Effects of Environmental Stressors on Photosynthetic Microorganisms in Geothermal Springs of Yellowstone National Park

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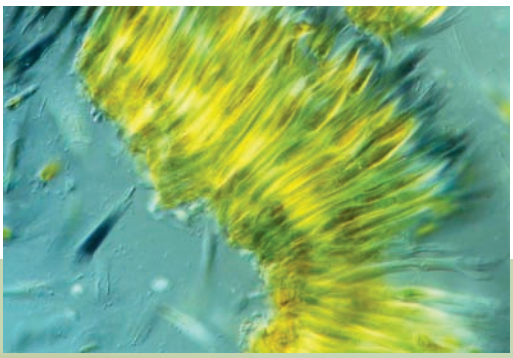


ABSTRACT

Much of the work by R.W. Castenholz and colleagues over the past 6-7 years has involved cyanobacterial communities in Yellowstone National Park or cultures of photosynthetic microorganisms obtained from the Park. The primary focus of these efforts was to elucidate the effects of UV radiation on cyanobacteria—both those protected by the UV-shielding pigment, scytonemin, and others that depend on more active metabolism to repair UV and high light damage. Results have demonstrated that the lower temperature (<35°C) cyanobacteria (i.e. *Calothrix* spp.) that possess scytonemin in the extracellular sheath show little negative effect of exposure to full solar radiation that includes UV. The degree of UV protection directly correlates with scytonemin content, and adverse field conditions appear to promote higher levels of this protective pigment. Other communities of *Synechococcus* and *Leptolyngbya*-like cyanobacteria at higher temperatures (40-47°C) contained no scytonemin or mycosporine-like amino acids. Long-term (2-3 months) exclusion of UV radiation showed little recognizable change in species composition in these communities (as measured by molecular methods), but the UV-minus cyanobacteria were far less competent photosynthetically when exposed to full solar irradiance than those that had continuously been exposed to UV. In another study, the high temperature *Synechococcus* biofilms (68-74°C) were increasingly inhibited by UV and visible solar irradiance during the course of a clear summer day, particularly at supraoptimal (70°C) and suboptimal temperatures (55°C). However, these populations (also lacking protective compounds) recovered by the following morning, presumably by overnight repair processes.

Key Words

Calothrix
cyanobacteria
denaturing gradient gel electrophoresis
microbial mat
scytonemin
Synechococcus
ultraviolet radiation

1.0 INTRODUCTION

Much of what is known about the microbiology of phototrophs and other microorganisms in hot springs of Yellowstone National Park (YNP) has come from the pioneering work of Thomas D. Brock and co-workers (Brock 1978) and from the laboratory of David M. Ward (e.g. Ward and Castenholz 2000; Ward et al. 2002). Most recently, the establishment of the Thermal Biology Institute (TBI) at Montana State University has helped to increase the research efforts in YNP, particularly interdisciplinary research.

Richard W. Castenholz and associates have also conducted research on phototrophs in YNP. They have contributed comparative studies with hot spring habitats in other regions, such as Oregon (e.g. Castenholz 1973a, 1996; Miller and Castenholz 2000), and have also established a culture collection of thermophilic cyanobacteria from many regions of the globe (**http://cultures.uoregon.edu**). Although some of the past research in Yellowstone has involved the effects of sulfide on cyanobacteria and anoxygenic *Chloroflexus* relatives (Castenholz 1973b, 1977; Giovannoni et al. 1987), work completed over the past 6-7 years has focused on the effects of ultraviolet (UV) radiation on cyanobacterial communities. These investigations have examined a number of different strategies for living under the stress of high solar UV radiation and the interaction between temperature and UV stress.

The anomaly of different microorganisms using different primary strategies for UV tolerance or avoidance is greatly influenced by other innate properties of the particular organism. For example, the UV-shielding pigment scytonemin occurs only as an extracellular compound in the sheaths of cyanobacteria (Garcia-Pichel and Castenholz 1991). However, no sheathed cyanobacteria are known to occur in geothermal habitats above ~45-50°C or grow in culture above 50-55°C (Wickstrom and Castenholz 1978). Therefore, no scytonemin for passive UV protection occurs in cyanobacteria that grow at higher temperatures. Another innate property of the sheathed cyanobacteria is a relatively slow growth rate (at least in culture and apparently in the field as well) that might also necessitate the use of the passive UV-shielding pigment as the predominant type of defense (e.g. as in *Calothrix* spp.). Thus, cyanobacteria that grow at temperatures above 45-50°C must rely on other means for withstanding the stress of UV radiation prevalent during days of high solar irradiance in most hot spring habitats. Such cyanobacteria (often also devoid of UVB mycosporine-like amino acids) must survive by establishing relatively high contents of carotenoids known to quench forms of reactive oxygen species that are produced with excess irradiance (e.g. in *Phormidium/Leptolyngbya* spp.), or by maintaining a very active metabolism capable of repairing daytime damage caused by high UV and visible solar radiation as evidenced in *Synechococcus* spp. (Castenholz and Garcia-Pichel 2000). Other thermophilic and mesophilic cyanobacteria survive by an active gliding motility response causing a move downward into soft microbial mats or sediments with increasing irradiance, thereby avoiding the stress almost entirely (Castenholz 1968; Richardson and Castenholz 1987; Kruschel and Castenholz 1998).

