THE EFFECTS OF TEMPERATURE AND OXIDATIVE STRESS ON THE SYNTHESIS OF SCYTONEMIN IN THE CYANOBACTERIUM *CHROOCOCCIDIOPSIS* M88-Vd-G(1)

by

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Scytonemin, an extracellular screening pigment, protects some cyanobacteria against UV wavelengths. Scytonemin synthesis is induced by exposure to blue light and UVA irradiance. The effects of other environmental stresses, namely temperature and oxidative stress, on the synthesis of scytonemin are unknown. In the temperature experiments, *Chroococcidiopsis* M88-Vd-G(1) cells were exposed to a range of temperatures at two different irradiance treatments. In the oxidative experiments, the cells were exposed to a range of concentrations of the oxidative reagent, methylene blue, under high irradiance. Temperature did not induce scytonemin under moderate irradiance. However, under high irradiance, total scytonemin content was higher at the highest temperature (35° C). Intermediate levels of oxidative stress caused the highest level of scytonemin accumulation. These findings indicate that scytonemin synthesis may be the result of a general stress response.

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To my parents, Ronnie and Mila,

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INTRODUCTION

It is well documented that ultraviolet radiation (UVR) is damaging to microorganisms (O'Brien and Houghton, 1982; Mitchell and Karentz, 1993). Scientific and public concern has increased over these negative effects due to the ongoing depletion of the ozone layer over both Antarctica and the high latitudes of the northern hemisphere. This depletion has resulted in an increase of harmful UVR reaching the Earth's surface (Madronich, 1992). UVR has both direct and indirect effects on all organisms, increasing in its toxicity as the UV wavelength decreases. UVB (280-320 nm) directly damages DNA, RNA, and proteins (Vincent and Quesada, 1994). UVA (320-400 nm) is associated with the production of reactive oxygen species (ROS) which results in DNA lesions (Dahl et al. 1987; Keyer et al. 1995). The effects of UV damage are especially harmful to photoautotrophs because they must expose themselves to damaging UVR when using solar irradiation to drive photosynthesis.

Cyanobacteria, also known as blue-green algae, are photosynthetic members of the domain Bacteria. They are widely distributed throughout nature in terrestrial, freshwater, and marine habitats. They frequently exist in extreme environments where they are subjected to unfavorable stress conditions such as heat, desiccation, and salt stress. They originated very early in planetary history, ~3.5 billion years ago, during a time when oxygen did not exist in the atmosphere (Schopf, 1996). Without oxygen to form an ozone layer, the amount of UVR reaching the earth's surface was much higher at that time. Cyanobacteria had to evolve mechanisms to survive which include motility, the ability to repair UV damage, the production of agents to quench ROS, and the ability to produce UV-screening pigments (Quesada and Vincent, 1997). These mechanisms are still found in modern cyanobacteria.

Scytonemin is an extracellular sheath pigment that is synthesized in some cyanobacteria upon exposure to high blue light and UVA conditions. It is a dimeric molecule of indole and phenolic subunits that absorbs strongly in the ultraviolet portion

of solar irradiance with peaks in the UVA (~384 nm) and UVC (~250 nm) regions (see Fig.1) (Proteau et al. 1993). Scytonemin acts as a UV sunscreen reducing the amount of UVA reaching cells by 90% (Garcia-Pichel and Castenholz, 1991). Scytonemin is very stable and serves as a passive screen even when cells are metabolically inactive (Garcia-Pichel et al. 1992). The biochemical pathway of scytonemin synthesis is still unknown at this time.

If multiple stresses activate a common pathway in an organism, then a general stress response is observed. These types of responses to multiple stresses have been observed previously (Volker et al. 1992). However, if a response pathway is specifically activated by a particular environmental stress, then a specific stress response is observed. Previous work has established that scytonemin is induced by blue-light/UVA radiation. This indicates that scytonemin may be a part of a specific stress response (Garcia-Pichel et al. 1992). However, the effect of other environmental stress factors on the synthesis of scytonemin is unknown. It is possible that stresses other than UVR may enhance the synthesis of scytonemin combined with exposure to UV or may even induce scytonemin synthesis on their own. If other stresses induce scytonemin, then they would be considered part of a general stress response. Testing the effect that temperature stress and oxidative stress have on scytonemin synthesis in the absence of and in conjunction with UVR has been the focus of my work.

Temperature stress occurs when a cyanobacterium is placed at a temperature outside of its optimal growth range, either below (suboptimal) or above (supraoptimal). Cyanobacteria respond to supraoptimal temperatures by altering the pattern of growth and protein synthesis (Borbely et al. 1985). A shift up in temperature nearly universally causes an increased frequency of denatured (misfolded) proteins (Neidhardt and VanBogelen, 1987). Treatment at non-lethal temperatures induces the synthesis of heat-shock proteins, which function as molecular chaperones to refold proteins denatured by heat (Nishimura, 1998; Venetianer et al. 1994). The induction of heat shock proteins by a variety of stresses (Hightower, 1991) points to their role as part of a general stress

response. While scytonemin is not a protein, the production of scytonemin may be linked to a general stress response involving temperature. By subjecting cyanobacteria to temperature stress in conjunction with UVR, I am simulating environmental conditions. Solar irradiation, which is required for photosynthesis, exposes these cells to damaging levels of UVR and temperature stress.

Oxygenic photosynthesis normally produces non-reactive oxygen (0_2) as a by-product. Reactive oxygen species (e.g. singlet oxygen, superoxide) are produced in cells through the interaction of O_2 and high irradiance. Oxidative stress occurs when the level of ROS surpasses the cell's ability to scavenge and remove ROS (Farr and Kogoma, 1991). ROS exposure results in numerous types of DNA damage (Dahl et al. 1987; Keyer et al. 1995). ROS also causes damage via lipid peroxidation, chlorophyll photobleaching, phycobiliprotein degradation, and growth inhibition (Castenholz, 1997). It is known that oxidative stress induces the synthesis of proteins that overlap significantly with those induced by other stresses such as heat shock (Morgan et al. 1986). Thus, oxidative stress may trigger a general stress response.

