Pl. Syst. Evol. 164, 263–272 (1989)

Plant Systematics and Evolution

Cell morphology and growth characteristics of *Porphyridium aerugineum* (*Rhodophyta*)*

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Received December 28, 1988

Key words: Algae, *Rhodophyta, Porphyridiales, Porphyridium aerugineum.* – Cell morphology, surface structure, cell size, growth parameters, photosynthetic pigments.

Abstract: Four unialgal strains of the freshwater coccoid red alga *Porphyridium aerugineum* GEITLER were cultivated under laboratory conditions. Cell morphology was studied with the light microscope. The cell surface was examined by means of electron microscopy in order to contribute to the knowledge of polysaccharide sheaths and cytoplasmic membranes. Optimum growth conditions were determined. The range of cell sizes and the average dry masses of single cells were compared in all four strains cultivated at exactly defined temperatures and irradiances. Photosynthetic pigment maxima were measured in intact cells. The red-coloured phycobiliprotein phycoerythrin was not found in any of the examined strains.

The freshwater coccoid red alga *Porphyridium aerugineum* is one of many algal taxa intimately associated with the work of Prof. Dr LOTHAR GEITLER. Since 1923, when he described the alga as a new species, numerous papers dealing with its morphology, ultrastructure, methods of cultivation, pigment analyses, production and composition of extracellular polysaccharide have been published.

In the present paper, some results of laboratory investigations of four strains of *Porphyridium aerugineum* are communicated. Morphology and surface of cells were examined with the light and electron microscope. Optimum growth conditions were established. Growth rates, range of cell diameters and average dry mass of individual cells were compared. Contents of photosynthetic pigments were measured and dependence of growth rates on the ratio of pigments phycocyanin and chlorophyll *a* was investigated.

Material and methods

Algal material. Three strains of *Porphyridium aerugineum* GEITLER – strains SAG 1380-2 of R. C. STARR, SAG B 110.79 of T. CHRISTENSEN, and SAG B 111.79 of F. AMBARD (SCHLÖSSER 1982) – were obtained from the Sammlung von Algenkulturen, Göttingen. The fourth strain – HINDÁK 1983/2 – was isolated from material collected at Piešťany, western

* Dedicated to Prof. Dr LOTHAR GEITLER on the occasion of the 90th anniversary of his birthday.

Slovakia (for the description of the locality, see PEKÁRKOVÁ & al. 1988) and is deposited in the collection (SAO) of the Institute of Botany, Třeboň, Czechoslovakia. For the experiments, non-axenic cultures were used.

Cultivation. Glass cuvettes with cultures (suspension layer 2 cm) were placed into water baths and illuminated from one side with bulbs Tungsram 500 W ("white" light). Irradiance was regulated with dim glass and measured with an integrating phytoactinometer (KUBÍN & al. 1985). Suspensions were stirred with sterile mixtures of carbon dioxide (2% v/v) and air.

Three kinds of liquid media were tested: the GORHAM's one modified by KOEMAN & HOEK (1980), the MCY II of RAMUS (1972) – the modification with higher concentrations of nutrients (PEKÁRKOVÁ & al. 1988), and the more concentrated (five times) Bold's BBM (BROWN & BOLD 1964). Vitamin B_{12} (10^{-6} g · l^{-1}) was always added into the media.

Electron microscopy. Washed cells were fixed with 2% (v/v) glutaraldehyde (GLA – pH 7.2). For scanning electron microscopy (SEM), fixed cells were washed, dehydrated with acetone of increasing concentrations, dried at the critical point, mounted on specimen stubs and coated with gold. Then they were examined in a Stereoscan Cambridge S 4 SEM. For transmission electron microscopy (TEM), fixed cells were washed and transferred stepwise into 30% (v/v) glycerol. Then they were freeze-fractured in a Balzer BA 360 M device. Carbon replicas of fractured planes were shadow-cast with platinum and examined in a Tesla BS 500 TEM.