Much of the work over the past 6-7 years on Yellowstone cyanobacterial communities and Yellowstone-derived cyanobacterial cultures has helped to differentiate strategies of UV tolerance, and these will be reviewed here, beginning with the conditions and possible advantages of scytonemin in nearly monospecific populations of *Calothrix* sp. in tepid springs, followed by the *in situ* demonstration of long-term stability of somewhat higher temperature carotenoid-rich cyanobacterial communities dominated by *Leptolyngbya* under summer solar irradiance with or without the UV component. Finally, the interaction of temperature, and high UV and visible radiation, on a high temperature population of *Synechococcus* will be reviewed with results that suggest dark repair of UV damage is necessary for population stability.

2.0 SCYTONEMIN'S ROLE IN UV PROTECTION OF *CALOTHRIX*-DOMINATED MATS

2.1 Method for Evaluation of Photosynthetic Performance

Throughout these studies a [^{14}C]-photoincorporation assay was used to evaluate photosynthetic performance of cyanobacteria subjected to various experimental

treatments. Field samples were transported to a research trailer located in West Yellowstone, MT, and processed immediately. Inoculum from mats was first uniformly dispersed by gentle homogenization in native spring water. Aliquots of the cell suspensions were then dispensed into nearly UV-transparent WhirlPak™ bags, and [^{14}C]$NaHCO_3$ was introduced to achieve a final activity in the range of 1.5 kBq (0.04 μCi). Samples were incubated for one hour in constant temperature water baths outdoors in natural light. Experiments were usually performed at midday when measured ambient irradiance was at its peak. Following incubations, the uptake of radiolabeled carbon was terminated by the addition of formalin. Samples were processed by filtration onto membrane filters, removal of excess radiolabeled [^{14}C], and scintillation counting. Dark controls were always included.

The experimental design often included the use of UV-modifying filters. The filter sheets were attached to rectangular wooden frames with adjustable swivel legs such that the filter could be placed roughly 2-3 cm above a geothermal stream for acclimation treatments. Three filter types were used. All of the filters transmitted about 90% of the visible and infrared portion of the spectrum. In addition, one filter type allowed the transmission of UVA and UVB, one allowed the transmission of UVA but not UVB, and one blocked both UVA and UVB. A description of spectral properties of filters is given in Dillon et al. (2003). In some cases neutral density screens were also added to filters to decrease the total irradiance. UV-modifying filters were also used during the photoincorporation assays by placing them over water baths during the incubation period.

2.2 Scytonemin as a UV-Screening Pigment

The first study to examine the role of scytonemin for UV protection in natural geothermal populations of cyanobacteria was undertaken by Brenowitz and Castenholz (1997) at Potts Hot Spring Basin, on the West Thumb shore of Yellowstone Lake (**Figure 1**). Geothermal streams in this area are populated by a nearly monospecific cyanobacterial mat at temperatures below ~35°C. The cyanobacteria that form these mats belong to the genus *Calothrix*, a moderately thermotolerant, filamentous cyanobacterium with scytonemin-containing sheaths. The *Calothrix* forms a biofilm comprised of from one to a few layers of upright filaments, and is tightly adherent to the siliceous substrate of the shallow outflow streams (**Figure 2**). The purpose of this work was to evaluate the extent to which UV radiation affected scytonemin production in a natural setting, and the extent to which the presence or absence of UV affects photosynthetic performance. In this study, UV-modifying filters were placed over the West Thumb outflow stream for an acclimation period of three months during the summer. One treatment also included mat that had been cleared of *Calothrix* to study recolonized populations. It was found that these recolonized mats accumulated high levels of scytonemin under all light treatments except when UV radiation was

Figure 1. *Calothrix* mat in a tepid stream of the Potts Basin (West Thumb area of Yellowstone Lake). The dark brown color is a result of the scytonemin in the sheaths of the filaments.