Although scytonemin is not a protein, its synthesis and/or transport is probably mediated by structural proteins and enzymes at some level. Thus, it is possible that UV-induced proteins aid in these processes and scytonemin induction may be part of a general stress response (Dillon, 1996). The main question that I will address concerns whether the production of scytonemin is a specific response to solar irradiation or part of a general stress response. My hypothesis is that scytonemin production is part of a general stress response and that temperature stress and/or oxidative stress enhance scytonemin production in combination with solar irradiation. Alternatively, temperature stress and/or oxidatve stress may not enhance scytonemin production either as a single environmental stress or in combination with solar irradiation.

MATERIALS AND METHODS

Strain and culture. The cyanobacterium *Chroococcidiopsis M88-Vd-G(1)* was used for all experiments. It is a clonal isolate cultured from a biofilm encrusted on a desert rock in Baja, California N., Mexico. The cells were grown at 23° in liquid D medium (Castenholz, 1988) under low levels of visible light (~8 W m^{-2}) and UVA (~.05 W m^{-2}) with cool-white fluorescent light (Sylvania, Danvers, PA) to obtain exponentially growing cell material for experiments. The cells grew in aggregates; therefore, in order to obtain approximately equal amounts of starting material for the experimental flasks, samples were homogenized with a glass tissue grinder. For all experiments, optical density (OD) readings of cell suspensions were measured and adjusted to equal density (~0.425) at 750 nm using a DU 640 recording spectrophotometer (Beckman, Fallston, CA). This ensures the cells were roughly equal cell suspensions to start the experiment after being inoculated into the appropriate experimental flasks or tubes.

Protein quantification. 5 or 10 mL cell samples were placed into 15 mL centrifuge tubes (Corning, Corning, New York) and spun down in a clinical tabletop centrifuge (International Clinical Centrifuge, Boston, MA). The supernatant was removed and the cells were resuspended in 1 mL of distilled water. 1 mL of 1 M NaOH was added and the samples were boiled for 20 min. to extract the protein from the cells. 1 mL of 1M HCl was added to neutralize the solution and then mixed with a vortex genie mixer (American Hospital Supply Corporation, Evanston, IL). Proteins were assayed using the bicinchoninic acid assay (Smith et al. 1985).

Determination of pigments. 10 mL cell samples were filtered onto GF/C glass microfibre filters (Whatman, England), rinsed with distilled water and then placed filter cell side up in the bottom of a 20 mL scintillation vial. 3 mL of acetone was added to the vial and then the filter material was ground with a glass tissue homogenizer. These filters

were then incubated at 4°C in the dark overnight. The acetone-pigment extract was pipetted into 1.5 mL polypropylene microcentrifuge tubes (Fisher, Santa Clara, CA) and then spun down in a microcentrifuge for 2 minutes to remove particulates. Absorption of the clarified samples was measured at 384, 490, 663, and 750 nm using a spectrophotometer. The value of absorbance at 750 nm was subtracted from all measured absorbances to account for any residual scatter due to particulates. Scytonemin, chlorophyll *a*, and total carotenoids were determined using the trichromatic equations and extinction coefficients developed previously (Garcia-Pichel et al. 1992).

Temperature experiments. Cells were inoculated into quartz flasks with 280 mL of D medium. The flasks were placed in water baths maintained at constant temperatures of 20°, 25°, 30°, 35°, and 40°C (± 1°C). During moderate irradiance experiments, visible irradiance (~20 W m^{-2}) illuminated the flasks from above. During high irradiance experiments light fixtures were set up on both sides of the water baths illuminating the flasks with high visible irradiance (~52 W m^{-2}). Cells tend to clump, so flasks were swirled daily to minimize self-shading effects. Triplicate 10 mL samples for protein and pigment determination were removed on days 0, 2, 5, 7, and 10 under sterile conditions. Cells were first resuspended from the bottom of the flask and then homogenized by pumping with a syringe before sampling.

Oxidative stress experiments. Cells were inoculated into quartz flasks with 175 mL of D medium augmented with the singlet oxygen generator methylene blue (MB) at concentrations of 0, 1, 2, and 5 μM and kept at a constant temperature of 23° C. The addition of MB causes an increase in singlet oxygen, a type of ROS. Visible irradiance (~8.4 W m^{-2}) illuminated flasks from above and UVA irradiance (~9 W m^{-2}) illuminated flasks from below. Flasks were swirled to minimize self-shading effects and rotated every day to account for irradiance differences. Methylene blue levels were checked daily with

a spectrophotometer with full spectrum scans and restored to starting levels as necessary. Duplicate 10 mL samples for protein and pigment determination were removed on days 0, 2, 5, 7, and 10 under sterile conditions. Cells were first resuspended from the bottom of the flask and then homogenized by pumping with a syringe before sampling.

