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Effects of solar UV and visible radiations on the spiral structure and orientation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta)

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Morphological characteristics of *Arthrospira* spp. are important for product quality and harvesting efficiency. Solar ultraviolet radiation (UVR) has been previously shown to break their spirals. To further explore the relationship of morphology and UVR, we examined the spiral length, helix diameter, helical pitch and orientation of *A. platensis* while exposing the cells to solar radiation with or without UVR. Although solar exposures initially led to breakage of the spirals, no significant difference was found among the treatments with or without UVR. During the long-term exposures to solar radiation, the spirals became more compressed in the outdoor strain (grown under sunlight for decades) but more loosened in the indoor strain [grown under artificial photosynthetically active radiation (PAR) for decades]. Spiral orientation was altered from the right- to left-handed only in the outdoor strain, with a frequency of less than 1%. Presence of UVR enhanced such an orientation change, and regrowing under indoor low PAR (UVR-free condition) reversed the orientation to right-handed. Structure of some membrane molecules was suggested to be altered by high solar PAR and UVR.

KEY WORDS: *Arthrospira platensis*, Cyanobacterium, Helix orientation, Morphology, Solar radiation, UVR

INTRODUCTION

Arthrospira (*Spirulina*) *platensis* (Nordstedt) Gomont is an economically important filamentous cyanobacterium commercially produced as a source of human health food, animal feed and cosmetic colorants (Ciferri 1983; Vonshak 1997; Lu *et al.* 2002). Morphological features of *Arthrospira* spp. are characterized by regular helical coiling or spirals. Traditional *Arthrospira* taxonomy has been based on the spiral characteristics, such as the degree of spiralization and arrangement of the spirals or helix orientations (Lewin 1980). Helical structure has also been considered as an important feature of product quality (Belay 1997). However, spiral length and helix pitch and orientation of *A. platensis* usually change under different conditions (Jeeji Bai & Seshadri 1980; Fox 1996; Kebede 1997; Wang & Zhao 2005). A temperature upshift from 30 to 32–34°C for 7 days led to a change in spiral orientation (left-handed to right-handed) for 3 of 10 tested *Arthrospira* strains, which however, reversed to its original orientation when the temperature conditions resumed (Mühling *et al.* 2003).

In nature or in commercially operated ponds, *A. platensis* cells are exposed to solar radiation and thus susceptible to effects of high levels of photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). This is especially important in view of the enhanced solar UV-B (280–315 nm) radiation due to stratospheric ozone having been reduced by atmospheric pollutants (Pieter 2007). UVR is known to cause pigment bleaching, protein degradation, enzyme inactivation and DNA photoproduct formation in many photosynthetic organisms (Kumar *et al.* 1996; Sass *et al.* 1997; Sinha *et al.* 2001; Buma *et al.* 2003; Häder & Sinha 2005). Wu *et al.* (2005) found that solar UVR led to breakage of the spiral of *A. platensis* during winter season. Gao *et al.* (2008) demonstrated that such UVR-induced damage to the spiral structure was temperature dependent. However, it is still unknown whether UVR would lead to a change in the helix orientation of *A. platensis*, which requires days to happen (Mühling *et al.* 2003). Previous findings about the spiral breakage were either based on indoor short-period experiments (Gao *et al.* 2008) or on the data obtained during winter season when the temperature was not optimal for growth (Wu *et al.* 2005). Therefore, the present study aimed to investigate long-term effects of solar UVR on the trichome length, helix diameter and helix orientation of *A. platensis* under the optimal temperature.

