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Exposure of *Fischerella* [*Mastigocladus*] to high and low temperature extremes: strain evaluation for a thermal mitigation process

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Abstract

In conjunction with a proposed algal cultivation scheme utilizing thermal effluent, twelve *Fischerella* strains were tested for tolerance to temperatures above and below their growth range. Exposure to 65 °C or 70 °C for 30 min caused bleaching and death of most or all cells. Effects of 60 °C exposure for periods of up to 2 h ranged from undetectable to severe for the various strains. Chlorophyll *a* content typically decreased 21–22% immediately following 60 °C or 65 °C (1 h) exposure. However, the 60 °C-shocked cultures regained normal Chl *a* content after 24 h at 45 °C, whereas Chl *a* in 65 °C-shocked cultures immediately lost visible autofluorescence and was later degraded. Exposure to 15 °C virtually stopped growth of all strains during a 48 h exposure period. Most strains grew as rapidly as 45 °C controls when restored to 45 °C, while a few strains recovered more slowly. Comparison with dark-incubated controls indicated that photooxidative damage did not occur during cold shock. Certain strains exhibited relatively rapid recovery from both heat and cold exposure, thus meeting the temperature tolerance criteria for the proposed algal cultivation process.

Introduction

The discharge of thermal effluents from fossil- or nuclear-fueled power plants or other facilities can have significant impacts on receiving waters. These detrimental effects are exacerbated when high nutrient levels are present in the effluent. The combination of nutrient loading and thermal enhancement of cooling impoundments typically favors the growth of cyanobacteria (blue-green algae), often resulting in nuisance blooms.

A conceptual process to mitigate such combined thermal/nutrient impacts was recently described by Wilde *et al.* (1991). It involves the use of the thermal effluents to cultivate filamentous, thermotolerant cyanobacteria, followed by harvesting of the cyanobacterial biomass to remove the nutrients responsible for eutrophication. In this concept, a 'cultivation zone' near the thermal discharge is created by means of floating plastic curtains or other barriers, selected strains are inoculated into the cultivation zone, and the biom-

ass is harvested by means of microstrainers, which selectively remove filamentous forms. Part of the harvested biomass would be recycled back to the influent, to help establish and maintain the cyanobacterial culture in the cultivation zone. The harvested biomass, containing most of the nutrients removed from the cooling water, could then serve as a source of fertilizer, fuels, and possibly other products.

The proposed process is based on a combination of known properties of algal cultures, such as the effectiveness of biomass recycle in maintaining dominance of selected species (Weissman & Benemann, 1979). However, it remains to be determined whether the overall concept can be realized in practice. The proposed process requires the use of strains that exhibit a combination of properties: nitrogen fixation (to allow complete removal of phosphate when fixed nitrogen is otherwise the limiting nutrient), filamentous growth (to allow harvesting by microstrainers), and a wide tolerance of temperature fluctuations.

The cultivation zone could exhibit a temperature gradient from as much as 60 °C at the influent to below 30 °C at the harvesting point. The cyanobacteria would experience this temperature range over an approximately 1-day period of growth in the cultivation zone, and would undergo a drastic, essentially instantaneous temperature upshift during recycling. In addition, the organisms must be able to survive for several days at very low temperatures (e.g. 15 °C) during shutdowns in wintertime. The filamentous cyanobacteria that are the most likely candidates for such a process are the thermophilic strains of the genus *Fischerella* sp. (formerly known as *Mastigocladus laminosus*) (Castenholz, 1989).

Temperature limits for the growth and survival of thermophilic cyanobacteria can be dependent on the specific strain used (Peary & Castenholz, 1964; Castenholz, 1969a) and the environmental conditions under which they are cultivated (Castenholz, 1969b), such as pH (Muster *et al.*, 1983). The factors governing the upper and lower temperature limits for growth are not well established: derangements of photosynthetic and carbon fixation mechanisms may be involved in the cessation of growth at high temperatures, and photooxidation of pigments or cessation of macromolecular synthesis may be implicated at low temperatures (Castenholz, 1972; Meeks & Castenholz, 1971, 1978; Sheridan & Ulik, 1976). Thermophilic *Fischerella* strains may tolerate temperatures a few degrees above or below the growth range with relatively little permanent damage (Castenholz, 1969a, b). Even relatively low temperatures (10 °C) are tolerated for extended periods by natural populations that exhibit optimal growth rates above 50 °C (Tison *et al.*, 1981).

However, the data available on the effects of temperature fluctuations and extremes are limited. The present investigation focuses on the growth and/or survival of a number of *Fischerella* cultures obtained from reactor cooling waters and other sources and subjected to temperature extremes beyond the normal growth range. Our major objective was the identification of resilient strains with potential for use in the proposed algal cultivation process.