Specific growth rates were determined in exponentially growing asynchronous batch cultures, saturated with light and nutrients and adapted to defined conditions: temperatures 25, 28, 30 °C; irradiances 30, 80, 120, 150 W · m^{-2} PhAR. Optical densities of these suspensions were measured at hourly intervals with a spectrophotometer Specol in 1 cm glass cuvettes at 750 nm, and were transferred on semilogarithmic paper. The doubling times, t_d, were estimated from these growth lines, and the specific growth rates, μ, were calculated from the formula $\mu = \ln 2/t_d$.

Cell sizes. The diameters of living cells (without sheaths) were measured with a Biolar light microscope. Cell numbers, N, were ascertained by counts in a Bürker chamber. Dry masses of cells, W, were estimated by weighing the dried cells, separated from known volumes of suspensions before drying (105 °C for 15 h). The average dry mass of a single cell, W_a, was calculated from the formula $W_a = W/N$.

Photosynthetic pigments. The method of DOUCHA & KUBÍN (1976) was used to estimate in vivo photosynthetic pigment absorption spectra. Pigment contents of intact cells were measured, while cells of the same suspensions, bleached with peracetic acid and "white" light 100 W · m^{-2} PhAR, served as reference samples. Pigment spectra were analysed with the double beam recording spectrophotometer Specord M 40 in 1 cm glass cuvettes.

Results and discussion

The most suitable medium for growing *Porphyridium aerugineum* was found to be MCY II (RAMUS 1972). Higher concentrations of nutrients according to PEKÁRKOVÁ & al. (1988) enabled to cultivate dense, nutrient-saturated suspensions. KOEMAN & HOEK's (1980) modification of GORHAM's medium could not be used for cultivation of dense suspensions. Nutrient deficiency was connected with the decrease of the amount of the blue-coloured pigment phycocyanin. It began in cultures with dry mass of about 0.3 g · l^{-1}, and the cell growth stopped when the dry mass of yellow-green cells of about 1 g · l^{-1} was achieved. The maximum growth rates, reached by cells of thin suspensions, were similar in both the media mentioned. Suspensions saturated with nutrients and light were of a characteristic blue-green colour. Growth rates of cells achieved in the more concentrated (five times) medium

