Applied and Environmental Microbiology, Nov. 2011, p. 7533–7540 Vol. 77, No. 21
0099-2240/11/$12.00 doi:10.1128/AEM.05392-11

Microcolony Formation by Single-Cell *Synechococcus* Strains as a Fast Response to UV Radiation▽

Cristiana Callieri,* Andrea Lami, and Roberto Bertoni

CNR-Institute of Ecosystem Study, Largo Tonolli 50, 28922 Verbania-Pallanza, Italy

Received 6 May 2011/Accepted 22 August 2011

UV radiation (UVR) has different effects on prokaryotic cells, such as, for instance, filamentation and aggregation in bacteria. Here we studied the effect of UVR on microcolony formation in two freshwater *Synechococcus* strains of different ribotypes (group B and group I) and phycobiliprotein compositions (phycoerythrin [PE] and phycocyanin [PC]). Each strain was photoacclimated at two light intensities, low light (LL) (10 μmol m^{-2} s^{-1}) and moderate light (ML) (100 μmol m^{-2} s^{-1}). The cultures were exposed for 6 days to treatments with UVR or without UVR. PE-rich *Synechococcus* acclimated to LL had a low carotenoid/chlorophyll *a* (car/chl) ratio but responded faster to UVR treatment, producing the highest percentages of microcolonies and of cells in microcolonies. Conversely, the same strain acclimated to ML, with a higher car/chl ratio, did not aggregate significantly. These results suggest that microcolony formation by PE-rich *Synechococcus* is induced by UVR if carotenoid levels are low. PC-rich *Synechococcus* formed a very low percentage of microcolonies in both acclimations even with low car/chl ratio. The different responses of the two *Synechococcus* strains to UVR depend on their pigment compositions. On the other hand, this study does not exclude that UVR-induced microcolony formation could also be related to specific ribotypes.

Freshwater *Synechococcus* strains appear as single cells with different morphologies and as microcolonies (2). Microcolonies have been observed as a consistent part of the total picocyanobacterial (Pcy) community in early summer in Lake Mondsee (6), in late summer in Lake Superior (20), and in spring and late summer in Lake Maggiore (34). In particular, in Lake Maggiore their appearance was coincident with the presence of large colonial species: *Microcystis* spp. in spring and *Aphanothece* spp. in late summer (3). Furthermore, peaks of microcolonies have been observed in summer or autumn in a variety of freshwater system (27, 33, 38).

The richness of morphotypes in freshwater *Synechococcus* may reflect a genotypic diversity among Pcy communities that accounts for the different compositions observed in spring and summer assemblages, including the presence of microcolonies (4, 7). The tendency to form microcolonies has been observed in *Synechococcus* strains belonging to freshwater groups H and B of the 16S rRNA phylogenetic tree (8).

To better understand genus-specific microcolony formation, the structural changes of Pcy single cells as well as the external factors acting as stressors should be taken into account. Examples of structural changes which may induce microcolony formation are (i) the surface S-layer, composed of regularly ordered globular protein layers that would facilitate the sticking of daughter cells (11), and (ii) the rigid spinae, induced by grazers, observed on the surfaces of *Cyanobium* cells (21). Structural modification of cells is generally the result of external factors acting on them. One of the most effective factors for photoautotrophic cells is solar radiation, which has generally deleterious effects when UV radiation (UVR) (280 to 400 nm) accompanies high levels of photosynthetically active radiation (PAR) (400 to 700 nm) flux (17). There is evidence indicating that high PAR has an aggregation effect on single-cell *Synechococcus*, due to a triggering effect of reactive oxygen radicals (26). In addition, the excretion of photosynthate-rich mucilage has been indicated as the reason for microcolony formation from Pcy single cells in surface water (6), where both PAR and UVR can be stressing factors. Nevertheless, no experiments have been performed to understand if *Synechococcus* could form microcolonies under exposure to solar radiation.

The effect of UVR has been considered important in the induction of different protective mechanisms in many phytoplankton and in particular in cyanobacteria (14, 17). Among others, the production of carotenoids and mycosporine-like amino acids (MAAs) has been indicated as responsible for the resistance of *Microcystis aeruginosa* to high UVR (29). The colonial *Microcystis* can synthesize substances such as D-galacturonic acid, which is the main component of its slime layer (37) and which hence may provide a protective function. In addition, *Nostoc* has been observed to produce a glycan sheet that acts as a matrix for MAAs (10). On the other hand, the *Synechococcus* response to UVR has not been investigated in detail yet.

To better understand the role of UVR in microcolony formation by *Synechococcus*, we selected monoclonal strains isolated from lakes and belonging to phycoerythrin (PE)-rich cells (ribotype group B) and to phycocyanin (PC)-rich cells (ribotype group I), with different photoacclimation, and exposed them to treatments with UVR (+UVR) and without UVR (−UVR) for 6 days. The numbers of microcolonies, of single cells, and of cells in microcolonies were monitored to see if UVR could induce a morphological transformation of single-

* Corresponding author. Mailing address: CNR-Institute of Ecosystem Study, Largo Tonolli 50, 28922 Verbania-Pallanza, Italy. Phone: 39 0323 518320. Fax: 39 0323 556513. E-mail: c.callieri@ise.cnr.it.

▽ Published ahead of print on 2 September 2011.

