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Influence of temperature and methyl jasmonate on *Scenedesmus incrassulatus*

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Abstract

The effect of the methyl ester of jasmonic acid (MeJA) in 10 μM concentration was studied on the development of the bacterial contaminants and on the content of some metabolites *in Scenedesmus incrassulatus* cultivated at temperatures 15, 20, 25, 30, and 36 °C. The number of bacteria on algae cells increased with the rise in temperature. Application of MeJA into nutrient medium inhibited the development of bacterial pathogens more than 3 times at 20 °C, 2.3 times at 30 °C, and 2.6 times at 36 °C without changing the species composition. MeJA caused an increase of the protein content in algae cells. The contents of palmitic and linoleic acids increased with the rise of temperature from 15 to 36 °C. At the same time the contents of linolenic and oleic acid decreased. At low temperatures, cultivation with MeJA induced more significant changes in the composition of C18 acids while at high temperature the changes were more pronounced in C16 acids. Treatment with MeJA decreased the activity of glutamate dehydrogenase at optimal and suboptimal temperatures and increased it at superoptimal temperature. Hence MeJA jasmonate had a positive effect on the tolerance of *S. incrassulatus* to stress temperatures, which was also demonstrated by better growth.

Additional key words: bacterial contaminants, fatty acids, glutamate dehydrogenase, growth, pigments, proteins.

Introduction

Jasmonic acid (JA) and its methyl ester (MeJA) are natural compounds of wide distribution in the plant kingdom. Their effects on senescence, germination, tuber formation, or ethylene biosynthesis draw these substances close to classical phytohormones (Sembdner and Parthier 1993, Creelman and Mullet 1997). Jasmonate modulates the expression of numerous genes and influences specific aspects of plant growth, development, and responses to abiotic and biotic stress (Sembdner and Parthier 1993, Creelman and Mullet 1997). However, the occurrence and role of jasmonate in lower plants remains quite obscure. JA was identified in the cyanobacterium *Spirulina* (Ueda *et al.* 1991a), in the green unicellular algae *Chlorella,* in *Euglena gracilis* (Ueda *et al.* 1991a,b), and in the red alga *Gelidium* (Krupika and Dathe 1991). Our investigations showed that considerable changes occur in the content of jasmonic acid like substances during the cell cycle of *Scenedesmus acutus* (Christov *et al.* 1996). Maximal quantity was observed in aged cells. Their content was gradually reduced in growing cells and particularly low was in autospores.

Methyl jasmonate reduced *Chlorella, Scenedesmus*, and especially *Nostoc* viability during long-term storage on agar medium and enhanced peroxidase and glutamate dehydrogenase activities. The action of MeJA on the activity of α-esterase was different in dependence on the strain or the physiological state of the cultures (Pouneva *et al.* 1994, 1995).

Except axenic algal cultures all others are contaminated with different number and species of bacteria. In the algal-bacterial association bacteria could be a source or consumers of plant growth substances

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Abbreviations: Chl - chlorophyll; GDH - glutamate dehydrogenase; JA - jasmonic acid; MeJA - methyl ester of jasmonic acid.

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(Evans and Trewavas 1991). For that reason in the investigations of the effect of MeJA on nonaxenic *Scenedesmus* culture it's important to take into consideration the presence of bacteria. The present paper shows the influence of MeJA on bacterial contamination, changes in the content of some metabolites, and on the activity of glutamate dehydrogenase in green alga cells cultivated at different temperatures.

Materials and methods

The unicellular green algae *Scenedesmus incrassulatus* strain R-83 from the collection of autotrophic organisms in the Institute of Plant Physiology at the Bulgarian Academy of Sciences were cultivated in a chemostat at temperatures in the suspension 15, 20, 25, 30 and 36 °C and continuous irradiance (520 μmol m^{-2} s^{-1}) supplied from two sides of 200 cm^3 culture vessels. The culture was bubbled with air (100 dm^3 h^{-1}) enriched by 2 % CO_2. The nutrient medium described by Georgiev *et al.* (1978) was used. After adding MeJA to the medium in final concentration 10 μM cultivation proceeded at the indicated temperatures. The alga suspension had an initial density of 0.3 mg cm^{-3}. Two experiments, each with 4 replications, were conducted. Samples used for determining yield, contents of protein, carbohydrates, pigments and lipids, glutamate dehydrogenase activity and bacterial contamination were assessed after three days.