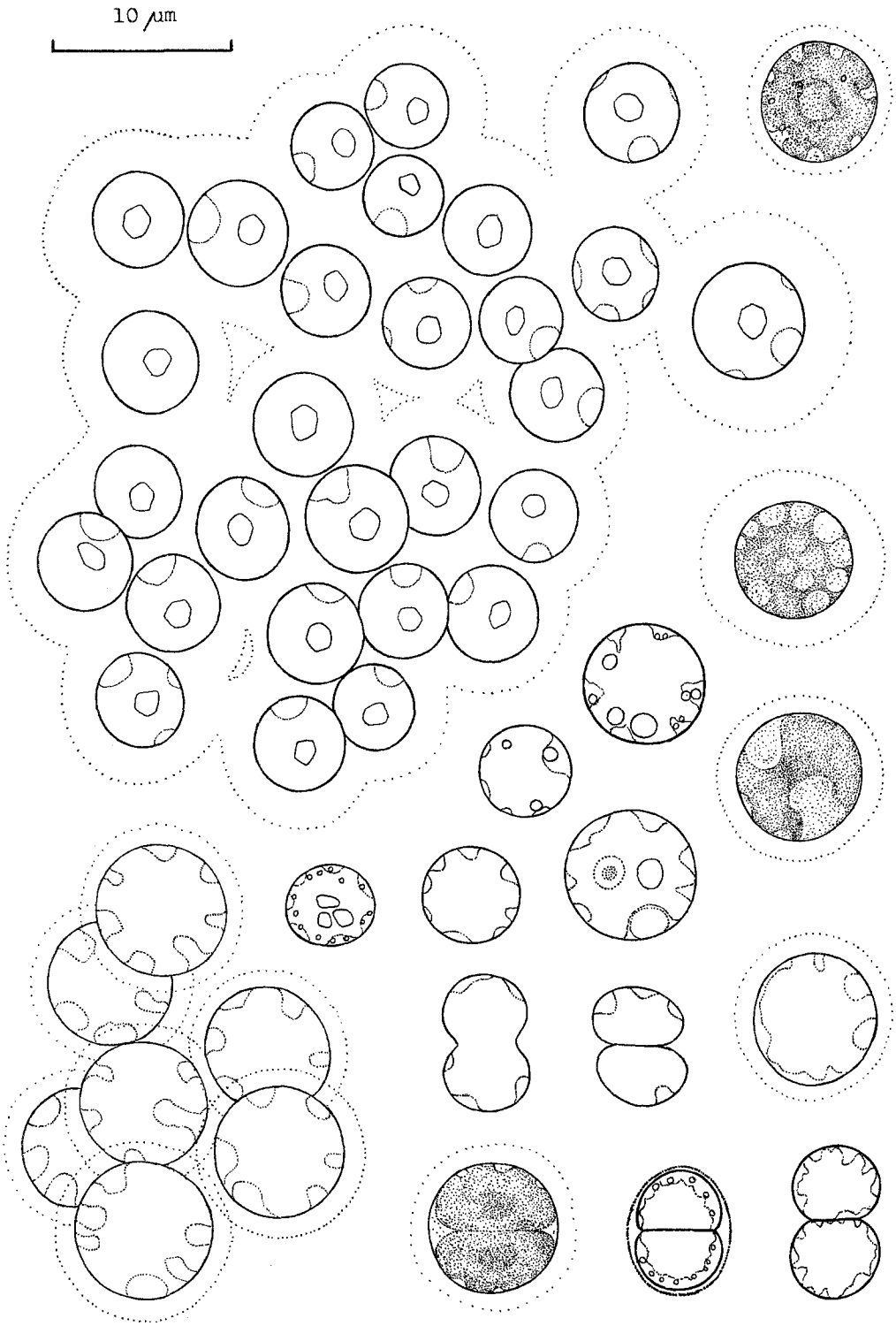

Fig. 1. *Porphyridium aerugineum*, strain HINDÁK 1983/2. Morphological variation of cells in culture. Orig. F. HINDÁK

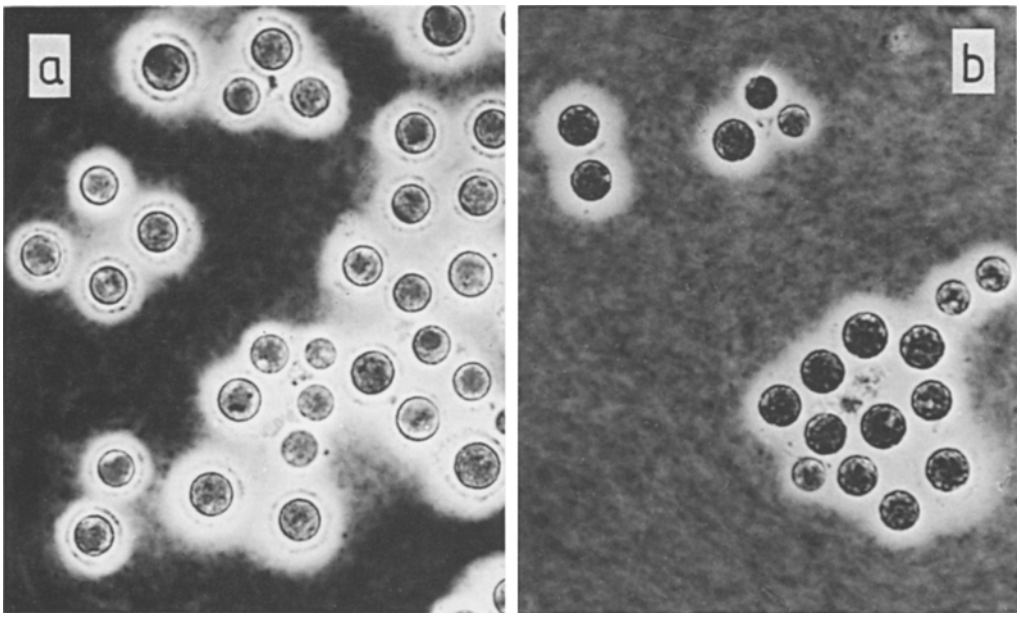

Fig. 2. Suspensions of *Porphyridium aerugineum* with Indian ink. *a* Strain SAG B 111.79. Rings, observed only in this strain, separate polysaccharide sheaths into two layers of different material densities. External polysaccharide fibres solubilize into the liquid medium. *b* Strain HINDÁK 1983/2. Differentiation of cell sheaths into layers is inconspicuous. Note the fibrous polysaccharide, solubilized in the medium. Photo J. KOMÁREK