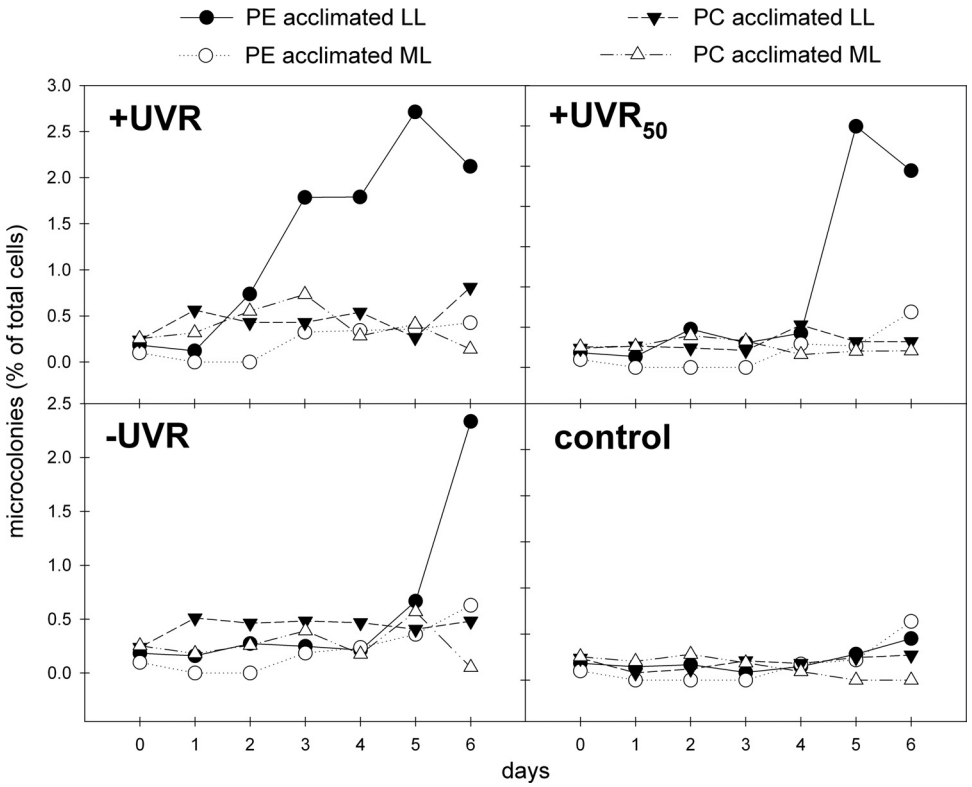

FIG. 1. Number of microcolonies as a percentage of total cell number (single cells plus cells in microcolonies). Results for PE- and PC-rich *Synechococcus* strains in the treatments (+UVR, $+UVR_{50}$, −UVR, and control) at two acclimations (LL, 10 μmol m^{-2} s^{-1}; ML, 100 μmol m^{-2} s^{-1}) during experiment times of 6 days are shown.

cell *Synechococcus* and if acclimation and photosynthetic pigment composition could alter the responses of the two different ribotypes.

MATERIALS AND METHODS

Strain selection. Monoclonal strains of *Synechococcus* were carefully selected in order to find those that did not clump as a secondary effect of culture growth. Aggregation is frequent in dense culture, and for this reason we used diluted culture without aggregates, gently sonicating the inoculum, and controlled its conditions before dilution in BG11 medium. We deliberately selected two *Synechococcus* strains with different spectral phenotypes: one with prevalent phycoerythrin (PE-rich *Synechococcus*) and the other with phycocyanin (PC-rich *Synechococcus*). These two *Synechococcus* types constitute a first classification of freshwater picocyanobacteria (4). The PE-rich strain belongs to the group B cluster (6), which includes strains able to form microcolonies. The PC-rich strain (kindly provided by N. Crosbie and T. Weisse) belongs to group I. The *Synechococcus* strains were maintained in BG11 medium, which is specific for freshwater strains, at 10 μmol m^{-2} s^{-1} with a 12-h/12-h light-dark cycle at 20°C. Under this condition of low light, both strains were present predominantly as single cells.

Acclimation. After strain selection, the diluted cultures were partly kept at the low light of maintenance (LL) (10 μmol m^{-2} s^{-1}) and partly photoacclimated to an higher irradiance, here considered moderate light (ML) (100 μmol m^{-2} s^{-1}), for 1 month in order to have many generations of acclimated cells. Thus, four strains, different in phylogenetic origin, photosynthetic pigment composition, and acclimation, were obtained. After acclimation, the cells were counted to prepare the inoculum to use in the experiments, starting at time zero (T_0) with a cell number not higher than 400×10^3 ml^{-1}, which is typical of summer peaks in oligotrophic lakes (3).

Experimental setup. Separate experiments using the four acclimated strains in turn were carried out in a walk-in chamber at 20 ± 1°C with a 12-h/12-h light-dark photoperiod and a PAR intensity of 100 μmol m^{-2} s^{-1} (cool white fluorescent tubes). The UVR source was provided by two UVR tubes (Q-Panel Lab Products, Cleveland, OH). The UV spectrum of these tubes closely resembles the solar spectrum (between 280 and 350 nm) but has low emission (compared to the solar spectrum) in the 350- to 400-nm range. The cultures were distributed in 12 150-ml quartz tubes, previously sterilized, that were placed in a slowly rotating cross supporting 12 tubes. The treatments were as follows: +UVR, exposed to 300 mW m^{-2} nm^{-1} (UVA, 340-nm band), 70 W m^{-2} nm^{-1} (UVB, 305-nm band), and 100 μmol m^{-2} s^{-1} PAR; $+UVR_{50}$, exposed to 50% of the +UVR treatment obtained using neutral screening, which lowered UVA to 150 mW m^{-2} nm^{-1}, UVB to 40 mW m^{-2} nm^{-1}, and PAR to 48 μmol m^{-2} s^{-1}; −UVR, exposed to 100 μmol m^{-2} s^{-1} PAR without UVR; and control, exposed to 10 μmol m^{-2} s^{-1} PAR without UVR. To eliminate UVR, a screen cutting the <380-nm light was used (E. Balseiro, personal communication). UVR exposure lasted for 4 h each day, centered in the 12 h of PAR exposure. We used two intensities of UVR which have been found to induce filamentation in freshwater bacteria (31). The highest UVR intensity used was equivalent to surface noon summer sunlight in mountain lakes, thus reflecting natural conditions (5).

