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THE EFFECT OF ILLUMINATION REGIMEN ON TEMPERATURE-INDUCED AND SPONTANEOUS BLEACHING IN *EUGLENA GRACILIS*

Mit deutscher und kroatischer Zusammenfassung
Sa sadržajem na njemačkom i hrvatskom jeziku

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Introduction

A variety of physical and chemical factors, among them subletal temperature (Pringsheim and Pringsheim 1951) and intense light (Leff and Krinsky 1967), are known to induce permanent chloroplast-loss in *Euglena gracilis* (the so-called »bleaching« factors). However, when plated on a »complex« organic medium a small percentage of spontaneously bleached colonies appear. According to De Deken-Grenson (1959) the percentage of spontaneously bleached colonies in *Euglena gracilis* var. *bacillaris* varies between 1 — 2 per cent; in *Euglena gracilis* strain »Z« according to Ebringer et al. (1969) 4 colonies out of 600, or generally 0,1—1 per cent (McCalla 1967). On synthetic medium no white colonies appear (Grenson 1964, Leff and Krinsky 1967). All these experiments were performed in continuous light.

In our experiments the effect of the light regimen on induction of temperature-induced and spontaneous bleaching has been studied.

Materials and Methods

Euglena gracilis strain »Z« was obtained from Dr. C. Birnboim (Chalk River Laboratories, Canada). Cultures were grown on a modified »complex« Pringsheim medium (1951), with beef extract omitted, sodium acetate raised to 0,04 M to be non-limiting (Grenson 1964) and acidified with N HCl to pH 4,5 to reduce the possibility of bacterial contamination. The content of the medium was as follows:

bactopeptone (Difco)	0,20 g
yeast extract (Difco)	0,20 g
sodium acetate	0,32 g
distilled water	100 ml

N HCl to pH 4,5.

The stock cultures were kept on agar slants.

a) In experiments on temperature-induced bleaching, test tubes with 5 ml complex medium, or complex medium with additional phosphates (0,08 per cent w/v KH_2PO_4 and 0,08 per cent w/v K_2HPO_4), were inoculated with ca 10^4 cells/ml and grown at 34,5° C in an incubator. The phosphorus in our complex medium without additional phosphates has been estimated to be 26 μg/ml by the Analitical Service of the Institute »Ruđer Bošković«. With additional phosphates the phosphorus content was raised to 350 μg/ml. After 72 hours the cultures were diluted and plated on the same medium (without HCl) solidified with 1,5 per cent agar in petri dishes.

b) In experiments on spontaneous bleaching *Euglena* was grown in the same P r i n g s h e i m - solution at room temperature for 2 to 3 days and after dilution plated as in the above experiments.

In both types of experiments (a and b) the cells were exposed to different light regimens. The difference in the light regimens consisted in the duration of the photoperiod only. One half of the petri dishes were illuminated continuously, and the other half illuminated for 14 hours daily. The light intensity was about 130—150 ftc. After 6—9 days the proportions of the green and white colonies were counted. A small proportion of mixed colonies were scored as green.

Results

In the first set of experiments the effect of the light regimen on temperature-induced bleaching was studied. The bleaching was performed in two ways:

a) During the bleaching the cells were grown for 3 days in the complex medium without additional phosphates.

b) During the bleaching the cells were grown for 3 days in the complex medium with additional phosphates.

In both cases the cells were illuminated continuously during the treatment. After the treatment *Euglena* cells were exposed to continuous light as well as to the 14 hours light periods.

In the case the cells were exposed to continous light after the treatment, a significantly higher proportion of white colonies has been detected in both experiments in comparison with the cycled illumination (Table I and II).

Table III shows the growth in the complex medium at 34,5° C with and without additional phosphates. The cell density in the medium with additional phosphates is even a little higher than in the simple complex medium, but the increase is statistically insignificant. Additional phosphates in the medium during the bleaching treatment enhance the recovery of the greening ability of heat-treated *Euglena* (Table II).

In the second set of experiments the effect of the light regimen on spontaneous bleaching was studied. In this case the experiments were performed at room temperature without any special bleaching agent. The results were as in the first set of experiments: a significantly higher proportion of bleached colonies were detected after growth at continuous illumination (Table IV).

