

Photosynthesis and Heat Response of the Green Alga *Micrasterias denticulata* (Desmidiaceae)

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Cells of the green alga *Micrasterias denticulata* cultivated at 15 °C, 20 °C or 25 °C were exposed to heat shocks at different temperatures (30–40 °C) for varying duration (5–90 min). Cell pattern formation, division rate as well as photosynthesis and respiration by measuring oxygen production and consumption have been studied. The degree of cell shape malformations was found dependent on the preceding cultivation temperature along with the mode of the heat shock. Cells cultivated at 15 °C and 20 °C could counteract a 90 min heat shock at 35 °C much better than those cultivated at 25 °C, which was seen by a less reduced young semicell. Cells cultivated at 15 °C and 25 °C reveal a reduced division activity compared to those grown at 20 °C even with a marked retardation when affected by a preceding heat shock.

Photosynthesis and the level of plastid pigments (carotenoids, chlorophylls, β -carotene, lutein) of controls determined by HPLC analysis reached a plateau after about 26 days when starting with 22-day old cultures. Photosynthesis and respiration were determined in a range between 15 °C and 40 °C in defined *Micrasterias* cell cultures of about this age (cultivation temperature 15 °C, 20 °C or 25 °C). Both processes rose steadily with increasing temperature starting with 15 °C and reached peaks between 30 °C and 32 °C, followed by a considerable drop when increasing the incubation temperature up to 40 °C. The experiments reveal that primary processes of energy formation and consumption are much less affected by temperature influences than cell shape formation and division rate.

Introduction

Unicellular algae of the family Desmidiaceae are confined to small acid moorland bog ponds found at different altitudes all over the world (Brook and Williamson, 1988). Thus the algae are exposed to altered diurnal and seasonal light and temperature conditions, from which they cannot escape. For example, as an extreme, various species of the desmids *Cosmarium* and *Staurostrum* have been observed in temporary pools at even 40 °C (Brook, 1981). Especially on sunny summer days an enormous amount of algae is visible as a green layer underneath the water surface, whereas at frosty conditions the algae seem to withdraw to the ponds ground. These movements are possible by excreting slime through numerous pores in the algal cell wall (Kiermayer, 1981; Neuhaus and Kiermayer, 1981).

Among the family Desmidiaceae particularly *Micrasterias denticulata* has been subject to nu-

merous cell biological and physiological investigations (for summary see Meindl, 1993). The large cell with an approximate diameter of 200 μ m is characterized by its extraordinary, highly ornamented cell pattern expressed in numerous lobes and indentations of the two semicells which are arranged like a mirror image. After each mitosis the cell pattern of one half cell has to be rebuilt within approximately 5 hours during which the alga is extremely sensitive to environmental influences (Meindl and Lütz, 1996; Lütz *et al.*, 1997). As any harmful influence may change cell size and symmetry accompanied by shifts in position of both lobes and indentations, *Micrasterias* is an ideal indicator for any physical or chemical impact.

Previous studies reveal the importance of temperature during cell shape formation and cell division, especially at very early stages (Meindl, 1990). Temperatures of 10 °C and more above optimum

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(20 °C) may induce malformations, reduced cell size and pattern, with the most striking effects in the range between 35 °C and 37 °C, and partial or total growth inhibition at temperatures beyond. At an ultrastructural level elevated temperature causes an increase in ER cisternae and leads to the formation of “heat shock granules” accumulated particularly in the area of the nucleus. Recently we could prove the presence of two heat shock proteins, HSP70 and HSP78, the latter also known as binding protein (BiP), in *Micrasterias* cells grown at different cultivation temperatures. Whereas a previous heat shock increases the amount of HSP70 in dependence of duration and temperature of heat treatment it has no effect on the content of BiP (Weiss and Lütz-Meindl, 1999).

