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The long-term effects of UV exclusion on the microbial composition and photosynthetic competence of bacteria in hot-spring microbial mats

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Abstract

The primary objective of this study was to determine whether the long-term exclusion of ultraviolet (UV) radiation (UVR) from hot-spring microbial mats resulted in an alteration of microbial composition, such as a shift to more UV-sensitive species. Over a 1–3-month period, microbial mats in two alkaline geothermal streams in Yellowstone National Park were covered with filters that excluded or transmitted UVR. Over some, 25% transmission neutral density screens were also used. In the 40–47°C range, there were no apparent changes in community composition during the summer with or without high or low UVR, as assessed by denaturing gradient gel electrophoresis (DGGE) profiles after polymerase chain reaction amplification of 16S-rRNA genes with general Bacteria and Cyanobacteria primers. Major bands were purified from the DGGE gels and sequenced. Only one of the cyanobacterial sequences matched known strains in the database; the others appear to be unique. Although the bacterial composition of these communities was apparently stable, surface layers of cyanobacteria protected from UVR were not as competent photosynthetically as those that had been maintained under UVR. This decrease in competence was expressed as a loss of the ability to perform at a maximum rate under full UVR plus visible irradiance. However, even +UV-maintained cyanobacteria performed better when UVR was excluded during the photosynthesis tests. It is probable that the large differences in photosynthetic competence observed reflect changes at the level of gene expression in the dominant species rather than changes in species composition.

Keywords: Ultraviolet radiation; Hot spring; Microbial mat; Cyanobacteria; Photosynthesis; Denaturing gradient gel electrophoresis

1. Introduction

Although the prevalence of perennial cyanobacterial mats today is mainly relegated to extreme environments in which grazers are absent (e.g. hot springs, hypersaline waters, frequently desiccated terrestrial habitats), a universal stress present or potentially present in all of these habitats is the inhibitory, and sometimes community-altering, stress of intense solar radiation, particularly the ultraviolet (UV) spectral region [1]. In some cases, it has been shown that the presence of the UV-absorbing pigment, scytonemin, in the extracellular sheaths of some cyanobacteria provides at least partial protection from UV radiation [1,2]. In others, the ability to react to UV radiation by a motility escape response has been demonstrated [3,4]. In most cyanobacteria, active repair and resynthesis of damaged constituents, the dissipation of excitation energy at the reaction center via triplet–triplet energy transfer to a carotenoid pigment, and quenching of UV-induced reactive oxygen compounds by carotenoids are also probably involved, but have yet to be demonstrated in natural situations (see [5,6]).

UV radiation as a stress or influencing factor may have had a great effect in determining mat species composition, development, maintenance, and evolution during the early Precambrian portion of the 'Age of Stromatolites' when fluxes of UV-B (and UV-C) radiation were considerably higher than current levels [7].

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Recently, Sheridan [8] found that aerial microbial mats of black mangroves required the UV component of irradiance to maintain the structure and physiological properties (e.g. N_2 fixation) of the mixed community found in the natural habitat. Without UV, opportunistic, less UV-tolerant cyanobacterial species overgrew and disrupted the mat. The conclusion based on these laboratory experiments is that UV-tolerant species normally occupy the mat surface, giving protection to the more sensitive species below. Thus, it is possible that the structure of natural microbial mats, in general, is a result of UV radiation being a component of solar irradiance. Since substantial changes occurred in the mangrove mats in less than a month, it was thought that similar changes might occur in hot-spring mats over a similar interval of time, and under full natural solar irradiance in which only UVR was excluded.

In the present study, the results of UV exposure and UV protection were compared over 1–3 months in intact hot-spring microbial mats of Yellowstone National Park. The objective was to determine whether the UV component of summertime solar irradiance influenced community composition in a manner similar to that found by Sheridan [8] under laboratory conditions. In the mat communities examined in this study, no scytonemin-containing cyanobacteria were present, but many of the mats were rich in carotenoid pigments, compounds known to provide indirect protection from high solar irradiance through their ability to quench various forms of reactive oxygen or inhibit their production.

2. Materials and methods

2.1. Location and nature of the mats

Microbial mats of thermal streams from two locations in the 40–47°C range were utilized. A mat in a thermal stream with this temperature range was located in one of the southernmost tributaries of Rabbit Creek (about 2.5 km from the highway; Midway Geyser Basin) and another in Octopus Spring (White Creek area, Lower Geyser Basin; elevation 2237 m). A higher temperature mat (58–62°C) in a backwater pool of Octopus Spring was also used. All streams had an alkaline pH (Rabbit Creek: 8.0–9.5; Octopus Spring outflow: 8.0–8.5). The macro ions of Octopus Spring water were: sodium ($\sim$320 mg l^{-1}), potassium ($\sim$16 mg l^{-1}), chloride ($\sim$250 mg l^{-1}), silicate ($\sim$250 mg l^{-1}), sulfate ($\sim$20 mg l^{-1}), fluoride ($\sim$20 mg l^{-1}), and less than 1 mg l^{-1} of calcium and magnesium. Arsenic (V) was about 1.4 mg l^{-1} [9]. The Rabbit Creek site is in an adjacent watershed and has a similar chemistry (J. Dillon, personal communication).

Both lower temperature mats were in flowing water and were a bright orange color due to high carotenoid to chlorophyll (and phycocyanin) ratios (Fig. 1). The higher temperature mat at Octopus Spring was a green to yellow-green color at the surface. All mats before filter coverage were exposed to full solar irradiance under 1–1.5 cm of clear spring water. Mats were chosen for the deployment of filters by possessing temperature and visual uniformity over an area, as well as Bacteria 16S-DNA denaturing gradient gel electrophoresis (DGGE) band-profile uniformity. In addition, two gray ceramic tiles (4.9×4.9 cm) were laid under each filter to measure new settlement and growth. However, only the two under each filter at the higher temperature Octopus Spring site acquired a microbial film or mat that remained attached.

2.2. Alteration of solar irradiance by filters and screens

Two types of filters were used: (1) OP-4 (3.2-mm thick; Cyro Industries, Woodcliff Lake, NJ, USA), which transmits about 90% of the visible and near-infrared spectrum (400–$>$800 nm) and 80–90% transmittance in the UVA/UVB region (i.e. 280–400 nm) and (2) UF-5 (3.2-mm thick; Plexiglass: Autohaas N. Am., Philadelphia, PA, USA), which transmits about 90% of the visible spectrum, roughly 5% of the near-UVA spectral region and none measurable in the UVB region. Filters (1) and (2) are equivalent in spectral transparencies to filters UV-T and OP-3, respectively, in Cockell and Rothschild [10]. The 46×35-cm filter sheets were mounted on wooden racks with four adjustable swivel legs attended with metal angles which were nailed into the siliceous sinter substrate with concrete nails (Figs. 1 and 2). In some areas, two additional racks were used with a triple layer of neutral density plastic window screen in addition to the two filter types; this reduced the total irradiance (visible and UV) by about 75% (Fig. 1). The rack legs were adjusted so that the filter was about 2–3 cm above the water surface and about 3–4 cm above the surface of the mat. Although condensation often occurred on the underside of the filters, measurements have shown that this had no effect on transmission at the wavelengths of interest (data not shown). Filters were cleaned with distilled water about every 2 weeks during the summer, but often this was unnecessary because of frequent afternoon rain showers. Samples were taken in the center two thirds of the area covered so that only solar radiation penetrating the filter affected the sampling area, regardless of irradiance angle. The filters were deployed at different dates between June 30 and July 5, 2000. Some were removed near mid-August; others on September 25, although sampling occurred usually at 2–3-week intervals (see Table 1).

2.3. Irradiance measurements

Visible and UV irradiances were measured for the photoincorporation experiments (see below) with a IL-1700 radiometer (International Light, Newburyport, MA, USA). Separate filters and sensors were calibrated to

read VIS+near-IR (~400 to >1000 nm), UVA (320–400 nm; primary sensor sensitivity 368 nm), and UVB (280–320 nm; primary sensor sensitivity 292 nm). Continuous recordings of total solar irradiance were made at Octopus Spring, using a Belfort Model 5-3850 recording pyrheliograph (Belfort Instrument Co., Baltimore, MD, USA). This was used primarily to detect and record clear versus overcast conditions. Solar irradiance values for clear and overcast days have been measured over several summers with typical values for a sunny midday in July and August of 800–1000 W m^{-2} (VIS+near IR), 30–40 W m^{-2} UVA, and 2–3 W m^{-2} UVB [5,11]. Midday values were about 15% lower in mid to late September. Photoincorporation experiments were conducted under clear skies near midday in order to utilize the highest intensities (see below).