The experiments lasted 6 days, and samples (10 ml) for determining *Synechococcus* abundance and morphology (single cells and microcolonies) were collected at the start of the experiment (T_0) and after 24 h of incubation for each day of the experiment. At each sampling time, a volume of 10 ml was replaced with sterile BG11 medium. At T_0 and at the end of the experiment, chlorophyll and carotenoid contents were measured.

Single-cell and microcolony counting. Samples from each treatment and day were fixed with 20% formaldehyde (0.2-μm filtered) buffered with 0.1 M sodium cacodylate (final concentration of 2%, vol/vol), stored in darkness at 4°C, and processed the day after. The replicates were counted on polycarbonate filters (Poretics; 0.2-μm pore size) by autofluorescence of phycoerythrin or phycocyanin (epifluorescence microscopy) (filter sets for blue light excitation, BP450-490 and FT510; filter sets for green light excitation, LP510-KP560, FT580, and LP590). For the total count, a minimum of 200 cells were counted in at least 10 fields at a magnification of ×1,250. For the numbers of microcolonies and of cells in microcolonies, a minimum of 50 fields were inspected at a magnification of ×787.5.

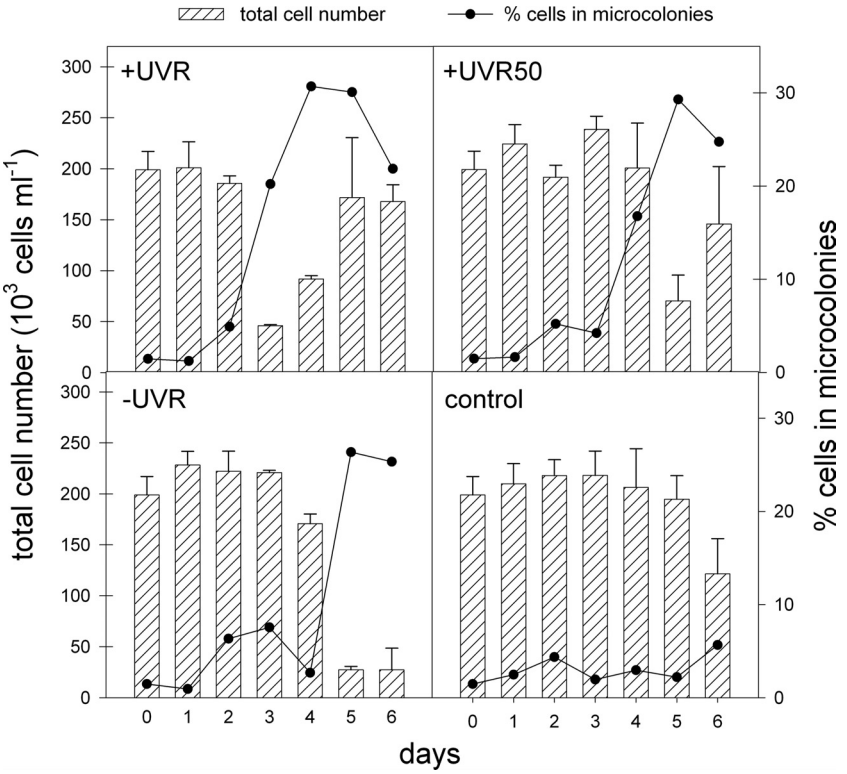

FIG. 2. Number of total cells and percentage of cells in microcolonies with respect to total cell number in PE-rich *Synechococcus* cultures acclimated to LL (10 μmol m^{-2} s^{-1}) with +UVR, +UVR_{50}, −UVR, and control treatments (see Materials and Methods).

Photosynthetic pigment analyses. Carotenoid and chlorophyll contents were measured with a high-pressure liquid chromatography (HPLC) system (Summit, Dionex) that consisted of a gradient pumping system and a dual-channel variable-wavelength UV-DAD detector (set at 460 nm and 665 nm for carotenoids and chlorophyll, respectively) controlled by a computer. The column used for the separation of the carotenoid and chlorophyll was a reverse-phase C_{18} octyldecyl silane (ODS) column (5-μm particle size; 250 mm by 4.6 mm [inner diameter]). The separation was obtained with a solvent gradient by the method described by Lami et al. (28).

Calculations and statistical analyses. The number of microcolonies was expressed as a percentage relative to the total number of cells (single cells plus cells in microcolonies) to compare 4 experiments in which the total cell numbers were different. The microcolonies were considered one morphotype of *Synechococcus* likely derived from one single cell.

To test for the significant differences between treatments in each experiment, one-way repeated-measures (RM) analysis of variance (ANOVA) tests were applied. The data were normally distributed and passed both the normality and the equal-variance tests. The *post hoc* multiple comparison (Holm-Sidak method) was used to separate the effects of +UVR and −UVR treatments on microcolony formation versus control. One-way ANOVA and the *t* test were used for comparing carotenoid contents in the different treatments at the initial and final times.

RESULTS

Microcolony formation in PE- and PC-rich *Synechococcus* cultures. In PE-rich *Synechococcus* cultures acclimated to LL, the microcolonies begun to appear from the third day in the +UVR treatment, reaching 1.8% of total cells (Fig. 1). In the same treatment the highest percentage was attained on the fifth day (2.7%). When UVR was halved (+UVR_{50}), microcolony formation was delayed and begun to appear on the fifth day. When UVR was excluded (−UVR), microcolonies were present at the end of the experiment and were almost absent in the control, irrespective of the acclimation used (Fig. 1). RM ANOVA applied to PE-rich *Synechococcus* cultures acclimated to LL confirmed the differences among treatments to be statistically significant ($P = 0.024$). The multiple comparison showed the +UVR treatment as the only one with results statistically different from the control ($P = 0.004$), suggesting an important role of UVR in microcolony formation. PE-rich *Synechococcus* cultures acclimated to ML produced a low percentage (<0.5%) of microcolonies in the +UVR treatment (Fig. 1). Furthermore, the difference between the treatments was not statistically significant (RM ANOVA, $P = 0.560$).

