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Growth Response and Heat Shock Protein (Hsp) Expression in *Chlorella vulgaris* Exposure to Elevated Temperatures

S. ZARGAR, K. KRISHNAMURTHI, S. SARVANA DEVI, T.K. GHOSH and T. CHAKRABARTI*

Environmental Biotechnology Division,
National Environmental Engineering Research Institute (NEERI),
Nehru Marg, Nagpur-20 (MS) India

Abstract

The present paper deals with the impact of various sublethal levels of temperature (26, 31, 33, 36, 39, 42 and 45°C) on growth and heat shock protein (Hsp) expression in freshwater green alga *Chlorella vulgaris*. Impact of select levels of temperature on growth rate (based on optical density), population count, chlorophyll-*a* and biomass, of the alga was evaluated in artificial growth medium for a period of 15 days. To determine the induction of Hsp in the alga, it was exposed to select temperature levels for 3 hrs. and further kept for 6 hrs at culturing conditions at 26°C. Induction of Hsp was confirmed by immuno detection followed by SDS-Polyacrylamide gel electrophoresis. The select growth parameters of the alga were reduced drastically at 39, 42 & 45°C. Temperatures below 39°C may be considered as the limit of safe exposure for thermal stress of the alga. The Hsp 70 expression was also observed only at 39°C.

Introduction

Temperature is one of the major environmental factors, which plays a critical role in growth, reproduction, migration, succession pattern, metabolism of organisms and health of ecosystem (Countant and Suffern 1979; Das et al. 2004). In general, elevated water temperature causes changes in species composition, species dominance, standing crop and productivity of biota including phytoplankton communities in any aquatic ecosystem. Thus, warm water discharges from the power plants into the receiving water bodies may adversely affect aquatic ecology. Phytoplankton, being placed at the bottom rung of the food chain in the aquatic biotope, their fluctuations in density and the biomass directly affect the entire biotic structure of the ecosystem. The range of temperature tolerated by the life form is completely wide but each species shows characteristic-limited temperature preference and tolerance (Richardson et al. 1994; Shulman and Love 1999). It is known that plants and animals are able to thrive best in certain temperature ranges and that changes in the temperature of a body of water will influence the types and numbers of organisms in the aquatic ecosystems.

* Corresponding author.
E-mail address: twmneeri_ngp@sancharnet.in

Thermotolerance can be induced by heat shock (exposure of cells to a sudden rise in temperature) Nover (1991) and the heat shock response of cells is characterized by a high rate of synthesis of heat shock proteins (Hsps).It is considered that heat shock proteins contribute to the ability of living organism to survive at a high temperature, at least temporally (Wollgiehn et al. 1994).In algae, heat shock treatment has been shown to induce protection against thermodamage in *Acetabularia mediterranea* (Kloppstech et al. 1986) and *Chlamydomonas eugametos* (Alexandrov 1979). Thermotolerance induced by heat shock has been studied (Shen and Lee 1997) in *Cholrella zofingiensis*, however, the same has not been reported in the alga *Chlorella vulgaris*. Still, little is known on the induction of the Hsp in algae under different environmental conditions.

The focus of this study was to investigate the impact of various sublethal levels of temperature on survival, growth and heat shock protein (Hsp) formation of the green alga *Chlorella vulgaris*.

Materials and Methods

Algal exposure and parameters

The alga *Chlorella vulgaris*, was isolated from the Kadra reservoir, Kaiga, Karnataka and its pure culture was maintained in Beckers' nutrient solution (Stein 1975). All cultures were kept at a light intensity of 35 µE (12:12 light/dark regimen) in 1-*l* glass flasks containing 500 mL of test culture solution with a cell density of 3 x 10^4 cell ml^{-1} for 15 days at seven different temperatures, viz.26, 31, 33, 36, 39, 42 and 45^{o}C. Impact of various levels of temperature on growth rate (based on optical density: at 560 nm), population count (Lackey Drop method), chlorophyll-*a* (spectrophotometric method) and biomass (gravimetric method) of the alga was evaluated. Experiments were run in triplicate. Details of the methodology have been referred in standard methods (APHA 1999).