2.4. Sampling methods

Samples were taken by two methods for two different purposes. Cores about 1 cm in length were taken with transparent plastic straws 5 mm in diameter or with a #1 cork borer (3.5-mm orifice). These were used primarily for the DGGE procedure, with a universal Bacteria polymerase chain reaction (PCR) primer, plus (in some cases) Cyanobacteria primers (described below). Some of these cores were also used for microscopic examination. Those used for molecular analysis were frozen with dry ice within an hour after collection. After transport to the laboratory at Montana State University, these samples were moved to a −80°C freezer and stored for later processing. Core samples were taken at all sites prior to the deployment of filters to assure that mats utilized were uniform with respect to microbial composition as determined by DGGE analysis.

The second sampling method was used to prepare inoculum used in ^{14}C-photosynthesis experiments, for pigment and DGGE analyses. In the case of the lower temperature mats (~40–47°C) the semi-translucent top orange layer (1–2-mm thick) (or green top layer under the filters+three screens) was peeled from the center area of the mats and later easily dispersed to uniformity by pumping with a 5- or 10-ml syringe (without needle). In the case of the higher temperature mat at Octopus Spring, the topmost, more opaque, loose greenish cover (primarily *Synechococcus* spp.) was vacuumed off the mat with a syringe. This material (again dispersed to uniformity) was also used for photosynthetic experiments, and for pigment and molecular analyses. Ramsing et al. [12] showed that there was a highly ordered vertical structure within the 1 mm-thick photic zone at 61°C in Mushroom Spring (0.5 km from Octopus Spring). In the case of the present study, the vertical zonation of the photic layer was disrupted and mixed when sampling. However, in the translucent lower temperature mats of Octopus Spring and Rabbit Creek, the photic zone extended to a depth well below the 1–2-mm peeled upper layer used for photoincorporation experiments (data not shown).

2.5. Nucleic acid purification

A modification of the method of Moré et al. [13] was used for extraction of DNA from mat cores or mat surface samples. For surface material (also used in photoincorporation experiments) 1.5 ml of material was centrifuged for 3 min at 13 000 × *g* and most of the supernatant removed, leaving a remainder of 250 μl. This or mat core samples weighing approximately 0.25 g were combined with 1.0 g of 0.1-mm diameter zirconia/silica beads (BioSpec Products, Bartlesville, OK, USA) in sterile 2-ml screw-cap polypropylene microcentrifuge tubes. Then, 375 μl of 120 mM sodium phosphate (pH 8.0) was added to each tube along with 190 μl of a buffer comprised of 10% sodium dodecyl sulfate, 0.5 M Tris–HCl (pH 8.0) and 0.1 M NaCl. Cell lysis was achieved by shaking the samples for 45 s at 6.5 m s^{-1} in a Bio101 Fast Prep bead-beater (Qbiogene, Carlsbad, CA, USA). Tubes were centrifuged for 3 min at 13 000 × *g* and 700 μl supernatant was collected. DNA was precipitated on ice for 5 min with 2/5 vol of 7.5 M ammonium acetate and then centrifuged as before. The supernatant was isopropanol precipitated and the pellet washed with 70% isopropanol and resuspended in 50 μl Tris–EDTA buffer.

RNA purification was done using the Ambion Totally RNA kit (Ambion, Austin, TX, USA). Cell suspensions were first centrifuged as above in sterile 2-ml screw-cap polypropylene microcentrifuge tubes and the supernatant removed leaving a volume of 250 μl behind. Next, 1 ml of denaturation solution from the Totally RNA kit and 1.0 g of 0.1-mm diameter zirconia/silica beads were added. Cells were lysed using the Fast Prep instrument and centrifuged as described above. The supernatant was split into two aliquots of 0.5 ml each and the remainder of the protocol was carried out according to the manufacturers instructions. In the final step pellets were resuspended in 25 μl H_2O with 0.1 mM EDTA. Possible DNA contamination was removed with RQ1 DNase treatment (Promega, Madison, WI, USA). RNA quality was verified on a 1% agarose gel.

2.6. DGGE analysis of community DNA and RNA

PCR amplification of Bacteria 16S-rRNA genes from community DNA was done using the Bacteria specific forward primer 1070F and the universal reverse primer 1392R [14] containing a GC-clamp, yielding a 320-bp product. Each reaction contained 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer, approximately 10 ng template DNA, 0.1 mg ml^{-1} bovine serum albumin (BSA), 2.5 U Taq polymerase and 1× buffer (Promega) in a total volume of 50 μl. The PCR amplification cycle was as follows: 2 min at 95°C, then 26 cycles of 45 s denaturation at

95°C, 45 s annealing at 43°C, 45 s extension at 72°C, and a final extension of 7 min at 72°C. In order to specifically amplify Cyanobacteria 16S-rRNA genes, the forward primer CYA106F (5′-CGCCCGCCGCGCCCCGCGCC-GGTCCCGCCGCCCCCGCCCGCGGACGGGTGAGT-AACGCGTGA-3′) containing a GC-clamp and the reverse primer CYA781R(a) (5′-GACTACTGGGGTATC-TAATCCCATT-3′) were used [15]. Reaction conditions were the same as described above except that 1.5 mM $MgCl_2$ was used. The PCR amplification cycle using Cyanobacteria primers was: 5 min at 94°C, then 26 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, 1 min extension at 72°C, and a final extension of 7 min at 72°C.

Reverse transcription (RT)-PCR was carried out using the Access RT-PCR system (Promega). Reaction mixtures contained 0.4 μM of each primer, approximately 10 ng template RNA, 0.1 mg ml^{-1} BSA, 1 mM $MgSO_4$, 0.2 mM dNTPs, 5 U AMV reverse transcriptase, 5 U T*fl* DNA polymerase, and 1× AMV/T*fl* reaction buffer. A pre-mix of primers and template was denatured at 95°C for 5 min, snap-cooled on ice and then added to the rest of the reaction mixture. The amplification cycle for the RT-PCR reaction was as follows: first strand synthesis for 45 min at 48°C, denaturation of AMV-RV for 2 min at 94°C, then 25 cycles of 30 s denaturation at 94°C, 1 min annealing at 55°C, 2 min extension at 68°C, and a final extension of 7 min at 68°C. Absence of DNA contamination was verified by assembling the same reactions without AMV-RT. PCR (or RT-PCR) products were quantified on a 1% agarose gel by comparison to mass ladder standards (Gibco BRL, Grand Island, NY, USA). Approximately 120 ng of PCR product per lane were loaded on DGGE gels.

DGGE was performed on a Bio-Rad Dcode system (Bio-Rad, Hercules, CA, USA) as described by Muyzer et al. [16]. An 8% polyacrylamide gel with a linear denaturant concentration from 40 to 70% was used (where 100% denaturant contains 7 M urea and 40% (v/v) formamide). Gels were electrophoresed for 17 h at a constant 60 V, and then stained for 30 min in SYBR Green I (Molecular Probes, Eugene, OR, USA), illuminated on a trans-illuminator, and photographed using Polaroid 57 film.

2.7. Sequencing of Cyanobacteria DGGE bands

DGGE bands were isolated from DGGE gels by carefully touching the band of interest with a sterile polypropylene pipet tip several times along the length of the band. The tip was then rinsed into a PCR reaction tube containing 10 μl sterile, nuclease-free H_2O. For band re-amplification, 2 μl of this 'band stab' was added to PCR reaction mixtures. The PCR reaction mixtures and the thermocycler program were the same as that used for the initial reactions, although the number of cycles varied from 25 to 30. The quality of the band purification was checked by DGGE and re-amplification was repeated if necessary until a single band of sufficient purity was attained. Then, another PCR reaction was done from the band stab which gave a clean product, but using the reverse primer CY-A781R(a) and a forward primer without the GC-clamp, CYA106Fshort. Reaction conditions were otherwise the same. The PCR product from Band OL10 was first TA-cloned into the pCR 2.1-TOPO vector according to the manufacturers instructions (Invitrogen, Carlsbad, CA, USA) and then vector inserts were re-amplified and sequenced. PCR products were purified using Amicon microcon centrifugal devices (Millipore, Bedford, MA, USA) and used as sequencing template. Sequencing was accomplished using the ABI Prism BigDye Terminator Cycle Sequencing reaction kit and an ABI 310 DNA sequencer (Perkin-Elmer, Norwalk, CT, USA). The following primers were used for sequencing; CYA781R(a) [15], 338F (5′-ACTCCTACGGGAGGCACG-3′), 338R (5′-GCTGCC-TCCCGTAGGAGT-3′), and 522R (5′-ATTACCGCGG-CKGCTG-3′)[17]. A BLAST search of the GenBank database was done to identify species or strains of closest similarity. The 16S-rDNA sequences from this study were submitted to GenBank with the accession numbers AF399857–AF399870.