As temperature changes at their natural habitats are of high relevance for occurrence and distribution of desmids we investigated the influence of elevated temperature of different degrees on cell division rate, photosynthesis and respiration of the green alga *Micrasterias* in the present study under varying cultivation temperature. The experiments under short-term laboratory conditions as well as the long term investigations are performed at temperature conditions comparable to those in the natural environment. The results reveal an optimum in gross photosynthesis of *Micrasterias* in a range between 30 °C and 32 °C depending on the previous cultivation temperature but indicate that the cell division rate decreases at any deviation from the normal cultivation temperature at 20 °C.

Materials and Methods

Culture conditions

Cells of the green alga *Micrasterias denticulata* Bréb. (Fig. 1, algae culture collection of the Institute for Plant Physiology, University of Salzburg) were grown under semi-sterile conditions in a “desmid” medium with soil extract (Schlösser, 1982) at temperatures of 15, 20 and 25 °C in an incubator. The culture flasks (Erlenmeyer flasks) were kept at a light-dark regime of 14 to 10 h and a growth light intensity of approx. 50 µmol photons m⁻²s⁻¹.

Light microscopy

For light microscopical investigations on temperature effects young developing cells of *Micrast-*

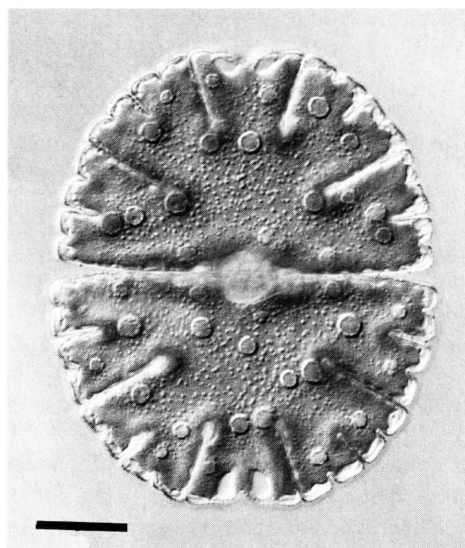


Fig. 1. Non-growing untreated cell of *Micrasterias denticulata* (cultivation temp. 20 °C). bar = 50 µm.

erias less than 15 min after mitosis were selected from the cultures (cultivation temperature 15 °C, 20 °C and 25 °C), transferred into glass tubes filled with distilled water and placed in darkness in a thermostated water bath (Julabo F10). After heat treatment in a range between 30 °C and 40 °C lasting for 5 to 90 min the cells were kept at their anterior cultivation temperature till cell shape formation was finished (5 h). The cells were examined and photographed with a Reichert Univar light microscope.

To investigate the influence of the heat treatment on the cell division rate a defined number of untreated cells (10 cells) as well as heat shocked ones (10 cells) both cultivated at different temperature (15 °C, 20 °C, 25 °C) were kept at their former cultivation conditions for several weeks. The number of cell divisions was determined every 24 h. For the calculation the day of the experiment start (heat shock) was set as zero.

Plastid pigment measurement

Over a period of two weeks plastid pigments of *Micrasterias denticulata* cells (50 cells per assay) were measured as previously described (Lütz *et al.*, 1997) every two or three days starting with 22 days old cultures. After centrifugation (5000×g) the pellet, containing 50 cells, was resuspended in

100 µl acetone containing 1 drop of 25% NH₃ per 50 ml. After centrifugation 20 µl of the supernatant was analyzed with a Waters HPLC equipped with a photodiode array (Type 996; Wildi and Lütz, 1996).

