina*) *platensis*, we examined the morphological changes and photosynthetic performance using an indoor-grown strain (which had not been exposed to sunlight for decades) and an outdoor-grown strain (which had been grown under sunlight for decades) while they were cultured with three solar radiation treatments: PAB (photosynthetically active radiation [PAR] plus UVR; 280 to 700 nm), PA (PAR plus UV-A; 320 to 700 nm), and P (PAR only; 400 to 700 nm). Solar UVR broke the spiral filaments of *A. platensis* exposed to full solar radiation in short-term low-cell-density cultures. This breakage was observed after 2 h for the indoor strain but after 4 to 6 h for the outdoor strain. Filament breakage also occurred in the cultures exposed to PAR alone; however, the extent of breakage was less than that observed for filaments exposed to full solar radiation. The spiral filaments broke and compressed when high-cell-density cultures were exposed to full solar radiation during long-term experiments. When UV-B was screened off, the filaments initially broke, but they elongated and became loosely arranged later (i.e., there were fewer spirals per unit of filament length). When UVR was filtered out, the spiral structure hardly broke or became looser. Photosynthetic O_2 evolution in the presence of UVR was significantly suppressed in the indoor strain compared to the outdoor strain. UVR-induced inhibition increased with exposure time, and it was significantly lower in the outdoor strain. The concentration of UV-absorbing compounds was low in both strains, and there was no significant change in the amount regardless of the radiation treatment, suggesting that these compounds were not effectively used as protection against solar UVR. Self-shading, on the other hand, produced by compression of the spirals over adaptive time scales, seems to play an important role in protecting this species against deleterious UVR. Our findings suggest that the increase in UV-B irradiance due to ozone depletion not only might affect photosynthesis but also might alter the morphological development of filamentous cyanobacteria during acclimation or over adaptive time scales.

The depletion of the stratospheric ozone layer, mainly due to anthropogenically released pollutants such as chlorofluorocarbons, has resulted in an increase in the solar UV-B radiation (280 to 320 nm) that reaches the Earth's surface (10, 26). Ozone depletion is expected to increase and to spread over a broader range of altitudes and latitudes throughout most of the current century (49). UV-B radiation is potentially detrimental to all forms of life but is more detrimental to photosynthetic organisms, including cyanobacteria (21, 47, 48). The cyanobacteria are the largest and most widely distributed group of photosynthetic prokaryotes on the Earth, and they contribute markedly to global CO_2 and N_2 fixation (7, 15). It has been reported that UV-B not only impairs the motility and photoorientation of cyanobacteria (13) but also affects a number of physiological and biochemical processes, such as growth, survival, pigmentation, nitrogen metabolism enzymes (28, 43), CO_2 uptake, and ribulose 1,5-bisphosphate carboxylase activity (27, 46). On the other hand, cyanobacteria are the oldest au-

totrophic inhabitants of the planet, and they may have been exposed to high UV radiation (UVR) levels during the early Precambrian era (12, 17). Therefore, they must have developed effective mechanisms to counteract detrimental effects of these highly energetic wavelengths (at present levels of UVR and possibly at higher levels that may occur in the future). Many species of cyanobacteria show wide variation in tolerance to UV-B and possess a variety of defense strategies, such as avoidance of brightly lit habitats (2, 8, 53), production of UV-absorbing compounds, such as mycosporine-like amino acids (MAAs) and scytonemin (19, 45), and active repair or de novo synthesis of DNA (41, 42). However, despite the large amount of literature on the effects of UVR on cyanobacteria, we are not aware of studies documenting the impact of solar UVR on their morphology.

Arthrospira (*Spirulina*) *platensis* is an economically well-known filamentous cyanobacterium that is commercially produced as a source of human health food (9), animal feed (31), and cosmetic colorants (11), and considerable efforts have been made to optimize the growth conditions of this organism for massive production (24, 40, 50, 52). The morphological features of *Arthrospira* species include characteristic regular

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* Corresponding author. Mailing address: Marine Biology Institute, Shantou University, Shantou, Guangdong 515063, China. Phone: 86-754-2903280. Fax: 86-754-2903977. E-mail: ksgao@stu.edu.cn.

helical coiling or spirals. The helicity has been used as a tax-onomic criterion (30), as well as a way to select for high-quality strains (3). Environmental factors, such as light, temperature, and salinity, can affect the helical structure (22, 23, 25, 30); for example, the filaments change from straight to the typical helical shape when they are shifted to growth with a high intensity of visible light (16). However, we are not aware of any study documenting the effects of solar UVR on the morphol-ogy of *Arthrospira*.

In this work, we investigated the effects of solar radiation on the morphology and photosynthesis of *A. platensis*. The infor-mation obtained not only could provide a potential basis for optimizing the growth conditions for this species with increas-ing solar UV-B radiation but also could have special impor-tance for understanding the overall impact of UVR on fila-mentous cyanobacteria.

MATERIALS AND METHODS

Organisms. Two strains of *A.* (*S.*) *platensis* were used in our experiments. These strains were (i) strain 439, an indoor strain that was grown in the labora-tory, had not been exposed to sunlight for decades, and was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences, and (ii) strain D-0083, an outdoor strain that has been grown under sunlight for decades in the commercial production base and was obtained from Hainan DIC Microalgae Co. Ltd., Hainan, China. For these two strains we used three different culture concentrations, 330, 590, and 780 mg (dry weight) liter^{-1}, in the experiments outlined below.

Solar radiation measurements and treatments. Incident solar radiation (UV-B [280 to 315 nm], UV-A [315 to 400 nm], and PAR [400 to 700 nm]) was continuously monitored using a broadband filter radiometer (ELDONET; Real Time Computer Inc., Germany) (20) permanently installed on a roof at Shantou University (116.6°E, 23.3°N).

Subsamples of *A. platensis* were harvested during the exponential growth phase, resuspended in Zarrouk freshwater medium, and dispensed into 1-liter UV-transparent quartz tubes (inside diameter, 6.4 cm; length, 40 cm). The cultures were aerated (0.35 liter min^{-1}) and maintained in a flowthrough water bath for temperature control at 18 to 20°C. The following three radiation treat-ments were used: (i) cells received full solar radiation (PAB treatment; 280 to 700 nm) in uncovered quartz tubes; (ii) cells received UV-A and PAR (PA treatment; 320 to 700 nm) in quartz tubes covered with Folex 320 (Montagefolie no. 10155099; Folex, Dreieich, Germany); and (iii) cells received only PAR (P treatment; 400 to 700 nm) in quartz tubes covered with Ultraphan film 395 (UV Opak; Digefra, Munich, Germany).