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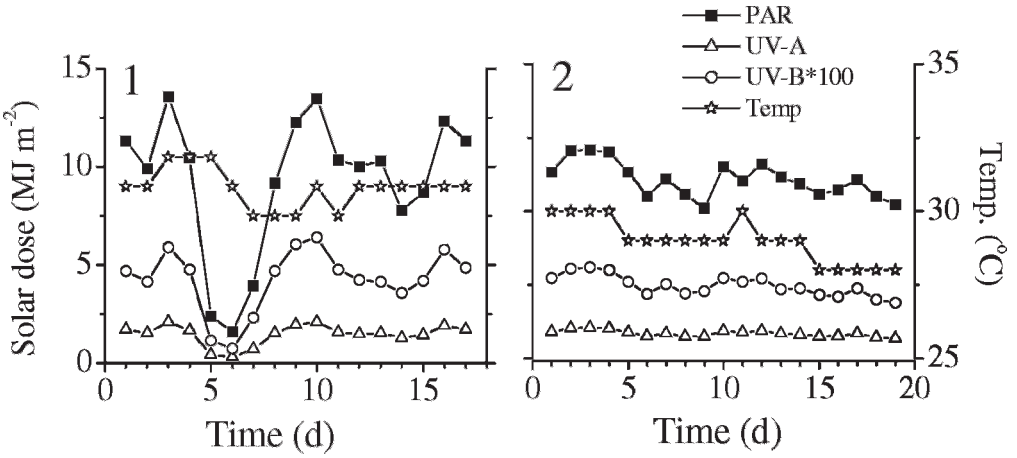
MATERIAL AND METHODS

Organism and culture conditions

Two strains of *A. platensis* were used in our experimentation. An indoor stain (439), obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences, was grown in the laboratory (40 μmol photons m^{-2} s^{-1}, 25°C) and had not been exposed to sunlight for decades. An outdoor-stain (D-0083) had been grown under solar radiation for decades in the commercial ponds (110°18′E, 20°0′N) of Hainan DIC Microalgae Co. LTD., Hainan, China, before its sustained growth under the indoor conditions (50 μmol photons m^{-2} s^{-1}, 25°C) in 2004. Single filaments of each strain were



Figs 1–2. Daily solar doses of PAR, UV-A and UV-B and the daily mean water temperature over the periods of experiments with the two strains: 23 July to 8 August 2004 for the culture of the outdoor strain (D-0083) (Fig. 1) and 24 September to 12 October 2004 for the culture of the indoor strain (439) (Fig. 2).

isolated and cultured to obtain the clonal cultures. The two strains had been grown in sterile Zarrouk's medium (Zarrouk 1966) in a growth chamber at 30°C with a 12 : 12 h light : dark period, at the same PAR level of 100 μmol photons m^{-2} s^{-1} for 10 days before being used for the experiments. Either the indoor or outdoor cultures were aerated with filtered (0.22 μm) ambient air at a rate of 0.2 litres min^{-1}.

Solar radiation measurements and treatments

Solar radiation was continuously monitored using a broadband filter radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany) with three wavelength bands for UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm), respectively (Häder et al. 1999). The instrument, located on the roof at Shantou University (116°36′E, 23°18′N), monitors every second and automatically records the irradiances in each channel as averages over 1 min intervals.

Solar exposures were carried out from 23 July to 8 August 2004 (strain D-0083) and from 24 September to 12 October 2004 (strain 439) at the campus of Shantou University (116°36′E, 23°18′N). Subsamples of *A. platensis* were harvested during the exponential growth phase, resuspended in sterilized Zarrouk's medium and dispensed in UV-transparent quartz tubes (Pacific Quartz Co. LTD, Lianyungang, China) (inner diameter 5.4 cm, length 35 cm) before the exposures. The initial biomass density was set at OD_{560} 0.3 (*c.* 280 mg dry-weight l^{-1}) for each culture and every 2–3 days the cells were partially subcultured with fresh medium to re-establish the initial biomass density. The cultures were maintained in a flow-through water bath for temperature control (29–31°C). Three different radiation treatments were implemented: (1) samples receiving PAR, UV-A and UV-B (PAB treatment, 280–700 nm), uncovered quartz tubes; (2) samples receiving PAR plus UV-A (PA treatment, 320–700 nm), quartz tubes covered with Folex 320 (Montagefolie, No. 10155099; Folex, Dreieich, Germany); and (3) samples receiving PAR alone (PAR treatment, 395–700 nm), quartz tubes covered with Ultraphan film 395 (UV Opak; Digefra, Munich, Germany). The transmission spectra of the filters were reported elsewhere (Korbee Peinado *et al.* 2004).