Oxygen measurement

Oxygen evolution and consumption were recorded using a Clark-type oxygen suspension electrode (Hansatech, England). For temperature controlled measurements the electrode unit was attached to a thermostated water bath (Julabo F10). For the measurement 1 ml of a solution containing *Micrasterias* cells at a density of 2000 cells per ml concentrated by gentle centrifugation and 1 ml of a 0.8% NaHCO₃ solution (pH 6.5), known to be sufficient to saturate photosynthesis according to previous tests, were added to the chamber. After a 5 min adaptation to the corresponding temperature, measurement was started following a distinct schedule lasting for 1600 s. A dark period lasting for 400 s was followed by a 400 s period at an illumination of 200 µmol photons m⁻²s⁻¹, and by another 400 s dark and light cycle. These dark/light cycles made it possible to record twice respiration as well as net photosynthesis in one sample. A typical record is given in Fig. 2. Gross photosynthesis was calculated by addition of average respiration values to average net photosynthesis values.

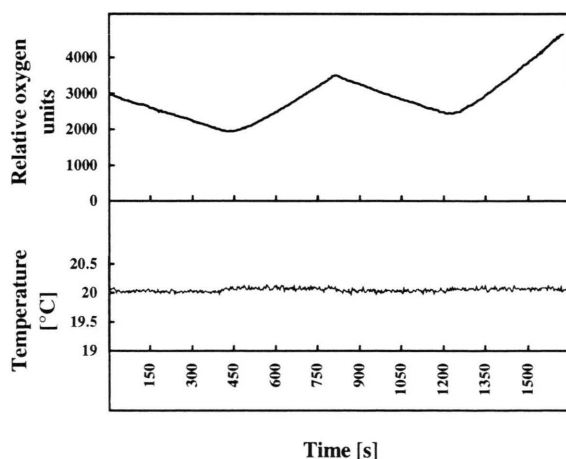


Fig. 2. The selected sample shows a typical record of an oxygen measurements. Two consecutive dark/light cycles were run to determine net photosynthesis and respiration of 2000 *Micrasterias* cells at 25 °C (cultivation temp. 25 °C).

All data were evaluated running a computer software designed for use with the electrode (for details see Lütz, 1996). For further processing gross data were calculated taking approximately three different measurements per temperature value.

The experiments to determine changes in photosynthesis, pigment composition and content in dependence on the age of the cultures were started with algae (cultivation temperature 20 °C) 22 days after the last medium exchange. During this analysis lasting for two weeks, the parameters were estimated every second or third day.

The effects of temperature on photosynthesis and respiration were recorded in the range between 15 °C to 40 °C by raising the temperature in steps of 1 °C, with measurements at every step. The cell cultures to be tested were grown at temperatures of 15 °C, 20 °C, 25 °C, respectively. Photosynthesis studies in relation to temperature (15 °C to 40 °C) were carried out in one degree steps using about 28 days old cultures (cultivation temperature 15 °C, 20 °C, 25 °C). At this age the cultures provide a sufficient number of algae for the measurements, reveal highest photosynthetic activity as shown by oxygen analyses and HPLC and the physiological stage of the cells corresponds to that used for a variety of earlier physiological and cell biological investigations.

Results

To investigate the influence of temperature on cell shape formation, growing *Micrasterias* cells less than 15 min after mitosis were exposed to short term heat treatments (5–90 min at 30–40 °C). From earlier studies it is known that at least long term temperature treatments (2–24 h) between 30 °C and 38 °C give rise to malformations, reduced cell size and cell pattern (Meindl, 1990). In the present study these results are confirmed. However, in our experiments the temperature applied, ranged from 30 °C to 40 °C and the duration of the treatment was cut down to 5–90 min.

In our study it turns out that even a heat shock at 34 °C for only 5 min affects cell morphology of young developmental stages (treatment approx. 15 min after mitosis). Two different types of morphological aberration are observed in cells cultivated at 20 °C after a 90 min heat shock at 34 °C.