Morphological examination. Morphological examinations were performed us-ing a compound microscope (Zeiss Axioplan 2; Carl Zeiss, Germany). Digital images were recorded with a Zeiss Axicam camera and were analyzed with a Vision Analysis system (AxioVision 3.0). The length and helix pitch of 20 to 50 *A. platensis* filaments were measured in at least five fields. The helix pitch was measured by using the ichnography of the spiral-shaped trichome (i.e., the distance between two neighboring spirals).

To study morphological changes, cultures of *A. platensis* at initial concentra-tions of 330, 590, and 780 mg (dry weight) liter^{-1} were exposed to the PAB and P treatments for 6 h centered on local noon (from 10 a.m. to 4 p.m.) on 2 and 3 December 2003. Subsamples were taken every 2 h and examined under the microscope to determine morphological changes. Since there were no morpho-logical changes in the two strains with the high biomass (780 mg [dry weight] liter^{-1}) in 1 day, we conducted long-term experiments for 15 to 20 days to monitor potential morphological changes in the strains. In this long-term exper-iment, subsamples were taken daily at sunset for microscopic observation.

Assessment of photosynthetic inhibition. Cultures of *A. platensis* at initial concentrations of 330 and 780 mg (dry weight) liter^{-1} were exposed to solar radiation by using the PAB and P treatments for 6 h (duplicate samples for each treatment). Oxygen evolution was measured at the beginning (i.e., time zero [t_0]) and then every 2 h during the incubation period, which was centered on local noon (from 9 or 10 a.m. to 3 or 4 p.m.). Photosynthetic O$_2$ evolution was determined with a five-channel oxymeter (Oxym 5; Real Time Computers Inc., Germany), which had five quartz chambers in a UV-transparent thermal jacket. Five Clark microelectrodes (model 5351; Yellow Spring Instruments Co.) were
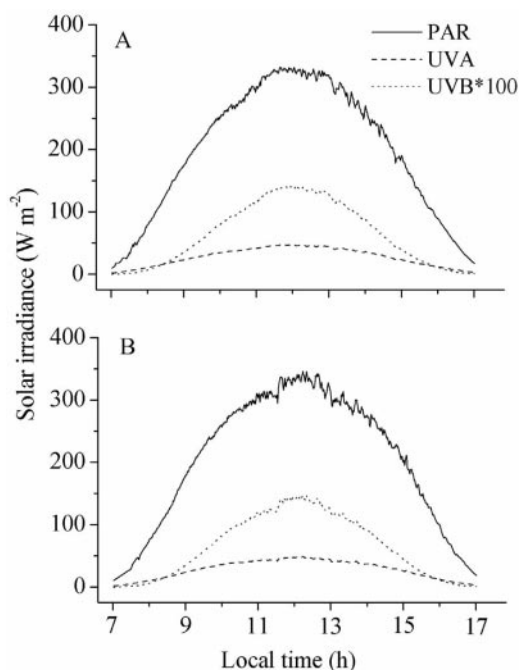

FIG. 1. Solar irradiance curves for PAR, UV-A, and UV-B on 2 December 2003 (A) and 3 December 2003 (B), when morphological changes were evaluated (from 10 a.m. to 4 p.m.).

connected to the quartz chambers and interphased to a laptop computer; data were acquired every 2 s. Complete measurement of photosynthesis for the two radiation treatments took approximately 12 to 15 min, and the rate of O$_2$ evolution was calculated from the slope of the curve of O$_2$ concentration versus time. The relative photosynthetic inhibition due to UVR was calculated as follows: $(P_{PAR} - P_{UVR}) \cdot (P_{PAR})^{-1}$, where P_{PAR} and P_{UVR} are the photosyn-thetic O$_2$ evolution rates in cells exposed to only PAR and to PAR plus UVR, respectively. In order to compare photosynthetic inhibition with different irradi-ances (i.e., on different days), the values described above were normalized to the mean PAR irradiance over the measurement period (i.e., 12 to 15 min).

Evaluation of UV-absorbing compounds and photosynthetic pigments. Sam-ples were taken from the cultures after 5 and 10 days of exposure and filtered onto Whatman GF/F glass fiber filters (25 mm). UV-absorbing compounds and photosynthetic pigments were extracted in 10 ml of absolute methanol overnight and centrifuged (5,000 × g for 5 min), and the extract was scanned at wave-lengths of 250 to 750 nm using a scanning spectrophotometer (Shimadzu UV 2501-PC). The total concentration of UV-absorbing compounds was estimated from the peak at 334 nm as described by Dunlap et al. (14). The chlorophyll *a* (Chl *a*) concentration was calculated as described by Porra (36), and the con-centration of carotenoids was determined using the equations of Parsons and Strickland (35).

Statistical analysis. Analysis of variance and a *t* test were used to establish differences among the different radiation treatments and strains. A confidence level of 95% was used in all analyses.