BBM were rather lower and suspensions saturated with nutrients and light were green in colour. The growth ability was better in cells with higher amounts of phycocyanin. The media containing organic compounds (Tris, citric acid in the GORHAM's medium, Tricine-buffer and glycerolphosphate disodium salt in the RAMUS' one) were then more suitable for cultivation of *Porphyridium aerugineum*. Trace amounts of vitamin B_{12} were necessary for successful growth of this alga. In all kinds of the media, solitary and geminated cells together with cell aggregates were observed. Cell aggregates frequency was higher in intensely illuminated suspensions.

Light microscopic features (Figs. 1 and 2). Cell morphology of the four mentioned strains fitted well with the original diagnosis and figures of GEITLER (1923). Daughter cells in couples were elliptical, while the older ones were spherical in shape, solitary or in small clusters, and always embedded in colourless hyaline polysaccharide mucilaginous sheaths, analysed by RAMUS (1972) and replacing rigid cell walls. Sheaths were 1 to 3 μm wide and prevented the cells from forming tight packed-like aggregates. The sheath material was separated to at least two layers of different polysaccharide density, visible best at the strain SAG B 111.79. Sheaths were conspicuous after adding Indian ink into the suspensions. From the external surface of sheaths, polysaccharide fibres solubilized into the liquid medium. As for cell ultrastructure, the chloroplast was central, bright blue-green or green in colour, lobed, with one central, spherical to slightly irregular naked pyrenoid, without orange osmiophilic globules. The nucleus was mostly peripheral. In the protoplast,

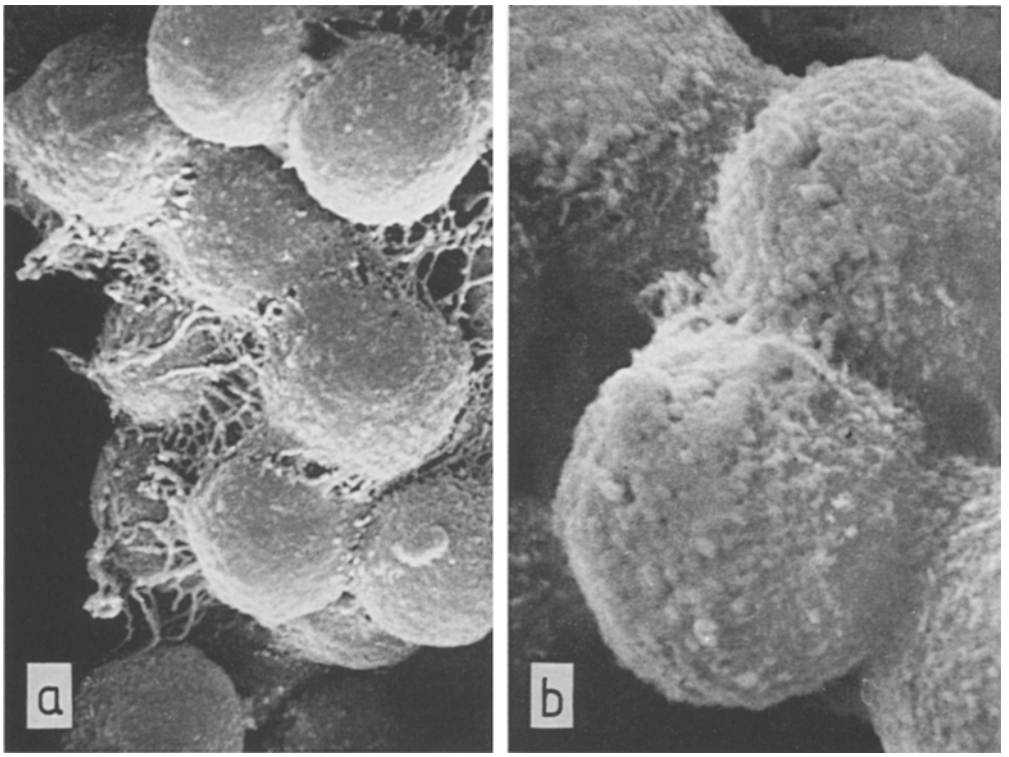

Fig. 3. SEM of *Porphyridium aerugineum*, strain HINDÁK 1983/2. *a* Round-shaped cells are interconnected with polysaccharide fibres, × 5 000. *b* Surfaces of cell sheaths are strongly wrinkled, × 10 000. Photo J. ŠMARDA