Materials and methods

Cultures and culture maintenance

Five unialgal cultures of thermophilic, filamentous cyanobacteria were derived from water samples collected from cooling reservoirs at the Savannah River Site (SRS) (Table 1). Collection temperatures (Table 1) reflect the fact that SRS reactors were not operating at full power levels at the time of sampling. During previous periods of reactor operation, the three SRS sites sampled had all received thermal input from reactor cooling water effluents, resulting in a maximum temperature of >55 °C in L Lake, 63 °C in Pond 4, and >55 °C in Pond C. It was therefore expected that selective enrichments would reveal the presence of thermotolerant or even thermophilic strains, although such strains would not dominate the microflora in the absence of thermal input. The cultures closely resembled each other and were classified as belonging to the species formerly known as *Mastigocladus laminosus* (now

Table 1. Sites, dates, and temperatures at which cultures were obtained.

Culture	Source	Date	Water temp., °C
T-7	Hawaii Island, Hawaii	Rec'd at SRS[a] 2 Mar 1989	–[b]
4L	SRS, Pond 4	15 Jun 1988	26
UTEX B 1931	Univ. of Texas culture collection	rec'd at SRS 23 Jun 1988	–
823	Media contaminant	23 Aug 1988	–
7	SRS, Pond C	14 Jun 1988	26
8	SRS, Pond C	14 Jun 1988	26
6H	Hawaii Island, Hawaii	rec'd at SRS 2 Mar 1989	–
113	Media contaminant	1 Jan 1989	–
1B	Hawaii Island, Hawaii	rec'd at SRS 2 Mar 1989	
2	SRS, L lake	15 Jun 1988	34.5
14	Media contaminant	23 Jun 1988	–
6L	SRS, Pond C	14 Jun 1988	26

[a] SRS denotes the Savannah River Site, Aiken, SC, USA.
[b] Data not available.

Fischerella spp.) based on morphological features which included the presence of primary and secondary trichomes, numerous heterocysts, and true branching (infrequent). Two other unialgal cultures, morphologically similar to the other local cultures, appeared as contaminants at various times in L Lake water that was used as culture media (Table 1). Three additional cultures were collected from a region of geothermal activity on Hawaii Island (Table 1). These cultures were contaminated with unicells and/or unbranched, nonheterocystous filaments in addition to the dominant *Fischerella*. One unialgal strain, (*Mastigocladus laminosus* UTEX B 1931) was obtained from the University of Texas algal culture collection. It exhibited more frequent true branching and a higher ratio of primary to secondary trichomes than the other cultures. A final culture appeared as a contaminant during transfer of the UTEX culture, but morphologically resembled the local cultures and hence was probably of local origin (Table 1).

Thermophilic, unialgal isolates were obtained from enrichment cultures maintained in a Psychrotherm G-27 temperature controlled incubator (New Brunswick Scientific Co.) at 45 °C with gentle shaking (150 rpm) and a light-dark cycle (12:12 h, 50 $\mu E\ m^{-2}\ s^{-1}$). Microwaved (Keller *et al.*, 1988), 0.45 μm membrane filtered L Lake water was used as the enrichment medium. Inocula from field sources (Table 1) were added to the enrichment culture medium and incubated until growth was visible. To eliminate non-thermotolerant forms, the cultures were periodically exposed for 2 h at 55 °C. Following growth and enrichment, cultures were transferred monthly using ND medium, a modification of Medium D (Castenholz, 1982), lacking KNO_3 and $NaHCO_3$ but with 0.7 $g\ l^{-1}$ Na_2HPO_4 and 0.036 $g\ l^{-1}$ KH_2PO_4 replacing normal phosphate additions. These stock cultures were maintained in Psychrotherm incubators at 45 °C under continuous illumination (50 $\mu E\ m^{-2}\ s^{-1}$) and gentle shaking (150 rpm).

Experimental procedures

Material from stock cultures was transferred to filtered (0.45 μm), microwaved L Lake water and allowed to grow 3–10 days at 45 °C to provide inoculum for all experiments. Experiments were performed using microwaved, 0.45 μm filtered L Lake water to which $NaHCO_3$ was added as a carbon source. Appropriate carbon levels (no added carbon, 3 mM $NaHCO_3$ with pH adjusted to 7.5, or 1 mM $NaHCO_3$, pH = 8.0–8.2) for each culture were determined in preliminary experi-

ments. Cultures 1B, 6H and 14 grew best when provided with 3 mM $NaHCO_3$ (LB3 medium; alkalinity = 2.7–3.1 meq l^{-1}), while the remaining cultures preferred 10 mM $NaHCO_3$ (LB10 medium, alkalinity = 9.0–9.4 meq l^{-1}). Unless stated otherwise, all work was done under 50 μE m^{-2} s^{-1} continuous illumination with gentle shaking (150 rpm).