In PC-rich *Synechococcus* cultures there was an increase in the percentage of microcolonies in the first day of +UVR treatment, particularly in the LL-acclimated culture (Fig. 1). Even though the percentage was low, there was a significant difference among the treatments for the differently acclimated cultures (RM ANOVA, $P = 0.001$ and $P = 0.012$ for LL and ML, respectively). The multiple comparison showed that the PC-rich *Synechococcus* cultures acclimated to ML respond to the +UVR treatment, forming a low number of microcolonies, but the value was significantly different from the control ($P < 0.001$). In PC-rich *Synechococcus* cultures acclimated to LL, the +UVR and −UVR treatments were both significantly different from the control ($P < 0.001$ for both), indicating a response of this culture to form microcolonies also without UVR.

Solitary cells and cells in microcolonies in PE- and PC-rich *Synechococcus* cultures. The total cell numbers (solitary cells

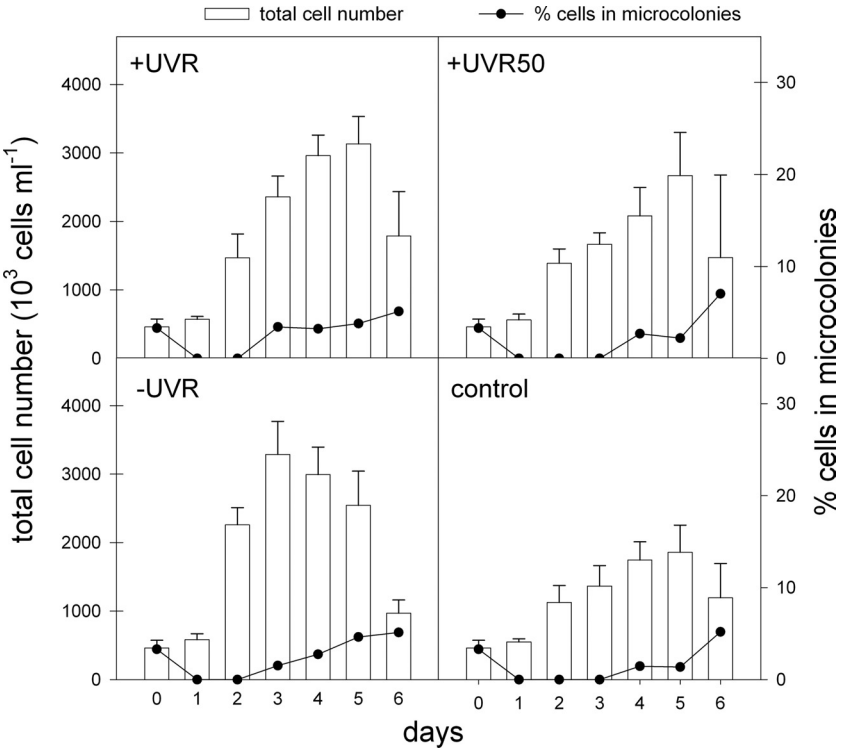

FIG. 3. Number of total cells and percentage of cells in microcolonies with respect to total cell number in PE-rich *Synechococcus* cultures acclimated to ML (100 μmol m^{-2} s^{-1}) with +UVR, +UVR_{50}, −UVR, and control treatments (see Materials and Methods).

and cells in microcolonies) of PE-rich *Synechococcus* acclimated to LL in the +UVR treatment remained constant during the first 2 days, abruptly decreased on the third day, and then increased again (Fig. 2). The percentage of cells in microcolonies increased particularly from the third day and reached 30% of cell population on the fifth day in the +UVR treatment. In this treatment the microcolonies were composed of a mean of 11 ± 5 cells. In the +UVR_{50} treatment the microcolonies were formed later than those in the +UVR treatment. RM ANOVA showed a significant effect of treatments ($P = 0.032$). In particular, the multiple comparison showed that the +UVR and +UVR_{50} treatments were significantly different from the control ($P = 0.006$ and $P = 0.022$, respectively). The −UVR treatment was not significantly different from the control.

The total cell numbers of PE-rich *Synechococcus* acclimated to ML showed a pattern different from that for cells acclimated to LL (Fig. 3). They increased gradually, reaching a high number on the fifth day, particularly in +UVR treatments, indicating the absence of a detrimental effect of +UVR. The percentages of cells in microcolonies were low in all the treatments and composed of a mean of 10 ± 3 cells. In general, the treatments had no significant effect on the formation of cells in microcolonies (RM ANOVA, $P = 0.629$) in PE-rich *Synechococcus* acclimated to ML.

The total number of PC-rich *Synechococcus* cells acclimated to LL and ML increased relative to the control from the fifth day in the +UVR and −UVR treatments (Fig. 4 and 5), indicating a growth-enhancing effect of irradiation. The percentage of cells in microcolonies was low (maximum value of 8.5%) but was significantly different from the control, resembling the results for the percentage of microcolonies. In the +UVR treatment the maximum numbers of cells per microcolony were 9 ± 2 and 11 ± 2 for PC-rich *Synechococcus* cells acclimated to LL and ML, respectively.

Photosynthetic pigments. HPLC analysis was focused to measure the composition and abundance of the photosynthetic pigments. The major photosynthetic pigments were chlorophyll *a* and zeaxanthin; other significant carotenoids identified were beta-carotene, echinenone, alpha-carotene, and a few other undetermined carotenoids were also present on the HPLC chromatogram. This photosynthetic assemblage is typical of cyanobacterial taxa (15, 18), and no significant difference was detected between the two *Synechococcus* strains. In none of the samples analyzed was a significant presence of degradation products of chlorophyll detected; this indicates that the cultures were in a good physiological status. At time zero the carotenoid/chlorophyll *a* (car/chl) ratio (Table 1) was significantly higher in the strains (both PE- and PC-rich) acclimated to ML than in those acclimated to LL (*t* test for PE, $P < 0.001$; *t* test for PC, $P < 0.002$); furthermore, when the strains with the same acclimation were compared, the car/chl ratio for PE was higher than that for PC only with ML acclimation (*t* test, $P < 0.001$).