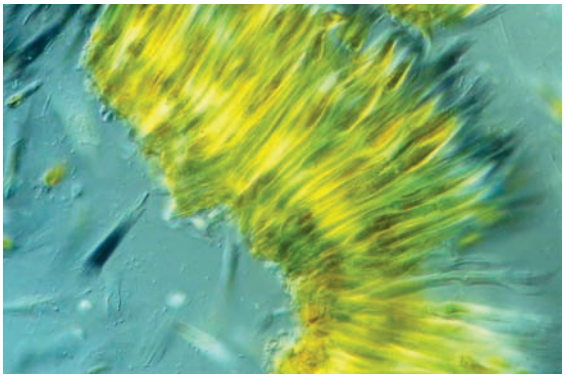


Figure 2. Photomicrograph of a *Calothrix* layer peeled from the siliceous substrate in the same stream as in Figure 1. The yellow color is due to the scytonemin in the extracellular sheaths. The left side of the strip is the basal side that was attached to the rock. Heterocysts may be seen forming the basal cell of a few filaments. The width at the base of most filaments is 4-5 µm.

excluded, thus pointing to the UV portion of the spectrum as the primary inducer of scytonemin production (Figures 4 and 5 in Brenowitz and Castenholz 1997). Earlier results showed that UVA was the principal wavelength-inducing scytonemin synthesis in all cyanobacteria tested (Garcia-Pichel and Castenholz 1991). Radiolabeled carbon photoincorporation experiments showed no inhibition of photosynthesis under any acclimation treatments except for *Calothrix* mat that was a result of recolonization under low visible and low UV irradiance (Figures 5d and 9c in Brenowitz and Castenholz 1997). As a passive protectant, scytonemin appears to remain in the extracellular sheath without being degraded. It follows then, that in the natural setting, low levels of scytonemin would only be found in new growth that had not yet been exposed to seasonally high levels of visible and UV irradiance.

2.3 How Natural Populations of *Calothrix* Respond to UV Radiation

Dillon and Castenholz extended work on scytonemin containing *Calothrix* mats by investigating the possibility that natural populations are phenotypically adapted to their environment (Dillon et al. 2003). This work was initiated as a result of observations of the Rabbit Creek hot spring outflow stream, which also contained monospecific *Calothrix* mats in the 30-35°C temperature range. The Rabbit Creek mats differed from West Thumb mats in that they were composed of longer filaments and formed thicker layers on the substrate. Also, Rabbit Creek mats were reddish brown to yellow green in color compared to the West Thumb filaments (**Figure 1**), which were dark brown. This coloration difference was due to considerably higher scytonemin content in the West Thumb filaments, and higher carotenoid and chlorophyll *a* content in the Rabbit Creek cells. Despite these obvious phenotypic differences, the two strains were found to be genetically identical over 900 bp of the 16S rDNA sequence, which prompted the hypothesis that each population was uniquely acclimated to its environmental conditions.

UV modification experiments were designed to test whether these two natural populations were similarly able to alter their phenotype in response to the presence or absence of UV stress. Acclimation treatments were performed in which natural mats were subjected to modified irradiance regimes by placing filters over the mat for periods of a few days to two months. Following acclimation treatments, photosynthetic ability was tested to compare the various treatments (**Figure 3, next page**). The photoincorporation assay also included filter treatments during the incubation period to assess the effects of UV radiation on photosynthetic performance. Contrary to the predicted outcome, there was no significant effect of acclimation for any of the treatments after two months. Also, there were no significant pigment changes during the acclimation period, again demonstrating the stability of scytonemin. The most significant outcome was the difference in photosynthetic rates between the two populations. The West Thumb population, which has a much higher scytonemin content, had lower photosynthetic rates than the Rabbit Creek population (Dillon et al. 2003). However, there was significant UV inhibition of photosynthesis as inferred by observed increases in [^{14}C]-photoincorporation when UVA/B was excluded. This inhibition was not significant for the West Thumb population, with its high scytonemin content (**Figure 3**).

2.4 Genetic and Environmental Factors

Additional experiments were conducted with two cultured *Calothrix* strains to further elucidate the potential