Antibiotic experiments. Cells were inoculated into 50 mL centrifuge tubes (Corning, Corning, New York) with 30 mL of D medium augmented with chloramphenicol and rifampicin. Stock solutions of chloramphenicol, an inhibitor of translation, and rifampicin, which inhibits transcription, were prepared according to Gerhardt et al. (1994). Chloramphenicol was dissolved in ethanol at a concentration of 34 mg/ml and rifampicin was dissolved as 2 mg/ml in 10 % aqueous methanol. The final concentration in the medium was 25 µg/ml for both antibiotics. Antibiotic solutions were stored in the dark at 4° C. Control cultures were exposed to the equivalent concentration of ethanol and methanol without antibiotic addition to test for growth inhibition caused by the solvent. Media were replaced daily because rifampicin and chloramphenicol have been found to be unstable over time periods of several days (Portwich and Garcia-Pichel, submitted for publication). Tubes were spun down, the media was suctioned off and then replaced from the original stock solutions of media. Visible light (~8.4 W m^{-2}) illuminated flasks from above and UVA (~9 W/m^2) illuminated flasks from below. Triplicate 5 mL samples for protein and pigment determination were taken on days 0 and 8 under sterile conditions. Cells were first resuspended from the sides of the flask, homogenized by vortexing then pumping with a syringe before sampling.

Statistical analysis and graphical analysis.. All statistical tests were made using the computer program SuperANOVA® (Abacus Concepts, 1989). Pairwise comparisons were based on the results of Tukey Compromise and Scheffe's S *post hoc* tests. Figures were produced using SigmaPlot® (Jandel Scientific, 1992).

RESULTS

Temperature experiments. Comparisons of cells grown at temperatures under high and moderate irradiance are shown in Figure 1 and Figure 2. Two-way ANOVA showed a significant irradiance treatment effect (P=.0001) on scytonemin production with the high irradiance experimental cells having much higher scytonemin values (Fig. 1) than the moderate irradiance experimental cells (Fig. 2). There was no significant effect of temperature on scytonemin synthesis under moderate irradiance and Fig. 2 shows that temperature alone does not trigger scytonemin synthesis. However, there was a significant effect of temperature treatment (P=.0001) of scytonemin synthesis for cells simultaneously exposed to high irradiance (Fig. 1).

In the high irradiance treatments, there was a lag in scytonemin production at 20°, 25°, and 30° C from day 0 to day 2, but this lag was not observed at 35° C. The amount of scytonemin synthesized at 35° C by day 2 was 112 μg per mg protein whereas the other treatments did not produce any significant amount of scytonemin. After day 2, the rate of scytonemin induction at 25° and 30°C increased to a rate nearly identical to 35° C, but the 35° C treatment had produced a significantly greater amount of scytonemin per mg protein (P<.05) than all other treatments. The rate of scytonemin synthesis at 20° C did not attain the high rate seen in the other treatments. After 5 days the increased rate of scytonemin production began to level off for the 25°, 30°, and 35° C treatments and either began to decrease or level off after day 7. This indicates that scytonemin production was slowing down relative to cell growth.

In Figure 3, the cellular growth rate at 40° C shows that growth was inhibited at this highly stressful temperature. The cellular growth rates at 25°, 30°, and 35° C were not significantly different over the experimental period, even through the amount of protein produced by day 10 was significantly higher at 30° C compared with 25 and 35 ° C. This difference can be accounted for by a slight increase in the rate of protein production from day 7 to day 10 at 30° C. Cells at the 20°C treatment were sampled over

a 12 day period to determine whether the amount of scytonemin synthesized would attain the higher levels seen in the other treatments. The 20° C treatment never achieved the same rate of scytonemin synthesis over the long-term period. The estimated doubling time of cells in exponential phase growing in the different treatments with exposure to 20°, 25°, 30°, and 35° C is respectively as follows: 6.3, 4.3, 3.8, and 4.2 days. These figures show that the optimal growth temperature for these cells is ~30° C.

Oxidative stress experiments. The reactive oxygen treatment had a significant effect (P=.0001) on the synthesis of scytonemin. As seen in Figure 4, after the lag in scytonemin production up to day 2, the intermediate treatment (1μM MB) had a significantly (P<.05) higher rate of scytonemin synthesized per mg of protein than the 0 μM and 2 μM treatments. Following day 5, the rate of scytonemin production across all treatments decreased indicating that scytonemin synthesis was slowing down relative to cell growth rate. However, there was a significant interaction effect (P<.05) of cell protein level by this treatment. As seen in Fig. 5, cell growth rate was significantly (P=.0001) higher at 0μM than the other treatments indicating that the addition of MB decreased cell growth. The estimated doubling time of cells in exponential phase with exposure to 0μM, 1μM, and 2μM is respectively as follows: 4.2, 8.5, and 18.1 days.

Antibiotic experiments. The amount of scytonemin synthesized in the different treatments is shown in Fig. 6. The synthesis and accumulation of scytonemin was significantly (P=.0001) inhibited by the addition of chloramphenicol and rifampicin to the growth medium. Scytonemin synthesis did occur in the MeOH control.

DISCUSSION

The results of the temperature experiments show that temperature alone does not trigger scytonemin synthesis under moderate irradiance. However, under high irradiance, temperature has an effect on the rate of scytonemin synthesis. Upon exposure to 35° C, cells did not undergo the commonly observed lag in synthesis and produced more scytonemin per mg protein as compared to cells exposed to 20, 25, and 30° C. Simple enzyme kinetics states a direct relationship between temperature and the rate of enzymatic reactions. As temperature rises, chemical and enzymatic reactions in the cell proceed at more rapid rates. Growth rate would continue to increase until an optimal growth rate is reached, however, once this optimal temperature is surpassed, growth is inhibited. Cells did not survive at 40° C indicating a high level of stress at this temperature. Cell growth at 35° C was not greatly inhibited and was similar to 25 and 30° C. Doubling times are used as indicators for the physiological state of a cell population. Doubling times for the different treatments show that optimal growth occurred near 30° C, therefore, 35° C may be considered a supraoptimal growth temperature. This finding indicates that high irradiance coupled with supraoptimal temperatures may signal for an immediate start to scytonemin production, that is the commonly seen lag is removed. *Chroococcidiopsis* M88-Vd-G(1) comes from an environment of high irradiance, high temperature, and long periods of metabolic inactivity during times of desiccation. It is possible that high irradiance and higher temperatures may be a signal for an upcoming period of metabolic inactivity. The ability of scytonemin to protect cells from UVA during periods of metabolic inactivity (Garcia-Pichel et al. 1992) would thus enable the cells to cope with upcoming stressful conditions.