Heat shock protein expression

In order to determine the induction/expression of heat shock protein in the alga, test culture solution (5 x 10^4 cell ml^{-1}) was exposed to select temperature levels, viz. 26, 31, 33, 36 and 39^{o}C for 3 hrs. After thermal exposure, the algal cultures were maintained at culturing conditions at 26^{o}C for 6 hrs. During this period equal volumes (100 ml) of the culture were taken out at different time intervals, viz. 0 (just after thermal exposure), 1, 3 and 6 hrs. Appropriate control (algal culture at 26^{o}C) was maintained during the experiment. Protein quantification of each sample was determined by Lowry's method (Lowry et al. 1951) after centrifugation (1000 xg for 10 minutes at 4^{o}C), lysing {50 mM Tris. HCl. (pH 6.8), 2 % SDS, 0.1 % Bromophenol blue and 10% Glycerol} and sonication (15s, 20 KHz, 75 W thrice in ice). The sample containing 50 µg of protein was loaded onto 10 % SDS polyacrylamide gel (Sambrook et al., 1989), which was then transferred to nitrocellulose membrane (Hybond ECL, Amersham Pharmacia, UK) using electroblotting apparatus. Further, immuno detection was carried out according to the instruction manual (Hybond ECL, Amersham Pharmacia, UK).

All the experiments were run in triplicates, and the results were presented as means, standard error, and levels of significance ($P<0.001$-0.05), following Student's 't' test using a statistical software Statistica version 5.0.

Results and discussion

Tolerance of alga to elevated temperature

Growth curves of the alga at various temperature levels are typical of the results obtained during the period of investigation. The impact of various levels of temperature on calculated percent growth rate (based on optical density values), population density, chlorophyll-*a* content and biomass of the alga are shown in Figs. 1 to 4. The values of all the selected growth parameters of the alga were reduced drastically at 39, 42 & 45°C. Miquel (1892) and Daletzkaya and Chulanovskaya (1964) found lethal temperature for green algae at 42°C and above. In the present study all the growth parameters of the alga at 26°C showed significantly higher value from those at 31 ($P<0.05$), 33 ($P<0.05$), 36 ($P<0.01$), 39 ($P<0.001$), 42 ($P<0.001$) and 45°C ($P<0.001$) and it conforms with the earlier finding of Sayed and EL-Shahed (2000). During the experiment, optimum growth temperature for the alga was estimated as 26°C, however, it showed considerable growth up to 36°C, which was found to be the limit of thermotolerence of the alga. The temperature 36°C may be considered as the maximum allowable temperature limit (MATL) to the test alga.

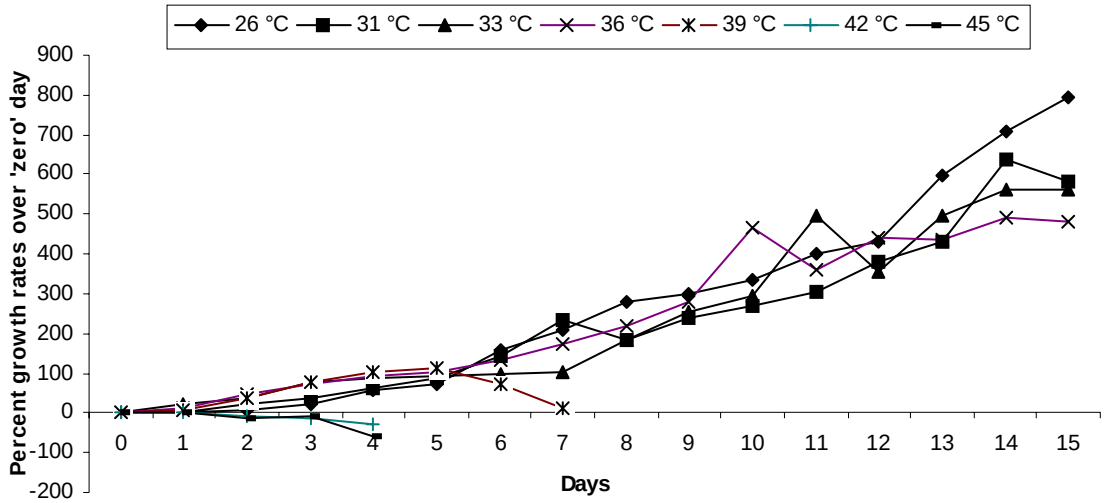


Fig. 1. Effect of different temperature levels on growth rate of the alga, *Chlorella vulgaris*