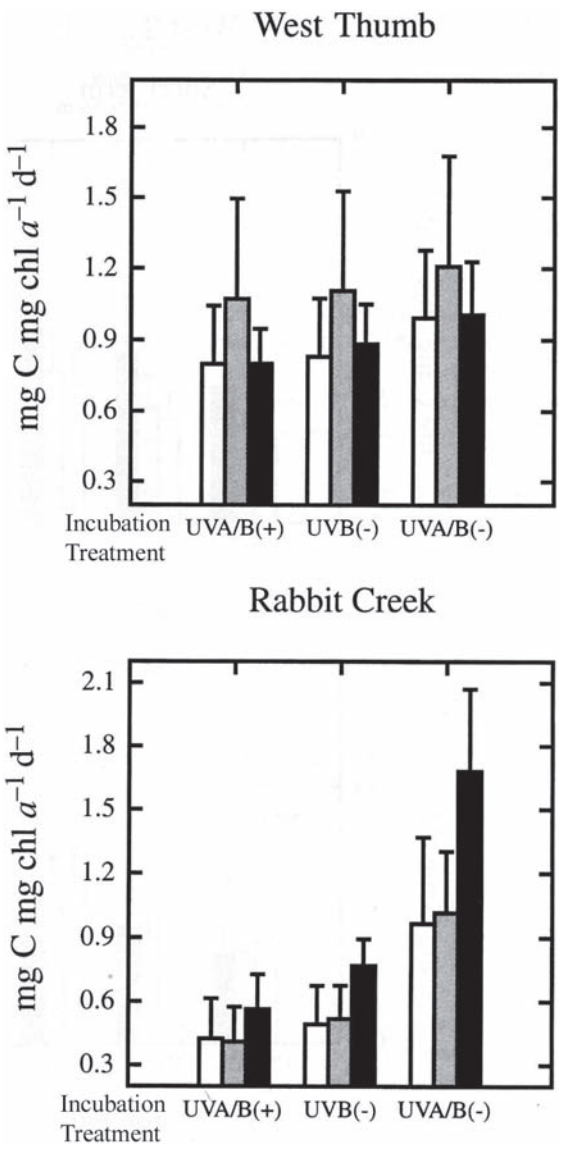


Figure 3. Photoincorporation of [^{14}C] bicarbonate for West Thumb (upper panel) and Rabbit Creek (lower panel) *Calothrix* populations, all acclimated for 3 days with UVA/B+ (open bars), UVB– (gray), or UVA/B– (black). All treatments were under natural solar irradiance with or without the UV components. Acclimated filaments from each of the acclimation conditions were then exposed to a 1hr [^{14}C]- incubation under all three conditions. Labels underneath indicate experimental photoincorporation treatment. Dark incorporation has been subtracted. (Dillon et al. 2003).

contributions of environment and genetics on scytonemin production (Dillon and Castenholz 2003). Culture isolates from the two streams were used in laboratory experiments to compare scytonemin production. Genetic differences between the two strains were confirmed by the fact that the Rabbit Creek strain does indeed produce scytonemin in laboratory culture, but not as much as the West Thumb strain under identical conditions.

A more extensive investigation into these issues was provided by reciprocal transplant experiments. In these experiments culture isolates from each stream were used to grow lawns of firmly attached filaments on agar plates under laboratory illumination. These plates were then placed in both streams for an incubation period before testing the photosynthetic ability of the cells. The result was that the two streams affected both photosynthesis and pigment content differently. In particular, the West Thumb stream water enhanced scytonemin production in both strains more than the Rabbit Creek stream water, although scytonemin production was greater for the West Thumb strain in both streams. In fact, the outplanted Rabbit Creek strain died in West Thumb stream water after 7-8 days. In terms of photosynthetic ability, both strains performed better when incubated in Rabbit Creek, although each strain exhibited an advantage in its native stream (**Figure 4**). Aqueous chemical analysis of major ions did not reveal differences in nutrients or levels of toxic substances that could account for these differences (Dillon and Castenholz 2003). However, the data support the existence of some as yet unidentified toxic property of the West Thumb stream that is inhibitory toward photosynthetic performance and growth. The fact that both cell types altered their pigment content and photosynthetic performance depending on which stream they were transplanted into confirms the ability to adapt physiologically in response to the local environment. Of broader significance is the implication that other environmental factors besides light can influence production of protective pigments, suggesting scytonemin production could be part of a general stress response pathway.

The result of two cell populations behaving differently under identical conditions in the natural setting is further evidence that genotypic factors are also important for

the phenotypic differences between these two strains. This work has provided preliminary evidence that the cyanobacterial response to UV is under genetic control, and provides a starting point for further physiological UV/stress response studies. An obvious additional step would be to make further genetic comparisons between these closely related populations with obvious differences in their UV response. The two strains are identical at the 16S rDNA sequence level (900 bp sequenced), suggesting that 16S rDNA analysis alone is not sufficient to resolve genetic differences. This type of discrepancy is not unique to our studies, and as a general observation has raised a host of new questions among microbial ecologists regarding bacterial species concepts, concurrent with a burgeoning of new phylogenetic methods to address such problems. In studies such as these, the ability to resolve phenotypic differences between closely related strains of cyanobacteria will certainly require a polyphasic approach that includes both genetic and physiological methods.