Results from the oxidative stress experiments show that under UV radiation and oxidative stress the rate of scytonemin synthesis is increased at the intermediate level of 1μM MB as compared to the rates at 0 and 2 μM MB. This finding indicates that oxidative stress may enhance the synthesis of scytonemin under UV stress at stress levels

where growth was not fully inhibited as in the 1μM MB treatment. It is possible that these cells use elevated levels of ROS as a signal for scytonemin synthesis in addition to UVR and blue light irradiance. The synthesis of scytonemin would then decrease the amount of UVR entering a cell and subsequently decrease the level of ROS in the cell.

The antibiotics, chloramphenicol and rifampicin, which interfere with protein synthesis, were shown to have an inhibitory effect on the induction of scytonemin. This result indicates that scytonemin synthesis and/or transport is probably mediated by structural proteins and enzymes. The production of heat-shock proteins in response to other stresses may be a signal for the cell to prepare for a stress condition such as UVR.

Simultaneous exposure to multiple stresses are common for this cyanobacterium in its natural desert crust environment. Previous work with this cyanobacterium has shown that some scytonemin is inducible by osmotic stress without UVR (Tatsumi, 1998). While temperature stress did not induce scytonemin synthesis by itself, supraoptimal temperature and an oxidative stress of 1μM MB did enhance the production of scytonemin. The effect of these other stresses makes it likely that the synthesis of scytonemin is part of a general stress response. In addition, the antibiotic experiments show that in order to induce scytonemin synthesis, protein synthesis is required.

Table 1. Light conditions for experiments.

	Visible (W m -2)	UVA (W m -2)	Light Energy (mE m-2 sec -1)
Culture Irradiance	7.54 - 8.45	.047 - .052	20 - 23
High Irradiance and temperature exp.	48.5 - 55.2	.982 - .960	175 - 200
Moderate Irradiance and temperature exp.	18 -22	NA	50-60
High Irradiance and singlet oxygen exp.	8.27 - 8.63	8.82 - 9.29	55 - 57
High Irradiance and antibiotics exp.	8.27 - 8.63	8.82 - 9.29	55 - 57

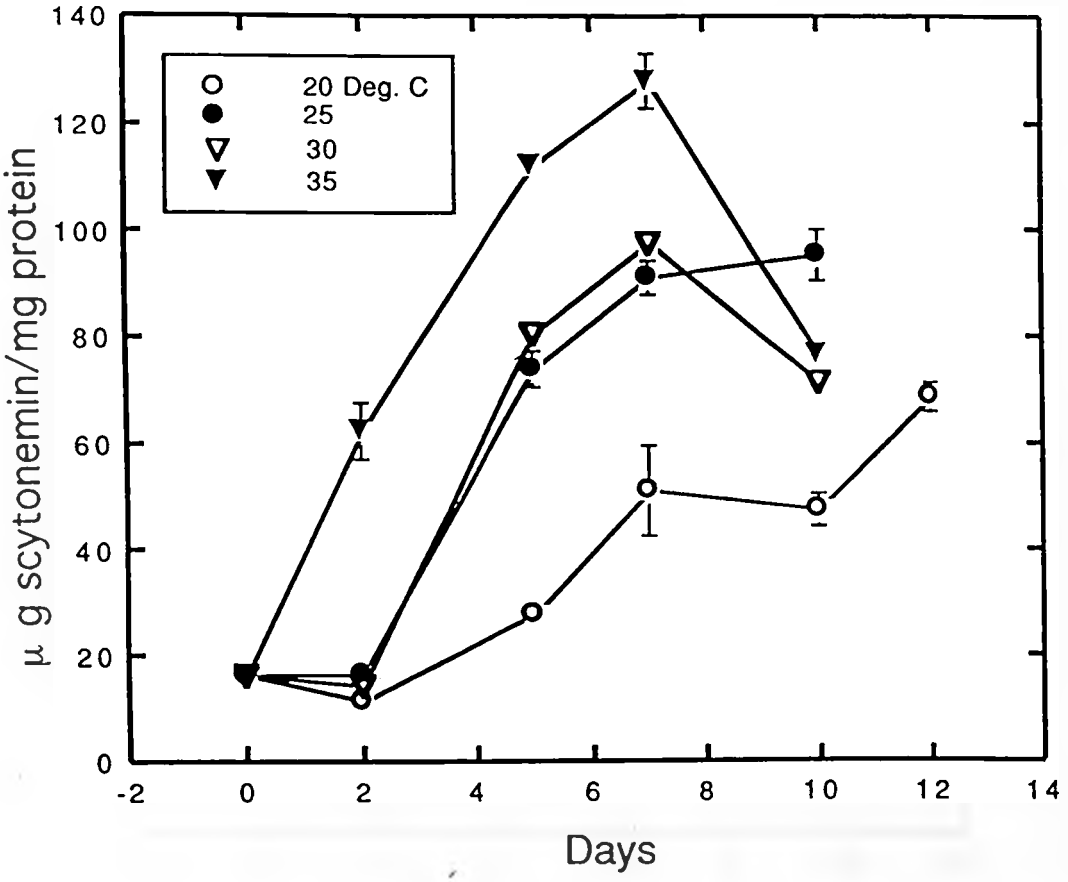

Figure 1. High irradiance and temperature experiment. Changes in scytonemin content in cells grown under high laboratory VIS irradiance at varying temperatures.