RESULTS

Morphological changes. Morphological changes were exam-ined for the indoor and outdoor strains on 2 December 2003 (Fig. 1A) and 3 December 2003 (Fig. 1B), respectively, when the maximum PAR irradiances were 329.2 and 341.1 W m^{-2}, respectively. On these days, the maximum values for UV-A varied between 46.3 and 48.1 W m^{-2}, whereas the maximum values for UV-B varied between 1.37 and 1.42 W m^{-2}. The cells of indoor strain 439 were originally arranged in spring-shaped filaments that were ~1.15 mm long with up to 12 spirals
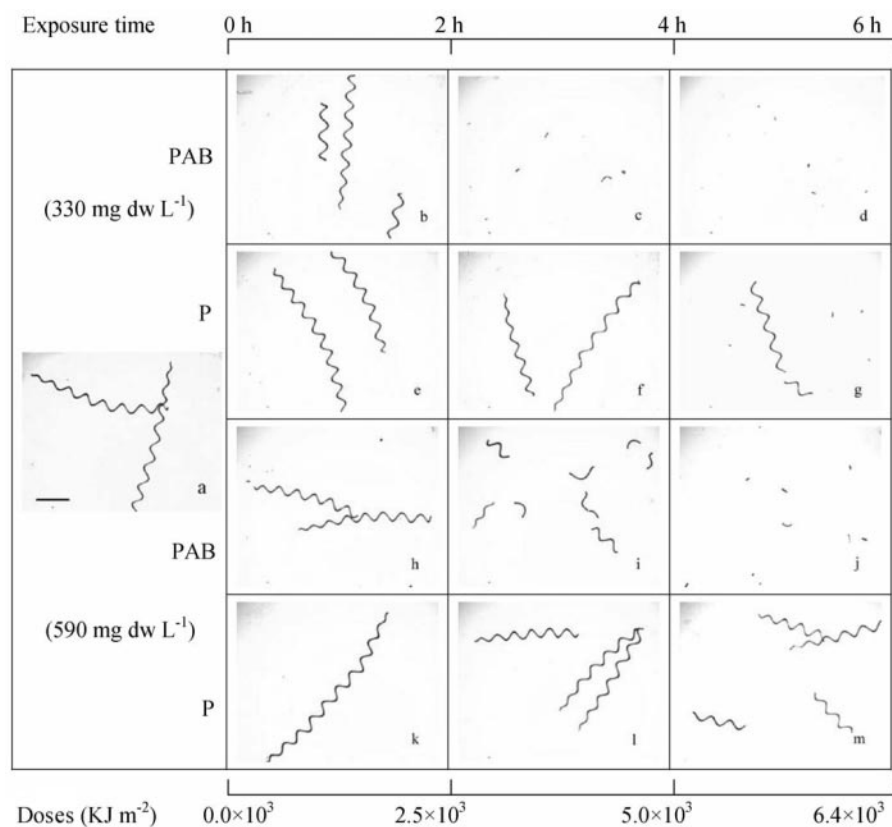
FIG. 2. Spiral structure of *A.* (*S.*) *platensis* indoor-grown strain 439 exposed to different solar radiation treatments for 6 h centered on local noon. (a) Spiral structure at the beginning of the experiment (before exposure to solar radiation). Scale bar = 0.2 mm. (b to g) Spiral structure in low-biomass cultures (330 mg [dry weight] liter^{-1}) exposed to full solar radiation (PAB) and only PAR (P) for 2, 4, and 6 h. (h to m) Spiral structure in high-biomass cultures (590 mg [dry weight] liter^{-1}) exposed to the PAB and P treatments. dw, dry weight.

(Fig. 2a). Exposure to full solar radiation (i.e., PAB treatment) for 2 h led to breakage of the filaments in the low-biomass (330 mg [dry weight] liter^{-1}) culture (Fig. 2b), whereas 4 to 6 h of exposure resulted in severe breakage of the spiral filaments into small pieces (Fig. 2c and d). In contrast, no significant changes in filament morphology were observed after 4 h of exposure to PAR (Fig. 2e and f), while breakage of filaments was induced by this treatment after 6 h of exposure (Fig. 2g) but to a lesser extent compared to the PAB treatment. In the culture with a biomass of 590 mg (dry weight) liter^{-1}, the filaments did not break after 2 h of exposure to the PAB treatment (Fig. 2h), but breakage to form one or two helices was observed after 4 h of exposure (Fig. 2i); 6 h of exposure resulted in further breakage (Fig. 2j). As observed in the low-biomass culture, exposure to PAR for 2 to 4 h did not result in changes in filament morphology (Fig. 2k and l), and 6 h of exposure to PAR resulted in only slight breakage (Fig. 2m).

The cells of the outdoor-grown strain (D-0083) were arranged in a much tighter configuration; the filaments were ~0.22 mm long with up to 19 spirals (Fig. 3a). Exposure to the PAB treatment for 4 h resulted in slight breakage of the filaments in the low-biomass (330 mg [dry weight] liter^{-1}) culture (Fig. 3b). Severe breakage was observed after 6 h of exposure to full solar radiation (Fig. 3c), but small helices were still present. When cells were exposed to PAR for 4 h, the filaments did not break (Fig. 3d), but some breakage occurred after continuous exposure for 6 h (Fig. 3e). In contrast, no change in filament morphology was observed with the PAB treatment after 4 h of exposure in the culture containing 590 mg liter^{-1} (Fig. 3f); however, 6 h of exposure resulted in slight breakage of the filaments (Fig. 3g), but the extent of breakage was less than that in the low-biomass culture. Similarly, samples exposed to the P treatment did not show breakage of the filaments after 4 h of exposure (Fig. 3 h), but 6 h of exposure resulted in less breakage than the breakage observed with the PAB treatment or in the low-biomass culture (Fig. 3i).