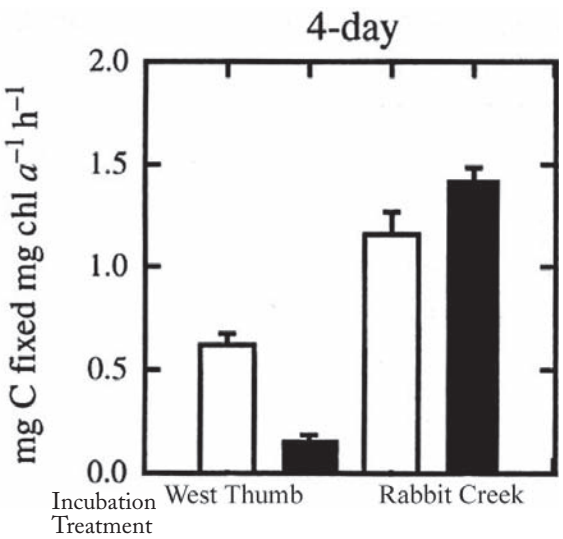


Figure 4. Photoincorporation of [^{14}C] bicarbonate in *Calothrix* cultures outplanted on agar plates reciprocally for 4 days in both the West Thumb stream and Rabbit Creek. Open bars depict the West Thumb culture; black bars depict the Rabbit Creek culture. The 1 hr [^{14}C] incubations were performed at 30°C in West Thumb water and Rabbit Creek water under full solar irradiance (~ 800 W m^{-2} visible, ~ 35 W m^{-2} UVA, and ~3 W m^{-2} UVB). (Dillon and Castenholz 2003).

3.0 EFFECT OF UV EXCLUSION ON CYANOBACTERIAL MATS

3.1 Changes in Community Composition in Response to UV Treatments

It is likely that ultraviolet radiation not only impacts phenotypic responses of individual cells, but also plays an important role in structuring the hot spring mat community. This includes both species richness and relative abundance. Evidence of UV radiation as a factor for structuring mat communities was advanced in a laboratory experiment by Sheridan (2001) in which aerial microbial mats of black mangroves required the UV component of irradiance in order to maintain the mat structure found in the natural habitat. In sharp contrast, however, Ferris and Ward (1997) did not observe significant seasonal variation in the community structure of the Octopus Spring mat using 16S rDNA analysis and denaturing gradient gel electrophoresis (DGGE), even though winter light intensity at that location is 25% of that in summer. Our goal was to discover whether UV played a role in structuring these lower temperature microbial mats that did not contain scytonemin or mycosporine-like amino acids.

Recent advances in molecular methods have made the study of whole microbial communities a more tractable problem. Among these methods, DGGE is one of the most useful techniques for tracking changes in microbial community structure. In this method, the sequence of a particular gene (usually 16S rDNA gene) is PCR amplified from a pool of template DNA. Differences in the base composition of individual amplicons allow them to be separated in a gel with a gradient of denaturant. According to convention, each band on the gel corresponds to a unique 16S rDNA sequence. For further details on DGGE methodology see Muyzer et al. (1993).

We used DGGE to study the effects of the exclusion of UV radiation on community composition in hot spring microbial mats. The two sites used in this study were 40-47°C reaches of thermal streams in the Rabbit Creek and Octopus Spring areas (**Figure 5, next page**). As described for previous experiments, UV-modifying filters were placed over the mats for 1-3 months. Samples were taken periodically for molecular analysis. Surveys of community

Figure 5. Lower temperature Rabbit Creek mat with filter setups. Orange mat color is typical of 40-47°C reaches of alkaline springs in YNP during summer and is due to the high carotenoid content of the cyanobacteria forming the mat. (Norris et al. 2002).

composition using mat core samples and bacteria-specific primers were not very informative because DGGE patterns were excessively complex. Consequently, further analysis was restricted to the photoautotrophic members of the community by limiting sampling to the upper 1-2 mm of the mat and using cyanobacteria-specific primers (Nübel et al. 1997).

It is logical to assume that the major changes in community structure would be observed in the upper layers of the mat to the depth that light and UV radiation are able to penetrate. Changes in the community structure following light treatments were not as dramatic as expected (**Figure 6**). After 40-65 days of treatment, the major changes in the Octopus Spring mat were the decrease in intensity or disappearance of a few bands in treatments where UV was excluded. The effects were most obvious in treatments that also included a reduction in total light reaching the mat. In contrast, no apparent changes were discernible in the Rabbit Creek mat, even after 65 days of treatment. Bands were purified from DGGE profiles and sequenced to learn more about the identity of community members. With one exception, the band sequences were only 89-92% identical to known sequences available in the GenBank database. The exception was a sequence that was 99% identical to a *Synechococcus* strain (C9) retrieved from Octopus Spring by Ferris et al. (1996). Sequence information did not give us much insight into the physiological basis for the changes we observed in community structure. Some effort was directed toward isolation of cultures from the mat community, but unfortunately the 16S rDNA sequence of the two cultivated strains did not correspond to sequences from our molecular analysis.