Due to the filamentous nature and strong clumping tendency of the cultures, growth measurements were performed on the entire contents (75 ml) of replicate 125 ml culture flasks. Uniform inoculum for the flasks in each experiment was provided by gentle homogenization of the inoculum culture (grown in L Lake water) using a hand-held tissue grinder with a glass or Teflon pestle. For determining the effect of brief high temperature shock, 12 flasks were inoculated with each homogenate and Hour 0 dry weight was measured in duplicate aliquots of the homogenates. Flasks were incubated at 45 °C until Hour 24 and dry weight was again measured in duplicate flasks. At this time, duplicate cultures of each strain were placed for 30 min at each of the following temperatures: 55, 60, 65, and 70 ± 1 °C. This treatment was carried out in a water bath under normal room illumination (approx. 5 μE m^{-2} s^{-1}) and flasks were swirled at ≤5-min intervals to aid in heat transfer. Controls were similarly treated using a 45 °C bath. Approximately 18 minutes were required for flask contents to reach 70 ± 1 °C, and 11–14 minutes were required to reach the lower temperatures. At Hour 24.5, cultures were returned to the 45 °C incubator and harvested at Hour 72 for dry weight measurements.

The effect of a longer exposure to 60 °C was tested similarly. Cultures were grown at 45 °C until Hour 24, then placed in a 60 °C water bath (swirled at ≤5-min intervals) for 2 h. Controls were simultaneously placed in a 45 °C under otherwise similar conditions. At Hour 26, all cultures were replaced into the 45 °C incubator. Dry weight was measured (in duplicate flasks) at Hours 0, 24, and 72.

In an experiment to determine the relationship between pigment loss and growth inhibition at elevated temperature, 40 replicate flasks of LB10 medium (75 ml) were inoculated with strain 4L (Table 1) and incubated (45 °C, continuous illumination) until Hour 24. Dry weight and chlorophyll *a* (Chl *a*) were measured in duplicate at Hour 0 and Hour 24. Measurements at Hour 0 were done on the inoculum, while the contents of 4 flasks were sacrificed at each subsequent time point to obtain duplicate dry weight and Chl *a* determinations. At Hour 24, 12 flasks were placed for 1 h in a 60 °C bath and swirled at 5-min intervals. Twelve flasks were similarly treated using a 65 °C bath, while the remaining twelve flasks remained in the 45 °C incubator to serve as controls. At Hour 25, dry weight and Chl *a* were each measured in 2 flasks that had been subjected to each treatment, and the remaining cultures were returned to the 45 °C incubator. Dry weight and Chl *a* were again measured at Hours 48 and 72. Chl *a* fluorescence was examined by means of epifluorescence microscopy at the various time points and the percentage of red-fluorescing vegetative cells was calculated. A portion of the experiment was later repeated to allow separate determination of Chl *a* and pheophytin levels immediately before and after heat shock. Rates of dry weight increase following 45 °C and 60 °C treatment were compared according to Zar (1984).

Tolerance to suboptimal temperature was tested using cultures grown for 24 h at 45 °C in microwaved, filtered L Lake water (75 ml/flask) with $NaHCO_3$ added. Dry weight was measured in duplicate samples at Hours 0 and 24. At Hour 24, four cultures of each strain were placed in a 15 °C incubator until Hour 72, while four control flasks remained at 45 °C during this time. Four additional flasks were wrapped in foil and incubated at 45 °C as a dark control (to test effects of stopping growth without exposure to cold). At Hour 72, dry weight was measured in duplicate flasks under each incubation condition, and the remaining flasks were restored to the 45 °C incubator in the light. All flasks were harvested for dry weight measurement at Hour 120.

Exponential growth rates at 30, 35, 45 and 55 °C were measured for two strains. Cultures

were inoculated at densities of approximately 1 mg l^{-1} dry weight and grown in 4-liter polycarbonate bottles maintained in water baths under continuous, unidirectional 200 μE m^{-2} s^{-1} illumination. They were magnetically stirred and aerated with 0.1% CO_2 in air. Duplicate samples for dry weight analysis were taken periodically over a 48-hour period and the exponential growth phase was identified by examination of graphs.

Analytical methods

Dry weight was measured using preweighed GF/C filters (U.S. EPA, 1979). Preliminary experiments indicated that ash content of the cultures was negligible. Sample pH was measured electrometrically, and alkalinity was determined by the low alkalinity method of EPA (U.S. EPA, 1979). Chl *a* content was routinely determined spectrophotometrically following methanol extraction (Weissman & Benemann, 1977). In one experiment, Chl *a* and pheophytin content were both measured following acetone extraction (Rand *et al.*, 1979). Chl *a* autofluorescence was determined by examining at least 200 vegetative cells from each replicate sample at 500× magnification, using epifluorescence microscopy with a Zeiss BP436 exciter filter, FT460 chromatic beam splitter, and LP470 barrier filter.

Statistical analysis

Results were analyzed statistically by means of the Statgraphics personal computer software package (STSC, Inc.). Overall effects of the various temperature treatments on mean dry weight increase by the 12 strains in the 48 h following treatments were tested using t-tests for paired comparisons, in which data points for the same strain were paired. In analyzing data from cultures in which growth was stopped by cold or dark treatment during the Hour 24–Hour 72 interval, mean dry weight increase of treated cultures between Hours 72 and 120 was compared with that of control cultures between Hours 24 and 72. Bonferoni's comparison was applied, and results of comparisons between 30 min temperature treatments (a total of 5 comparisons) were judged significant if $P < 0.01$. Differences between cultures treated for 2 h at 60 °C and control cultures (1 comparison) were considered significant if $P < 0.05$, while those between controls, dark-treated cultures, and 15 °C-treated cultures (3 comparisons) were considered significant at $P < 0.017$.