Morphological examination

Morphological examinations were carried out with an inverted microscope (Olympus IX51, Olympus, Tokyo, Japan) immediately after sampling around sunset time (local time, 1800 h). For orientation determination, 300 filaments in 10–20 observation fields were examined and analyzed. A right-handed orientation was defined as a spiral around a central axis in a clockwise direction when seen from the viewer's side (Mühling *et al.* 2003). Digital images were recorded with a Canon digital camera (S50, Canon, Tokyo, Japan) and spiral length (L), helix pitch (P) and helix diameter (D) were determined randomly on the basis of the recorded images of 30–100 filaments. For helix pitch and diameter, only the trichomes that had more than two helices were measured.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to establish differences among the different radiation treatments and strains. A confidence level of 95% was used in all analyses.

RESULTS

Daily solar doses during the exposure period are showed in Figs 1–2. The mean of maximal irradiances of PAR, UV-A and UV-B were 505.4, 75.4 and 2.4 W m^{-2} during the period of 23 July–8 August 2004 and were 426.8, 65.5 and 2.1 W m^{-2} during the period of 24 September–12 October 2004, respectively. The daily mean water temperatures in the water bath were 31 ± 1°C for the former and were 29 ± 1°C for the later period, respectively (Figs 1, 2).

In the outdoor strain (D-0083), trichome length significantly decreased ($P < 0.05$) at day 3 either with or without