3.2 Changes in Photosynthetic Competence in Response to UV Treatments.

To measure the physiological changes associated with modified UV radiation treatments in the non-scytonemin-containing mat communities, photosynthetic carbon fixation experiments were conducted as described earlier. As in previous studies, the experiments included a UV-filter treatment for both a prolonged acclimation period in the field and also during the short photoincorporation assay. In contrast to the scytonemin-containing microbial mats, here there was a pronounced effect of UV exclusion during the prolonged acclimation period.

For the low temperature Octopus Spring mat, a one to three month acclimation that included UV radiation resulted in better photosynthetic performance than acclimation without UV radiation (**Figure 7**). The addition of neutral density screens to reduce total light followed the same trend but with less total photoincorporation. This implies that the UV component of irradiation is required to maintain cells in a state that is best adapted physiologically to UV stress. It is likely that UV radiation is a signal for changes in gene expression that ameliorate UV stress. Such changes in gene expression are likely to involve modifications of the photosystem proteins and/or UV repair systems. The results from the Rabbit Creek mat were similar, although

not as dramatic. It seems then, that the modification of UV radiation mainly results in changes at the level of gene expression rather than changes in community structure. Microbial mat communities such as these are probably very stable in terms of community composition, and the UV-adapted populations are probably not very easily displaced. In fact, an experiment by Ferris et al. (1997) on the high-temperature Octopus mat demonstrated that a very significant disturbance of the mat is required to initiate changes in community structure.

4.0 EFFECTS OF HIGH SOLAR IRRADIANCE AND HIGH TEMPERATURE STRESS

4.1 Optimal Temperature for Photosynthesis

Temperature is another obvious stressor encountered by photosynthetic hot springs microorganisms. The question of the upper temperature limit for life in general, and photosynthetic life in particular, has been pondered since the earliest work on hot springs. In Brock's pioneering work (1967, 1968) it was noted that there was a positive correlation between the mean temperature of collection and optimal temperature for photosynthesis of *Synechococcus* sp. collected from Mushroom Spring. This led Brock to the conclusion that the optimal temperature for photosynthesis and growth matched the mean environmental temperature, including the *Synechococcus* biofilms at 70-72°C. However, Meeks and Castenholz (1971) found that a cultured *Synechococcus* strain from Hunters Hot Springs in Oregon

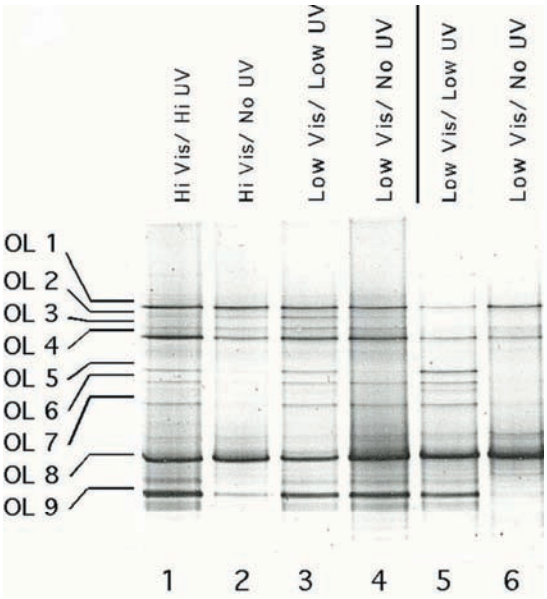


Figure 6. DGGE profiles of Octopus Spring (40-47°C) mat communities subjected to different UV and visible light regimes. Cyanobacteria-specific primers were used in the PCR amplifications of mat surface layer. Prominent bands (OL series) were isolated and sequenced: lanes 1 and 2 after 40 days of acclimation; lanes 3 and 4 after 19 days; lanes 5 and 6 after 65 days of acclimation. The acclimation conditions are indicated on the top of each lane. (Norris et al. 2002).