there were numerous spherical vacuoles of different sizes, and spherical to slightly asymmetrical bodies, probably grains of floridean starch. Cell division resulted in two equal parts. Daughter cells remained geminated only for a short time and then they separated.

Electron microscopy (Figs. 3 and 4). SEM of round-shaped cells revealed a wrinkled surface of their polysaccharide sheaths. The cells were usually interconnected by fixed polysaccharide fibres and their bunches.

Most freeze-fractured cells were devoid of their sheaths which got denaturated during GLA fixation. Some cells exposed the rather smooth outer surface of their plasma membrane, while others were devoid also of their outer leaflet of this membrane, and exposed its protoplasmic fracture face (PF) or its exoplasmic one (EF). In both cases, the plasma membrane showed a general wartlike appearance. The warty protrusions were of two ranges of size and seemed to indicate the localization of pores serving for the transport of the polysaccharide material outwards the cell.

Cell ultrastructure was the same in all media used. However, the method of freeze-fracturing proved to be less suitable for observations of the cell inner ultrastructure than cross sections of cells published by GANTT & al. (1968) and RAMUS (1972).

Growth characteristics (Fig. 5). The optimum temperature for intensely growing

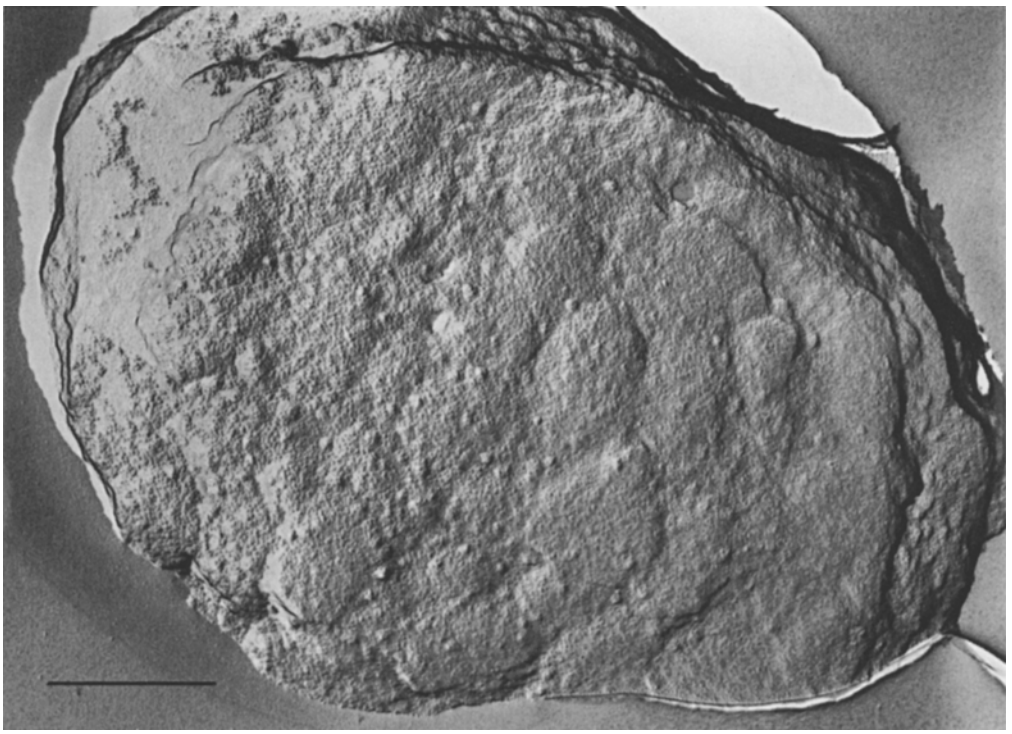

Fig. 4. TEM of *Porphyridium aerugineum*, strain HINDÁK 1983/2. Freeze-fracturing of an elliptical mother cell before its division. This cell was devoid of the sheath; note the cytoplasmic membrane with many tiny protrusions. Bar: 1 μm. Photo J. ŠMARDA

