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Effect of cultivation temperature and light intensity on fatty acid production in the red alga *Porphyridium cruentum*

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The spectrum of fatty acids was studied in a unicellular red alga *Porphyridium cruentum*, strain 1380-1a, grown in a photostat at 21, 24 and 28 °C and irradiance 30 and 60 W · m^{-2}. Basic growth characteristics were determined for the strain under these conditions.

The proportion of unsaturated fatty acids (18:2, 20:4, 20:5) increased with decreasing cultivation temperature, their relative proportion changing depending on irradiation intensity; the proportion of 18:2 and 20:4 acids was higher at a subsaturation irradiance (30 W · m^{-2}) while at saturation irradiance (60 W · m^{-2}) the proportion of the 20:5 acid was substantially increased. The production of saturated acids (16:0) declined with decreasing temperature.

The current knowledge of the cell components of algae indicates that algae could become a potential source of lipids or essential fatty acids, especially unsaturated acids with higher numbers of carbon atoms. According to Ahern *et al.* (1983) an important producer of biologically interesting unsaturated fatty acids is the red alga *P. cruentum*.

We used the alga *P. cruentum* as a model organism for studying the regulation of fatty acid production by light intensity and temperature.

Materials and methods

Organism and growth: The unicellular red alga from the species *Porphyridium cruentum* (strain 1380-1a) was obtained from the Algal Culture Collection, Institute of Plant Physiology, Göttingen, FRG. The culture was grown in a modified seawater medium (Brody and Emerson 1959) which was added to the plate-parallel culture vessels of 1 l volume (Doucha 1979). A continuous culture regime was used. The suspension density (determined by measurement at 750 nm) was held constant by means of a phyto-actinometer, a device provided with an energy-proportional detector sensitive in the region of photosynthetically active radiation (PhAR). This PhAR-detector was placed on the back side of the plate-parallel vessel.

Irradiation density on the surface of the vessels (from one side only) was 20, 30, 40, 60 or 80 W · m^{-2} of PhAR from incandescent lamps. The culture units were aerated with filter-sterilized 2% CO_2 (by volume) in air. The specific growth rate μ of the cultures was estimated in steady-state phytoactinometer-controlled photostatic cultures growing under non-light-limited conditions (Doucha 1985). The amount of suspension overflow per time unit (h^{-1}) was measured in a steady-state regime, when the dilution rate equals the specific growth rate, $D = \mu$.

Isolation of fatty acids: 100–500 mg biomass separated by centrifugation was hydrolyzed by boiling 3 N NaOH and the liberated acids were converted to methyl esters after acidification with 5 N HCl (Řezanka *et al.* 1983). Identification of fatty acids: methyl esters of fatty acids were separated and identified by comparing their elution characteristics with those of standards using gas chromatography on a PU 4900 instrument (Pye Unicam, Great Britain) with a fused silica capillary column 10 m × 0.32 mm ID × 0.2 mm CP-Sil 57 CB film (Chrompack, The Netherlands). The carrier gas (hydrogen) flow was 2.5 ml/min, injector temperature 250 °C, split 1:25, programmed temperature from 110 to 200 °C at a rate of 8 K/min, temperature of FID injector 260 °C.

Results and discussion

Table 1 shows that the photosynthesis proceeds at an optimal rate at 24 °C and saturation irradiation intensity of about 40 W · m^{-2}. The μ_{max} under these conditions is 0.055 h^{-1}. The growth characteristics for 21 and 28 °C are similar but the μ_{max} values are lower and at 28 °C the optimum PhAR is shifted to lower values (20 W · m^{-2}).

Table 1
Dependence of specific growth rate of *P. cruentum* 1380—1a on photosynthetically active radiation (PhAR) and cultivation temperature

Temperature (°C)	Specific growth rate μ (h^{-1}) for PhAR (W. m^{-2})				
	20	30	40	60	80
21	0.035	0.041	0.045	0.042	0.040
24	0.045	0.051	0.055	0.052	0.048
28	0.030	0.033	0.029	0.020	0.011

We identified a total of 19 acids (Fig. 1) with certain quantitative differences affected by temperature and light intensity for the major acids 16:0, 18:2, 20:4 and 20:5 at irradiation 30 or 60 W · m^{-2} (Tab. 2). The separation regime resolved also fatty acids with different numbers of double bonds, e.g. the 20:4 acid was separated from the 20:5 one.