2.8. Microscopy

In the lower temperature mats, microscopic examination was used to elucidate the large number of predominant bacteria that was indicated by DGGE analyses. Nomarski photomicrography was used to demonstrate the large diversity of morphotypes, a few of which could be affiliated with known species or genera of the domain Bacteria. An examination of the higher temperature material was also done. The nomenclature in Bergey's Manual of Systematic Bacteriology [18] is used, when possible, with traditional 'botanical' names in addition when appropriate.

2.9. ^{14}C-Photoincorporation experiments

Photosynthesis experiments were run on August 9 and September 25, 2000 in order to determine whether the long-term +UV or −UV environment had resulted in changes in the ability to tolerate high-intensity UV radiation. Inoculum was obtained from the various mats by the methods described above. All collections for use in photoincorporation experiments were made between approximately 09.00 and 10.00 h. After dispersal to a uniform suspension by syringe pumping, the suspension was diluted with native spring water from each source to an optical density (OD) at 750 nm that varied from 0.15 to 0.30, but was uniform for each source inoculum and experiment. Samples from each source and from under all filter and screen conditions were dispersed and diluted as above; then 7.5 ml from each was dispensed into 20-ml capacity nearly UV-transparent 60-ml capacity Whirl-Pak™ bags (Nasco, Ft. Atkinson, WI, USA). WhirlPak

bags transmit about 83–88% of the UVB and UVA, respectively. [^{14}C]$NaHCO_3$ was added as 0.5 ml to attain a final activity of 1.48 kBq (0.04 μCi). Photosynthesis ^{14}C-incorporation incubations were performed at 40–42°C in constant-temperature water baths (Catalog 9820E35, A.H. Thomas, Swedesboro, NJ, USA) located outside the research laboratory trailer at West Yellowstone, Montana, USA. The water baths have low sides and the WhirlPak bags were placed near the top over submerged stainless steel screens in order to assure no shading with changing sun angles. Photosynthesis was stopped by adding 0.5 ml of formalin (37% formaldehyde solution). Processing consisted of filtering the entire sample with GN-6 (Gelman, Ann Arbor, MI, USA) filters (0.45-μm pore size), washing with 2% (v/v) HCl, washing with water, and immersing the dry filters overnight in 7.5 ml Ecolume scintillation cocktail (ICN, Costa Mesa, CA, USA). The radioactivity was measured with a Beckman LS6000SE scintillation counter. Since only relative measurements in cpm were relevant within each spring experiment, absolute rates of carbon incorporation were not calculated.

Significance of results between various pairs was analyzed by one-way ANOVA, using Standard Least Squares Contrast with a program of JMP, Version 4 (SAS Inst., Cary, NC, USA).

2.10. Pigment measurements

A portion of the inocula used for both ^{14}C-experiments was saved under refrigeration and used for quantitative pigment measurements. Extraction was with absolute methanol saturated with $MgCO_3$ to maintain neutral pH. After 12-h extraction in the dark and clarification by GF/F (glass-fiber filters), the OD at the red maximum of chlorophyll (Chl) *a* (662–666 nm) was determined, as well as the peak primarily caused by carotenoids which varied from 471 to 474 nm. The ratios of these two peaks (carotenoids to the Chl *a* red peak) may be used as a rough measure of carotenoid absorption relative to Chl *a*. Mycosporine-like amino acids (MAAs) were assayed by the method described by Garcia-Pichel and Castenholz [19] and were determined for several inocula from different UV environments. None of the cyanobacteria involved in these studies produced sheaths containing the UVA-absorbing pigment, scytonemin, although these were common in many other warm-spring mats in YNP (see [1]).

3. Results

3.1. Visual and pigment changes in mats

The lower temperature mats at Octopus Spring and Rabbit Creek were a bright orange color at the beginning of the experiments and remained so throughout the experimental period, regardless of UV filtering (Figs. 1 and 2). In addition to no obvious visual difference between the two filter types, the 'carotenoid to chlorophyll' ratio values were similar between paired filters at each time point (Table 2). In all cases, orange colored mats had a high OD ratio of 473 to 665 nm (up to 9.6) (Table 2). Phycocyanin was present in a very low cell content in all but the higher temperature mat at Octopus Spring and was not routinely measured (data not shown). The two filter treatments allowed penetration of approximately 90% of the visible irradiance. Darker pigmentation occurred only along the

Fig. 1. Low-temperature Rabbit Creek mat with filter setups (29 July). Orange mat color is typical of alkaline springs of this region in 40–47°C range. Filters from background to foreground: visible+UV, vis−UV, 25% vis+25% UV, and 25% vis−UV.

Fig. 2. Low-temperature Octopus Spring mat on 20 August with similar orange color but after about 4 weeks of treatment under filters. Area to left: visible+UV; area to right: vis−UV. Both filters raised.

borders where the wooden frame caused greater shading (Fig. 2).

The mats that were covered in the lower temperature Octopus Spring and Rabbit Creek sites by neutral density screens (three layers with about a 75% reduction over all wavelengths) in addition to the +UV or −UV filters, showed an obvious surface layer with greatly reduced 'carotenoid to Chl *a*' OD ratios. These changes became obvious visually within a few days after screen/filter placement, but the first sampling was done about 1–3 weeks afterwards (Tables 1 and 2). On August 9, the surface layers from under both filters with three neutral density screens at the low-temperature Octopus (+ or −UV) were quite green and had a 'carotenoid to Chl *a*' ratio of only 4.13 and 3.51 for +UV and −UV, respectively (Table 2). By contrast, in the absence of screens (filters only), the +UV- and −UV-treated mats remained orange, which is evidenced by high carotenoid to Chl *a* ratios (Table 2). With continued screening, the triple-screened mats became even greener, and on September 25 showed 'carotenoid to Chl *a*' ratios of 2.78 (+UV) and 3.01 (−UV) (Table 2, Fig. 3). Pigment samples from Rabbit Creek at the end of the season (September 25) again demonstrated large differences between screened and unscreened mats (Table 2). Decreased 'carotenoid to Chl *a*' ratios were a consequence mainly of increases in Chl *a* content rather than a great decrease in carotenoids (data not shown).

The −UV filter covering the higher temperature mat at Octopus Spring resulted in a mat slightly greener than under the +UV filter after 4 days, but this was not reflected in any major way by pigment analyses on August 9 (after 40 days) and not at all on September 25, using dispersed surface inoculum from the photoincorporation experiments (Table 2). Material from the ceramic tiles placed under the two filters on August 9 and harvested quantitatively on September 25 (34 days), showed a slightly higher Chl *a* content from under the −UV treatment, and thus a slightly lower 'carotenoid to chlorophyll' ratio (Table 2).

MAAs, if present, were in very small quantities in all material used for the photosynthesis experiments. A small absorption peak occurred at 334–337 nm, which is indicative of some species of these compounds [19]. It is, therefore, likely that one or more species of cyanobacteria in all

Table 1
Sampling schedule for research sites in year 2000

	Octopus low temperature	Octopus high temperature	Rabbit Creek
Pre-filter	1 July	1 July	25 June
Filter on	1 July	1 July	5 July
Screens on	21 July	n.a.	21 July
1st sampling	10 July	10 July	21 July
2nd sampling	29 July	29 July	8 August
3rd sampling	9 August	9 August	19 August
4th sampling	20 August	20 August	n.a.
Final sampling	25 September	25 September	25 September

Table 2
'Carotenoid' (473 nm) to Chl *a* (665 nm) OD ratios in methanol

Site	Filter	9 August	25 September	4 October
Octopus low temperature	+UV	9.59	n.a.	n.a.
	−UV	8.47	n.a.	n.a.
	+UV/three filters	4.13	2.78	n.a.
	−UV/three filters	3.51	3.01	n.a.
Post-removal	+UV/three filters	n.a.	n.a.	5.07
Post-removal	−UV/three filters	n.a.	n.a.	4.41
Octopus high temperature	+UV	2.73	2.29	n.a.
	−UV	2.24	2.44	n.a.
	+UV/tile	4.99	3.83	n.a.
	−UV/tile	4.52	3.02	n.a.
Rabbit Creek	+UV	n.a.	4.94	n.a.
	−UV	n.a.	5.67	n.a.
	+UV/three filters	n.a.	3.27	n.a.
	−UV/three filters	n.a.	2.41	n.a.

the mats investigated contained very low contents of such compounds. However, the peaks were so small compared to well-known mycosporine-rich species that essentially no UV protection could be expected (data not shown). In any case, no difference was seen between the mat-surface communities of those exposed and those not exposed to UVR.