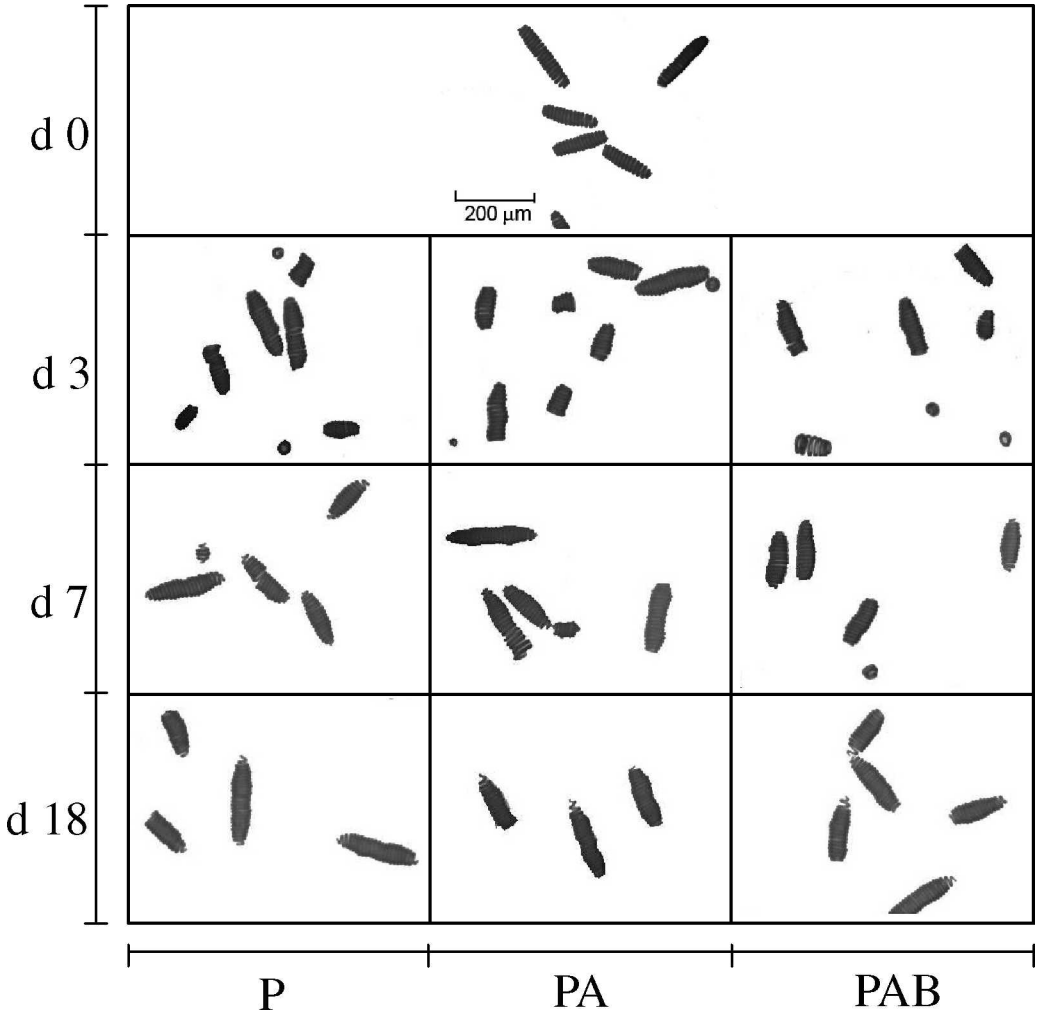


Fig. 3. Spirals of the outdoor strain (D-0083) of *A. platensis* while exposed to different solar radiation treatments (PAR, PA and PAB). Scale bar represents 200 μm.

UVR by about 20–25% (Figs 3, 5). The trichomes started to elongate after day 3 and increased to their original value at day 8 under all radiation treatments. At the end of the exposure (day 17), they were about 3–10% longer compared with the initial length, with the longest filaments found under PAR alone and the shortest under PAB (Fig. 5). The helix diameter significantly ($P < 0.05$) increased in 3 days (Fig. 6). At the end of the experiment (day 17), it increased by 24%, 29% and 23% under PAR, PA and PAB, respectively (Figs 3, 6). The helix pitch decreased significantly ($P < 0.05$) by 10–12% at the end of the cultures (day 17), and no significant changes were found among P, PA and PAB treatments (Figs 3, 7).

For the indoor-strain (439), the trichome length decreased by 18–27% at the end of the cultures (day 19), but no significant changes were found among PAR, PA and PAB treatments (Figs 4, 8). The helix diameter also decreased. At the end of the culture, it decreased by about 35% in both PA and PAB treatments and by 20% in PAR treatment (Fig. 9). The difference between the treatments with and without UVR was significant ($P < 0.05$) (Figs 4, 9). The helical pitch increased by 127.1% and 114.4% in 5 days in the presence of UVR or UVA, respectively. However, at the end of the culture, such a difference became insignificant compared with the original value (Figs 4, 10). We did not measure the growth rates; however, since the cultures were diluted by 50–80% of their volumes, biomass increase for each of the strains appeared to double in less than 2 days.

Both strains of *A. platensis* were configurated with a right-handed orientation under the indoor condition (Figs 3, 4, 11). Left-handed helix trichomes were found only in the outdoor strain (D-0083) from day 5 under PAB, from day 9 under PA and after 13 days under PAR alone (Table 1, Fig. 12). The percentage of the left-handed helix was less than 1% for all the radiation treatments at the end of the experiment. When a single left-handed filament was isolated and recultured under indoor conditions at 50 μmol photons $m^{-2}\,s^{-1}$ of PAR and 30°C, the propagated filaments were all right-handed when examined 30 days later (Fig. 13), indicating that the left-handed helix was reversed to its original configuration. However, no orientation change was observed in the indoor strain (439) when it was either cultured under solar radiation with or without UVR or regrown under indoor conditions.