To determine growth rates of cultures grown at various temperatures in 4-liter bottles, dry weight values measured during exponential growth were subjected to logarithmic transformation followed by linear regression analysis. Slopes of the resulting regression lines were tested for similarity and multiple comparisons were performed according to Zar (1984).

Results

Response to elevated temperature

Control cultures inoculated with 2.3–4.5 mg l^{-1} dry weight of algal homogenates and incubated at 45 °C reached densities of 3.7–14.0 mg l^{-1} immediately prior to heat shock at Hour 24 and 12.3–46.7 mg l^{-1} at Hour 72, corresponding to doubling times of 24.5–41.8 for the Hour 24–Hour 72 interval. It should be noted that the light intensity (ca. 50 μE m^{-2} s^{-1}) used in these experiments was not saturating for growth, since more rapid growth has been observed at 200 μE m^{-2} s^{-1}. Incubation at 55 °C for 30 min at Hour 24 did not significantly affect overall growth compared to that of controls ($P = 0.39$), with dry weight increases ranging from 72 to 126% of those in control cultures (45 °C = 100%) for the 48 h period following (and including) the 30 min temperature shock (Fig. 1). Incubation for 30 min at 60 °C, however, did cause a statistically significant ($P = 0.0037$) decline in growth compared to controls, with ten out of the twelve cultures achieving lower mean final dry weights at Hour 72 than the control cultures (Fig. 1). Strains 113 and T-7 showed no inhibition, while

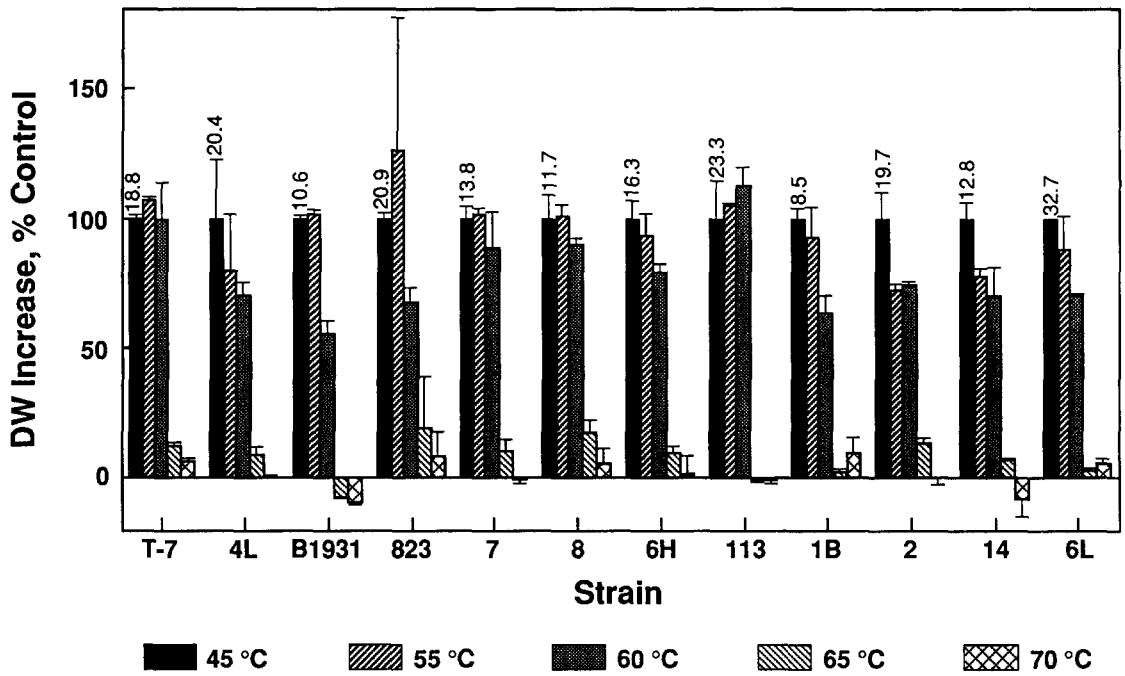

Fig. 1. Recovery from 30 min heat exposure. Replicate cultures were inoculated at Hour 0 and incubated at 45 °C, exposed to temperature treatments (55 °C, 60 °C, 65 °C, 70 °C) for 30 min at Hour 24, then incubated at 45 °C until Hour 72. Control cultures were maintained at 45 °C throughout the experiment. Graph bars show mean dry weight increase of duplicate heat-exposed cultures between Hour 24 and Hour 72, expressed as a percentage of mean dry weight increase in duplicate control cultures. Error bars show ranges of duplicate observations. Numerals above control bars indicate actual mean dry weight increases (mg l^{-1}) by control cultures between Hour 24 and Hour 72.

Strains 7 and 8 were among the least inhibited of the remaining strains. Strain UTEX B1931 was the most severely inhibited, while other strains appeared to show various degrees of mild inhibition.