3.2. Molecular analysis of community composition

PCR amplification of 16S-rDNA sequences and subsequent analysis by DGGE were used to monitor changes in the microbial communities in response to the various filter treatments. Uniformity of mat samples prior to filter placements was verified by taking 12 different samples across an area of mat encompassing roughly 0.1 m^2. All of these pre-treatment samples yielded essentially identical results for each site (data not shown). DGGE profiles obtained from Bacteria-specific PCR of DNA acquired from low-temperature Octopus Spring after filter treatments yielded complex patterns, with at least 25 distinct bands for each treatment (Fig. 4). The samples from the Rabbit Creek mat before and after treatment were equally complex (data not shown). Because irradiance effects would most likely occur at mat surfaces, subsequent (RT)PCR-DGGE analysis was restricted to 1–2-mm mat depth, and to photoautotrophic members of the community with the use of Cyanobacteria-specific primers [15]. This was the same mat material used for the photoincorporation experiments and this allowed a more direct comparison of results obtained from molecular and physiological analyses.

Typical DGGE patterns using Cyanobacteria-specific primers are shown in Fig. 5. From the Octopus low temperature (40–47°C) mat there are nine distinct bands (Fig. 5A, OL 1–9). After approximately 19–40 days under the various filter treatments there was very little apparent

Fig. 3. Low-temperature Octopus Spring mat on 10 August from under 25% visible−UV (to left) and 25% vis+25% UV (to right) after about 3 weeks of treatment under filters plus screens. The dark area was a deep green color; the lighter area surrounding was a bright orange as in Fig. 2.

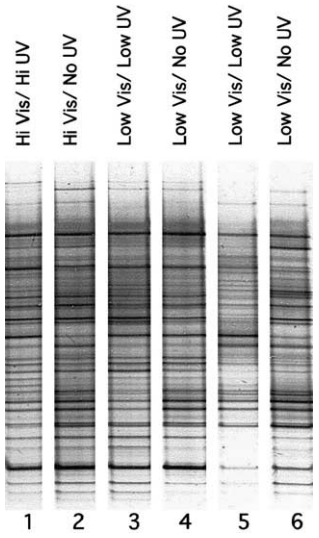

Fig. 4. DGGE profiles of low-temperature mat communities of Octopus Spring subjected to different UV/visible light treatments. A Bacteria-specific primer was used in the PCR amplification from the mat-surface material, also used in the photoincorporation experiments. Lanes 1–4 from 9 August sampling (1 and 2 after 40 days of treatment; lanes 3 and 4 after 19 days). Lanes 5 and 6 from 25 September sampling (after 65 days of treatment). Hi Vis/Hi UV = sample from mat covered by filter transmitting in both visible and UV region of the spectrum. Hi Vis/ no UV = sample from mat covered by filter transmitting visible and excluding 95% UVA and 100% UVB. Low indicates the addition of neutral density screens to filters which reduced total irradiance by ~75%.

change in the community composition in response to UV exclusion (Fig. 5A, lanes 1–4). One difference is that band OL 9 (lane 2) is much less prominent under −UV than in all the other treatments. After 65 days under the various treatments there were more obvious differences. The DGGE pattern from the +UV treatment (with neutral density screens) looked identical to that of 6 weeks earlier (Fig. 5A, lanes 3 and 5). In the case of the −UV (with three neutral density screens), bands OL 5, 6, 7 and 9 had completely disappeared. Samples from the Rabbit Creek mat, also in the 40–47°C range, were analyzed in a similar manner. The DGGE patterns of the Rabbit Creek mat yielded seven distinct cyanobacterial bands for each treatment (Fig. 5A, lanes 7–10, RC 1–7). Even after 65–80 days under the filters there were no discernible differences among the treatments.

The DGGE fingerprints of the Octopus high-temperature (58–62°C) mat were strikingly different from those of the low-temperature mats (Fig. 5B). DGGE profiles were less complex, with only ~five cyanobacterial bands observed for either +UV or −UV treatments. The appearance of fewer dominant populations is likely a result of the increased negative selective pressure of higher temperature. Furthermore, the banding patterns were identical for the two treatments, suggesting no change in community composition with alterations in UV exposure.

Some mat samples were also analyzed by RT-PCR-based DGGE. As the half-life of RNA is relatively short compared to DNA in actively growing populations, RT-PCR would likely be more sensitive to changes in the metabolically active fraction of the studied communities in response to environmental perturbations. A comparison of RT-PCR and PCR DGGE from the Octopus Spring low-temperature mat is presented in Fig. 6. All of the bands that are present in the DNA-based profiles are also present in the RNA-based DGGE profiles, although notable differences in intensity are clearly seen for some bands. However, one additional band (OL10) is present in

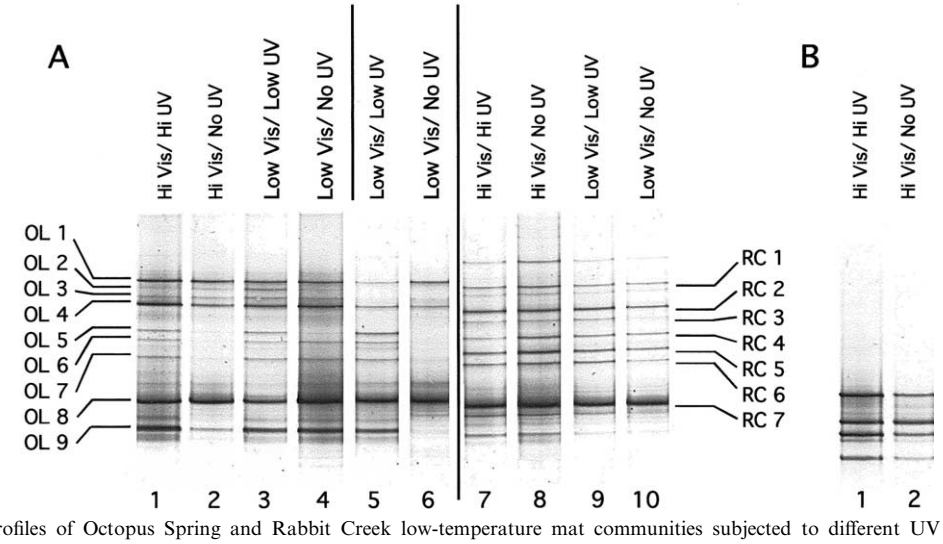

Fig. 5. (A) DGGE profiles of Octopus Spring and Rabbit Creek low-temperature mat communities subjected to different UV and visible light treatments. Cyanobacteria-specific primers were used in the PCR amplification of mat-surface material. Lanes 1–4, Octopus Spring 40–47°C mat sampled 9 August (lanes 1 and 2 after 40 days of treatment; lanes 3 and 4 after 19 days); lanes 5 and 6 Octopus Spring 40–47°C mat sampled 25 September (after ~65 days of treatment). Lanes 7–10, Rabbit Creek 40–47°C mat sampled 25 September (lanes 7 and 8 after 80 days; lanes 9 and 10 after 65 days). Filter treatments were as described in Fig. 4 and Section 2. Bands from both mats indicated in this figure were re-amplified for sequencing and are discussed in the text. (B) DGGE profiles of Octopus Spring high-temperature mat surface communities using Cyanobacteria-specific primers after 40 days of treatment on 9 August.

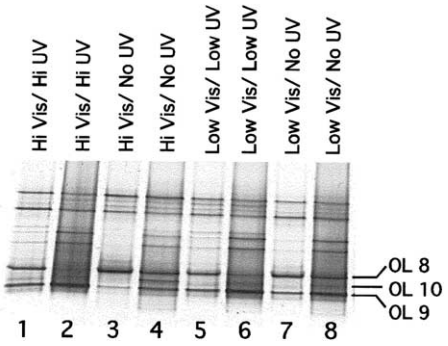

Fig. 6. DGGE profiles of low-temperature Octopus Spring surface community derived from either DNA or RNA templates. For lanes 1, 3, 5 and 7 DNA was used as PCR template; for lanes 2, 4, 6 and 8 RNA was used as RT-PCR template. All were from the 9 August collection date. An additional band, OL 10, generated from RT-PCR, is also indicated in the figure. Lanes 1–4 are from 40 days under filters; lanes 5–8 are from 19 days. Filter treatments were as described in Section 2.

the RNA-DGGE fingerprint. Band OL10 is more intense in the −UV treatments, with or without the addition of neutral density screens (Fig. 6). Another notable difference is the absence of band OL8 from the +UV samples (with and without screens) in the RNA-derived profiles, but which is present in the corresponding DNA-derived profiles. Conversely, band OL9 is very weak in the DNA-based −UV sample compared to its RNA counterpart.