As mentioned above, no changes were observed in the short term (i.e., 6 h) for the two strains when the high-biomass cultures (i.e., 780 mg liter^{-1}) were used. Thus, we investigated changes that could occur over longer time scales (i.e., weeks) when both strains of *A. platensis* were exposed to solar radiation using the three radiation treatments (i.e., PAB, PA, and P) (Fig. 4). Exposure to full solar radiation resulted in breakage of the spiral filaments within 5 and 10 days for the indoor and outdoor strains, respectively (Fig. 4c and d). Only cell debris was observed in the indoor strain culture after 5 days of exposure to PAB, and the culture became almost pellucid (Fig. 4c); on the other hand, shortened filaments of the outdoor strain had a tighter spiral structure (Fig. 4d). When UV-B was screened off, the spiral structure of *A. platensis* also broke during the initial period (1 to 2 days) (data not shown), but it sustained, elongated, and became loose later (Fig. 4e and f).
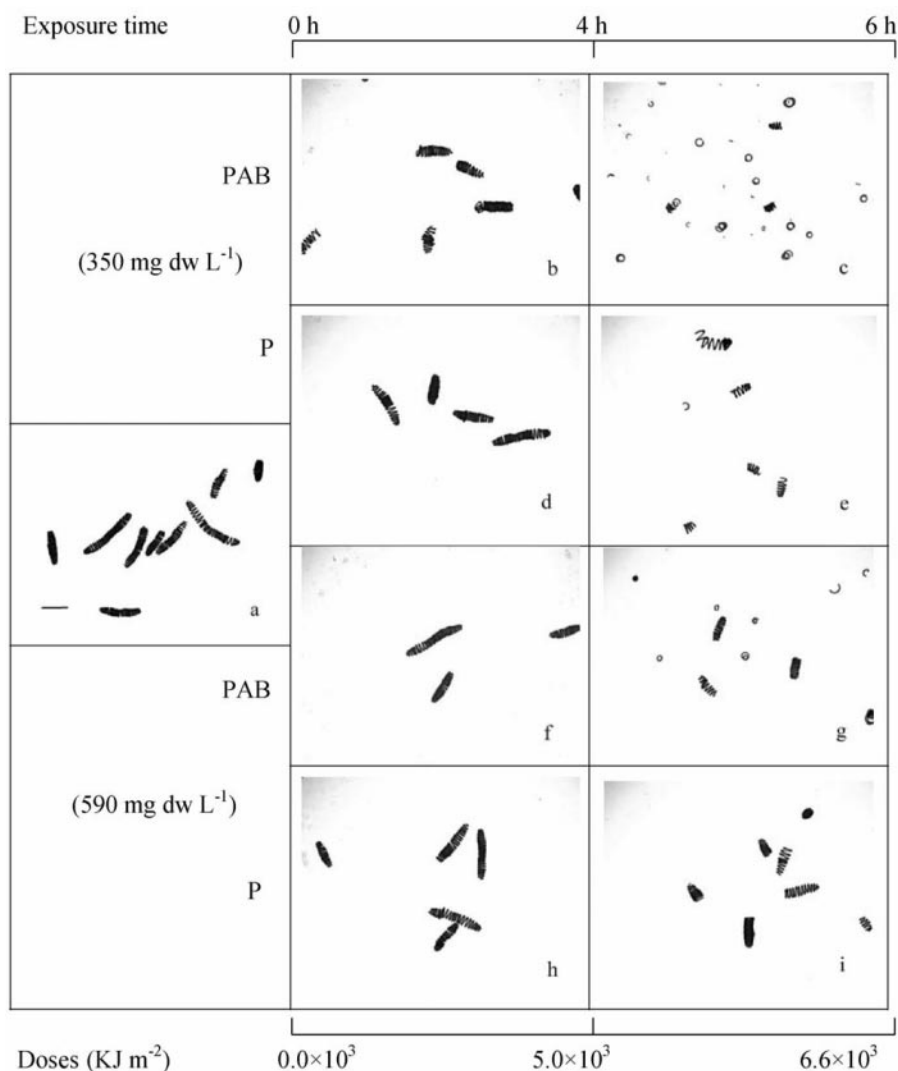
FIG. 3. Spiral structure of *A. platensis* outdoor-grown strain D-0083 exposed to different solar radiation treatments for 6 h centered on local noon. (a) Spiral structure at the beginning of the experiment (before exposure to solar radiation). Scale bar = 0.1 mm. (b to e) Spiral structure in low-biomass cultures (330 mg [dry weight] liter^{-1}) exposed to full solar radiation (PAB) and only PAR (P) for 4 and 6 h. (f to i) Spiral structure in high-biomass cultures (590 mg [dry weight] liter^{-1}) exposed to the PAB and P treatments. dw, dry weight.

When both UV-B and UV-A were filtered out, the spiral structure hardly broke, but it became loose (Fig. 4g and h).

The trichome length (Fig. 5A and B) and the helical pitch (Fig. 5C and D) of *A. platensis* varied according to the radiation treatment imposed on the samples. In the indoor strain, the trichome length decreased significantly ($P < 0.01$) during the first 3 days of exposure to solar radiation and was 9.6% and 15.7% of the original mean value in the samples exposed to the PAB and PA treatments, respectively (Fig. 5A). The decrease in trichome length continued in the samples exposed to the PAB treatment until the length was almost zero on day 5. In the samples exposed to the PA treatment, the trichome length started to increase after day 3, but the difference was significant ($P < 0.01$) only after day 7. In the outdoor strain, however, the trichome length decreased to one-half of the initial value after 3 days of exposure to UVR (Fig. 5B). Continuous exposure to UVR did not result in a