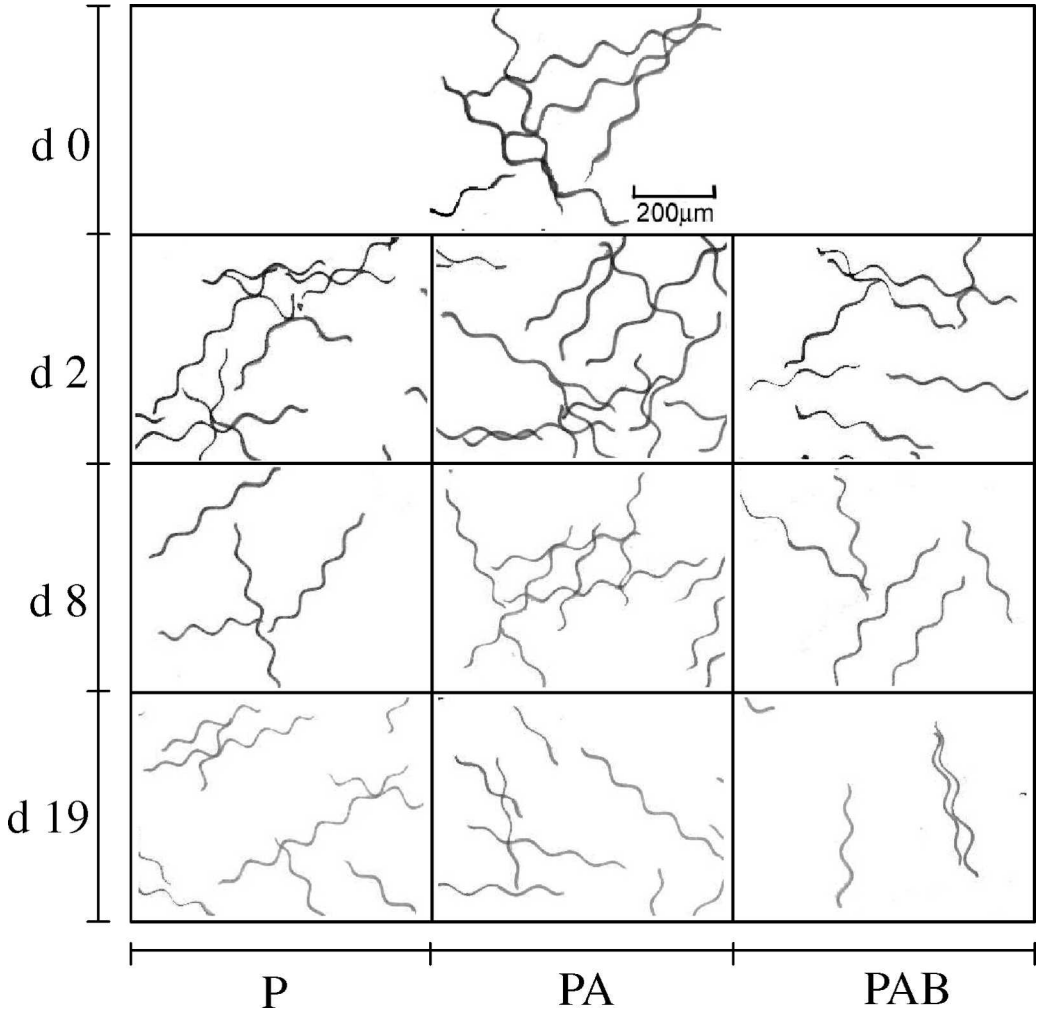


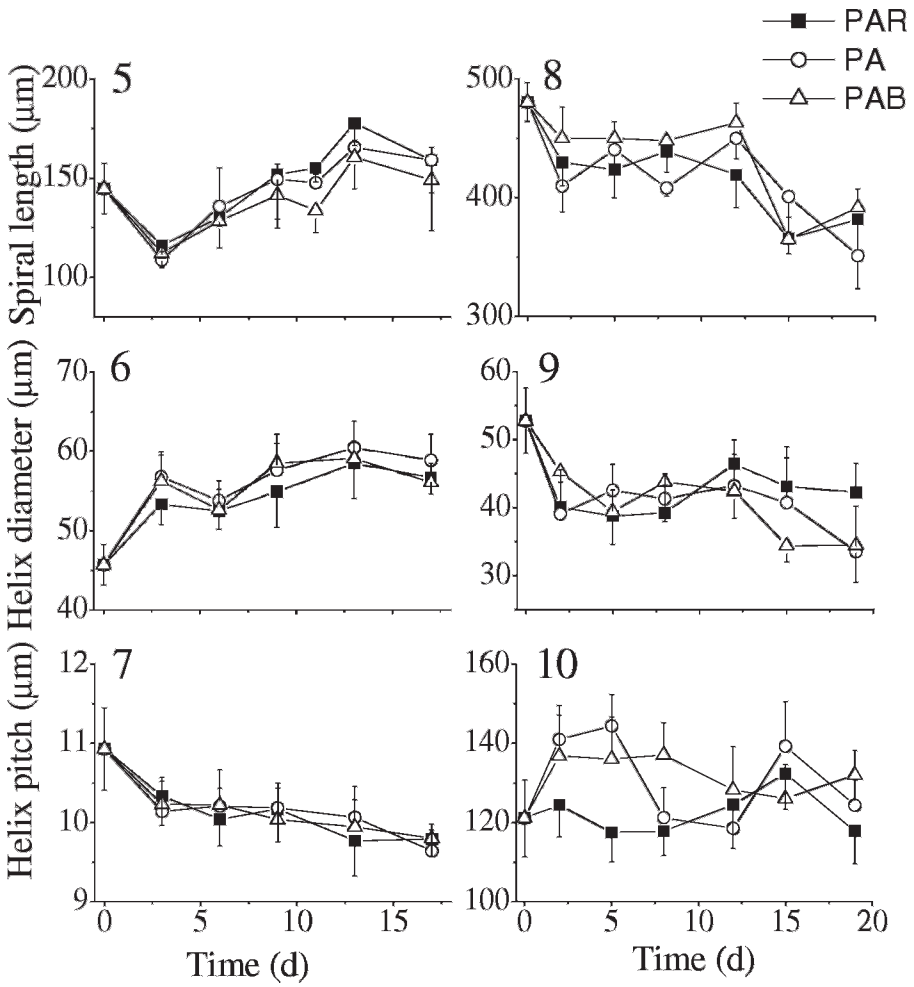
Fig. 4. Spirals of the indoor strain (439) of *A. platensis* while exposed to different solar radiation treatments (PAR, PA and PAB). Scale bar represents 200 µm.

DISCUSSION

The present study indicates that solar radiation can affect the morphology of the outdoor and indoor strains of *A. platensis* in a different way. Helix orientation was altered only in the outdoor strain during the exposures to solar PAR, and presence of UVR accelerated the orientation change.

Change in temperature can alter the PAR-induced as well UVR-induced effects on the morphology and physiology of *A. platensis*. Lower temperature in the morning induced higher photoinhibition under solar radiation, while heating up the culture to higher temperature levels raised its biomass production (Vonshak 1997). *Arthrospira platensis* filaments were broken when exposed to solar PAR + UVR at 18–20°C during the winter season (Wu *et al.* 2005). Under controlled conditions, it was shown that such UVR-related breakage only happened at lower levels of temperature (15°C and 20°C) but not at 30°C, at which DNA damage was also negligible (Gao *et al.* 2008). In the present study, presence of UVR did not result in significant difference in the spiral length when the cells were cultured during the summer season, with the temperature range 28–32°C (daytime temperature equal to its optimal level). We assume that *A. platensis* could efficiently counteract the damage caused by UVR since the repair mechanisms were temperature dependent (Roos & Vincent 1998; Pakker *et al.* 2000; Gao *et al.* 2008).