In order to identify the populations represented by the prominent bands from the low-temperature Octopus Spring and Rabbit Creek mats, individual bands were purified and then sequenced. The PCR-purified bands accounted for most of the community DGGE profile for the low-temperature mats from both Octopus Spring and Rabbit Creek. With one exception, sequences derived from these purified bands demonstrated very low similarity to previously reported cyanobacterial sequences (Table 3). Within each of the low-temperature mats, there were groups of two or more sequences that differed by less than 1%, suggesting the possibility that these mats were dominated by several very closely related populations of cyanobacteria. For example, RC1 and RC3 are each only distantly related to *Pseudanabaena* PCC6802, but differ from each other by only four positions over 600 bp sequenced. Interestingly, three sequences were retrieved from both of the low-temperature mats. The Octopus Spring bands OL1, OL5 and OL8 had identical counterparts in the Rabbit Creek bands RC1, RC4 and RC7. Fig. 5A shows that the bands from these sequence pairs of the two mats migrate to the same position on a DGGE gel, as would be predicted for identical sequences. The band OL8/RC7, the only one that was closely related to a previously described species, had just three disagreements with the sequence for *Synechococcus* ATCC700244 over 600 bp analyzed. This was the C9 type *Synechococcus* from Octopus Spring first described by Ferris et al. [14].

For a few of the bands (OL3, OL9, OL10, RC2 and RC6) it was not possible to obtain good data by direct sequencing of the re-amplified DGGE bands. As an alternative approach, the PCR product from band OL10 was first cloned into the pCR 2.1 TOPO vector, transformed into *Escherichia coli* and then individual colonies were picked for amplification and sequencing of vector insert. This method revealed that band OL10 was comprised of at least three different sequences. Apparently these sequences have similar melting profiles and co-migrate under our DGGE conditions. It is possible that the other bands that could not be sequenced were also comprised of multiple co-migrating sequences, although this was not confirmed.

Two enrichment clones from the Octopus low temperature were also subjected to 16S sequence analysis. Neither of these clones were related to the DGGE band sequences from the field samples. Clone 1 was a filamentous type with very low similarity to a *Phormidium* strain or species, while Clone 5 was an exact match for *Synechococcus* PCC6716 (C1 type), another *Synechococcus* strain or species that was previously described from Octopus Spring [14].

Table 3
Nearest GenBank relatives of 16S-rDNA DGGE band sequences from Fig. 5

Band	Nearest GenBank relative	Accession no.	% Identity
OL 1	*Pseudanabaena* PCC6802	AB039016	90
OL 2	*Pseudanabaena* PCC6802	AB039016	91
OL 4	*Pseudanabaena* PCC6802	AB039016	90
OL 5	*Spirulina subsala*	AB003166	91
OL 6	cyanobacterium WH12	AJ007375	91
OL 7	cyanobacterium (OS type 1)	L04709	89
OL 8	*Synechococcus* ATCC700244 (C9 type)	AF132773	99
RC 1	*Pseudanabaena* PCC6802	AB039016	90
RC 3	*Pseudanabaena* PCC6802	AB039016	89
RC 4	*Spirulina subsala*	AB003166	91
RC 5	*Spirulina subsala*	AB003166	91
RC 7	*Synechococcus* ATCC700244 (C9 type)	AF132773	99
clone 1	*Phormidium autumnale*	AF218371	92
clone 5	*Synechococcus* PCC6716 (C1 type)	AF216942	100

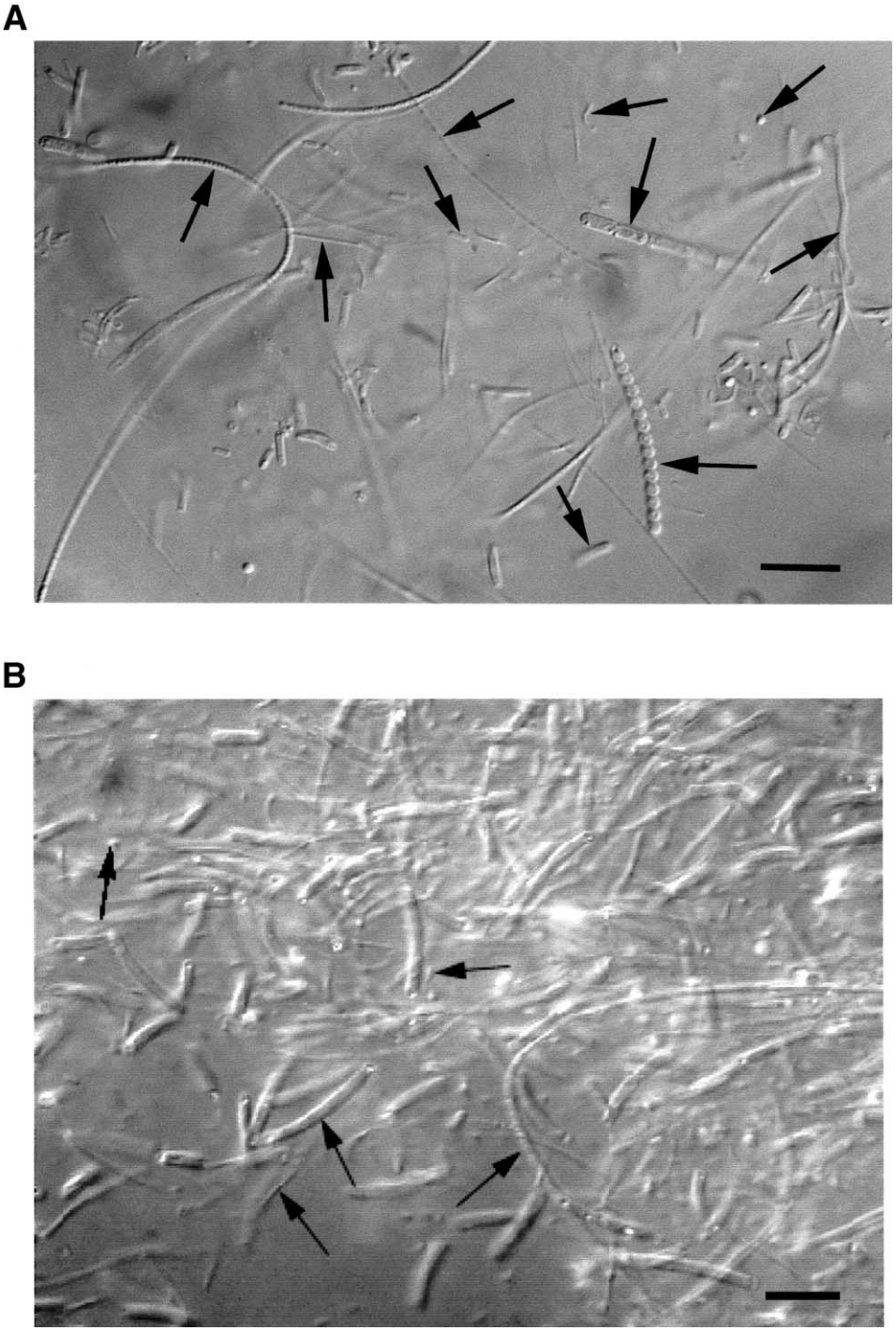

Fig. 7. Selected photomicrographs (Nomarski optics) of inocula used for photoincorporation experiments of August 9. (A) From the low-temperature Octopus Spring mat. Arrows indicate 10 distinct morphotypes of microorganisms. The two lower right-hand arrows correspond with *Synechococcus* sp. and *Isosphaera pallida* (left to right). (B) From the higher temperature Octopus Spring mat. Arrows indicate five distinct morphotypes. The second arrow in the lower left points to a *Synechococcus*-shaped cell. The bar is 10 μm for (A) and (B).

3.3. Microbial composition by microscopic examination

Microscopic examination of many of the same samples that were used for molecular analyses showed in a non-quantitative manner about 10 varieties of prokaryotes in the lower temperature (40–47°C) sites, both in the Octopus Spring (with 10 arrows) (Fig. 7A) and Rabbit Creek samples (photomicrograph not shown). Arrows in the figures indicate different morphotypes. Examination of the higher temperature samples revealed that only about five clear morphotypes could be distinguished, which agrees with the lower numbers of predominant populations or 'species' suggested by DGGE bands (Fig. 7B).

3.4. Photosynthesis experiments

During the August 9 clear-sky experiment, 'visible' irradiance (VIS+near IR) was ~800–900 W m^{-2}, UVA ~33 W m^{-2}, and UVB ~2.5 W m^{-2}. Figs. 8 and 9 show the results of these experiments expressed as cpm per μg Chl *a* (*y*-axis).