suspensions of *P. aerugineum* was 28 °C and the optimum irradiances (for thin suspensions) were 70 to 100 $W \cdot m^{-2}$ PhAR. The maximum growth rates, μ_{max}, were similar in the three strains, HINDÁK 1983/2 (0.133 h^{-1}), SAG B 110.79 (0.130 h^{-1}) and SAG B 111.79 (0.116 h^{-1}). The slowest growth was found in strain SAG 1380-2 (0.093 h^{-1}).

Cell sizes (Table 1). In general, cell diameters (without sheaths) increased in cells cultivated at the temperature of 30 °C and at the irradiance of 150 $W \cdot m^{-2}$ PhAR, in agreement with higher values of the average dry masses of one cell ascertained under the mentioned conditions. Extremely large cells were occasionally observed. The smallest cells were found in strain SAG 1380-2. For this strain, SOMMERFELD & NICHOLS (1970) stated a diameter range of 4.5 to 7.0 μm for cells cultivated at lower temperatures (maximum 22 °C).

Photosynthetic pigments (Fig. 6, Table 2). Attention was paid to the content of phycocyanin, a light-harvesting blue phycobiliprotein located in disc-shaped phycobilisomes (GANTT & al. 1968, GLAZER 1985, GANTT 1986) for its influence on algal growth.

Shapes of curves of in vivo photosynthetic pigment absorption spectra depended on the composition and nutrient concentration of the media. The ratios of absorbances of phycocyanin (maximum at 635 nm) and chlorophyll *a* (maximum at 683 nm) of nutrient-saturated cells differed in the media with organic compounds

Table 1. Ranges of cell diameters, in μm, and average dry masses of one cell, W_a (10^{-10} g), reached by cells of *Porphyridium aerugineum* grown under various temperature and irradiance conditions in the medium MCY II. * Exceptionally large diameters. *T* Temperature in °C, *I* irradiance in $W \cdot m^{-2}$ PhAR

Strain	T	I	Cell diameter		W_a	Strain	Cell diameter		W_a
SAG	25	30	4.4–6.9	8.8*	0.55	SAG	5.6–10.0		0.77
1380-2		80	4.4–6.9	8.1*	0.47	B 110.79	5.0–8.8		0.80
		120	4.4–6.9		0.50		5.0–9.4		0.94
		150	4,4–6.9		0.60		5.6–10.0		1.09
	28	30	4.4–6.3	11.9*	0.63		6.3–9.4	12.5*	1.16
		80	5.0–6.9		0.65		6.3–8.8		1.10
		120	5.0–6.9		0.63		6.3–8.8		1.12
		150	5.0–6.9		0.72		5.6–10.6	15.6*	1.20
	30	30	4.4–6.9	10.6*	0.74		5.0–10.6	12.5*	1.18
		80	4.4–6.9	8.1*	0.80		5.0–10.0	13.8*	1.14
		120	4.4–8.1	9.4*	0.88		5.0–10.6	13.2*	1.11
		150	4.4–7.5	11.3*	0.95		5.0–11.9	15.0*	1.32
SAG	25	30	5.6–10.0		0.69	HINDÁK	5.0–8.1		0.84
B 111.79		80	4.4–7.5		0.57	1983/2	5.0–9.4	16.9*	0.86
		120	5.0–8.8		0.59		5.0–10.0		1.03
		150	5.0–11.9		0.63		6.3–12.5		1.22
	28	30	5.0–10.0		0.68		6.3–12.5		1.26
		80	4.4–9.4		0.68		6.3–11.3	13.1*	1.18
		120	5.0–9.4		0.67		6.3–8.8	12.5*	1.23
		150	5.0–10.6		0.82		5.6–10.0	13.7*	1.28
	30	30	5.0–12.5		0.86		6.3–12.5		1.42
		80	5.0–13.1		0.92		6.3–10.6		1.34
		120	5.6–13.1		0.93		6.3–11.9		1.38
		150	5.0–13.8		1.13		6.3–12.5	16.9*	1.48

(1.15 to 1.4) and without them (approx. 0.9). Cells from the nutrient-deficient medium lost phycocyanin first, afterwards the amount of the chlorophyll *a* lowered, too.