further decrease in the trichome length; exposure to UV-A, on the other hand, resulted in a significant ($P < 0.01$) increase in the trichome length after 6 days of exposure, and the length reached the original length on day 10. In samples exposed to only PAR there was an initial decrease in the trichome length after 1 and 2 days of exposure in the indoor and outdoor strains, respectively, but after this the trichome length did not vary significantly ($P > 0.1$). The helix pitch increased significantly ($P < 0.01$) with the PA and P treatments after 9 days of exposure to solar radiation in the indoor strain from the original value (146.6 ± 8.7 µm) to 176.2 ± 6.9 and 179.4 ± 6.1 µm, respectively (Fig. 5C), indicating that there was a decrease in the number of spirals per unit of filament length. A significant decrease ($P < 0.01$) in the helix pitch (about 8%), however, was observed in this strain with the PAB treatment after day 1 (Fig. 5C), indicating that the cell structure was compacted (i.e., there was
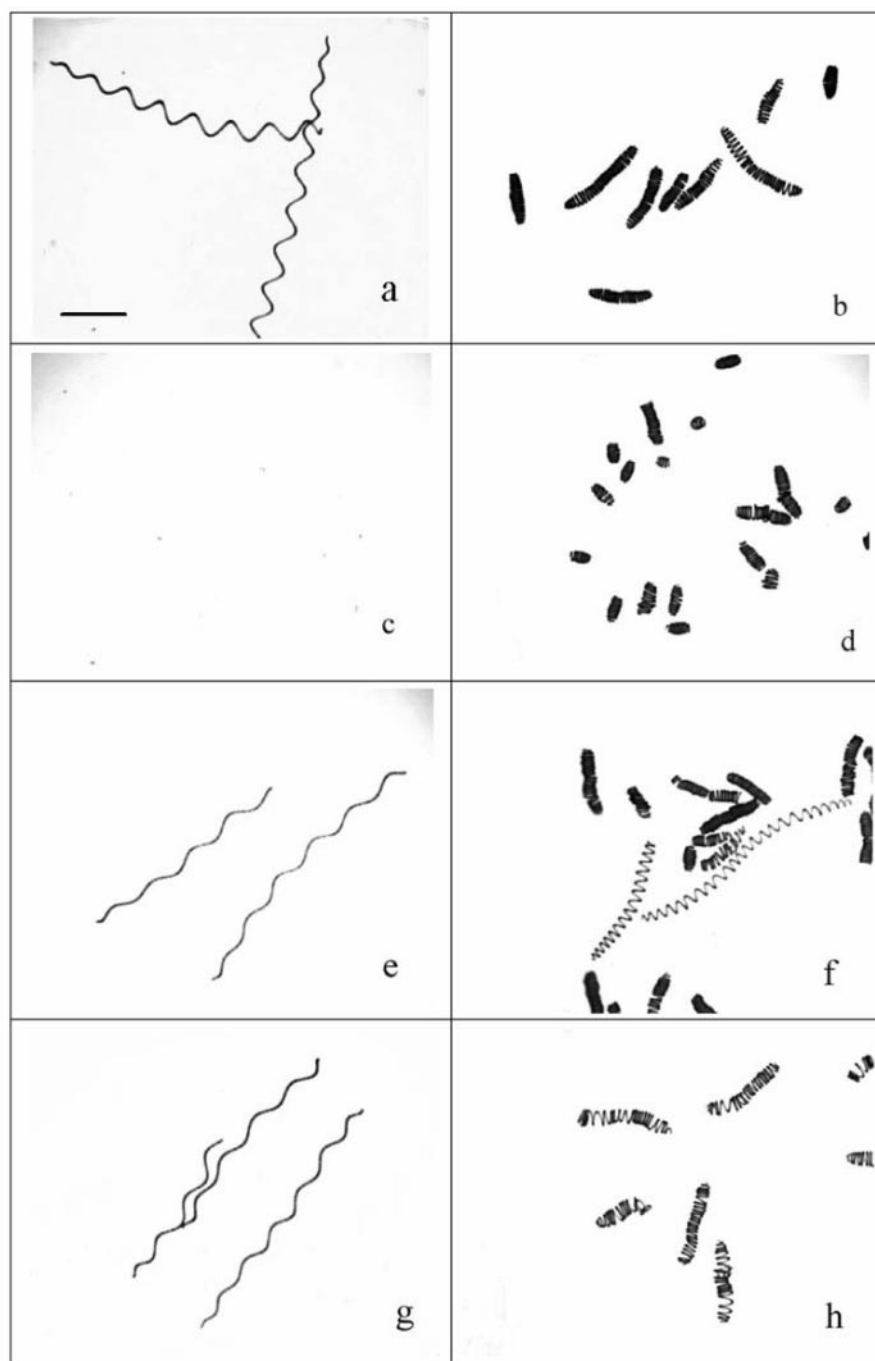
FIG. 4. Spiral structure of *A. platensis* indoor-grown strain 439 (a, c, e, and g) and outdoor-grown strain D-0083 (b, d, f, and h) exposed to different types of solar radiation in long-term cultures with a high level of biomass (780 mg [dry weight] liter^{-1}). (a and b) Spiral structure at the beginning of the experiment (before exposure to solar radiation). Scale bar = 0.2 mm. (c and d) Death of cells of the indoor strain and breakage of the filaments of the outdoor strain exposed to the PAB treatment for 5 and 10 days, respectively. (e and f) Arrangement of the filament helix in the two strains exposed to the PA treatment for 15 days. (g and h) Loose helical structure when cells were exposed to only PAR for 10 and 20 days, respectively.

an increased number of spirals per unit of filament length) in the presence of UVR. Breakage of trichomes into small pieces with the PAB treatment precluded measurement of the helix pitch after 1 day. Helix pitch in the outdoor strain also decreased significantly ($P < 0.01$) with the PAB treat-ment from the original value (11.8 ± 0.3 μm) to 10.3 ± 0.4 μm after day 5 (Fig. 5D), but it increased when UV-B or UVR was screened off. The increases, however, were signif-icant only after day 9 (16%) and on day 10 (20%) ($P < 0.01$) with the PA and P treatments, respectively.
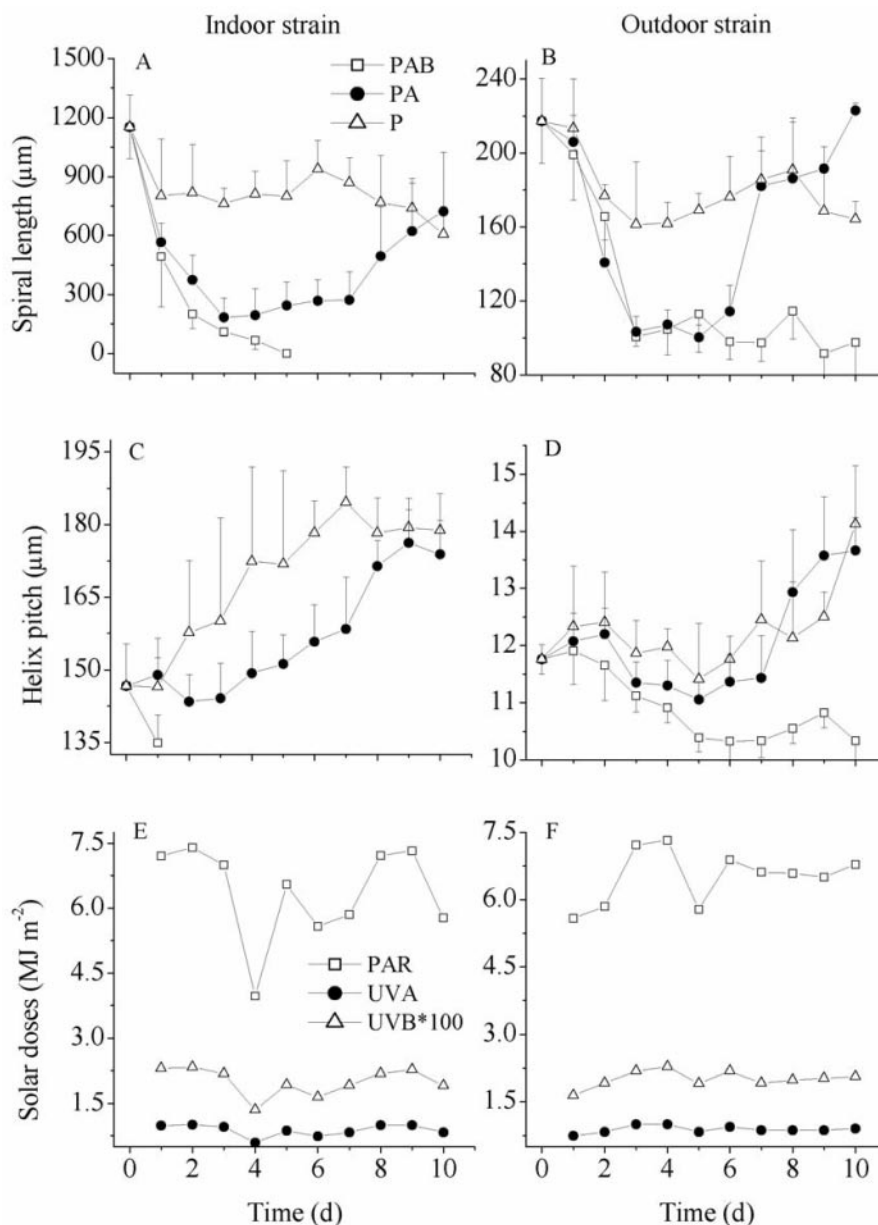
FIG. 5. Structural changes in the spiral filaments of *A. platensis* indoor-grown strain 439 (A and C) and outdoor-grown strain D-0083 (B and D) as a function of time of exposure to solar radiation (E and F). (A and B) spiral length; (C and D) helix pitch; (E and F) daily solar doses during the exposure period. The vertical bars indicate the standard deviations around the means ($n = 20$ to 50).