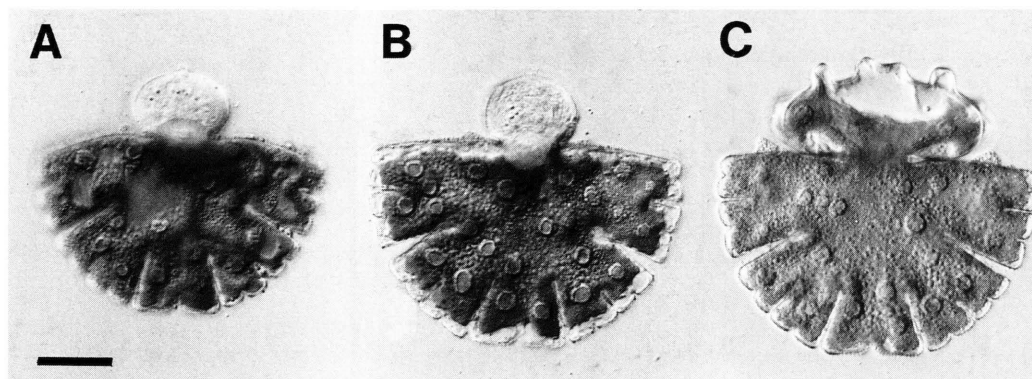


Fig. 3. (A-C) Developing *Microsterias* cells after heat shock (90 min at 35 °C), growth inhibited. (A) cultivation temp. 15 °C, (B) cultivation temp. 20 °C, (C) cultivation temp. 25 °C. bar = 50 μ m.

One cell type is characterized by a reduced symmetric or asymmetric cell pattern developed in the main plane of the cell. In the second type the lateral lobes may be arranged in different directions without any preferential orientation. When rising the temperature of the heat shock to 35 °C, cell growth is markedly inhibited (Fig. 3B). However, cells cultivated at 25 °C cope with the temperature stress of 35 °C much better (Fig. 3C). Up to 34 °C their temperature response is similar to cells cultivated at 20 °C, whereas between 35 °C and 36 °C the morphological aberrations are less severe in cells cultivated at 25 °C compared to those cultivated at 20 °C. On the contrary cells cultivated at 15 °C are much more affected by high temperatures (above 34 °C), since their cell size is much more reduced by the heat shock (Fig. 3A). Regardless to the preceding cultivation temperature, heat shocks between 36 °C and 40 °C completely inhibit cell growth.

Based on the results mentioned above the effect of heat shock (35 °C, 90 min) on division rates of cells cultivated at 15 °C, 20 °C and 25 °C were carried out. Cells both untreated and heat shocked ones grown at 20 °C (standard cultivation temperature) are dividing every 5th to 7th day, with a slight almost insignificant delay in cell division of the untreated ones (Fig. 4B). In contrast cells both untreated and heat shocked ones cultivated at 15 °C (Fig. 4A) or at 25 °C (Fig. 4C) reveal a reduced division activity. However, the delay is much more significant for the heat shocked cells than for the untreated ones. Untreated cells cultivated at 15 °C or 20 °C both divide for the first

time 7 days after the last division, whereas for heat shocked ones the first division is observable on the 9th (15 °C) or the 11th (25 °C) day after the beginning of the experiment. At the 26th day of observation cells grown at 20 °C count 24 (controls) and 25 (heat shocked cells), whereas cells cultivated at 15 °C count 17 (controls) and 14 (heat shocked) and those cultivated at 25 °C 18 (controls) and 8 (heat shocked). Remarkably the final number of heat shocked cells grown at 25 °C amounts only one third of the controls.

As basis for the temperature dependent oxygen measurements the optimum in photosynthetic activity in respect to the age of the cell cultures was determined by recording gross photosynthesis over a period of two weeks starting with 22 days old cultures (20 °C cultivation temperature). The studies revealed an increase of about 10% up to day 26, where the measurements reach a plateau up to day 33 of cultivation. This small but significant increase indicates activities in the chloroplast, because the calculation basis is equal cell number and therefore plastid number. Thereafter gross photosynthesis dropped slightly till the end of the experiment (Fig. 5).