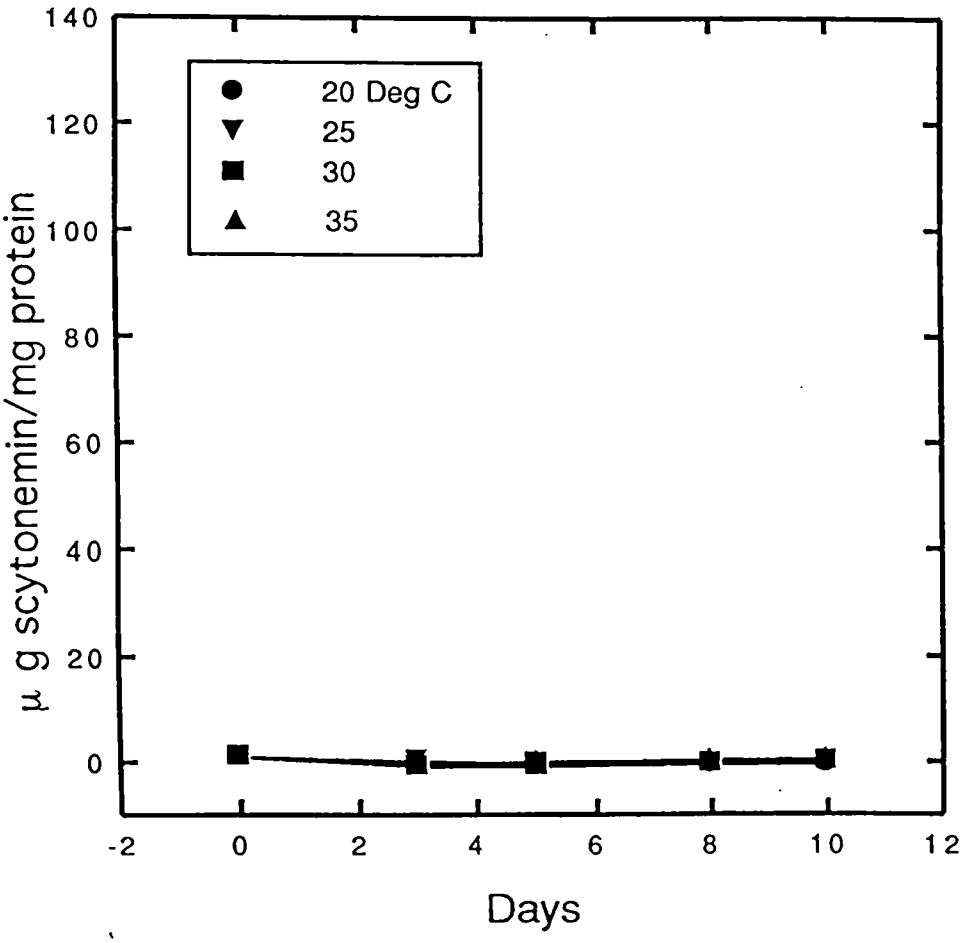

Figure 2. Moderate irradiance and temperature experiment. Changes in scytonemin content in cells grown under moderate laboratory VIS irradiance at varying temperatures.

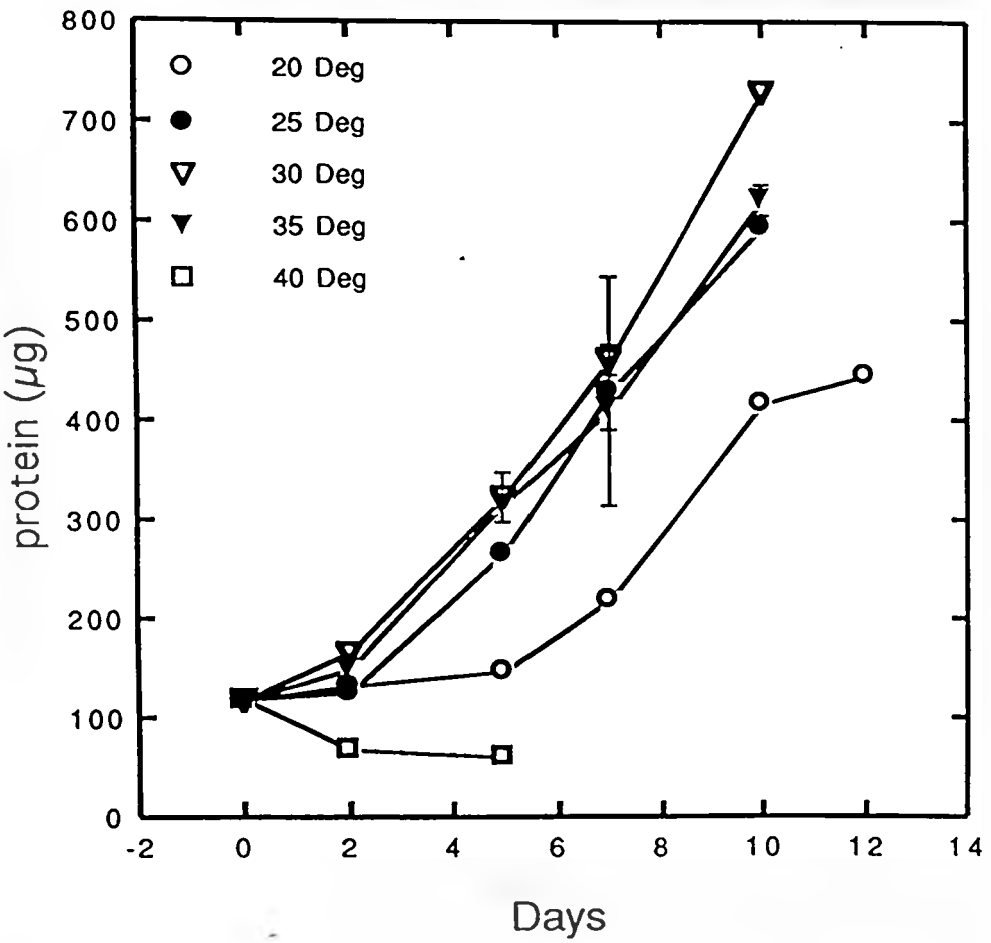

Figure 3. High irradiance and temperature experiment. Changes in total protein levels of cells grown under high laboratory VIS irradiance at varying temperatures.