Photosynthetic inhibition. Together with the continuous breakage of the spiral structure of *A. platensis* exposed to full solar radiation during the short-term experiments (6 h) (Fig. 2 and 3), we observed significant UVR-induced reduction in photosynthetic O_2 evolution (Fig. 6). Photosynthetic O_2 evolution in the indoor strain was significantly suppressed ($P < 0.01$) in the presence of UVR at the beginning of the experiment (t_0) (Fig. 6A) and after subsequent exposure to solar radiation (Fig. 6B, C, and D). Exposure to UVR for more than 2 h resulted in oxygen consumption (i.e., negative O_2 evolution values) (Fig. 6B, C, and D). The outdoor strain also showed photoinhibitory effects when it was exposed to UVR, but to a

lesser extent (Fig. 6E, F, G, and H). This strain displayed O_2 production (i.e., photosynthesis) even at the highest UVR irradiance registered at noon (Fig. 6F and G). Photosynthetic O_2 evolution was also suppressed in both strains when they were exposed only to PAR; however, the extent of suppression was much less than that observed with cells exposed to UVR plus PAR.

To better describe the inhibitory effects of UVR, we used the photosynthetic rate differences between cultures exposed to PAR plus UVR and cultures exposed to only PAR. Photosynthetic inhibition in the indoor strain reached a peak at noon and then decreased, following approximately the shape of the
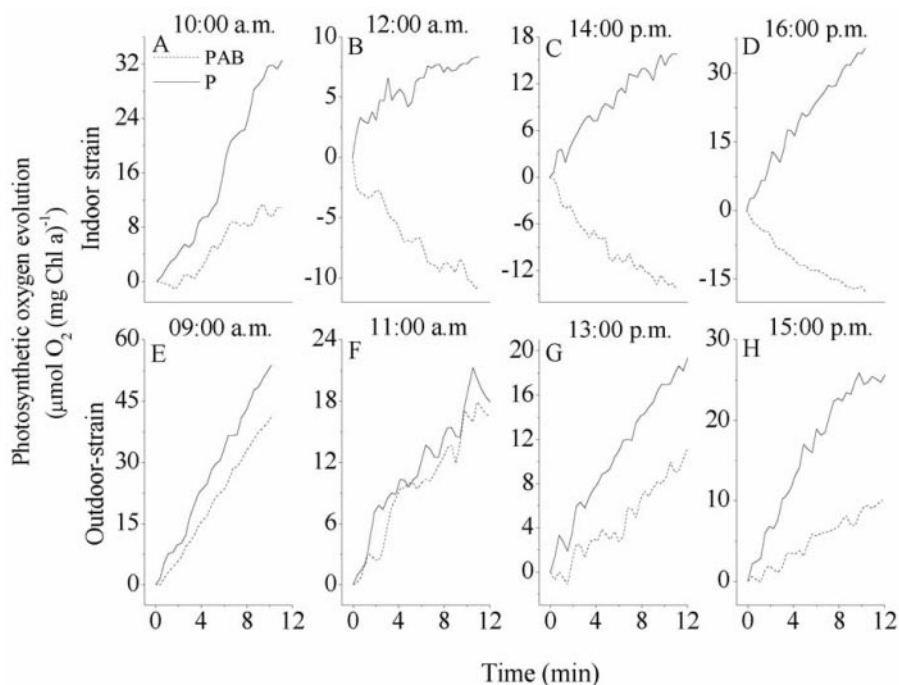
FIG. 6. Photosynthetic oxygen evolution in *A. platensis* indoor-grown strain 439 (upper panels) and outdoor-grown strain D-0083 (lower panels) exposed to the PAB and P treatments for 6 h centered at local noon (from 10 a.m. to 4 p.m. or from 9 a.m. to 3 p.m.). Oxygen evolution was measured for 12 to 15 min every 2 h during the experiment. The initial biomass density for both strains was 780 mg (dry weight) liter^{-1}.

irradiance curve (Fig. 7A and B). On the other hand, in the outdoor strain, inhibition did not decrease in the afternoon (Fig. 7C). When we compared UVR-induced inhibition under different solar radiation conditions (i.e., different days) (Fig.

7D), we found that the inhibition increased with exposure time, and this was much more evident for the indoor strain ($P < 0.01$) and low biomass ($P < 0.05$) than for the outdoor strain and high biomass.
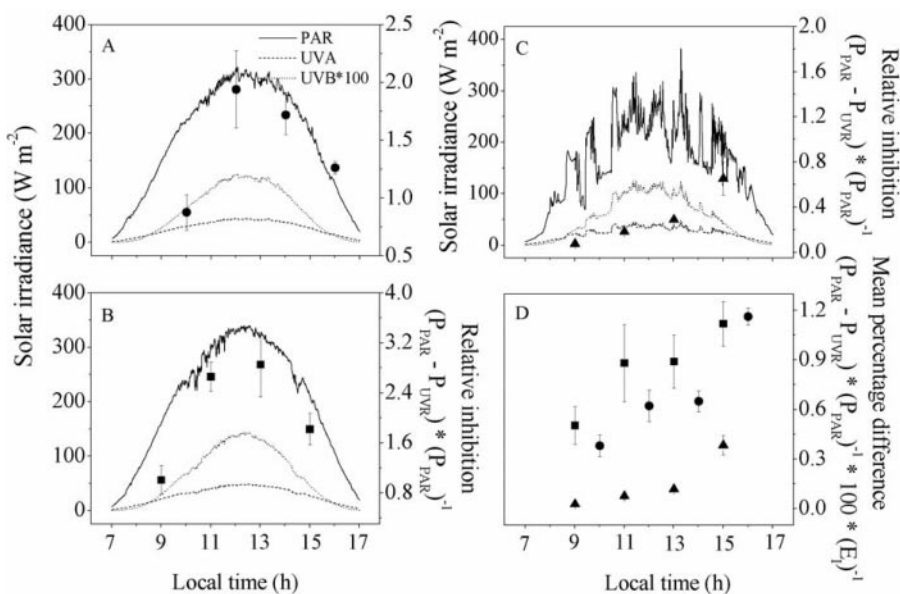