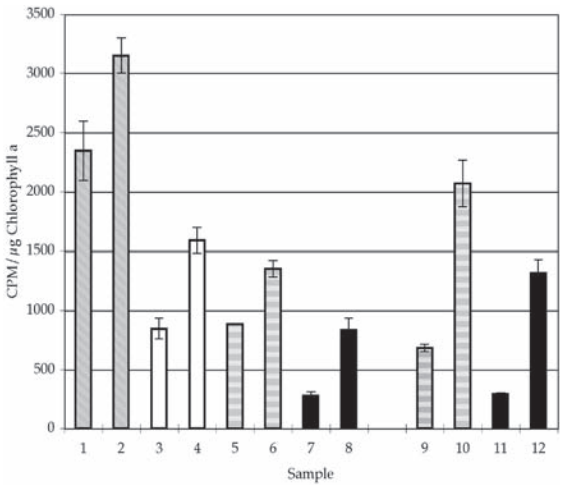


Figure 7. Photoincorporation of [^{14}C] bicarbonate for 60 min at 40-42°C by the surface layer of the low temperature mat community of Octopus Spring that had been acclimated as follows: dark gray bars = high visible/ high UV; white bars = high visible/no UV; light gray bars = low visible/low UV; black bars 5, 6 = low visible/no UV. The acclimation periods varied (bars 1-4: 40 days; 5-8: 19 days; 9-12: 65 days). Photoincorporation was measured under two conditions (high visible/high UV – odd numbered bars 1-11; high visible/no UV – even numbered bars 2-12). Error bars indicate standard deviation of the mean (n=3). All photoincorporation values are with dark rates subtracted (<10% of light values). The p values for comparing the following pairs were significant at <0.0001: column 1 vs 2, 1 vs 3, 2 vs 4, 3 vs 4, 5 vs 7, 7 vs 8. A comparison of 5 vs 6 was significant at a p value of 0.0002. In the September experiment (columns 9-12) the p values for the following pairs were significant at < 0.0001: 9 vs 10, 10 vs 12, and 11 vs 12. 9 vs 11 was significant at p = 0.0011. (Norris et al. 2002).

had a significantly lower temperature optimum for growth and photosynthesis than the highest environmental temperature of collection when tested in the laboratory. Miller et al. (1998) recently revisited this question in a study with the high temperature *Synechococcus* sp. of Octopus Spring (**Figure 8**). [^{14}C]-photo incorporation assays were performed using material collected from the biofilm at a mean temperature of ~70˚C. The assays were conducted at three temperatures: 55˚C, 65˚C, and 70˚C. The highest photosynthetic carbon uptake rates were seen at 65˚C (**Figure 9**), confirming that in nature some cyanobacterial populations survive under conditions of chronic temperature stress.

4.2 Interaction of Temperature and Irradiance

In the natural setting, cyanobacteria must simultaneously cope with the stress of both temperature and irradiance. The experiments performed by Miller et al. (1998) were designed to investigate the interaction between temperature and irradiance. Carbon uptake assays were conducted under a series of irradiances (in addition to multiple temperatures) by adding neutral density screens to the UV-modifying filters under which cells were incubated. A significant effect of irradiance was found in that there was a plateau in the carbon uptake rate at a value above ~ 400 Wm^{-2} (**Figure 9**). Additionally, statistical analyses demonstrated a significant interaction between temperature and irradiance. Below the saturating irradiance, the temperature dependence of carbon incorporation increased linearly with irradiance; above the saturating irradiance the temperature dependence reached a plateau. The most striking result shown in **Figure 9** is that temperature was a stressor at 70˚C (supraoptimal) and at 55˚C (suboptimal), and that 65˚C was close to optimal, although cells originated from a biofilm at ~70˚C.

Figure 8. View of one of the two outflow streams of Octopus Spring. The yellowish biofilm in the foreground is composed of *Synechococcus* sp. (a unicellular cyanobacterium). The water temperature over this biofilm varied from 67°C to 74°C.

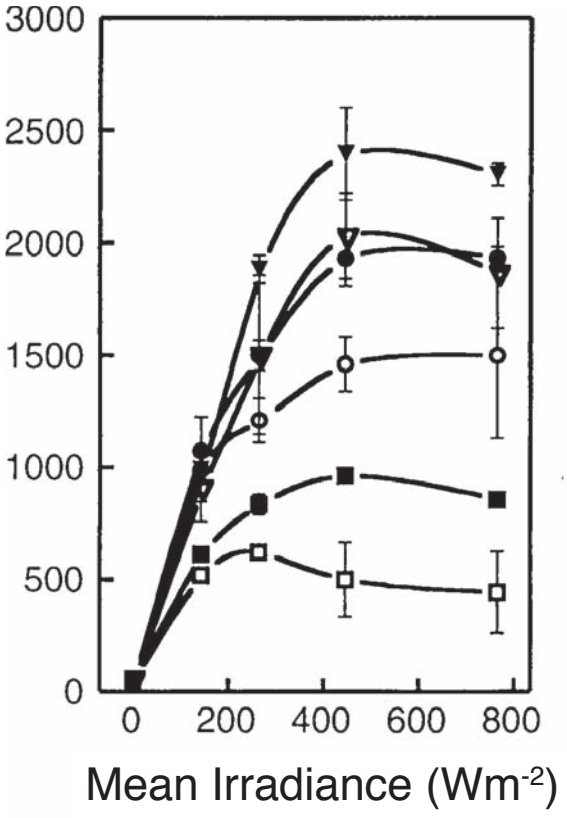
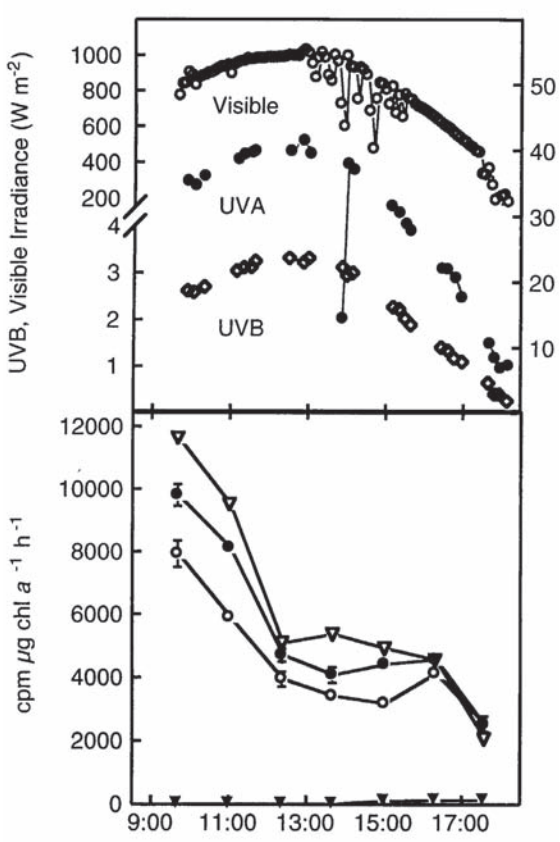