At the final time in PE- and PC-rich *Synechococcus* cells acclimated to LL, the car/chl ratio remained low (Fig. 6), with values comparable to those measured at T_0 and showing no significant difference among treatments (PE, $P = 0.092$; PC, $P = 0.452$ [one-way ANOVA]). In PE-rich *Synechococcus* cultures acclimated to ML, the car/chl ratio was significantly dif-

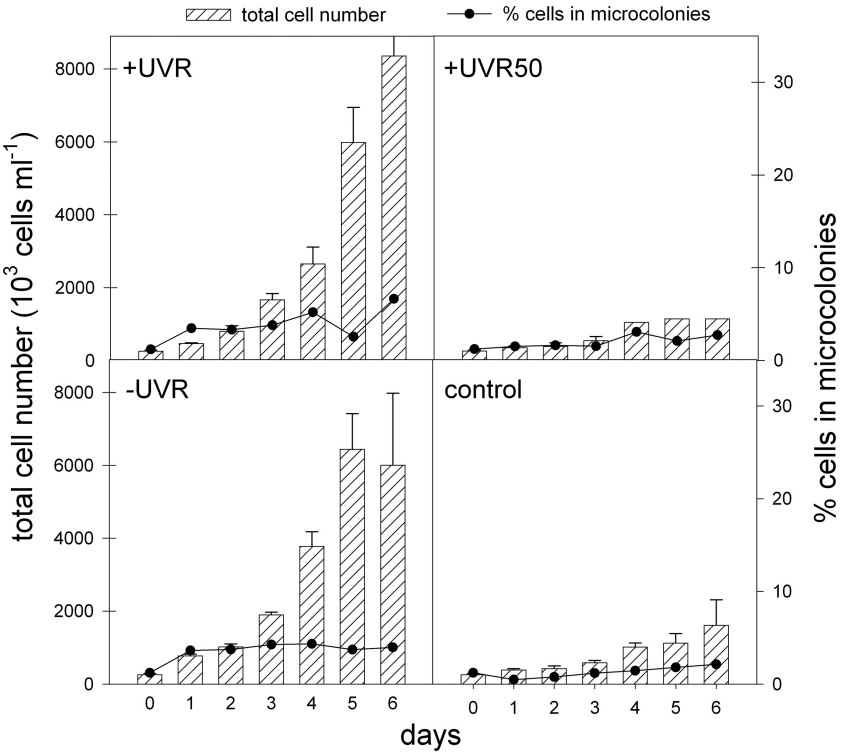

FIG. 4. Number of total cells and percentage of cells in microcolonies with respect to total cell number in PC-rich *Synechococcus* cultures acclimated to LL (10 μmol m^{-2} s^{-1}) with +UVR, +UVR_{50}, −UVR, and control treatments (see Materials and Methods).

ferent among treatments (one-way ANOVA, $P = 0.003$), and the multiple comparison versus the control showed a significant increase of the car/chl ratio in the +UVR and −UVR treatments ($P = 0.001$ and $P = 0.023$, respectively). In PC-rich *Synechococcus* acclimated to ML, the car/chl ratio did not show any significant difference among treatments (one-way ANOVA, $P = 0.343$).

DISCUSSION

Acclimation to LL and ML. Acclimation to different light intensities resulted an important factor strongly affecting the growth patterns of single-cell PE-rich *Synechococcus* exposed to the +UVR and −UVR treatments. In contrast, the acclimation did not greatly influence the response of PC-rich *Synechococcus*. From the results of previous experiments (32), we used as "high" PAR the intensity of 100 μmol m^{-2} s^{-1}. Although this is lower than the light intensity present on the surfaces of many lakes, it was effective in promoting a response in the cultures. Indeed, during the acclimation to ML, all *Synechococcus* cells were able to increase the total carotenoids, and in particular the xanthophyll zeaxanthin. This pigment has been identified as a major carotenoid involved in photoprotection (9, 12). In cyanobacteria, carotenoids exert their protective function as antioxidants to inactivate UVB-induced radicals in the photosynthetic membrane (16). In prokaryotes, the xanthophyll cycle is absent so that zeaxanthin is slowly formed from beta-carotene (19). In *Microcystis*, zeaxanthin is known to be involved in the dissipation mechanism to eliminate energy excess from the antenna (19), giving *Microcystis* the ability to survive near the lake surface during blooms. Also, in different species of *Anabaena*, zeaxanthin accumulated slowly during longer periods of high light exposure, for energy dissipation (36). In marine *Synechococcus*, the cellular content of zeaxanthin was found to be relatively constant at different light irradiances (23, 30). Conversely, in our experiments, the PE-rich *Synechococcus* culture at 1 month of acclimation to ML had increased the car/chl ratio from 0.8 to 2.6 (mainly due to an increase in zeaxanthin); this condition of pigment content was helpful to overcome the detrimental effects of the +UVR treatment on growth. Similarly, other studies revealed that a carotenoid increase had a protective effect against UVB radiation in *Synechococcus* strain PCC 7942 (16). In our study, the *Synechococcus* strain without phycoerythrin, although exhibiting a lower car/chl ratio, was not negatively affected by the +UVR treatment. The different behaviors of the two *Synechococcus* strains in response to acclimation and +UVR treatment may be linked to the phycobilisome structure and composition of phycobilins (PE or PC). Indeed, it is known that in cyanobacteria much of the solar radiation is absorbed by phycobilisomes; therefore, their regulation could be regarded as another protective mechanism. Jiang and Qiu (22) found that *Microcystis aeruginosa* 854 adapted different strategies to cope with UVB stress: synthesis of carotenoids and degradation of phycocyanin and allophycocyanin to avoid damage to DNA and to reaction centers. The different compositions of phycobilins of the two *Synechococcus* strains used in our experiments may explain their different responses to acclimation and to UVR irradiation.