Exposing the cultures for 30 min to 65 °C caused a visible yellowing of the cultures during the incubation period. Subsequent growth at 45 °C declined by about 90% on average, with some strains even exhibiting an overall decline in dry weight during this period (Fig. 1). A 70 °C temperature shock had an even more drastic effect. The inhibition of growth by both 65 °C and 70 °C treatments was significant ($P = 0.0036$ for 65 °C treatment, $P < 0.0001$ for 70 °C treatment), with 70 °C exposure producing a significantly ($P = 0.007$) more severe effect than 65 °C treatment.

The effect of a longer exposure to 60 °C was tested by incubation for 2 h, with other experimental conditions remaining the same. As before, the subsequent growth of 60 °C-exposed cultures differed significantly ($P = 0.004$) from that of controls, with most strains showing a slight decline in mean growth (Fig. 2). UTEX B-1931, the only strain derived from a culture collection, appeared much less heat tolerant than the other 11 strains, with an 80% decline in growth compared to 45 °C controls. Strains 4L and 1B also performed relatively poorly, with declines in growth of more than 40% compared to control cultures. Several strains (7, 113, and 6L) showed little or no growth impairment.

A more detailed study of the effects of 60 °C and 65 °C exposure was carried out using Strain 4L. Cultures were exposed to a 1 h incubation at 60 °C and 65 °C, following which analyses for dry weight, Chl *a*, and vegetative cell epifluorescence were carried out at Hours 48 and 72 (Fig. 3). It can be noted that 60 °C had only a

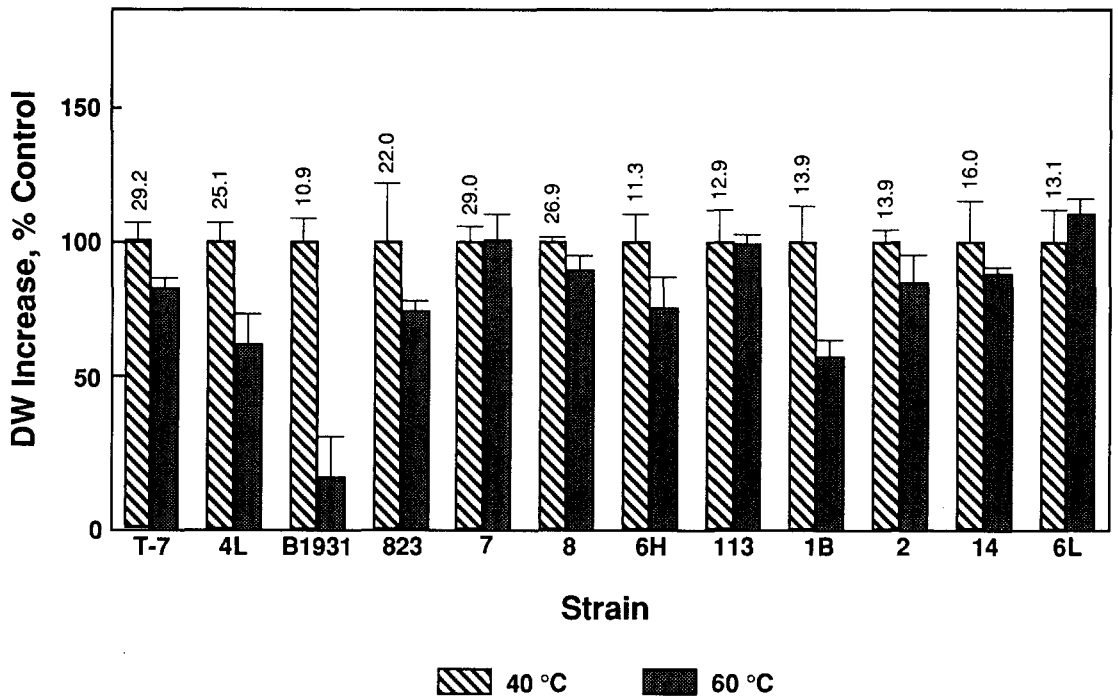

Fig. 2. Recovery from 2 h heat exposure. Replicate cultures were inoculated at Hour 0 and incubated at 45 °C, exposed to 60 °C for 2 h beginning at Hour 24, then incubated at 45 °C until Hour 72. Control cultures were maintained at 45 °C throughout the experiment. Graph bars show mean dry weight increase of duplicate heat-exposed cultures between Hour 24 and Hour 72, expressed as a percentage of mean dry weight increase in duplicate control cultures. Error bars show ranges of duplicate observations. Numerals above control bars indicate actual dry weight increases (mg l^{-1}) by control cultures between Hour 24 and Hour 72.

relatively minor effect, with a slight but significant ($P<0.05$) depression in growth (doubling time increased from 28 h to 37 h) and a 15% decrease in Chl *a* levels immediately after heat exposure. Dry weight-specific Chl *a* levels (not shown) at Hour 25 were 26% less than those of controls, but were equal to those of controls at Hour 48. The number of cells exhibiting lack of fluorescence was not affected. Less than 3% of the Chl *a* degraded to pheophytin (data not shown).