Light-saturated and light-limited suspensions differed in pigment amounts. In general, the maximum values of phycocyanin (635 nm) and chlorophyll *a* (683 nm) in cultures of identical densities were more than twice higher in light-limited cells compared with light-saturated ones. On the contrary, carotenoid contents (maxima at 470 and 500 nm) were less than double in light-limited cells. This means that relative carotenoid contents increased in intensely illuminated cells. Similar pigment behaviour was described in *Porphyridium cruentum* (BRODY & EMERSON 1959, JAHN & al. 1984), *Rhodella grisea* and red-coloured *Porphyridium* spp. (PEKÁRKOVÁ 1989), and in *Anacystis nidulans* (MASAMOTO & al. 1987). The red-coloured phycobiliprotein phycoerythrin (maxima at 545 and 565 nm) was not found in any of the investigated strains of *P. aerugineum*.

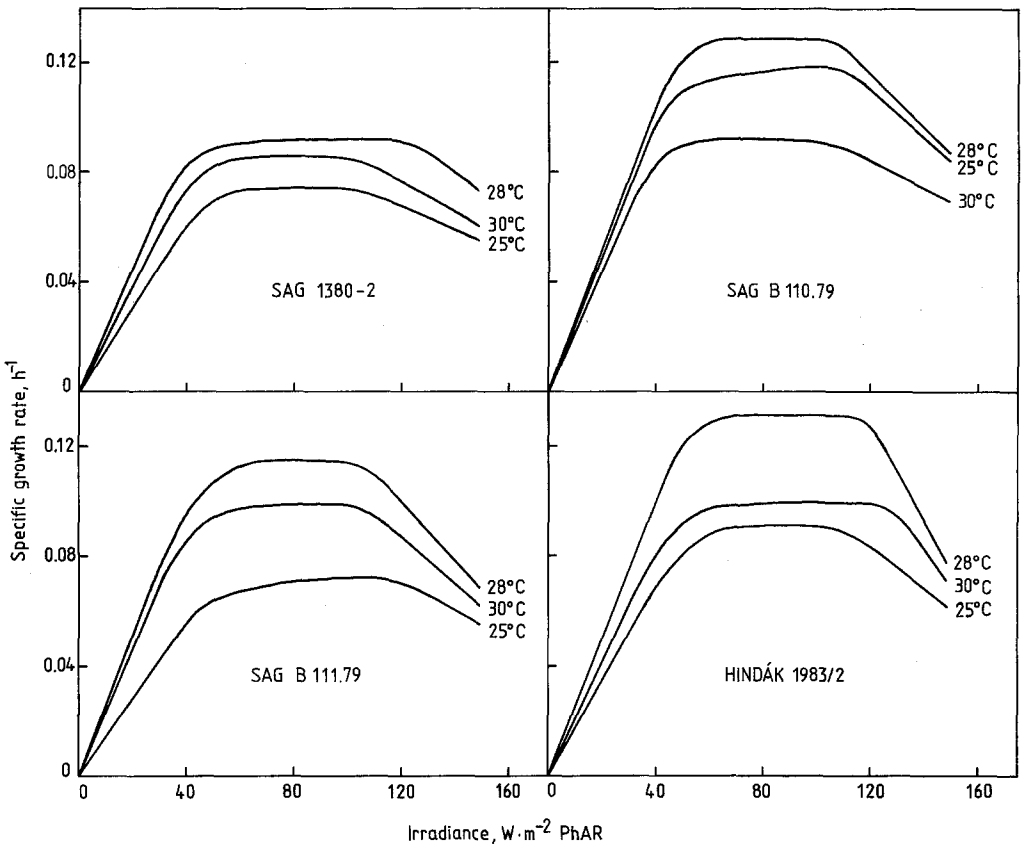

Fig. 5. Specific growth rates of four strains of *Porphyridium aerugineum* cultivated in the medium MCY II