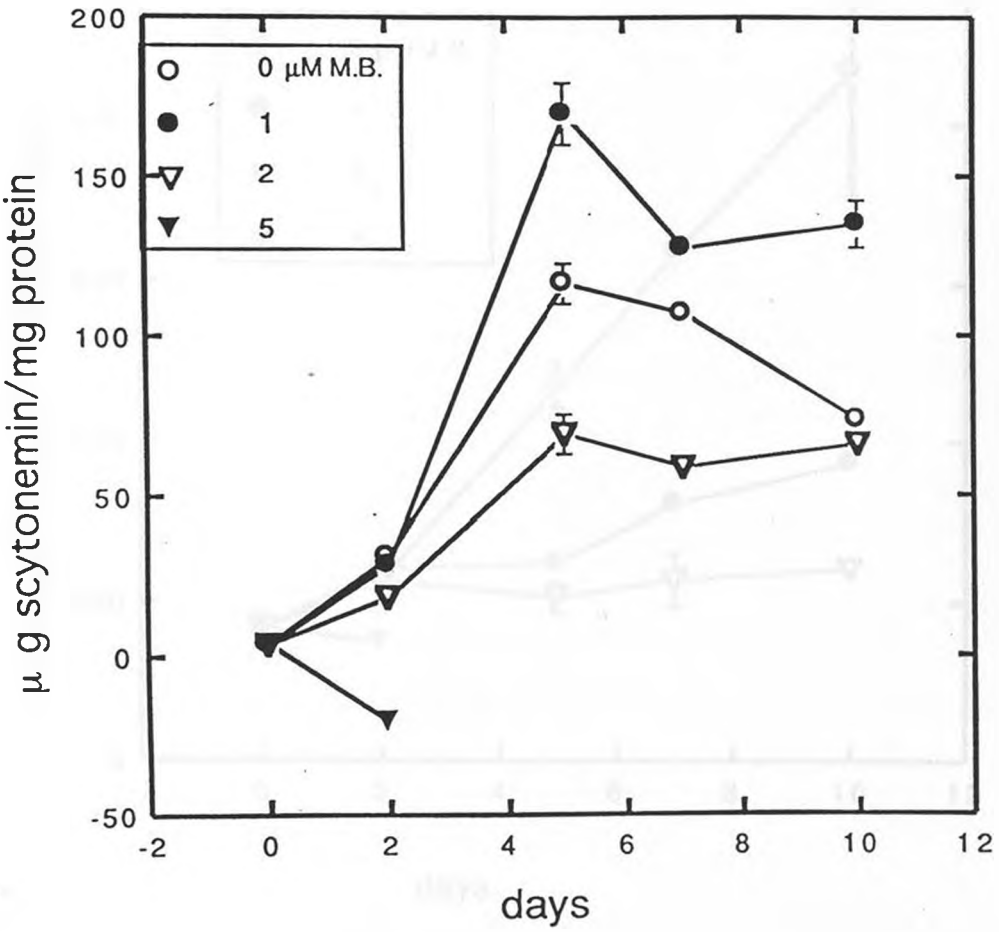

Figure 4. High irradiance and singlet oxygen experiment. Changes in scytonemin content in cells grown under high laboratory UVA irradiance at varying concentrations of methylene blue.