The outdoor-grown strain was configurated with compressed spirals, and these spirals became more compressed (reduced helical pitch) after being grown under solar radiation again (it had been grown under indoor conditions since 2004). Such a tightened configuration was considered as a protective strategy for *A. platensis* to cope with UVR or high PAR (Wu *et al.* 2005). However, the indoor-grown strain, which was configurated with loose spirals, showed more loosened structures after being grown under solar radiation even in the presence of UVR (Fig. 4). This differed from what was observed at about 20°C for the same strain by Wu *et al.* (2005), who showed broken but compressed spirals in the presence of UVR. Nevertheless, the two strains of *A. platensis* appeared to have distinct strategies to acclimate to high PAR or UVR at the high level of temperature in view of their morphological responses during the long-term cultures.

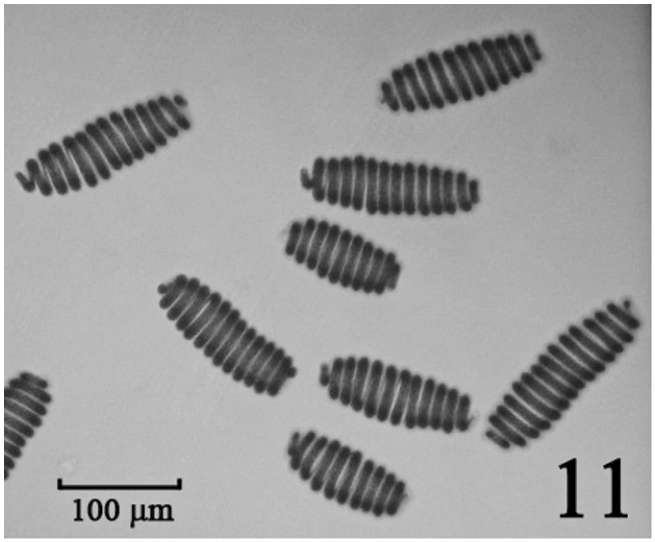


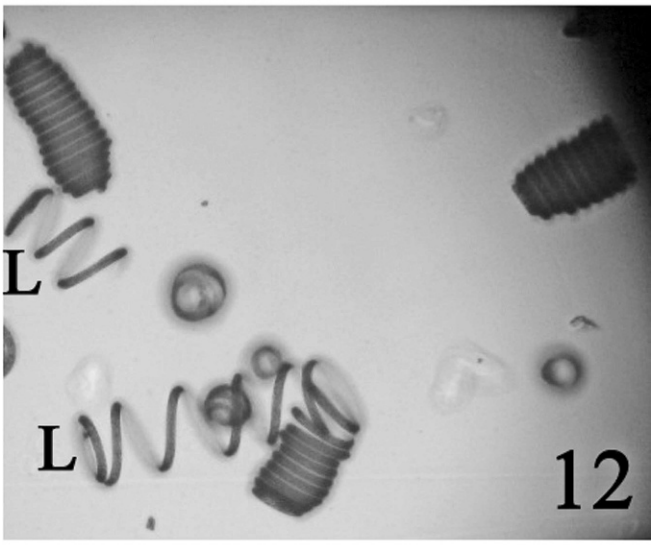
Figs 5–10. Morphological changes of the outdoor strain (Figs 5–7) and indoor strain (Figs 8–10) with time. The vertical bars indicate standard deviations around the means (n = 20–40).