Correspondingly the concentration of plastid pigments determined by HPLC analysis (Fig. 6A-D) reached its maximum at the 27th day of cultivation and decreased during the following measurement period. The pigment classes "carotenoids" and "chlorophylls" were chosen to describe separately the main pigment domains in the thylakoids; both increase by about 50% from the value measured at day 22 to day 27. For a more detailed

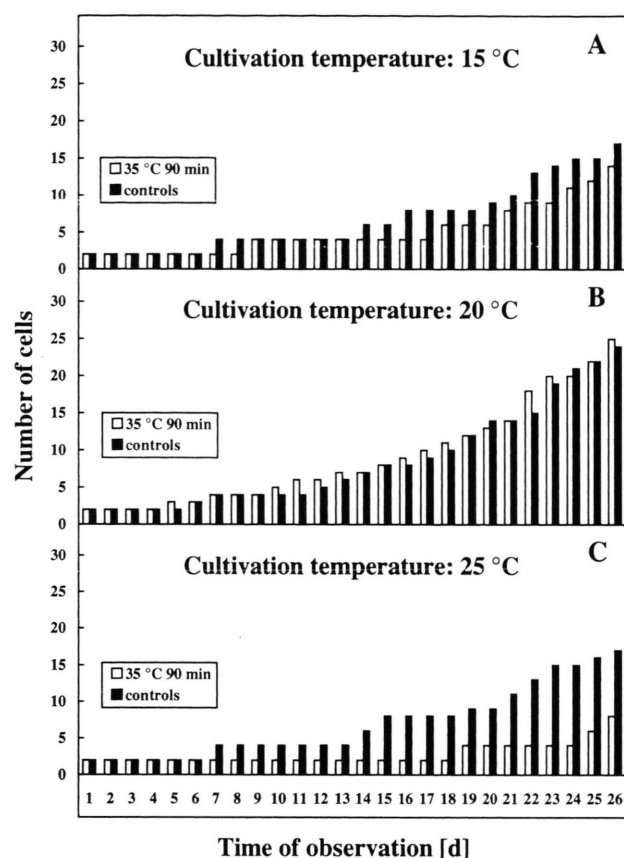


Fig. 4. Cell division rate of *Micrasterias* cells after heat shock (90 min at 35 °C) in comparison to control cells. (A) cultivation temp. 15 °C, (B) cultivation temp. 20 °C, (C) cultivation temp. 25 °C.

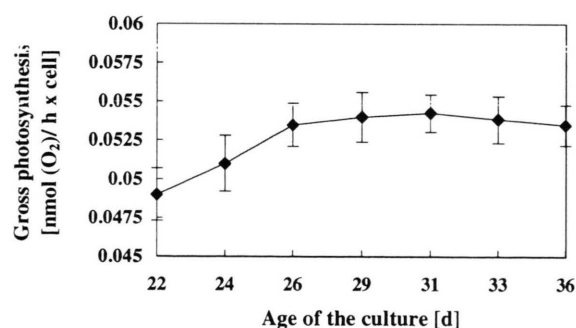


Fig. 5. Gross photosynthesis of *Micrasterias* cells (cultivation temp. 20 °C) recorded over a period of two weeks. Data are presented as means \pm standard error.

characterization, two single pigments were selected: β -carotene serves as a marker for the reaction centers of both photosystems, and lutein indicates the light harvesting complex. While β -carotene synthesis amounted to additional 23%, lutein showed the same 50% increase like both

pigment classes (days 22–27). At the end of the cultivation period (day 35), the amounts of all pigments were found higher or in the range of the first assays at day 22. As a consequence of these results 28 days old *Micrasterias* cultures were used for measuring oxygen production/consumption after temperature exposure.