In the lower temperature Octopus Spring photoincorporation experiments, quite obvious differences may be seen between the mat community maintained under +UV and that maintained under −UV conditions (Fig. 8). On August 9, the photosynthetic community from under +UV maintenance (from July 1 to August 9; see Table 1) was much better able to cope with +UV conditions for the 1-h photoincorporation exposure than was the community from under the −UV, long-term maintenance conditions (Fig. 8: columns 1 versus 3). However, even this 'UV acclimated or adapted' community showed photosynthetic improvement when exposed to the −UV incubation (columns 1 versus 2), as did the community acclimated under full light/−UV (columns 3 versus 4).

The communities maintained under 25% VIS irradiance with or without 25% UV (i.e. under each filter type plus three neutral density screens) showed a trend similar to the photoincorporation results of the unscreened communities described above. In contrasting columns 5 and 7, it may be seen that the 19 days of exposure to even this low-UV radiation allowed better photosynthetic performance than with no exposure to UV during this period when tested under 'full' solar irradiance. However, all of these lower light communities showed lower photoincorporation rates when incubated under 'full' irradiance (with UV) than their counterparts that had been maintained under high light and full UVR (Fig. 8: compare columns 5, 6, 7, 8 with 1 and 2).

Similar results were obtained in the September 25 experiment, where the community maintained under 25% UV and 25% VIS since July 21 was more competent photosynthetically than the community maintained under no UV and 25% VIS (Fig. 8: compare columns 9 and 11). The mats under the non-screened filters at this low-temperature site had bleached out because of damming by mat growth upstream between August 20 and September 25. However, both maintenance treatments with 25% transmittance and low UV or no UV showed considerable photosynthetic improvement when incubated for 1 h under −UV conditions than when exposed to 'full' solar irradiance with UVR (compare columns 9 and 11 plus 10 and 12). Again, as on August 9, the surface community maintained under 25% UV and 25% VIS seemed better able to cope with 'full' solar irradiance than those that had not been exposed to UV during this long-maintenance period (compare columns 9 and 10 with 11 and 12).

In the higher temperature Octopus Spring experiments of August 9 little difference could be seen in the photoincorporation rates between the surface community (primarily composed of *Synechococcus* spp. and *Chloroflexus*-like organisms) maintained under +UV and −UV conditions (data not shown). Cells from under both filters showed similar rates when exposed to +UV. However, when cells maintained under −UV were exposed to the 1-h −UV treatment, a great improvement in photoincorporation was seen as compared to the 1-h +UV treatment (data not shown). This may indicate that the cells from under −UV conditions were in a somewhat 'healthier' condition, since continued relief from UVR exposure assured the highest photosynthetic rates. Cells maintained under +UV conditions also showed a slight increase when exposed to the −UV treatment.

The low-temperature Rabbit Creek mats were also tested for photosynthetic competence on September 25 (Fig. 9). The +UV and −UV filters had been over the mat since July 5; the filters with screens since July 21 (see Table 1). The results agree for the most part with those of the lower temperature Octopus Spring (compare Figs. 8 and 9). No matter what the conditions of maintenance over the many weeks, exposure to 'full' solar ir-

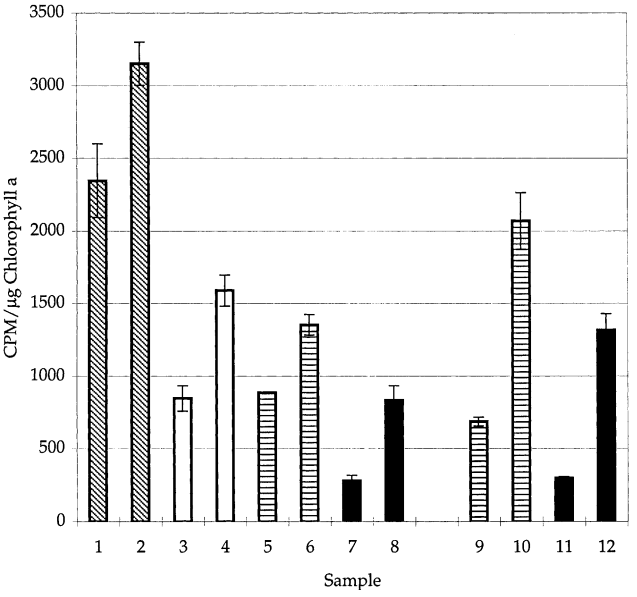

Fig. 8. Photoincorporation of [^{14}C]bicarbonate at 40–42°C by surface population of low-temperature mat community of Octopus Spring subjected to different UV and visible light treatments; columns 1–4 on August 9 (after 40 days), columns 5–8 on August 9 after 19 days; columns 9–12 on September 25 after 65 days, all with dark incorporation rates subtracted (< 10% of light values). Error bars indicate standard deviation of the mean ($n = 3$). Maintenance conditions: columns 1 and 2 (oblique hash marks) high visible/high UV; columns 3 and 4 (clear) high visible/no UV; columns 5, 6, 9 and 10 (horizontal hash marks) low visible/low UV; columns 7, 8, 11, and 12 (black) low visible/no UV. Photosynthesis incubation conditions: columns 1, 3, 5, 7, 9, 11 high visible/high UV. Columns 2, 4, 6, 8, 10, 12 high visible/no UV. The *P*-values for comparing the following pairs were significant at < 0.0001: 1 versus 2, 1 versus 3, 2 versus 4, 3 versus 4, 5 versus 7, 7 versus 8. A comparison of 5 versus 6 was significant at a *P*-value of 0.0002. In the September 25 experiment (columns 9–12) the *P*-values for the following pairs were significant at < 0.0001: 9 versus 10, 10 versus 12, and 11 versus 12. 9 versus 11 was significant at $P = 0.0011$.

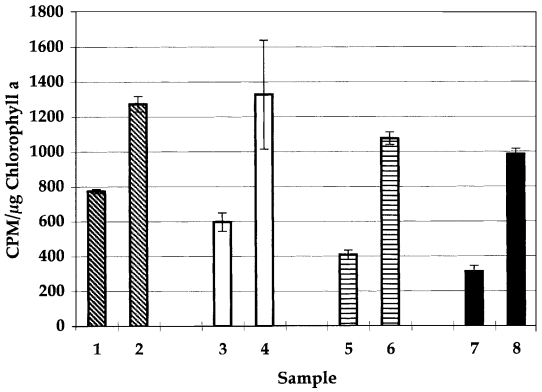

Fig. 9. Photoincorporation of [^{14}C]bicarbonate at 40–42°C by the low-temperature Rabbit Creek mat surface community on September 25, 2000. Maintenance conditions were the same as indicated by column markings in Fig. 8. However, columns 1–4 after 80 days; columns 5–8 after 65 days. Photosynthesis incubation conditions: columns 1, 3, 5, 7 high visible/high UV. Columns 2, 4, 6, 8 high visible/no UV. The *P*-values for comparisons of the following pairs were significant at <0.0001: 1 versus 2, 3 versus 5, 5 versus 6, and 7 versus 8. 1 versus 3 was significant at $P=0.0535$. The differences between 2 versus 4 and 5 versus 7 were not significant ($P=0.5377$ and 0.2781, respectively).

radiance with UVR was inhibitory to photosynthesis as compared to exposure to 'full' solar irradiance with no UVR (Fig. 9). In some cases the photoincorporation rates were more than doubled simply by the elimination of the UV component during the photoincorporation incubation. However, the better photosynthetic competence after long-term exposure to irradiance that included UVR (whether 'full' or 25%) was not as dramatic as in the lower temperature Octopus Spring experiments. Although maintenance with UVR showed better photoincorporation rates than cells maintained without UVR (Fig. 9: compare columns 1 and 3 and 5 and 7), the differences were not as great as the large differences observed in the lower temperature Octopus Spring mats.

4. Discussion

4.1. Molecular analyses

In the present study there was little evidence of change, as analyzed by DGGE, in the composition of populations of Bacteria in hot-spring mats exposed to or excluded from UVR and/or high light for over 2 months. When the analysis was limited to the photoautotrophic cyanobacterial members of the community in the upper 1–2 mm of the mat, only minor differences between treatments could be detected. The few changes that were observed occurred under the screened filters in the low-temperature Octopus Spring mat after 65 days. The DGGE pattern for the +UV treatment (with neutral density screens) had not changed, but for the −UV filter (with neutral density screens) four bands (OL 5, 6, 7 and 9) disappeared (Fig. 5A, lane 6). Three of the remaining bands were distantly related to *Pseudanabaena* strains and one band was closely related to *Synechococcus* C9 strain. These environmental sequences have related or identical counterparts in the low-temperature Rabbit Creek mat and so may represent the dominant populations in cyanobacterial mats in Yellowstone springs with this temperature range and chemistry. The significance of the 16S gene sequences that disappeared is unclear, especially considering that no similar changes were observed in the Rabbit Creek mat. With the exception of the C9 type *Synechococcus*, these sequences share little similarity with cultivated cyanobacterial strains, so there is little known about physiological differences. Further characterization of the UV tolerance of these cyanobacteria will have to await their cultivation.