FIG. 7. UVR-induced photosynthetic inhibition in *A. platensis* indoor-grown strain 439 and outdoor-grown strain D-0083. Cultures of *A. platensis* were exposed to the PAB and P treatments for 6 h centered at local noon. Each point represents the photosynthetic rate difference between cultures exposed to PAR plus UVR (P_{UVR}) and cultures exposed to only PAR (P_{PAR}). (A and B) Relative inhibition in the indoor strain cultured at densities of 780 and 330 mg (dry weight) liter^{-1}, respectively. (C) Relative inhibition in the outdoor strain cultured at a density of 780 mg (dry weight) liter^{-1}. (D) Percent inhibition normalized to the mean PAR irradiance (E_I) received during measurements. Superimposed are the irradiance curves for PAR, UV-A, and UV-B during the experiment (9, 10, and 11 December 2003). The vertical bars indicate the standard deviations around the means ($n = 4$).
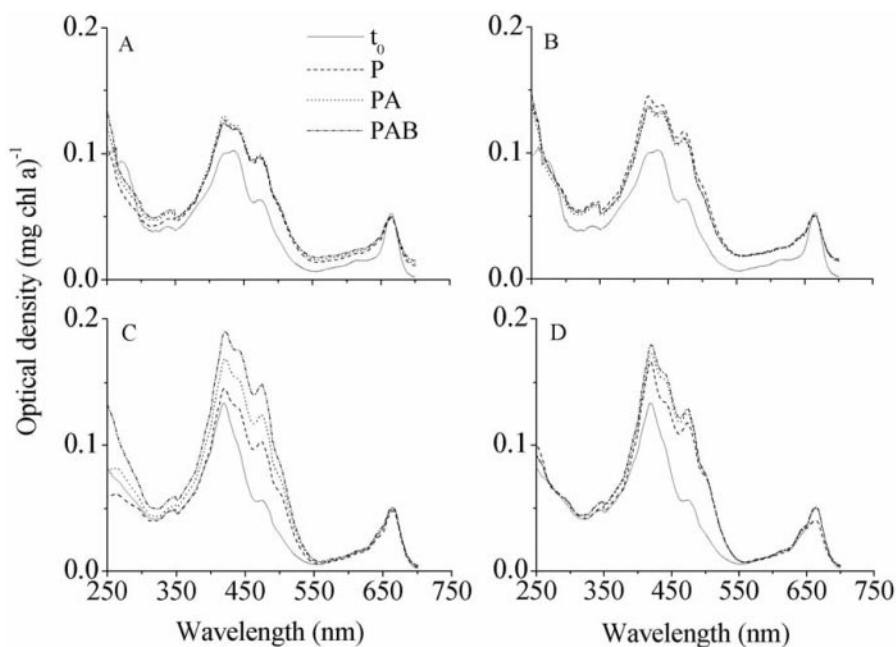
FIG. 8. Spectral characteristics of *A. platensis* indoor-grown strain 439 (A and B) and outdoor-grown strain D-0083 (C and D) exposed to PAB, PA, and P solar radiation. The ordinate is the optical density normalized to the concentration of Chl *a*. (A and C) Samples exposed for 5 days; (B and D) samples exposed for 10 days.

UV-absorbing compounds and pigments. The spectral characteristics of the two strains of *A. platensis* subjected to the PAB, PA, and P treatments at t_0 and after 5 and 10 days of exposure to solar radiation are shown in Fig. 8. There was a very small absorption peak of UV-absorbing compounds (absorbance between 310 and 360 nm) in these two strains (Fig. 8). Although the concentration of UV-absorbing compounds was very low in the indoor strain, it increased significantly during the PA and PAB treatments ($P < 0.01$) compared to the concentration at t_0. In contrast, there was a much lower amount of UV-absorbing compounds in the outdoor strain, and no significant increase in UV-absorbing compounds was observed when this strain was exposed to UVR (Table 1). Similarly, the ratio of UV-absorbing compounds to Chl *a* increased in the indoor strain ($P < 0.05$) when cells were exposed to the PA and PAB treatments, but it remained constant ($P > 0.05$) in the outdoor strain (Table 1). There were no significant differences between radiation treatments with regard to the concentration of carotenoids for both strains (data not shown). However, a significant ($P < 0.05$) increase in the ratio of

carotenoids to Chl *a* was observed with the PAB treatment for both strains compared to the ratio at t_0.

DISCUSSION

Cyanobacteria date back to the Precambrian era, when UVR levels were much higher than they are today due to the lack of the ozone layer; thus, their light-harvesting proteins absorbed more than 99% of the incident UV-B radiation to shield the DNA molecule (29, 51). However, studies have shown that the DNA molecule is indeed degraded in cyanobacteria (6, 34) and that the physiology of these organisms responds negatively when they are exposed to elevated levels of UV-B (44). In particular, it has been shown that UV-B affects the photosynthetic electron transport and pigment-protein complexes of *A. platensis* (38). However, it is also now known that aquatic organisms have mechanisms to minimize and counteract the deleterious effects produced by highly energetic short wavelengths (1, 32).