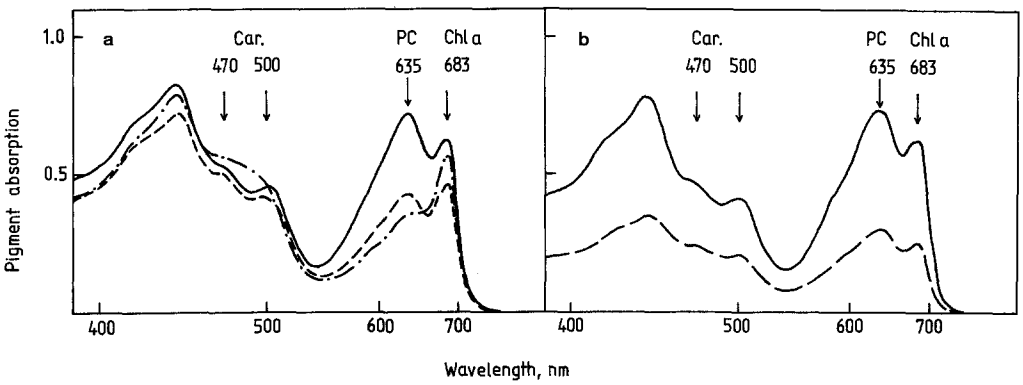

Fig. 6. Pigment characteristics of *Porphyridium aerugineum*, strain HINDÁK 1983/2. *a* Pigment absorbances of cells cultivated in three kinds of media: MCY II (——), 5× BBM (– –), GORHAM's medium, nutrient deficiency (– · –). *b* Different absorbances of cells cultivated in the medium MCY II. Light-limited cells (——) with dry mass of $1.7\,g \cdot l^{-1}$ and irradiance $40\,W \cdot m^{-2}$ PhAR reach much higher maxima than light-saturated ones (– –) with dry mass of $4 \times 0.33\,g \cdot l^{-1}$ and irradiance $130\,W \cdot m^{-2}$ PhAR

Table 2. Differences in maximum absorbances of photosynthetic pigments in light-limited and light-saturated cells of *Porphyridium aerugineum*, cultivated in the medium MCY II. *A* In vivo photosynthetic pigment absorption maxima in suspensions with identical dry mass of $1\,g \cdot l^{-1}$ (relative values). *M* Multiples of chlorophyll *a* maximum at 683 nm

Strain	Cultivation conditions	Carotenoid 470 nm		Carotenoid 500 nm		Phycocyanin 635 nm		Chlorophyll *a* 683 nm	
		A	M	A	M	A	M	A	M
SAG 1380-2	Light limitation	3.1	0.72	2.6	0.60	5.5	1.28	4.3	1
	Light saturation	1.8	1.00	1.6	0.89	2.5	1.39	1.8	1
SAG B 110.79	Light limitation	3.1	0.79	2.7	0.69	4.7	1.21	3.9	1
	Light saturation	1.8	1.38	1.5	1.15	1.6	1.23	1.3	1
SAG B 111.79	Light limitation	3.2	0.76	2.8	0.67	5.2	1.24	4.2	1
	Light saturation	1.5	0.88	1.3	0.76	2.2	1.29	1.7	1
HINDÁK 1983/2	Light limitation	2.8	0.76	2.5	0.68	4.4	1.19	3.7	1
	Light saturation	1.8	0.95	1.6	0.84	2.2	1.16	1.9	1

The authors are grateful to Prof. U. G. SCHLÖSSER for his kind supply of three strains of *Porphyridium aerugineum*. They also gratefully acknowledge the technical assistance of Mrs V. TRÁVNÍČKOVÁ in freeze-fracturing preparations and of Mr P. HNILIČKA in SEM, Dr J. KOMÁREK, Ph.D., for his help in light microscopic photography, and Mrs V. DUFKOVÁ for reading the manuscript. Laboratory cultivations were performed at the Institute of Microbiology, Czechoslovakian Academy of Sciences, Třeboň, Czechoslovakia.

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