Other differences were observed between the RNA- and DNA-derived profiles for bands OL8, OL9 and OL10. Unfortunately, bands OL9 and OL10 were among those that posed difficulties in sequencing. One explanation for sequencing difficulties is that these bands were actually heteroduplexes, but this possibility was excluded by the fact that multiple rounds of amplification consistently yielded a single band by DGGE analysis, whereas heteroduplex molecules should produce multiple bands. TA cloning revealed that band OL10 was composed of multiple sequences, but 70% were of the *Synechococcus* C9 type, the same as band OL8. Band OL10, and perhaps the others that could not be sequenced, may have been composed of multiple co-migrating sequences. With the exception of the differences described above, the RNA- and DNA-derived DGGE profiles were essentially identical, indicating that DNA DGGE profiles from the upper layer of the mat are those of actively metabolizing populations.

There are a number of possible explanations for why only minor differences were observed in the DGGE profiles. Changes in the mat community composition in response to irradiance perturbations may be relatively slow compared to the time scale of our observations, especially if the 'climax' species are essentially inactive or very slow growing as has been suggested for some thermophilic *Synechococcus* populations [20]. In a previous study Ferris and Ward [21] did not observe significant seasonal changes in the Octopus higher temperature mat, using general Bacteria-specific primers, even though winter light intensity at that location is only about 25% of that in summer. This light reduction is similar to that transmitted by the triple screens used on some of the filters, although daylength differs considerably from that of winter. Another possibility for the lack of change is that the high UV-adapted populations are not easily displaced or overrun. A disturbance of the mat structure may be required for a successful invasion and colonization by species or strains that were previously minor components. This idea is supported by a disturbance experiment of Ferris et al. [22]. After removal of the top layer of the Octopus Spring mat in the temperature ranges of 58–62°C, new *Synechococcus*

populations colonized the surface, while undisturbed adjacent cyanobacterial populations remained unchanged. In terrestrial systems, it is well known that many stable communities do not readily give way to or allow invaders, unless there is a major disturbance. The release of the surface layer of hot-spring microbial mats from UV stress may not constitute a sufficiently potent disturbance to allow overgrowth or enhanced growth of opportunistic species or strains that were present in the original mat. Another consideration is that potential recruitment from upstream in these low-temperature hot-spring populations may not have included novel cyanobacteria, and therefore recruitment of the same species of the original mat would not have been reflected in the analyses.

Comparing community profiles between the different mats apart from our UVR experiments also led to some interesting observations. In particular, we noted that there were fewer predominant community members in the higher temperature mat using either general Bacteria or Cyanobacteria primers (Fig. 5B and unpublished data). These results are consistent with those of Ferris et al. [14] who used DGGE to describe the changes in the microbial community of Octopus Spring across a thermal gradient. In both 40–47°C mats (Octopus and Rabbit Creek) we saw upwards of 25 bands by using general Bacteria primers (Fig. 4), while in the higher temperature mats of 58–60°C we observed 4–6 bands using the general Bacteria primers (data not shown) or Cyanobacteria primers (Fig. 5B). At the higher temperatures of 48–54°C Ferris et al. [14] also detected only six bands using the same general Bacteria primers, although under different PCR reaction conditions. Since temperature is a particularly strong selective force, it follows that community complexity would decrease with increasing temperature. We never attempted to amplify Archaea sequences, so we do not know if these would have shown a similar pattern.

In this work we have discovered a number of novel cyanobacterial sequences. Most of these sequences have very low homology ($<91\%$) to other cyanobacterial sequences reported in the literature and genetic databases. The discovery of novel sequences was facilitated in part by our choice of Cyanobacteria-specific primers, which have very different template specificity and cover a different region of the 16S-rDNA gene than the general Bacteria primers used by Ferris et al. [14]. Since there are very few cyanobacterial strains for which full length 16S sequence data are available, it is not surprising that for most of our sequences there were no close database matches in the region covered by the Cyanobacteria primers. Also, Cyanobacteria primers have not previously been used to probe these mats. Furthermore, the temperature range of the mats in our experiments was lower than that of the mats examined by molecular methods by Ferris et al. [14], and it is likely that the dominant community members are very different.

In each of the two lower temperature mats there are three sequences that differ by less than 1%. These closely related sequences could be considered ecotypes of the same species, as has been proposed for *Synechococcus* species at higher temperatures [23,24]. Alternatively, the similar sequences might represent a single species with multiple copies of ribosomal genes as discussed by Ward et al. [24]. These authors claim that the unique distribution of closely related sequences in the mat and existence of cloned cultures with a single gene copy number argues against this idea. We have also amplified 16S sequences from cloned cultures that reveal a single copy number as determined by DGGE and sequencing. Of course, this does not exclude the possibility that there are multiple 16S genes that are identical or that have differences in other parts of the molecule other than the sequenced region. Another possibility is that the observed species differing by less than 1% are actually the result of PCR amplification error. This is an underestimated problem that was recently investigated by Speksnijder et al. [25], who attributed microvariation in sequence clusters of environmental clone libraries to this type of artifact. We employed direct sequencing of DGGE bands in order to avoid some of these errors associated with cloning. More important was the fact that identical, closely related sequences were retrieved from both Octopus and Rabbit Creek mats, indicating that the sequence variations were real and not PCR artifacts. Our results are consistent with the conclusions of Ward et al. [24] that there are closely related ecotypes existing in the mat community. These species or strains may have adapted to slightly different growth conditions, such as different temperature ranges or chemistry, which ensures their success by filling a unique niche in the community. As an example, two strains of thermophilic *Synechococcus*, which have essentially identical 16S-rDNA sequences, differ from each other in that one lacks the ability to use nitrate and nitrite, and is therefore restricted to hot springs with high ammonium content while the other (strain C9) is not [26].

Although most of the sequences we determined were unique, one of the sequences we identified was a close match (99% similarity) to *Synechococcus* strain C9 (ATCC 700244). Ferris et al. [27] did not find the C9 strain in their molecular analyses of different temperature zones of the Octopus mat and to date this strain had only been detected as culture isolates from dilution enrichments of mat samples. Ferris and Ward [21] demonstrated that there was bias against amplification of the C9 sequence, using general bacterial primers. The reason we were able to detect C9 sequences from mat samples is probably due both to the specificity of our primers toward cyanobacterial sequences and that we were working at lower temperatures in the Octopus Spring mat, where C9 may be numerically more abundant. We have not done experiments to verify that C9 is a dominant community member at this temperature. However, Miller and Castenholz [28] determined that the optimal growth temperature of the culti-

vated strain of C9 is 45°C, and that growth does not occur above about 55–57°C.

Certainly, the community profile that is generated by DGGE is dictated by the choice of primers used. Although we did not sequence the Cyanobacteria bands from the higher temperature mat we did find a similar number of (presumed) cyanobacterial sequences as did earlier workers. Although the advantages of DGGE for describing microbial communities are numerous, this technique has inherent bias that must be taken into consideration when considering the data generated. Differences in the species retrieved by molecular and culture methods are also significant. In this case, two species were isolated from enrichment cultures from the low-temperature Octopus Spring mat. One was identical to a previously cultivated species from this mat, C1 type *Synechococcus*, and the other was only distantly related to a *Phormidium* species. However, neither was closely related to any of the species retrieved by DGGE. It is very clear that in order to get the most accurate picture of microbial communities as they exist in nature it is necessary to take advantage of the complementary information obtained from the combination of microscopy, molecular and culture methods.

4.2. Effects of UV on photosynthesis

Although the mat composition at each site, as measured by DGGE, appears to have remained stable (with a few minor changes) over the course of the summer and early fall, with or without UVR inclusion, there was an obvious effect of + or −UVR on the ability of the photosynthetic species to tolerate UV radiation during the short exposure photosynthesis experiments. In general, those maintained at high or even low UVR over the long term were better able to tolerate UVR during photosynthesis tests than those in which UVR had been absent (see Figs. 8 and 9). This was shown most prominently in the lower temperature Octopus Spring surface community (Fig. 8). Species of cyanobacteria exposed to long-term UVR (under UV/VIS-transmitting filter or in the open) over several weeks or months were presumably adapted to a fairly great extent to the ambient high solar irradiance of summer and early fall, although it appears from at least one other study that this is not a perfect adaptation; i.e. thermophilic cyanobacteria from their normal environment are still inhibited photosynthetically by the UV component of irradiance over most of the course of a summer day (see [5]). However, in the present study those photoautotrophs 'deprived' of UVR (and in some cases of high visible irradiance as well) for several weeks or months may have suffered a considerable loss of UV tolerance.