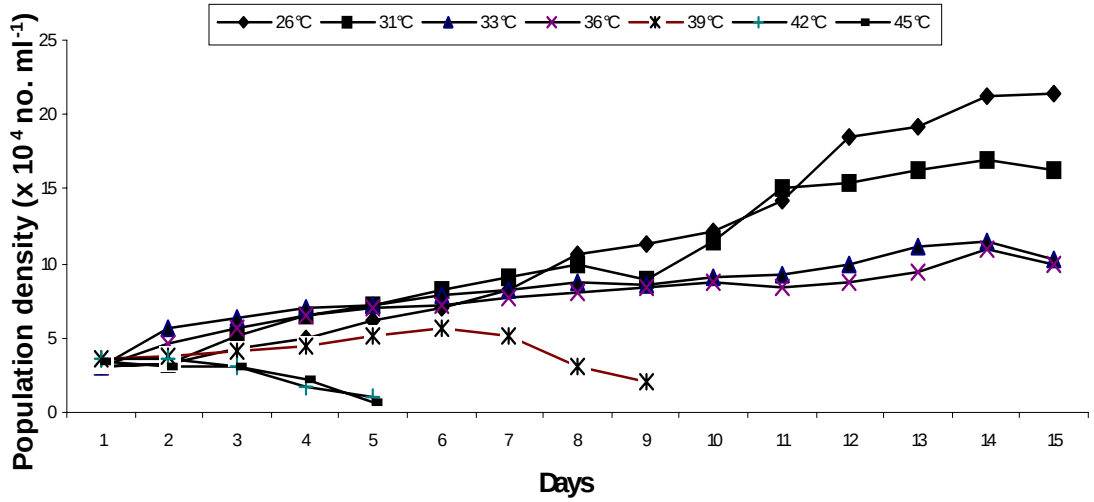


Fig. 2. Effect of different temperature levels on population density of the alga, *Chlorella vulgaris*

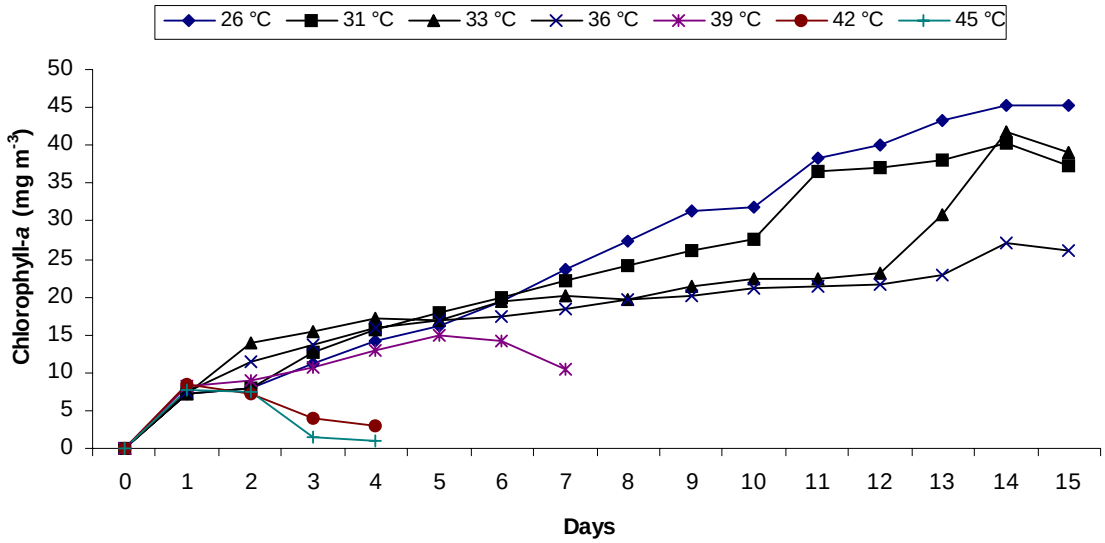


Fig. 3. Effect of different temperature levels on chlorophyll-*a* of the alga, *Chlorella vulgaris*