When raising the temperature in one degree steps from 15 °C to 40 °C oxygen production (gross photosynthesis, Fig. 7A) and consumption (respiration, Fig. 7B) both reached culmination points between 30 °C and 32 °C at the illumination of 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. However the lane of the graphs and the peaks varied slightly among cells cultivated at different temperatures. Oxygen production as well as consumption both rose steadily up to a temperature of 26 °C. From that on photosynthetic activity and respiration, respectively, increased steeper with peaks at 30 °C (cultivation temp. 15 °C), 31 °C (cultivation temp. 20 °C) and 32 °C (cultivation temp. 25 °C). A fur-

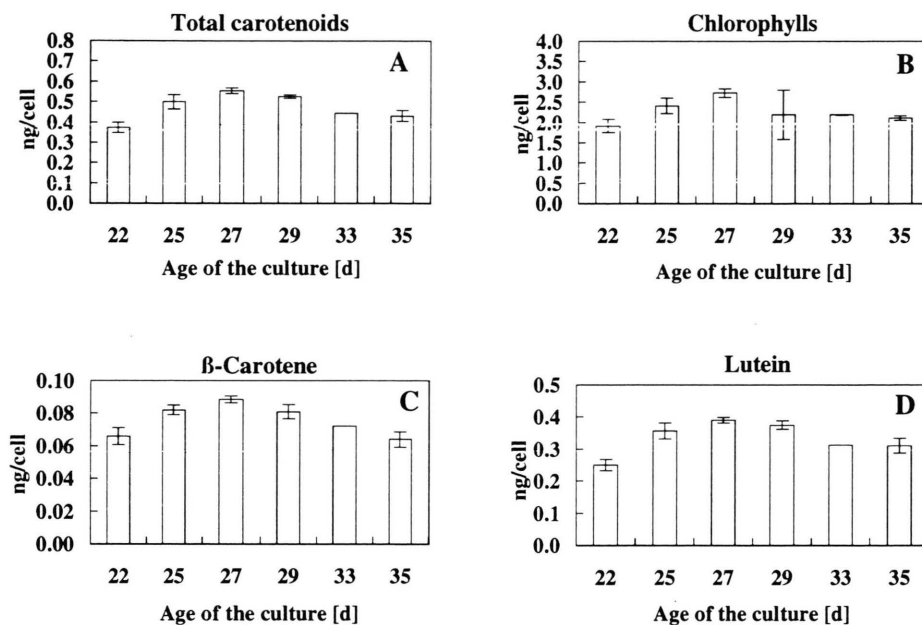


Fig. 6. Dependence of main chloroplast pigments on the age of the algal cultures grown at 20 °C. (A) total carotenoids, (B) chlorophylls, (C) β -carotene, (D) lutein.

ther increase in temperature up to 40 °C led to a considerable drop in oxygen rates reaching quantities comparable to those obtained at 15 °C or even less. Cells cultivated at 20 °C for example revealed higher photosynthetic rates than cells grown at 15 °C but less than the one cultivated at 25 °C.

However, the amounts of oxygen development at temperatures higher than 32 °C seem to be more related to the preceding cultivation conditions which means that cells cultivated at 25 °C reveal the highest photosynthetic activity. A similar adaptation to the different growth temperatures could be found for rates of respiration.

Discussion

The results provide evidence that *Micrasterias* cells are extremely sensitive to temperature changes in cell pattern formation and that the degree of cell shape malformation occurring after a heat shock depends on the preceding cultivation temperature. Cells cultivated at 25 °C seem to be less sensitive against elevated temperature. In contrast, photosynthesis up to approx. 32 °C is remarkably stable independently from the previous cultivation temperature but decreases continu-

ously when raising the temperature up to 40 °C. Highest photosynthetic activity is reached when the cells are grown at 20 °C. Furthermore it could be demonstrated, that concerning cell division rate, cells grown at 20 °C are more efficient than cells cultivated at 15 °C or 25 °C. In general it is suggested that the physiological requirements of *Micrasterias* cells are much better achieved at the cultivation temperature of 20 °C, than at a temperature of 15 °C or 25 °C.