By contrast, incubation at 65 °C for 1 h resulted in an immediate 80% reduction in the number of vegetative cells displaying Chl *a* autofluorescence (without any increase in pheophytin content), followed within 24 h by a 75% decline in Chl *a*. No subsequent Chl *a* production or growth was detected within a 2 day time period. Although the proportion of autofluorescent cells recovered somewhat at Hour 72, this was probably due to the preferential disintegration of non-fluorescing cells, as evidenced by the large amount of cell debris observed.

Response to cold treatment and darkness

The effects of a 48 h exposure to 15 °C or to total darkness (at 45 °C) on subsequent growth at 45 °C are shown in Fig. 4. Figure 5 presents the data for Strain 4L, demonstrating that almost no change in culture density occurred during the period of exposure to cold or darkness. After cultures were returned to the control conditions (45 °C, illumination), growth resumed at about its prior rate. In Fig. 4, therefore, growth during the 48 h following exposure to suboptimal conditions (Hours 72–120) is compared with that of control cultures over the Hour 24–72 period. Most strains recovered at near-expected rates (Figs 4, 5) and overall there were no significant

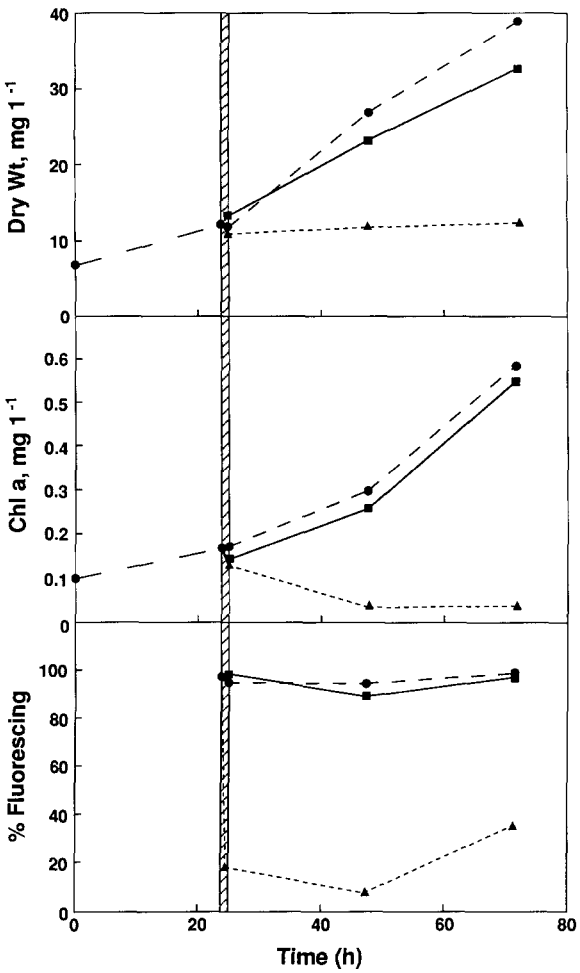

Fig. 3. Dry weight, Chl *a* content, and the percentage of cells showing visible autofluorescence during exposure to elevated temperatures and subsequent recovery of Strain 4L. Replicate cultures were inoculated at Hour 0 and incubated at 45 °C, exposed to elevated temperature for 1 h beginning at Hour 24 (shaded area), then incubated at 45 °C until Hour 72. Control cultures were maintained at 45 °C throughout the experiment. Fluorescence was not measured at Hour 0. Data points represent mean values for duplicate cultures. Symbols: ●, control cultures; ■, cultures exposed to 60 °C; ▲, cultures exposed to 65 °C.

differences between the various treatments ($P > 0.017$ for all 3 pairwise comparisons). However, Fig. 4 suggests that the strains differed in their response to cold treatment, with strains T-7, 6H, and 14 recovering more slowly from 15 °C incubation than did other strains. Cultures of these three strains also exhibited less growth than other strains after dark incubation.

Influence of temperature on growth rate

Among the locally obtained strains, Strain 113 appeared to be among the more resistant to high temperature, while Strain 4L was among the less resistant. The growth rates of these strains were determined as a function of temperature (Fig. 6). At intermediate temperatures, both strains grew at similar rates (at 200 $\mu E\, m^{-2}\, s^{-1}$), but Strain 4L grew significantly faster at 55 °C ($P \leq 0.05$), while Strain 113 grew somewhat more rapidly at 30 °C ($P \leq 0.10$), the reverse of what might be expected from their relative resistance to 60 °C exposure.

Discussion

Most physiological studies of cyanobacteria deal with cultures exposed to constant temperature conditions. In nature, organisms often encounter temperature fluctuations, which may extend beyond their permissible growth ranges for varying periods. It is of interest to determine not only the permissible (maximum, minimum, optimum) growth temperatures, but also the ability of specific strains to tolerate extremes that do not allow growth. Although the present study was inspired by a need to determine such limits as part of the development of an algal cultivation process (Wilde *et al.*, 1991), the data may be of broader physiological and ecological interest.