The loss of tolerance may be due to a change in gene expression. For example, it is known that *psbA* genes (*psbA2 and psbA3*) that code for a UVB-tolerant, PS II D1 protein may be induced in *Synechocystis* PCC 6803 under even low levels of UVB exposure [29,30]. Another study, using *Synechococcus* PCC 7942, has demonstrated that an ATP-dependent Clp protease is essential for acclimation to UVB, and results in a modified D1 PS II protein (D1:2) that is more resistant to UVB stress [31]. The level of this protein rose dramatically under low levels of UVB. In the present work the lower photosynthetic rates by cyanobacterial cells that had been completely protected from UVB (and UVA) radiation over a long period may reflect the loss of such adaptations or acclimations, perhaps a reversion to the *psbA* products typical of a low UVR environment. It is possible that these large differences in photosynthetic competence reflect changes at the level of gene expression in one or more predominant species, and do not reflect differences in species composition or abundance.

Numerous publications emphasize the inhibition and deterioration (e.g. of PS II) caused by UVB radiation in various species of cyanobacteria (see [1]). However, it should be pointed out that these studies were with laboratory strains of cyanobacteria that had not been exposed to UVR for innumerable generations. Natural populations of these and many other cyanobacteria are normally exposed to UVR on a daily basis and therefore should show at least partial adaptations to full solar irradiance. The laboratory strains used in most studies are probably those that have lost these attributes, at least temporarily. Thus, the 1–3-month UV-shielded populations of this study may be comparable in many ways to laboratory populations.

It would have been instructive to have measured the time course of recovery of photosynthesis of the −UV cells after re-exposure to full insolation, if indeed full or partial recovery would occur (see [32]). Logistic problems, however, prevented such tests during 2000. However, during the summer of 2001 the experiment with +UV and −UV filters at the low-temperature Octopus Spring site was repeated and similar results were obtained (data not shown). However, after 5 additional days of exposure of both mats to +UV, the photoincorporation rates were essentially the same, suggesting that recovery of normal UV tolerance had occurred.

It is well known that cyanobacteria grown under relatively low light, such as those in this study maintained under filters with triple screens, acquire higher cell contents of Chl *a* and phycobilins than those under high light [33,34,35]. It is also known that cells with high contents of these pigments (which act as photosensitizers) are more susceptible to photoinhibition (and UV inhibition) than those with lower contents [36]. Thus, it is to be expected that the screened cells (with ∼75% reduction in irradiance) would be more susceptible to photo- and UV-inhibition, and this is borne out by the results shown in Figs. 8 and 9. The higher Chl *a* content in all screened communities (and with lower 'carotenoid/Chl *a*' ratios) was a result not only of a loss or 75% reduction of UVR, but also of the 75% reduction in visible irradiance, since there was only a small difference in pigment ratios and color be-

tween those screened communities that included UVR and those with none (Table 2, Fig. 3). The lowering of the photosynthetic rates in the screened communities when exposed to full visible solar irradiance with or without UVR, relative to the rates of those maintained under high light or high light+UV is likely to be a result of photo-inhibition as well as UV-inhibition (Figs. 8 and 9). The greater inhibition of photosynthesis by high irradiance in the screened green mats is probably not only a result of a higher chlorophyll content per se, but also a result of the decreased 'carotenoid to Chl *a*' ratios. The untreated, lower temperature mats of these Yellowstone springs in summer are bright orange with a high 'carotenoid to Chl *a*' ratio (Table 2, Figs. 1 and 2). It is known that UVR exposure causes the disassembly of phycobiliproteins [37] which were in extremely low concentrations in these mats. This results in a loss of energy transfer to PS II and thus may be beneficial under photoinhibitory light intensities [38]. Carotenoids are well known to protect against high-light and high-UV damage in cyanobacteria, not so much through screening, but through their ability to intercept the triplet chlorophyll state, thus preventing the formation of singlet oxygen and other reactive oxygen species that are produced by UV and high visible irradiance, as well as by quenching singlet oxygen directly (see [6,36]). The content of thylakoid carotenoids relative to the sensitizing pigment, Chl *a*, is important. This relationship is highly regulated in cyanobacteria and in anoxygenic bacteria by light intensity. Protection by the passive UV shielding compound, scytonemin, was not involved in the mats of this study, and MAAs, if present, were in insignificant concentrations.

It seems that the lower temperature mats used in this study were already in a rather favorable condition to withstand the high insolation of summer at over 2200 m elevation, as a consequence of their high-carotenoid content. It should be pointed out again that the use of suspensions of the topmost layer results in averaging exposure of cells to radiation from the absolute surface to about 2 mm depth, but the orange summer mat is so translucent that even the lowermost cells in these layers receive high radiation. Thus, a comparison with an intact mat is not unreasonable.

The higher temperature Octopus Spring surface cells were much greener, and presumably tolerate UV and high light, to the extent that they do, by possessing efficient repair and resynthesis capabilities, which are probably used most efficiently during periods of darkness or low light (see [5]).

Essentially all of the photoincorporation of inorganic carbon in this study must have been by cyanobacteria. The only other phototrophs present would be bacteriochlorophyll-containing *Chloroflexus* or relatives of this genus which are primarily photo- and chemo-heterotrophs which show little incorporation of inorganic carbon in these waters. *Chloroflexus* as a photoautotroph requires sulfide (or H_2) as electron donor [39,40,41], and the soluble sulfide levels downstream in the low-temperature Octopus Spring are very low and then only below 3 mm depth in the mat [42]. Thus, photoautotrophic green sulfur bacteria and purple sulfur bacteria would not be expected, except as minor components. More importantly, spectroscopic scans of pigment extracts showed little or no evidence of bacteriochlorophylls in the surface biofilms used for the photosynthesis experiments (data not shown). Microscopic examination revealed no eukaryotic algae in the samples.

4.3. Comparisons with other long-term studies employing UV exclusion

Brenowitz and Castenholz [11] found that nearly monospecific and stable biofilms of the cyanobacterium, *Calothrix* sp., in a tepid spring in Yellowstone National Park showed little apparent change in species composition or in photosynthetic competence after 3 months of UVR exclusion. However, this species had a high sheath content of the UV-shielding pigment, scytonemin, which remained in these UV-protected populations throughout the experimental period. No molecular analyses were included in this study.

As mentioned briefly in Section 1, Sheridan [8] observed large differences in species arrangement and abundance when epiphytic cyanobacterial mats from tropical mangrove communities were subjected to long-term +UV and −UV treatments (i.e. 27 days with laboratory UV-B+UVA+PAR, or UVA+PAR, or PAR only). The top layer of scytonemin-containing *Nostoc commune* and *Scytonema* sp. (both fix N_2) were maintained (as in the natural mats) under the treatment that included UVB and UVA. When UVR was excluded, the more UV-tolerant *Nostoc* was over-run or overgrown by a less UV-tolerant species of *Phormidium* which was not capable of N_2-fixation. Under the latter treatment overall N_2-fixation decreased greatly. As may now be seen, no dramatic changes of the sort observed by Sheridan occurred in the present study.

Another study [43] was somewhat similar to ours, although it was done in Antarctica at $\sim$78° S. Lat. These investigators covered mats under water in several Antarctic ponds with UV-transparent and UV-opaque filters and looked for changes in species composition, pigments, MAAs, and photosynthetic capability after 3–4 weeks of treatment. Essentially no differences were found among the various treatments. No molecular analyses were included. As in our lower temperature mats, the mats were compact assemblages composed primarily of filamentous, non-heterocystous cyanobacteria. In Antarctica 3–4 weeks of treatment was probably too short a period in which to expect significant changes, especially at the low temperatures (<8°C) of these ponds.

In contrast to the strictly microbial food web of these

and the present studies, the long-term (3–5 weeks) influence of UVR on a complex lotic community colonizing artificial substrate was studied by Bothwell et al. [44,45]. In this community composed of several trophic levels, many changes occurred with enhanced UVR. Although both UVA and UVB could reduce photosynthesis and growth of the benthic diatoms, greater amounts of diatoms accumulated with enhanced UVB. Some of the results were explained by the greater sensitivity of chironomid larvae to UVB, which otherwise exhibited a great grazing pressure on the diatom community. However, it is probable that the +UV treatment would be closer to the natural condition in the field.

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