The range of photosynthetic activities is predetermined not only by growth temperature, but also by growth light intensity (Baker and Long, 1986; Berry and Björkman, 1980; Larcher, 1994). At every cultivation temperature, the cells were exposed to approx. 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ growth light, and photosynthesis was measured at the saturating intensity of 200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. As shown previously (Lütz *et al.*, 1997), even light incubation for several hours with approx. 600 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (at 24 °C) resulted only in a slight adaptive reduction in photosynthesis. We can assume that the measured culmination temperatures of oxygen evolution will not be markedly influenced by irradiations up to 600 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

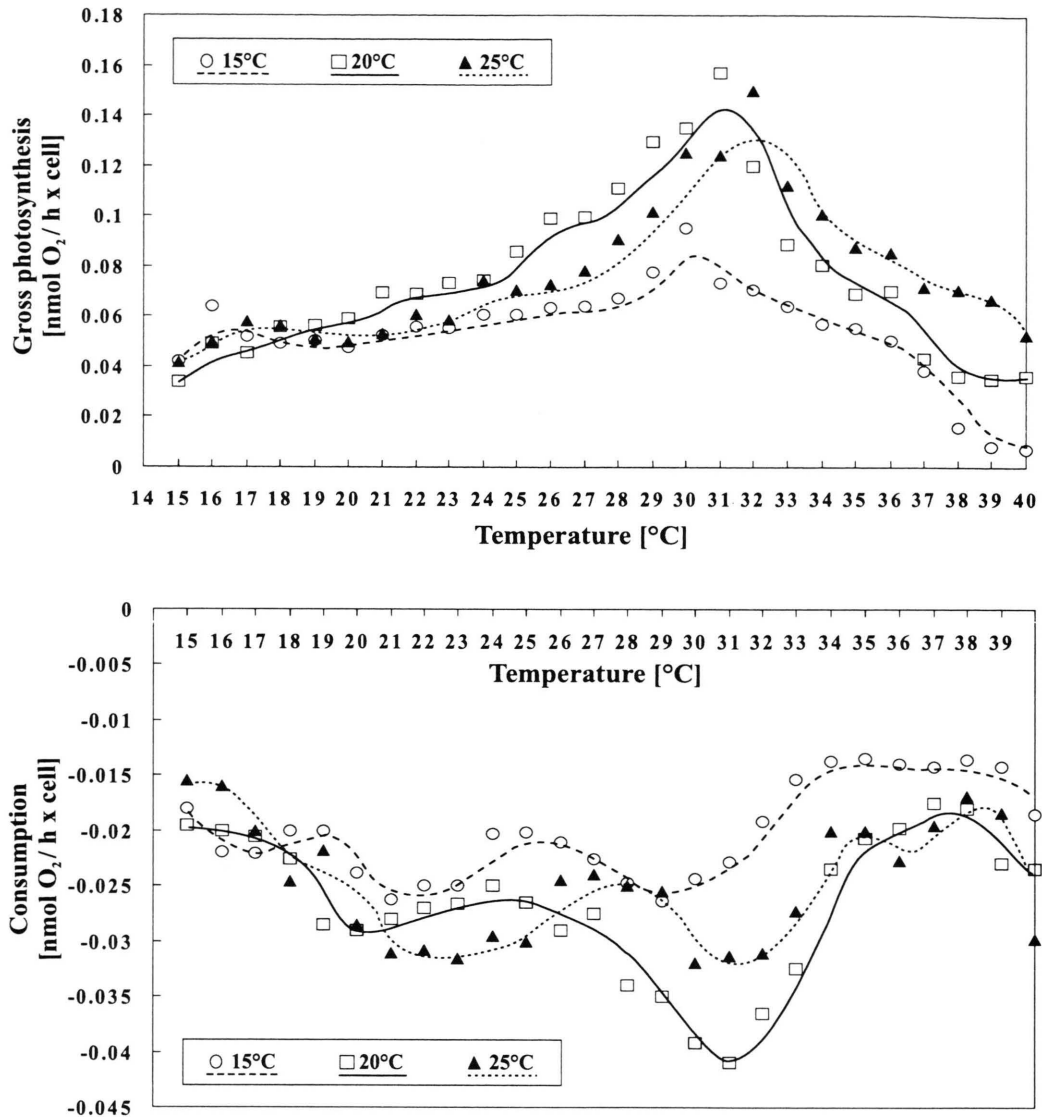


Fig. 7. Influence of temperature on (A) oxygen formation (gross photosynthesis; standard error approximately 0.0056) and (B) oxygen consumption (dark respiration; standard error approximately 0.00375) of *Micrasterias* cells grown previously under three different temperature conditions. ○ circle = cultivation temp. 15 °C, □ square = cultivation temp. 20 °C, ▲ triangle = cultivation temp. 25 °C.

Photosynthesis recorded over a period of two weeks starting with 22 days old cultures (20 °C cultivation temperature) revealed a slight (10%) increase in gross photosynthesis reaching a plateau after 26 days of cultivation followed by a subsequent even smaller decline. The concentration of main plastid pigments determined by HPLC analysis such as chlorophylls and carotenoids showed an equal and much stronger increase over

time than photosynthesis, reaching its peak earlier. The content per cell of both pigment classes developed very similar indicative of a formation of additional thylakoid membranes in the chloroplast. However, the separation into individual pigments showed that lutein as a marker for the light harvesting complex accumulated stronger than β -carotene indicating reaction centers. This is a response to growth light conditions. Furthermore,

both pigments comprise for nearly 80% of the carotenoids found in these cells, while neoxanthin is about 7-10% and especially the pigments of the xanthophyll cycle contribute much less than the 25–40% described for e.g. *Chlorella* (Thompson, 1996) or higher plant chloroplasts (Lütz, 1996; Wildi and Lütz, 1996).