Wilson et al. (39) demonstrated, in *Synechocystis* strain PCC

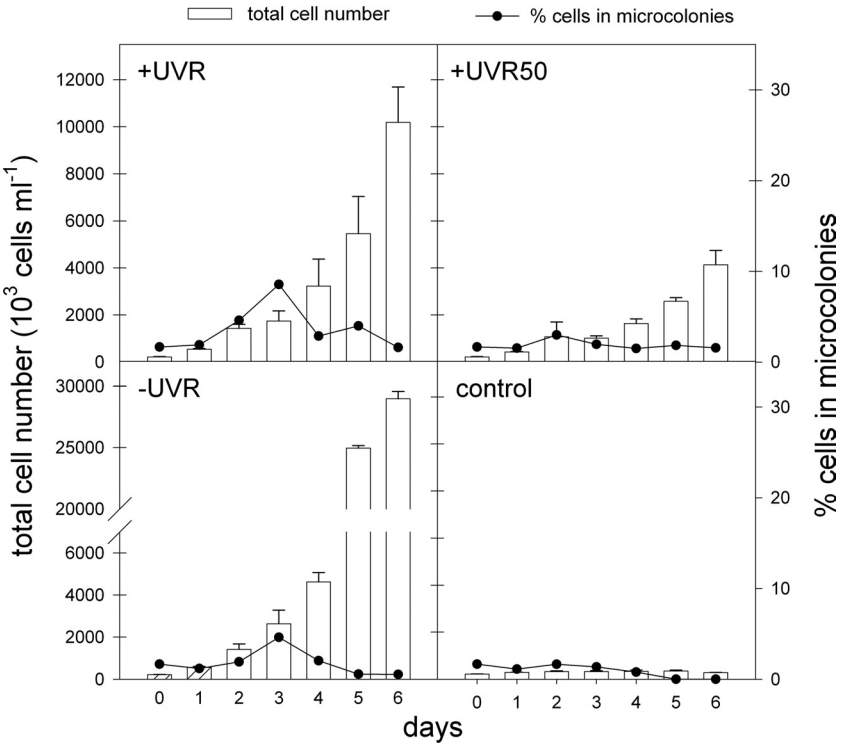

FIG. 5. Number of total cells and percentage of cells in microcolonies with respect to total cell number in PC-rich *Synechococcus* cultures acclimated to ML (100 μmol m^{-2} s^{-1}) with +UVR, $+UVR_{50}$, −UVR, and control treatments (see Materials and Methods).

6803, a photoprotective mechanism related to phycobilisome involving a soluble carotenoid binding protein (OCP) encoded by the slr1963 gene. At present we have no data on this gene in our strains, but future work could be addressed to investigate the presence of this gene in PC group I and whether it gives higher UVR protection.

Microcolony formation and its role in protection against UVR. The formation of microcolonies by *Synechococcus* as induced by UVR has been observed in this study for the first time. The two cultures of different ribotypes used in the experiments differed in their responses to +UVR and −UVR treatments. PE-rich *Synechococcus* cells of group B formed microcolonies only if they were acclimated to LL, having a low car/chl ratio. This low content of protective pigments together

TABLE 1. Carotenoid/chlorophyll *a* ratios for the two cultures (PE-rich *Synechococcus* group B and PC-rich *Synechococcus* group I) after acclimation

Acclimation	car/chl ratio[a] in:			
	PE-rich *Synechococcus*		PC-rich *Synechococcus*	
	Mean	SD	Mean	SD
LL (10 μmol m^{-2} s^{-1})	0.85	0.03	0.62	0.05
ML (100 μmol m^{-2} s^{-1})	2.58	0.09	0.75	0.03

[a] PE-rich *Synechococcus* acclimated to ML had a higher car/chl ratio than PE-rich *Synechococcus* acclimated to LL (*t* test, $P < 0.001$), PC-rich *Synechococcus* acclimated to ML had a higher car/chl ratio than PC-rich *Synechococcus* acclimated to LL (*t* test, $P < 0.002$), and PE-rich *Synechococcus* acclimated to ML had a higher car/chl ratio than PC-rich *Synechococcus* acclimated to ML (*t* test, $P < 0.001$).

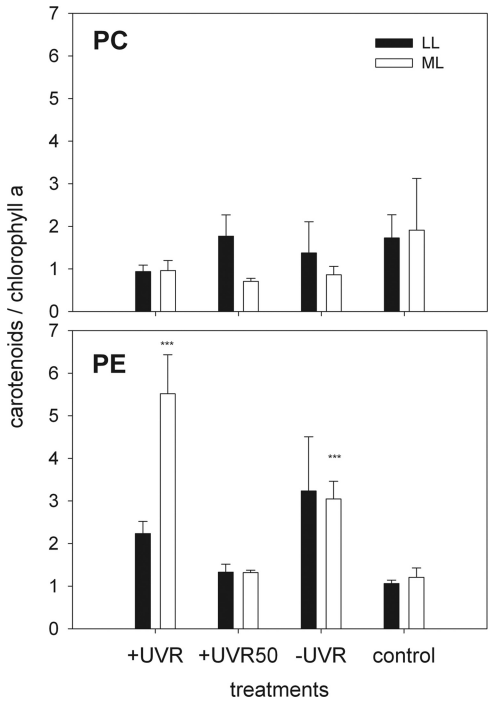

FIG. 6. Carotenoid/chlorophyll *a* (car/chl) ratios in PC-rich and PE-rich *Synechococcus* cultures acclimated to LL (10 μmol m^{-2} s^{-1}) and ML (100 μmol m^{-2} s^{-1}) in the +UVR, $+UVR_{50}$, −UVR, and control treatments, measured at the end of the experiment. The results of one-way ANOVA are described in the text. Significant differences between the treatment and control groups are indicated (***).