Protein content was determined according to Lowry *at al.* (1951), following a preliminary treatment with boiling methanol for the extraction of pigments. Chlorophyll content was assessed spectrophotometrically (spectrophotometer *Shimadzu UV-1601*, Tokyo, Japan) and estimated by the formula of McKinney (Arnon 1949). The amount of sugars was determined by the anthrone method. Lipids were extracted with acetone. Fatty acid methyl esters were separated by preparative TLC on 20 × 20 cm glass plates coated with silica gel (*Merck*, Darmstadt, Germany). Chromatography was carried out using the solvent mixture hexane:diethylether (10:1, v/v). The separation was achieved on a 180-cm glass column, packed with 10 % diethylene glycol succinate on *Chromosorb G/AW-DMCS* and temperature 180 °C on a gas chromatograph *3920 B* (*Perkin-Elmer*, Germany) (Petkov and Furnadjieva 1988). The activity of the enzyme glutamate dehydrogenase (GDH) was determined cytochemically as the percentage of cells in which a positive enzyme reaction had taken place (Lojda *et al.* 1979). The total number of contaminated bacteria was determined by seeding 0.2 cm^3 algal suspension from the different experiment on meat peptone agar (Tontcheva-Panova *et al.* 1997). For study the direct effect of MeJA on algal bacterial contaminants MeJA sterilised through *Sartorius-Membranfilter* (Gottingen, Germany) filter with pores of 0.2 μm was applied to medium containing only agar and were seeded with the isolated bacteria. The presence or absence of growth was compared to the growth of bacteria on meat peptone agar. The morphological status of the cells was microscopically controlled.

Results and discussion

MeJA treatment influenced alga cell growth. An enhancement of the biomass was observed at optimal and high temperatures. Only at low temperatures the stimulation was insignificant (Fig. 1). Microscopic observation showed a certain delay of cell division at extreme temperatures because enlarged, granulated and vacuolized alga cells predominated. At 30 °C homogenous cell content and normal cell size were observed. No differences in cell morphology as a result of MeJA treatment were found.

Changes related to the cultivation temperatures were significant. Lower temperatures led to reduced chlorophyll (Chl) *a* and Chl *b* content, but at the same time the quantity of saccharides and of lipids increased (Fig. 2). Chl *a* content at 15 °C was 4 times lower than at 30 °C. The same trend was evident for Chl *b*, but the decrease was 3 fold. In contrast, carotenoid content increased more than 2 times at the lowest temperature.

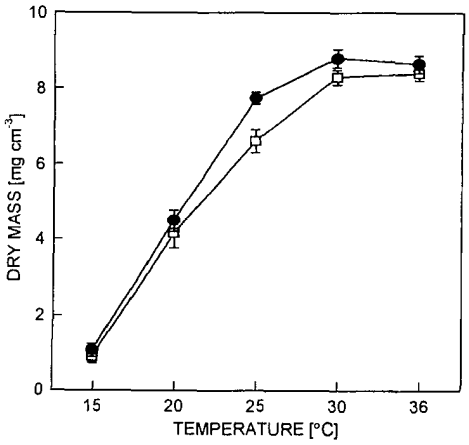

Fig. 1. Influence of MeJA (*closed circles*) and different temperatures on accumulation of biomass in *Scenedesmus incrassulatus*. *Vertical bars* indicate standard errors, $n = 6$.

The highest temperature contributed to an insignificant decrease in pigment content. Extreme temperatures caused an increase of more than 30 % in the content of sugars and lipids. Reduction in protein content within the range of 9 - 13 % was observed at temperatures lower

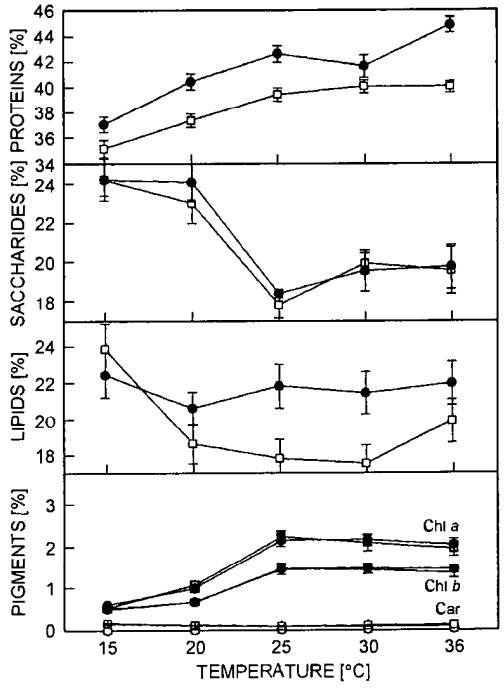

Fig. 2. Content of proteins, saccharides, lipids, and pigments expressed in percentage of dry mass in *Scenedesmus incrassulatus* treated with MeJA (*circles*) and cultivated at different temperatures (*squares* - control plants). *Vertical bars* indicate standard errors, $n = 4$.

than 25 - 30 °C. Application of MeJA did not induce changes in pigment and saccharide contents (Fig. 2). MeJA in concentration applied did not lead to growth inhibition and to senescence response, which are the typical reactions of higher plants (Creelman and Mullet 1997, Sembdner and Parthier 1993). Probably, this concentration was low as it did not induce loss of chlorophyll (Fig. 2). Increased protein and lipid content by MeJA was assessed, which was more pronounced at optimal and higher temperatures (Fig. 2). The enhanced content of proteins by MeJA in algae could be due to enhanced content of defence proteins or other proteins with still unknown function (Reinbothe *et al.* 1994).