It is known that thermophilic cyanobacteria can tolerate brief periods of incubation at temperatures above the stable growth limit (Castenholz, 1969a, b). For MTF *Fischerella*, this upper growth limit appears to be 58 °C (Castenholz, 1969b, 1973). Data presented in this paper indicates that, while growth of some strains was slightly reduced during a 2-day period following exposure to 60 °C for up to 2 h, this inhibition was not severe in most cases. Observations on Chl *a* content and autofluorescence of strain 4L suggest that 60 °C exposure led to a temporary decline in cellular Chl *a* levels, but probably did not reduce viability. The most severely affected strain, UTEX B1931, was derived from a stan-

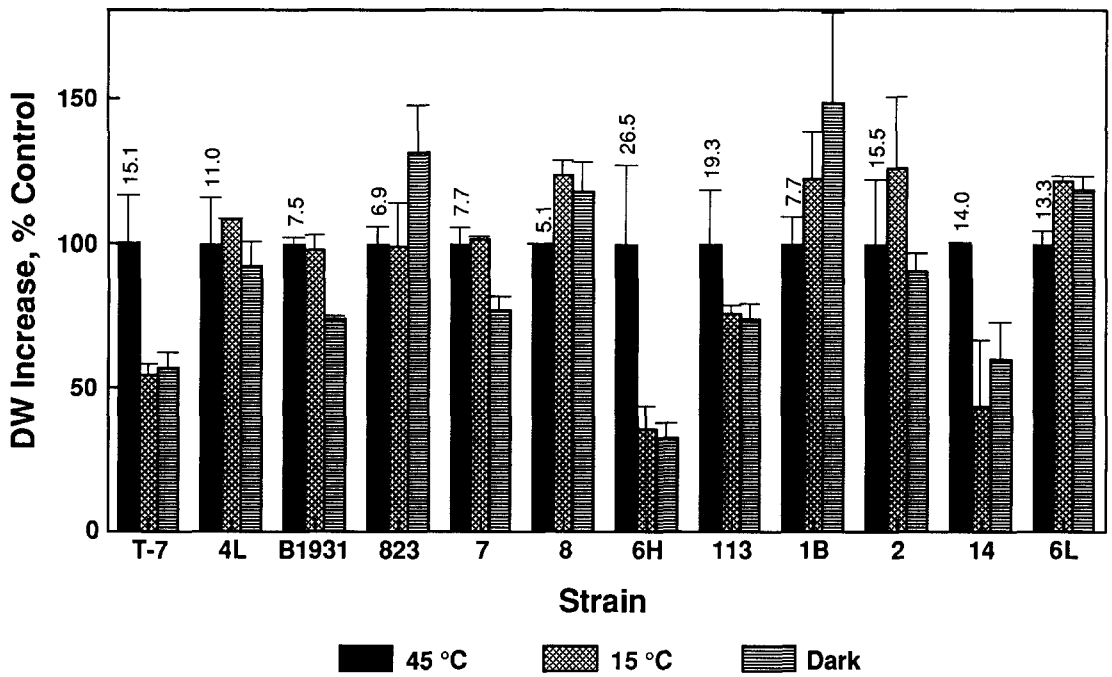

Fig. 4. Recovery from 15 °C or dark treatment. Replicate cultures were inoculated at Hour 0 and incubated at 45 °C, exposed to 15 °C or darkened for 48 h beginning at Hour 24, then incubated at 45 °C in the light until Hour 120. Control cultures were maintained at 45 °C in the light throughout the experiment. Graph bars represent mean dry weight increase of duplicate treated cultures between Hour 72 and Hour 120, expressed as a percentage of mean dry weight increase in duplicate control cultures between Hour 24 and Hour 72. Error bars show ranges of duplicate observations. Numerals above control bars indicate actual mean dry weight increases (mg l^{-1}) by control cultures during the 48-hour period.

dard culture collection and could have experienced a loss of heat tolerance during prolonged maintenance at constant temperature.

However, even a brief exposure to 65 °C (possibly as short as 15 min, considering the time required for flask contents to reach this temperature) was sufficient nearly to suppress subsequent growth. Although some cells probably survived (as indicated by the presence of a small number of chlorophyll-containing cells even after 1 h at 65 °C), most had clearly died.

The death of cultures at 65 °C and above was clearly associated with extensive pigment loss. Although chlorophyll levels declined only moderately during the high temperature incubation period, subsequent chlorophyll loss was severe. Vegetative cell autofluorescence, on the other hand, was immediately affected. This, together with the absence of subsequent growth, clearly indicates an irreversible process. Although it is possible and even likely that a small number of cells could have survived the treatment and eventually recovered sufficiently to initiate cell division, overall it is evident that even a relatively short exposure to temperatures only a few degrees above the maximum for growth results in irreversible growth inhibition and culture loss.