with the observation of a culture recovery parallel to the increase of microcolonies leads us to consider the microcolony formation a further adaptive strategy for a single-cell population to survive under UVR. The increase of microcolonies was parallel to culture recovery. In addition, the number of cells in the morphotype "microcolony" increased beginning from the fourth day in the +UVR treatment of PE-rich *Synechococcus* acclimated to LL. The same PE-rich *Synechococcus* strain acclimated to ML showed an increase of growth followed by a sharp decrease at the sixth day, without any significant formation of microcolonies in all treatments. The high car/chl ratio (due mainly to high zeaxanthin) made the culture able to dissipate the excess energy from the phycobilisome, as in all the photosynthetic prokaryotes (39). In this case a significant formation of microcolonies would not be advantageous for the survival of the population, also considering that the increased self-shading in the microcolony will decrease the photosynthetic performance under low-light conditions.

To explain the presence of *Synechococcus* microcolonies in lakes, two hypotheses, related to solar radiation effect, have been proposed (reviewed in reference 3). The first was that mucilage production could facilitate clump formation (24), particularly during nutrient limitation, suggesting a possible strategy for more efficient nutrient recycling (25). This interpretation fails to explain how diffusive uptake could advantage cells located in the center of the microcolony (6, 35). The other idea was that at near-surface depths, in a nutrient-depleted environment with high solar radiation, the large amount of photosynthate excreted could favor microcolony formation and give the cells a refuge from photochemical damage (6). Our experiments under controlled conditions with single-cell *Synechococcus* strains investigated more deeply the effect of solar radiation, in particular that of UVR, to verify the formation of microcolonies under different acclimation conditions. It is likely that the first reaction of unprotected cells to UVR is the induction of photoprotective metabolism with excretion of mucilage and formation of microcolonies. On the other hand, the presence of photoprotective pigments such as carotenoids inactivates UVR-induced radicals and constitutes an effective protection *per se*, so that the mucilage production would not add any advantage.

Not all the *Synechococcus* strains tested formed microcolonies. There was a significant effect of the +UVR treatment on LL-acclimated cells of PE-rich group B and of PC-rich group I. Nevertheless the PE-rich strain produced many more microcolonies than the PC-rich strain, and the effect of microcolony formation as a photoprotective strategy was evident only in PE-rich cultures (LL acclimated), in which, from the fourth day, the single-cell culture begun to recover (Fig. 2). In evaluating the response of the strains, we must consider that we exposed them for 4 h to moderate UVR, thus giving the cells time to recover. We simulated as much as we could the cell irradiation in natural systems, using a moderate UVR exposure each day and allowing time to recover to follow the growth pattern for 6 days. In other studies, higher UVR doses (in general UVB) have been used to study short-term responses in inducing screening compound production (13) or testing the survival of cyanobacterial cells (1). Instead, we used the same moderate UVR exposure that we had already successfully applied in studying the filamentation effect of UVR on bacteria (5).

The response of PC-rich *Synechococcus* group I acclimated to LL appeared to be much like the response to any irradiation higher than during acclimation. Indeed, the statistical tests showed a significant difference from the control for the +UVR and −UVR treatments, leaving some doubts about the interpretation of the results of UVR effects on this strain. It must be emphasized that PC-rich *Synechococcus* group I formed microcolonies in both acclimations in very low numbers and with few cells per microcolony, so that in spite of the significant difference from the control, the strategy of microcolony formation does not appear to prevail in this strain.

In conclusion, in this study we demonstrated for the first time the effect of UVR to foster microcolony formation in two strains of freshwater single-cell *Synechococcus*. The slow acclimation to ML increases the car/chl ratio. This change can be considered a slow defense strategy against UVR. Conversely microcolony formation appears as a fast defense strategy of the LL-acclimated culture. However, this study does not exclude the possibility that the prevalence of one or the other strategy for UVR protection, for the two *Synechococcus* strains used in the experiments, could be influenced by phylogenetic clade membership.

ACKNOWLEDGMENTS

We thank N. Crosbie and T. Weisse for providing the cultures.

REFERENCES

1. **Aráoz, R., and D. P. Häder.** 1997. Ultraviolet radiation induces both degradation and synthesis of phycobilisomes in *Nostoc* sp.: a spectroscopic and biochemical approach. FEMS Microbiol. Ecol. **23:**301–313.
2. **Callieri, C.** 2008. Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. Freshwater Rev. **1:**1–28.
3. **Callieri, C.** 2010. Single cells and microcolonies of freshwater picocyanobacteria: a common ecology. J. Limnol. **69:**257–277.
4. **Callieri, C., et al.** 2007. Photosynthetic characteristics and diversity of freshwater *Synechococcus* at two depths during different mixing conditions in a deep oligotrophic lake. J. Limnol. **66:**81–89.
5. **Corno, G., et al.** 2009. Bacterial diversity and morphology in deep ultra-oligotrophic Andean lakes: role of UVR on vertical distribution. Limnol. Oceanogr. **54:**1098–1112.
6. **Crosbie, N. D., K. Teubner, and T. Weisse.** 2003. Flow-cytometric mapping provides novel insights into the seasonal and vertical distributions of freshwater autotrophic picoplankton. Aquat. Microb. Ecol. **33:**53–66.
7. **Crosbie, N. D., M. Pöckl, and T. Weisse.** 2003. Rapid establishment of clonal isolates of freshwater autotrophic picoplankton by single-cell and single-colony sorting. J. Microbiol. Methods **55:**361–370.
8. **Crosbie, N. D., M. Pöckl, and T. Weisse.** 2003. Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and cpcBA-intergenic spacer sequence analyses. Appl. Environ. Microbiol. **69:** 5716–5721.
9. **Demmig-Adams, B.** 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll cycle. Biochim. Biophys. Acta **1020:**1–24.
10. **Ehling-Schulz, M., W. Bilger, and S. Scherer.** 1997. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. J. Bacteriol. **179:**1940–1945.
11. **Ernst, A., C. Postius, and P. Böger.** 1996. Glycosylated surface proteins reflect genetic diversity among *Synechococcus* spp. of Lake Constance. Arch. Hydrobiol. **48:**1–6.
12. **Falkowski, P. G., and J. A. Raven.** 1997. Aquatic photosynthesis. Blackwell Science, Malden, MA.
13. **Fleming, E. D., and R. W. Castenholz.** 2007. Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. Environ. Microbiol. **9:**1448–1455.
14. **Garcia-Pichel, F., and R. W. Castenholz.** 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. **59:**163–169.
15. **Goodwin, T. W.** 1980. The biochemistry of the carotenoids, vol. 1. Plants. Chapman and Hall, New York, NY.
16. **Götz, T., U. Windhövel, P. Böger, and G. Sandmann.** 1999. Protection of