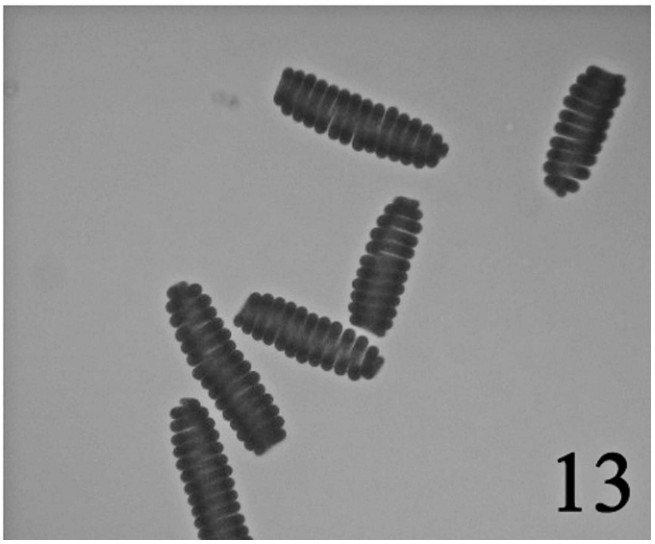
Fig. 5. Spiral length of outdoor strain (D-0083).
Fig. 6. Helix diameter of outdoor strain (D-0083).
Fig. 7. Helix pitch of outdoor strain (D-0083).
Fig. 8. Spiral length of indoor strain (439).
Fig. 9. Helix diameter of indoor strain (439).
Fig. 10. Helix pitch of indoor strain (439).

Cell wall structures of cyanobacteria are mainly composed of outer membrane proteins, lipopolysaccharide and peptidoglycan, which determines shapes (Atlas 1994; Nester *et al.* 2004). The orientation change observed in the outdoor strain could be due to changes in the structure or amount of newly synthesized peptidoglycan molecules in the cell wall layers, which could cause different cross-linking of individual layers or the degree of cross-linking. Presence of UVR might have accelerated such changes, resulting in reversed helix orientation. A ''right-handed-twist'' molecule (protein, polysaccharide or peptidoglycan monomer) was supposed necessary for *Arthrospira* spp. to maintain right-handed helix orientation (Mühling *et al.* 2003). Such a molecule might have been altered under solar radiation, resulting in left-handed orientation filaments, which occurred much faster in the presence of UVR. However, it was also possible that the morphology-controlling plasmids (if there are any) changed or were lost during the exposures. In the present study, orientation change caused by solar radiation was reversed when the spirals were regrown under the indoor condition. Therefore, it is much likelier that the structural change of some membrane molecules rather than genetic mutation is responsible for the alteration of spiral orientation.

Changes in morphological characteristics of *A. platensis* caused by UVR or high-PAR are usually temperature dependent. At reduced levels of temperature, the morphological changes, such as breakage of the spirals under the full spectrum of solar radiation, can be disadvantageous in terms of harvesting efficiency. On the other hand, compression of spirals or change in their orientation might play an important role in regulating the irradiance levels reaching the cells due to the shading provided by the spiral structure and hence, influence their photo-physiological performance. Since the morphology of *A. platensis* changes in response to PAR, UVR, temperature and possibly, availability of nutrients in the environment, attention needs to be paid to application of the morphological features as







Figs 11–13. Microscopic images of the outdoor-grown strain (D-0083) under PAB treatment. 'L' in the image indicates left-handed trichomes while the ones without an L are all right-handed filaments.
Fig. 11. Spiral structure on day 0.
Fig. 12. Spiral structure on day 10.
Fig. 13. Trichomes grown from single irradiance-induce left-handed filament (day 10).

Table 1. The frequency of left-handed helix trichomes in *A. platensis* D-0083 grown under different solar radiation treatments.

	No. of left-handed helix (every 300 trichomes)[1]			
Time (d)	Indoor	PAR	PA	PAB
1	0	0	0	0
3	0	0	0	0
5	0	0	0	1
7	0	0	0	1
9	0	0	1	1
11	0	0	3	1
13	0	1	1	1
15	0	1	0	3
17	0	3	2	2

[1] PAR, samples receiving photosynthetically active radiation alone (395–700 nm); PA, samples receiving PAR plus UV-A (320–700 nm); PAB, samples receiving PAR, UV-A and UV-B (280–700 nm).

taxonomical criteria, though the involved mechanisms for the observed changes are still uncertain.

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