Our results show that the temperature optimum for photosynthesis in *Micrasterias* is similar to that found in higher plants of temperate areas (Larcher, 1994). A further increase in temperature up to 40 °C led to a considerable drop in oxygen rates, but a zero oxygen evolution was nearly reached only in the cultures cultivated at 15 °C. The processes resulting in the decline in photosynthesis seen after the culmination points remain speculative, but it is possibly an imbalance in regulation of the carbon metabolism (Weis and Berry, 1988). It is known that temperature affects photosynthetic rates of C3 plants, via a reduction of the activation state of Rubisco (Long, 1991), whereas the specificity for CO₂ and the solubility of CO₂ relative to O₂ is reduced.

Photosynthetic rates of *Micrasterias* cells grown at different temperature make it obvious that there is a direct correlation between the amount of oxygen formed and the growth temperature of the cell cultures. This is most evident in cases where the heat shock temperature is higher than the temperature inducing maximum photosynthetic activity. The system tends to acclimate in

any pretreatment for maximum photosynthesis between 30-32 °C. Similarly, respiration is maximal between 29-31 °C, which may therefore be seen as a general metabolic peak.

It is not entirely clear why cells cultivated at 25 °C on the one hand show reduced division activity and even division inhibition. On the other hand these cells have a photosynthetic optimum above that of cells cultivated at lower temperature. This comparative view indicates that processes like cell division cannot adapt to changed growth temperature regimes as successful as photosynthesis, even when the temperature elevation is still moderate.

It is likely that temperatures above 40 °C have a detrimental affect on processes in the cell and even prevent normal cell functions by mediating enzymatic and/or membrane functions (Li and Christersson, 1993). Results obtained recently indicate the heat induced augmentation of a particular set of proteins, the so-called heat shock proteins (Weiss and Lütz-Meindl, 1999), which function as protective tools activated to cope with stress. However, there are also other mechanisms possible to function as chaperones and which we like to elucidate in future.

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