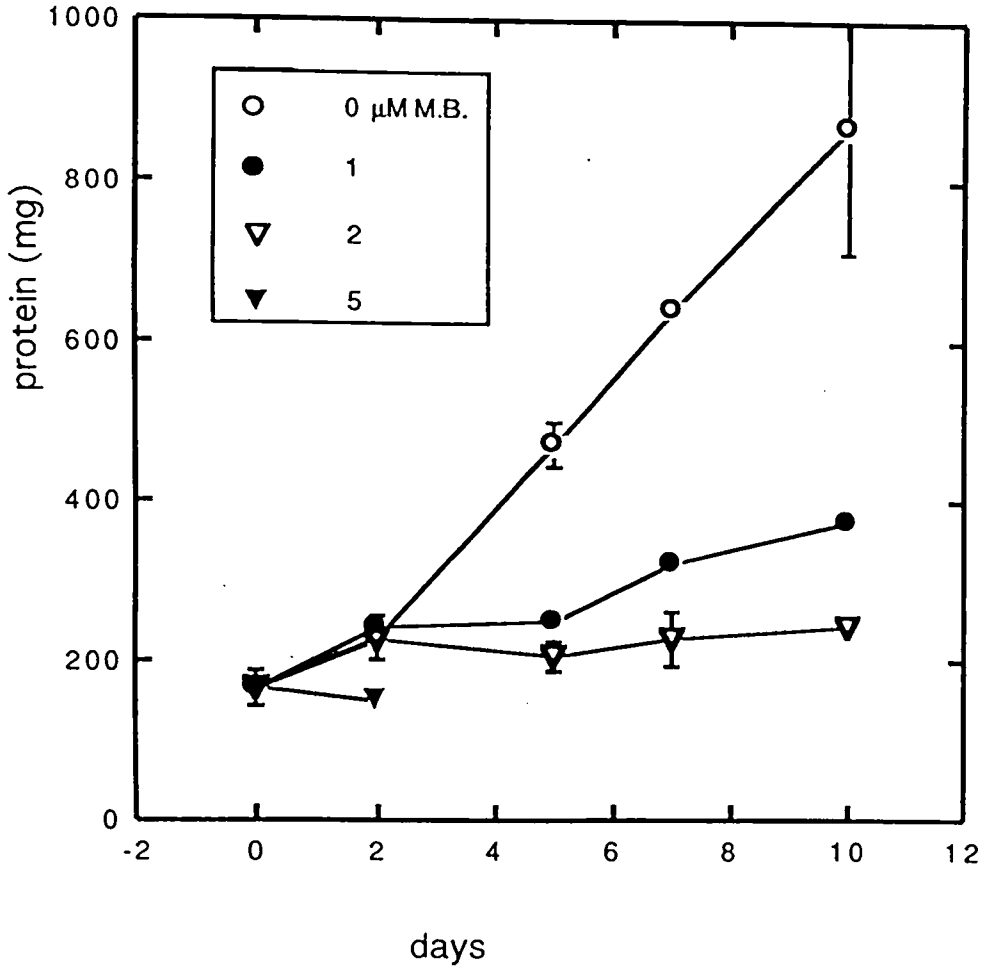

Figure 5. High irradiance and singlet oxygen experiment. Changes in total protein levels of cells grown under high laboratory UVA irradiance at varying concentrations of methylene blue.

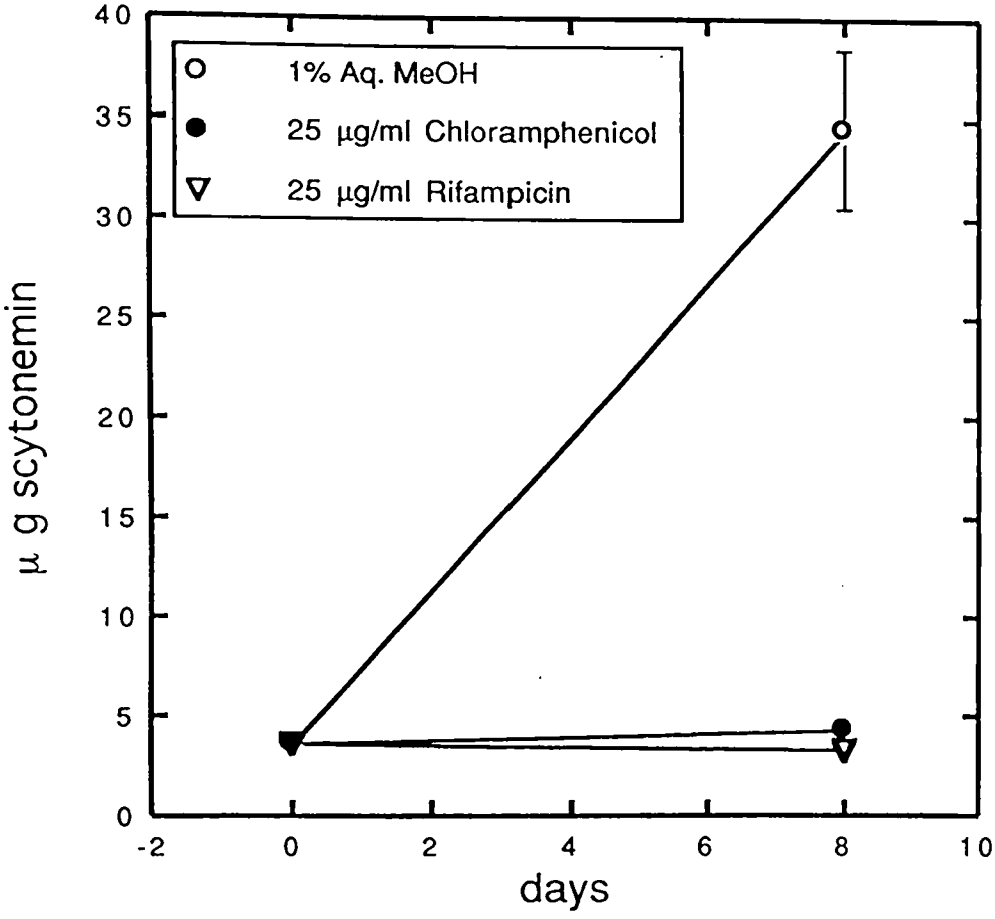

Figure 6. High irradiance and antibiotic experiment. Changes in total scytonemin content of cells grown under high laboratory UVA irradiance in different antibiotic treatments.

GLOSSARY OF TERMS

General stress response: A type of reaction that takes place upon exposure to multiple unfavorable environmental conditions.

Heat-shock proteins: Molecular chaperones induced by multiple environmental signals which refold proteins denatured by heat.

Motility: Ability of an organism to move.

Oxidative stress: An unfavorable environmental condition that occurs when the level of ROS surpasses the cell's ability to scavenge and reduce ROS.

Passive screen: A mechanism of cell protection that remains active even when a cell is metabolically inactive.

Photosynthesis: The use of light energy to drive the incorporation of carbon dioxide ($C0_2$) into cell material and the production of energy (ATP) with the release of oxygen (0_2) as a by-product

ROS: Molecules of oxygen (e.g. singlet oxygen and superoxide) that have been made more reactive through chemical reactions. In nature they are commonly a byproduct of photosynthesis and are the main products of UVA wavelength radiation. They may also be created through the use of artificial chemical reagents. These harmful types of oxygen molecules are capable of inflicting damage on DNA, proteins, and other cellular components including the photosynthetic machinery.