Figure 9. Photoincorporation of [^{14}C] bicarbonate (normalized to chlorophyll *a* content) of "high temperature" *Synechococcus* sp. for 60 min under different temperatures and irradiance levels of visible light and UV in outdoor, filtered constant temperature baths. The values on the ordinate are cpm µg^{-1} chl *a*. ▼ = 65°C, UV– ; ▽ = 65°C, UV+; ● = 70°C, UV– ; ○ = 70°C, UV+; ■ = 55°C, UV– ; □ = 55°C, UV+. (Miller et al. 1998).



↑ **Figure 10. Photoincorporation for 40 min of the "high temperature" *Synechococcus* sp. at ~ 67°C over the course of a day in summer, with and without UV (lower panel).** All cells were newly collected for each experimental time point. ∇ = UVA/B–; ● = UVB–; □ = UVA/B+. The diurnal course of visible irradiance, UVA, and UVB radiation (upper panel). Ordinate scale in Wm^{-2} for visible (upper left), UVA right ordinate, UVB lower left. (Miller et al. 1998).

4.3 UV Inhibition of Photosynthesis in High Temperature Forms of Cyanobacteria

The effects of UV on high temperature *Synechococcus* were also considered. During the photoincorporation assays, there was some inhibition by UV at all temperatures tested, as measured by comparisons of incubations with and without UV (**Figure 9**). However, there was no significant dependence of UV inhibition on temperature. The most obvious UV effect was related to the diurnal pattern of photosynthesis. Photoincorporation assays performed at intervals throughout the daylight hours at the Octopus Spring field site show a pattern of decreasing photosynthetic rate from a morning peak, to a plateau that coincides with the daytime peak of irradiance, followed by a late afternoon decline in productivity (**Figure 10**). New cells were collected for each time point. In these experiments there was also a significant UV inhibition effect, although this inhibition disappeared in late afternoon with declining irradiance. Presumably, UV inhibition may not be important unless light is at saturation level or higher. Since photosynthetic rates were highest in the morning, it is presumed that photosystem damage that may accrue during the course of a bright day in this natural population would be repaired during periods of low light or darkness.

5.0 ADDITIONAL STRESSORS FOR PHOTOSYNTHETIC MICROORGANISMS

Two additional stress factors that are of current interest to our collaborators and to us are that of desiccation/freezing tolerance and low pH.

Travertine terraces have been deposited by hot springs in Yellowstone from as early as 365,000 years ago to the present. Many of these $CaCO_3$ rocks contain a 1-2 mm-thick greenish band about 1-3 mm below the surface. These bands are composed of cyanobacteria and, sometimes, unicellular green algae. Desiccation and freezing are important stresses faced by these cryptoendolithic microorganisms. We are using molecular- and culture-based methods to survey cryptoendolithic community diversity and to investigate physiological aspects of desiccation and freezing tolerance.

The eukaryotic alga *Cyanidium* and its relatives (*Galdieria* and *Cyanidioschyzon*) are the only photosynthetic microorganisms that grow in acid environments (pH 0.5-3.5) at temperatures from about 40°C to 57°C. We are currently involved in characterizing the genetic variation among numerous strains from various acid springs and soils in Yellowstone as well as other geographic regions. One of our goals is to correlate genotypes of these "cyanidial"

unicells with geographic location (local and global) and with the specific chemistry of these habitats. This polyphasic approach allows us to assess the physiological tolerance of various strains to the concentrations of various metals and metalloids (e.g. Hg, Al, Cu, Fe, As) that vary greatly among Yellowstone habitats.

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