Relatively little is known about the mechanisms producing pigment loss and death at high temperatures. *Synechococcus lividus* undergoes disturbances in photosynthetic mechanisms near its upper growth temperature limit, and bleaches entirely when incubated slightly above its growth range (Meeks & Castenholz, 1971). However, such bleaching need not be the direct cause of death. Our results are consistent with the idea that growth impairment of *Fischerella* shocked at 60 °C is not directly due to pigment loss, since growth was significantly slower than that of controls even after normal cellular levels of Chl *a*

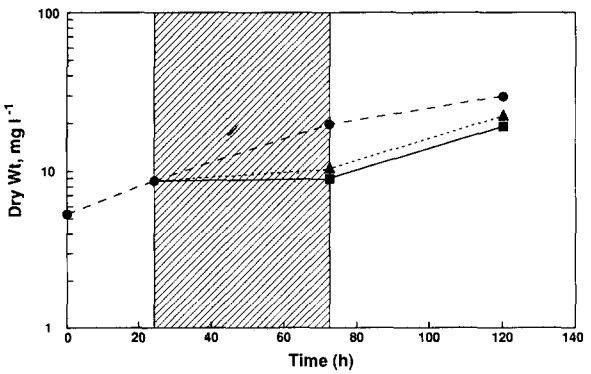

Fig. 5. Effect of cold or dark treatment upon growth and recovery of Strain 4L. Cultures were maintained at 45 °C, 50 μE m^{-2} s^{-1} except during cold or dark treatment (shaded area). Data points represent mean dry weight contents of duplicate cultures. Symbols: ●, control cultures (45 °C, 50 μE m^{-2} s^{-1}); ■, darkened cultures (45 °C, foil-wrapped); ▲, cold-exposed cultures (15 °C, 50 μE m^{-2} s^{-1}). Growth rates during recovery are summarized in Fig. 4.

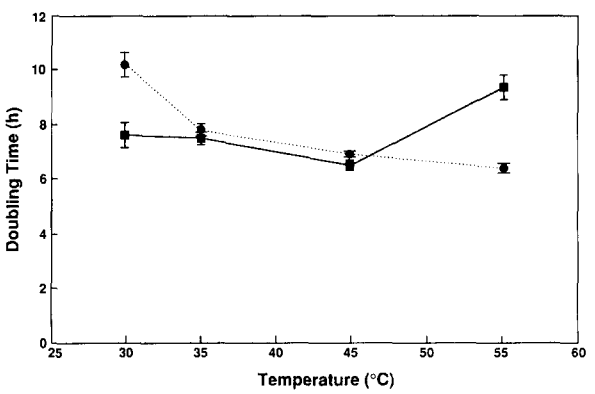

Fig. 6. Effect of growth temperature upon doubling time for Strain 113 (■) and Strain 4L (●). Cultures were grown under continuous 200 μE m^{-2} s^{-1} illumination. Error bars represent ± 1 standard deviation from the mean.

were restored. A similar long-lasting effect of heat shock has been noted in *S. lividus* (Sheridan & Ulik, 1976). The apparent nonviability of chlorophyll-containing cells immediately after 65 °C treatment also indicates that pigment loss alone does not account for the detrimental effects. The absence of Chl *a* fluorescence under these conditions suggests, rather, that damage to photosynthetic mechanisms had occurred. There were obvious differences between strains with regard to the effect of 60 °C exposure, some undergoing little or no growth inhibition, but others affected by even a brief exposure. This could indicate slight differences between strains in their maximum temperature tolerances, such as have been established for *S. lividus* (Peary & Castenholz, 1964). The most sensitive strain was UTEX B1931, while local and Hawaiian strains showed various degrees of resistance. The Hawaiian cultures, which contained cyanobacteria other than *Fischerella*, were not among the best performers, although in some cases they were more resistant than certain local strains. Thus, as would be expected, strain selection for upper temperature tolerance is both necessary and practical.

The fact that most of the 12 strains showed no lasting damage from cold incubation is in accordance with previous reports. *Fischerella* strains have been known to survive 15 °C for as much as 3 months, although the reported growth minimum is about 25 °C (Castenholz, 1969b). A few strains appeared to show reduced growth rates during recovery from the cold treatment. However, comparison of these rates with those of darkened cultures indicate that photooxidative or photodynamic damage was probably not involved. It is possible that, when returned to growth-permitting temperatures, these cultures simply experienced a brief lag period before resuming growth, as is known to occur in *S. lividus* (Meeks & Castenholz, 1971).

In addition to differing in their ability to survive extreme temperatures, two strains of thermophilic *Fischerella* differed substantially in their growth temperature optima. The data suggest that, at least among strains of MTF *Fischerella*, a higher optimal growth temperature may not invariably translate into a correspondingly greater ability to tolerate 60 °C exposure. Similarly, resistance to high temperature need not correlate with sensitivity to low temperature, as seen from the observation that some strains with high tolerance to the 2 h 60 °C exposure (e.g. strains 7 and 113) were also little affected by exposure to cold temperatures.

In conclusion, this study has demonstrated that the physiological responses of these thermophilic

cyanobacteria generally meet the temperature tolerance requirements of the algal cultivation process proposed by Wilde *et al.* (1991) and described in the Introduction. Further work is in progress to demonstrate the ability of selected strains to take up excess nutrients from effluent cooling water under varying environmental conditions.

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