Scytonemin: A UV-sunscreen pigment that accumulates in the extracellular sheath of some cyanobacteria. It absorbs strongly in the UV spectrum, especially in the UVA and UVC, and is induced by UVA and blue light wavelengths.

Specific stress response: A type of reaction that takes place upon exposure to one unfavorable environmental condition.

Temperature stress: An unfavorable environmental condition that occurs when an organism is subjected to temperatures either below (suboptimal) or above (supraoptimal) its optimal growth range.

Transcription: Synthesis of an RNA molecule complementary to one of the two strands of a double-stranded DNA molecule.

Translation: The synthesis of protein using the genetic information in a messenger RNA as a template.

UVR: Solar radiation of wavelengths from 190-400 nanometers (nm). Artificially divided into three wavebands: UVA (320-400 nm), UVB (280-320 nm), and UVC (190-280 nm).

REFERENCES

Abacus Concepts. 1989. Super ANOVA. Abacus Concepts, Inc., Berkeley, CA.

Borbély, G., Surányi, G., Korcz, A., and Pálfi, Z. 1985. Effect of heat shock on protein synthesis in the cyanobacterium *Synechococcus* sp. strain PCC 6301. *J. Bacteriol.*. Mar: 1125-1130

Castenholz, R.W. 1988. Culturing methods for cyanobacteria. In Cyanobacteria (Eds. Packer, L. and Glazer, A.N.). *Methods Enzymol.* 167: 68-100.

Castenholz, R.W. 1997. Multiple strategies for UV tolerance in cyanobacteria. *The Spectrum*. 10: 10-16.

Dahl, T.A., Midden, W.R., and Hartman, P.E. 1987. Pure singlet oxygen cytotoxicity for bacteria. *Photochem. Photobiol.*. 46: 345-352.

Dillon, J. 1996. Mechanisms for the induction and synthesis of scytonemin, a UV-sunscreening pigment in cyanobacteria. Ph.D. Proposal.

Dillon, J. and Castenholz, R.W. submitted for publication. Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: Implications for early photosynthetic life.

Farr, G.B. and Kogoma, T. 1991. Oxidative stress responses. In *Escherichia coli* and *Salmonella typhimurium* (Ed. Neidhardt, F.C.). p. 561-585.

Garcia-Pichel, F. and Castenholz, R.W. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *J. Phycol.*. 27: 395-409.

Garcia-Pichel, F., Sherry, N.D. and Castenholz, R.W. 1992. Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. *Photochem. Photobiol.* 56: 17-23.

Gerhardt, P., Murray, R.G.E., Wood, W.A. & Krieg, N.R. 1994. *Methods for General and Molecular Bacteriology.* American Society for Microbiology, Washington D.C.

Hightower, L.E. 1991. Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell.* 66: 191-197.

Jandel Scientific. 1992. SigmaPlot. Jandel Corp.

Keyer, K., Gort, A.S., and Imlay, J.A. 1995. Superoxide and the production of oxidative DNA damage. *J. Bacteriol.*. Dec.: 6782-6790.

Madronich, S. 1992. Implication of recent total atmospheric ozone measurements for biologically active ultraviolet radiation reaching the Earth's surface. *Geophys. Res. Letters.* 19: 37-40.

Mitchell, D.L. and Karentz, D. 1993. The induction and repair of DNA photodamage in the environment. In *Environmental UV Photobiology* (Eds. Young, A., Bjorn, L., Moan, J., and Nultsch, W.). p. 345-377.

Morgan, R.W., Christman, M.F., Jacobson, F.S., Storz, G., and Ames, B.N. 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. *Proc. Natl. Acad. Sci. USA.* 83: 8059-8063.

Neidhardt, F.C. and VanBogelen, R.A. 1987. Heat shock response. In *Escherichia Coli and Salmonella Typhimurium* (Ed. Neidhardt, F.C.). p. 1334-1345.

Nishimura, M. 1998. Molecular chaperones and temperature stress. In *Stress Responses of Photosynthetic Organisms* (Eds. Satoh, K. and Murata, N.). p. 83-91.

O'Brien, P.A. and Houghton, J.A. 1982. UV-induced DNA degradation in the cyanobacterium *Synechocystis* PCC 6308. *Photochem. Photobiol.* 36: 417-422.

Portwich, A. and Garcia-Pichel, F. submitted for publication. Induction and regulation of mycosporine synthesis by ultraviolet radiation and osmotic stress in the cyanobacterium *Chlorogloeopsis* PCC 6912.

Proteau, P.J., Gerwick, W.G., Garcia-Pichel, F., and Castenholz, R.W. 1993. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. *Experientia.* 49: 825-829.

Quesada, A. and Vincent, W.F. 1997. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *Eur. J. Phycol.* 32: 335-342.

Schopf, W.J. 1996. Cyanobacteria: Pioneers of the early earth. *Nova Hedwigia, Beiheft.* 122: 13-32.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, M.N., Olson, B.J., and Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. *Analyt. Biochem.*. 150: 76-85.

Tatsumi, C. 1998. The effects of multiple environmental stresses on the induction of scytonemin in cyanobacteria. Honors College Thesis.

Venetianer, A., Pirity M., Hever-Szabo, A. 1994. The function of heat-shock proteins in stress tolerance. *Cell Biol. Intl.*. 18: 605-614.

Vincent, W.F. and Quesada, A. 1994. Ultraviolet radiation effects on cyanobacteria: Implication for antarctic microbial ecosystems. *Antarctic Research Series.* 62: 11-124.

Volker, U., Mach, H., Schmid, R., and Hecker, M. 1992. Stress proteins and cross-protection by heat shock and salt stress in *Bacillus subtilis*. *J. Gen Microbiol.*. 138: 2125-2135.