Among the mechanisms that might play a significant role in protecting organisms against solar UVR is the production of

TABLE 1. UV-absorbing compound contents and ratios of the UV-absorbing compounds and carotenoids to Chl *a* in *A. platensis* cells of indoor-grown strain 439 and outdoor-grown strain D-0083 exposed to PAB, PA, radiation for 10 days

Radiation treatment[a]	Concn of UV-absorbing compounds (μg g [dry wt]$^{-1}$)		UV-absorbing compounds/Chl *a* (μg/mg)		Carotenoids/Chl *a* (μg/μg)	
	Strain 439	Strain D-0083	Strain 439	Strain D-0083	Strain 439	Strain D-0083
Control	0.43 ± 0.04^b	0.34 ± 0.02^c	0.10 ± 0.01	0.08 ± 0.01	0.32 ± 0.03	0.32 ± 0.01
PA	0.62 ± 0.01	0.32 ± 0.03	0.17 ± 0.01	0.11 ± 0.01	0.40 ± 0.02	0.43 ± 0.02
PAB	0.67 ± 0.01^b	0.33 ± 0.05^c	0.18 ± 0.01	0.11 ± 0.01	0.43 ± 0.01	0.49 ± 0.03

[a] The control data are the data for time zero.
[b] Significant at $P < 0.01$.
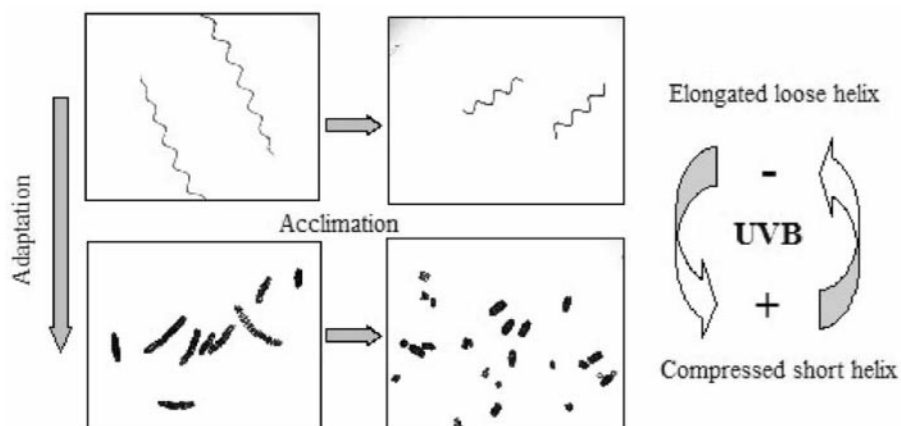[c] Not significant at $P > 0.05$.

FIG. 9. Schematic representations of short- and long-term morphological changes in *A. platensis* as a function of exposure to UV-B.

photoprotective compounds, such as MAAs and scytonemin (1, 4, 18, 19). It has also been shown that cells with high concentrations of MAAs are more resistant to UVR than cells with small amounts of these compounds (18). In fact, MAA concentrations varying between 0.9 and 8.4 μg mg (dry weight)$^{-1}$ have been measured in cyanobacterial isolates (18), and ratios of MAAs to Chl *a* in the range from 0.04 to 0.19 have been reported in cyanobacterial mats (37). In our study, we found that *A. platensis* contained a much lower concentration of UV-absorbing compounds (the highest value was 0.67 μg g [dry weight]$^{-1}$ [Table 1]) and that the ratio of these compounds to Chl *a* was much lower than that in the cyanobacterial mats. Moreover, when the time period necessary to synthesize UV-absorbing compounds was assessed, it was found that in *Nodularia* spp. the levels were increased fivefold when the organisms were exposed to the full spectrum of solar radiation for 72 h (45). In contrast, in our study, an increase of 56% in the concentration of UV-absorbing compounds was observed in the indoor strain after 10 days of exposure to full solar radiation. This increased amount of UV-absorbing compounds might have played a small role in protection against UVR-induced damage, but it did not prevent the cells from being harmed, as seen in the breakage of filaments and photoinhibition (Fig. 2, 3, 6, and 7).

The low levels of UV-absorbing compounds found in the outdoor strain compared to those in the indoor strain (Table 1) or other related species indicate that the outdoor strain might have developed an alternative strategy for protection against harmful UVR levels. In fact, the effective self-shading produced by compressed spirals, seen in both strains when they were exposed to UV-B or produced in high-biomass cultures (Fig. 7), seems to be much more important for protecting the cells against high UVR levels. In particular, we propose that decreasing the helix pitch in the presence of UV-B (Fig. 5C and D) to obtain a more compact structure (i.e., minimum helix pitch) of spirals (Fig. 3a) is an effective protective mechanism, which results in self-shading and thus less photoinhibition (Fig. 6 and 7). Our results also suggest that there are different scales of adaptation and acclimation when cells receive full solar radiation (Fig. 9). Over short time scales (i.e., days) the trichome length decreases, resulting in breakage of filaments into small pieces. However, over long time scales

(i.e., decades), adaptation to solar UVR can bring about changes in the spiral structure from a rather elongated helix to a very compressed helix. This compressed structure results in protection against high solar UV-B levels and provides an obvious advantage during growth in outdoor culture ponds or natural water. In addition, the increased ratio of carotenoids to Chl *a* with PAB treatment (Table 1) might indicate a protective function due to quenching of the highly reactive oxidants produced by UVR, as seen in other cyanobacteria (5).

Morphological changes in *A. platensis*, such as helix pitch, spiral width, or orientation, have been reported under laboratory conditions (22, 23, 25, 30, 33). It seems that laboratory conditions, without any impact of UVR, favor the development of straight forms (22, 23, 30). In addition, it was found that *A. platensis* strains with a tight helical structure could tolerate higher light intensity than strains with loose spirals (23) and that loose straight filaments could be transformed to tight coiled shapes when the cells were shifted to high-light conditions (16). Our study further demonstrates that *A. platensis* responds to solar UVR by changing its spiral structure. It appeared that such morphological changes from a loose helix to a tight helix, induced by UVR (as seen in our work) or by high levels of visible light (as determined by another researcher [16]), are associated with the protective strategy of this cyanobacterium to counteract solar UVR or high PAR levels by increasing self-shading.

Morphological characteristics of *Arthrospira* or *Spirulina* species are often related to product quality. In addition, the length of the spiral filament is very important for harvesting efficiency. Thus, breakage of filaments by solar UVR in outdoor cultures negatively influences the quality and yield of the harvested biomass. On the other hand, an increase in UV-B radiation due to ozone depletion, as forecast for the early 21st century, might alter the morphological development of cyanobacteria. Consequently, this effect on the shapes and sizes could ultimately affect higher trophic levels, as both metabolic rates (39) and grazing behavior in aquatic ecosystems highly depend on these parameters.

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