photosynthesis against ultraviolet-B radiation by carotenoids in transformants of the cyanobacterium *Synechococcus* PCC 7942. Plant Physiol. **120:** 599–604.
17. **Harrison, J. W., and R. E. H. Smith.** 2009. Effects of ultraviolet radiation on the productivity and composition of freshwater phytoplankton communities. Photochem. Photobiol. Sci. **8:**1218–1232.
18. **Hirschberg, J., and D. Chamovitz.** 1994. Carotenoids in cyanobacteria, p. 559–579. *In* D. A. Bryant (ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
19. **Ibelings, B. W., B. M. A. Kroon, and L. R. Mur.** 1994. Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes. New Phytol. **128:**407–424.
20. **Ivanikova, N. V., L. C. Popels, R. M. L. McKay, and G. S. Bullerjahn.** 2007. Lake Superior supports novel clusters of cyanobacterial picoplankton. Appl. Environ. Microbiol. **73:**4055–4065.
21. **Jezberová, J., and J. Komárková.** 2007. Morphological transformation in a freshwater *Cyanobium* sp. induced by grazers. Environ. Microbiol. **9:**1858–1862.
22. **Jiang, H., and B. Qiu.** 2005. Photosynthetic adaptation of a bloom-forming cyanobacterium *Microcyctis aeruginosa* (Cyanophyceae) to prolonged UV-B exposure. J. Phycol. **41:**983–992.
23. **Kana, T. M., P. M. Glibert, R. Goericke, and N. A. Welschmeyer.** 1988. Zeaxanthin and beta-carotene in *Synechococcus* WH7803 respond differently to irradiance. Limnol. Oceanogr. **33:**1623–1627.
24. **Kiørboe, T., and J. Hansen.** 1993. Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. J. Plankton Res. **15:**993–1018.
25. **Klut, E. M., and J. G. Stockner.** 1991. Picoplankton associations in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. **48:**1092–1099.
26. **Koblížek, M., J. Komenda, J. Masojídek, and L. Pechar.** 2000. Cell aggregation of the cyanobacterium *Synechococcus elongatus*: role of the electron transport chain. J. Phycol. **36:**662–668.
27. **Komárková, J.** 2002. Cyanobacterial picoplankton and its colonial formations in two eutrophic canyon reservoirs (Czech Republic). Arch. Hydrobiol. **154:**605–623.
28. **Lami, A., et al.** 2009. Sedimentary pigments in 308 alpine lakes and their relation to environmental gradients. Adv. Limnol. **62:**217–238.
29. **Liu, Z., D. P. Häder, and R. Sommaruga.** 2004. Occurrence of mycocporine-like aminoacids (MAAs) in the bloom-forming cyanobacterium *Microcystis aeruginosa*. J. Plankton Res. **26:**963–966.
30. **MacIntyre, H. L., T. M. Kana, T. Anning, and R. J. Geider.** 2002. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J. Phycol. **38:**17–38.
31. **Modenutti, B., et al.** 2010. Ultraviolet radiation induce filamentation in bacterial assemblages from North Andean Patagonian lakes. Photochem. Photobiol. **86:**871–881.
32. **Moser, M., C. Callieri, and T. Weisse.** 2009. Photosynthetic and growth response of freshwater picocyanobacteria are strain-specific and sensitive to photoacclimation. J. Plankton Res. **31:**349–357.
33. **Mózes, A., M. Présing, and L. Vörös.** 2006. Seasonal dynamics of picocyanobacteria and picoeukaryotes in a large shallow lake (Lake Balaton, Hungary). Int. Rev. Gesamt. Hydrobiol. **91:**38–50.
34. **Passoni, S., and C. Callieri.** 2000. Picocyanobacteria single forms, aggregates and microcolonies: survival strategy or species succession? Verhandlung. Int. Vereining. Limnol. **27:**1879–1883.
35. **Ploug, H., W. Stolte, and B. B. Jørgensen.** 1999. Diffusive boundary layers of the colony-forming plankton alga *Phaeocystis* sp.—implications for nutrient uptake and cellular growth. Limnol. Oceanogr. **44:**1959–1967.
36. **Schagerl, M., and B. Müller.** 2006. Acclimation of chlorophyll a and carotenoid levels to different irradiances in four freshwater cyanobacteria. J. Plant Physiol. **163:**709–716.
37. **Sommaruga, R., Y. Chen, and Z. Liu.** 2009. Multiple strategies of bloom-forming *Microcystis* to minimize damage by solar ultraviolet radiation in surface waters. Microb. Ecol. **57:**667–674.
38. **Szelag-Wasielewska, E.** 2003. Phytoplankton community structure in non-stratified lakes of Pomerania (NW Poland). Hydrobiologia **506–509:**229–236.
39. **Wilson, A., et al.** 2006. A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. Plant Cell **18:**992–1007.