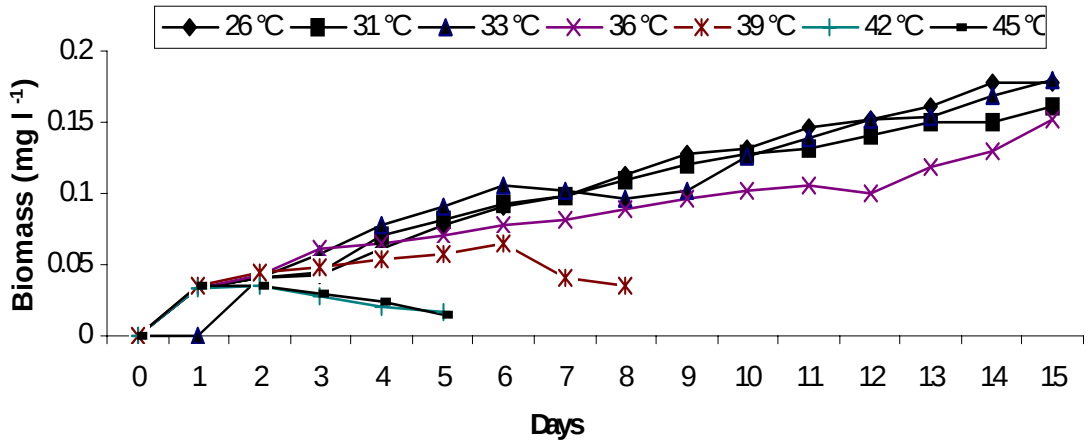
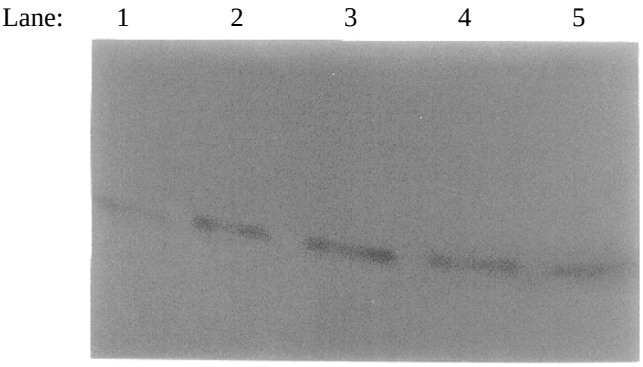


Fig. 4. Effect of different temperature levels on biomass of the alga, *Chlorella vulgaris*

Heat shock protein expression

In another experiment, the expression of Hsp 70 was assessed at 26, 31, 33, 36, and 39°C. The higher level of expression was observed only at 39°C in comparison with control (Fig. 5). The induction of Hsp 70 has been confirmed in a wide range of organisms, from microorganisms to humans (Lindquist and Craig, 1988).The role of Hsp in survival and/or adaptation of microorganisms at different stress conditions have been reported. Bierkens et al. (1998) conducted a study to detect the synthesis of Hsp 70 in *Raphiodocelis subcapitata* (a green alga) in response to changes in pH, temperature, humic acids, nitrates and phosphorus, and found that only temperature and pH were able to induce acquired tolerance. In another study,



1: Control; 2: 0 hour; 3: 1 hour; 4: 3 hour; 5: 6 hour (Lanes 2-5 indicate Hsp 70 expressions after exposure of the alga at 39°C; however, Lane 1 indicates the same of the alga at 26°C)

Fig. 5.Western blots (Hsp 70) of freshwater alga, *Chlorella vulgaris* at different hours of exposure at 39°C

Nicole (2000) had tested thermotolerance of *Synechocystis* sp. by withdrawing the Hsp and found the alga became sensitive to temperature.

Hsp's function was seen mainly in blue green algae, especially of the genera *Oscillatoria*, *Synechocystis*, *Anabaena* and *Synechococcus* in various countries (Gour et al. 1997; Porankiewicz and Clarke 1997; Voronova et al. 1997; Horvath et al. 1998). At both the cellular and organism levels, induction of synthesis of stress protein correlates with acquired tolerance, a phenomenon, which increases the ability of the exposed organism to survive a subsequent more severe stress that would have otherwise be lethal (Lindquist and Craig 1988). The induction of response in the alga may be due to conformational changes in protein caused by the exposed temperature. Therefore, the result suggests that Hsp 70 expression may be a sensitive indicator of exposure to nontolerable temperature of the test alga.

Conclusion

The outcome of this research suggests that the temperature 36^{o}C may be considered as the maximum allowable temperature limit for the alga. Since applications of stress protein to environmental monitoring are still limited, the present study provides validation for the development of Hsp as a biomarker of exposure and effect. The data base generated will serve as a reference source for initiation of review/reaffirm the thermal water quality standards and would also be useful for the management of thermally stressed fresh water reservoirs.

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