Cultivation of algae at various temperatures and treatment with MeJA had an effect on the fatty acid composition (Fig. 3). An increase in the amounts of palmitic, hexadecadienoic and linoleic acids was observed along with the rise of temperature from 15 to 36 °C. At the same time the relative share of oleic, linolenic and octadecatetraenoic acids decreased. MeJA increased the amount of palmitic, hexadecatetraenoic and linoleic acids and decreased palmitoleic and hexadecatrienoic acids at high temperatures.

Changes in fatty acid unsaturation play a role in the resistance of algae to different temperatures (Harwood and Jones 1989). An increase in the ratio of unsaturated to saturated fatty acids at low temperature was evident. Treatment with MeJA increased this ratio mainly due to the increased content of linoleic and linolenic acids. Established changes in fatty acid composition appear to be useful in extension of temperature tolerance of algae. Moreover linolenic acid is the precursor in JA biosynthesis (Sembdner and Parthier 1993).

Table 1. Effect of MeJA and different temperatures on glutamate dehydrogenase activity [GDA, % of cells with a positive cytochemical reaction] in *Scenedesmus incrassulatus* and number of bacteria [$\times 10^6$ cm^{-3}] in suspension.

Temperature	GDA		Number of bacteria cells	
	Control	MeJA	Control	MeJA
20 °C	71.8 ± 3	59.7 ± 2	30 ± 2.6	11 ± 2.1
30 °C	60.1 ± 4	35.0 ± 3	68 ± 3.0	29 ± 3.6
36 °C	43.1 ± 1	49.7 ± 2	115 ± 3.5	45 ± 4.0

Treatment with MeJA reduced the activity of glutamate dehydrogenase in the algae cells cultivated at 20 or 30 °C (Table 1). At temperature of 36 °C MeJA enhanced the enzyme activity. Microalgal cultures are a favourable medium for the development of bacteria. They affect not only the normal growth and development of algal cells, but also all substances which are found in the alga suspension. The results showed that the amount of bacteria in the control was within the range of 30 - 115 × 10^6 cell cm^{-3}, while in the MeJA treated ones this range was 11 - 45 × 10^6 cell cm^{-3} which indicates a certain reduction of bacterial contamination (Table 1). The bacterial contamination was represented mainly by *Pseudomonas, Flavobacterium* and *Bacillus*. It is most likely that increase resistance of algae to bacterial contamination is closely related to stimulation effect of MeJA on the concentration of unsaturated fatty acids (linoleic and linolenic) known for antibacterial effect. Besides that jasmonate activates expression of genes involved in biosynthesis of proteins, phytoalexin and phenols that are involved in plant defence (Creelman and Mullet 1997).

Exogenous application of jasmonate indicates that this plant growth regulator plays a role in alga growth and development, including the protein and fatty acid metabolism and a resistance to bacterial contamination. It shows a positive effect on the adaptation ability of green algae to stress temperatures, which is demonstrated through more vigorous growth. Understanding of the physiological role of JA in lower plants could be also a step toward the elucidation of the evolution of hormonal regulation and its action on a molecular level.

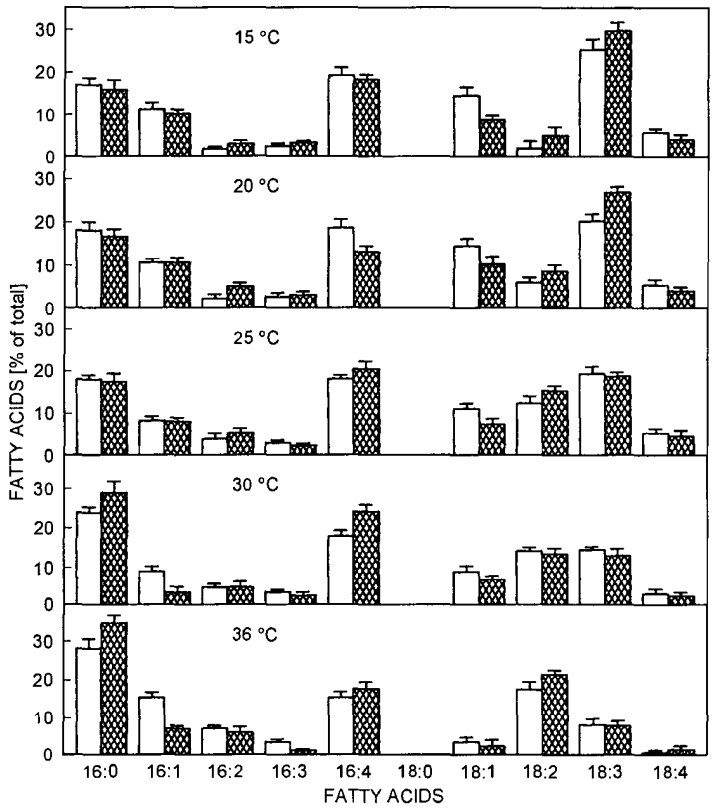

Fig. 3. Changes of fatty acid composition (% of total fatty acids) of lipids in *Scenedesmus incrassulatus* under the influence of methyl jasmonate (filled columns) and different temperatures. *Vertical bars* indicate standard errors, $n = 4$.

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