Table 2 shows that a maximal synthesis of the 20:5 acid takes place at a higher energy flux (60 W · m^{-2}) and a lower temperature (21 °C) than the optimal parameter

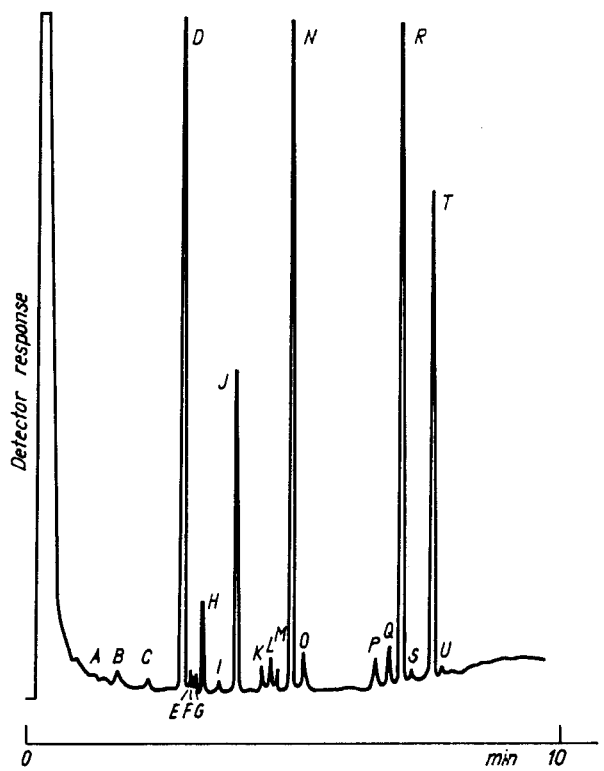

Fig. 1
Fatty acids profile of *Porphyridium cruentum* 1380-1a, cultivated at 21 °C under irradiation 60 W · m^{-2}.
A = 14:0, B = 14:1n7, C = 15:0, D = 16:0, E = 16:1n9, F = 16:1n7, G = non-identified, H = 16:2n6, I = 17:0, J = inner standard (ethylester of 17:0 acid), K = 18:0, L = 18:1n9, M = 18:1n7, N = 18:2n6, O = 18:3n3, P = 20:2n6, Q = 20:3n6, R = 20:4n6, S = 20:4n3, T = 20:5n3, U = 22:1n11

Table 2
Content of fatty acids (relative %) in the alga *P. cruentum* 1380—1a

Temperature (°C)	Fatty acids							
	16:0		18:2		20:4		20:5	
	A	B	A	B	A	B	A	B
21	29.4	22.4	19.2	8.7	36.0	27.9	11.0	32.3
24	33.7	33.1	17.5	9.9	28.7	23.3	11.9	26.8
28	42.5	35.3	11.5	8.8	29.7	23.5	10.9	26.7

A: irradiation intensity 30 W · m^{-2}
B: irradiation intensity 60 W · m^{-2}

for photosynthesis (specific growth rate μ). Maximum growth rate, $\mu_{max} = 0.055\ h^{-1}$, is attained at an energy flux of about 40 W · m^{-2} and a temperature of 24 °C. However, acids 20:4 and 18:2 displayed an inverse dependence on light intensity, the temperature dependence being unchanged. The maximum production of these acids is attained at a lower illumination (30 W · m^{-2}) and temperature (21 °C) as compared with the optimum conditions for photosynthesis. Biosynthesis of the 16:0 acid is crucially dependent on cultivation temperature; with increasing temperature its content also increases at both illumination intensities, the proportion of the 16:0 acid being higher at 30 W · m^{-2} than at 60 W · m^{-2}.

Our results fully confirmed the widely publicized relationship between the number of double bonds in molecules of fatty acids and cultivation temperature (Pohl and Zurheide 1982). The proportion of unsaturated fatty acids decreased and that of unsaturated acids increased with rising temperature. The unsaturation of fatty acids is associated with facilitation of transport through cell membranes caused by their increased permeability. This phenomenon was described also in other organisms, e.g. *Saccharomyces* (Welch and Burlingame 1973). In this context let us mention the interesting finding of Bild *et al.* (1978) that 47% of all cellular 20:6 n-4 acid in the genus *Porphyridium* is in chloroplasts in which it should be a component of lipoprotein membranes (Garnieri *et al.* 1970). The relationship between the increased production of unsaturated fatty acids and the cultivation temperature can be tentatively interpreted as reflecting an increased amount of the lipid-soluble molecular oxygen (at a lower temperature) necessary for the oxygen-dependent enzymes catalyzing the desaturation of long-chain fatty acids (Brown and Rose 1969). The relationship between desaturase and the occurrence of unsaturated fatty acids has been experimentally verified. According to Nichols and Appleby (1969) the biosynthesis of the 20:4 n-6 acid in *P. cruentum* involves the desaturation of the 18:2 acid. The desaturase was observed to be less efficient on lowering the fatty acid production (Morris *et al.* 1968). The activity of desaturation enzymes and of enzymes participating in fatty acid chain elongation can be closely associated with changes in cultivation temperature.

The studies of the effect of light intensity on fatty acid production are still on a descriptive level and the data published by individual authors are controversial (cf. e.g., Ahern *et al.* 1983, Kljačko-Gurvič *et al.* 1985). Our results are in accordance with those Kljačko-Gurvič *et al.* (1985) only in the case of the 20:5 acid.

Arachidonic acid is taken to be a biosynthetic precursor of desirable compounds such as leucotrienes and prostaglandins (Bild *et al.* 1978). The 20:5 acid has a similar importance since, apart from participating in the biosynthesis of prostaglandins (Seto *et al.* 1984) it probably plays an important role in the metabolism of lipid as a substance preventing blood platelet aggregation (Dyerberg *et al.* 1978).

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