Discussion

Recovery from the effects of the bleaching process in *Euglena gracilis* is influenced by the physiological conditions during and after the bleaching treatment. Growth rate (Brawerman and Chargaff 1960), the composition of the medium (De Deken-Grenson 1959, Mego and Buetow 1967) and light (Uzzo and Lyman 1969) were found to be important. No bleaching was found in the dark (during the bleaching treatment) at the bleaching temperature of 32 °C (Uzzo and Lyman 1969). Intense light itself is an effective bleaching agent (Leff and Krinsky 1967). Although the light intensities used in our experiments approached optimal values for growth of *Euglena* (the optimal for chloroplast development being 100 ftc. according to Stern et al. 1964), and no spontaneously bleached cells were found by Leff and Krinsky (1967) at optimal light intensities on synthetic medium, continuous illumination itself had an effect on the temperature-induced and even spontaneous bleaching of *Euglena* cells in our experiments. In this respect the light rhythm with day and night cycles, which induces synchronous growth in *Euglena* (Edmunds 1964), seems to be favourable to the recovery process.

The rate of multiplication determines the rate of bleaching in *Euglena* treated by a bleaching agent. Rapidly growing cells become devoid of chloroplasts faster than the slowly growing or not growing cells (Brawerman and Chargaff 1960). The growth of *Euglena* in a phospate rich medium at the bleaching temperature is not inhibited, but the proportion of green cells is even lowered (Table III). Optimal phosphorus content for growth of *Euglena* at 20° C has been estimated by Buetow and Schuit (1968) to be 4—5 μg/ml and inhibitory over 650 μg/ml. In the media used the content of phosphorus was in the range of optimum values for growth. The results indicate that: a) there are additional phosphate requirements for recovery of the greening ability in heat-bleached *Euglena*; b) there is an enhancement of recovery in the phosphate rich medium.

Summary

The temperature-induced and spontaneous bleaching of the growing cells in *Euglena gracilis* is influenced by the light regimen (the length of the photoperiod) at the time after the bleaching treatment.

The percentage of the heat-bleached as well as spontaenously bleached colonies is significantly higher in continuously illuminated cultures than in the periodically illuminated ones (14 hrs light: 10 hrs darkness).

Table I. Effect of Illumination Regimen on Temperature-Induced Bleaching of *Euglena gracilis* after Plating on a Complex Medium

CONTINUOUS LIGHT			14 hrs LIGHT — 10 hrs DARK	
No. of experiment	No. of green colonies	No. of white colonies	No. of green colonies	No. of white colonies
1.	320	842	534	460
2.	86	1600	104	686
3.	900	838	772	290
4.	614	574	580	382
Σ	1920	3854	1990	1818
	Per cent of white colonies 66,7		Per cent of white colonies 47,7	

Cells were grown at 34,5 °C in the complex medium for 3 days (inoculated with 10^4 cells/ml). During the bleaching treatment cultures were illuminated continuously. After 3 days the cultures were diluted and plated on agar. The petri dishes were kept at room temperature for 6—9 days at different illumination regimens.

Table II. Effect of Illumination Regimen on Temperature-Induced Bleaching of *Euglena gracilis* after Plating on a Complex Medium with Additional Phosphates

CONTINUOUS LIGHT			14 hrs LIGHT — 10 hrs DARK	
No. of experiment	No. of green colonies	No. of white colonies	No. of green colonies	No. of white colonies
1.	729	438	1072	218
2.	743	1283	821	239
3.	690	187	749	60
Σ	2162	1908	2642	517
	Per cent of white colonies 46,9		Per cent of white colonies 16,3	

Cells were grown and plated as in experiments in table I. The medium was supplemented by additional phosphates (0,08 per cent w/v K_2HPO_4 and 0,08 per cent KH_2PO_4).

Table III. Effect of Additional Phosphates on Growth of *Euglena gracilis* in a Complex Medium at 34,5 °C

	Cell density after 3 days growth at 34,5 °C inoculated with ca 10^4 cells/ml	
	Complex medium (P content 26 µg/ml)	Complex medium with additional phosphates (P content 350 µg/ml)
No. of cells	$2{,}0 \cdot 10^5 \pm 1{,}56 \cdot 10^4$/ml	$2{,}34 \cdot 10^5 \pm 1{,}57 \cdot 10^4$/ml

The values represent the mean of 20 experiments.

Table IV. Effect of Illumination Regimen on Spontaneous Bleaching of *Euglena gracilis*.

	CONTINUOUS LIGHT		14 hrs LIGHT — 10 hrs DARK	
No. of experiment	No. of green colonies	No. of white colonies	No. of green colonies	No. of white colonies
1.	2576	1	1000	0
2.	1100	1	900	0
3.	1021	1	1035	0
4.	3300	4	3300	0
5.	3000	3	3000	1
6.	3300	6	4200	1
Σ	14297	16	13435	2
	Per cent of white colonies 0,11		Per cent of white colonies 0,015	

Cells were grown for 2—3 days in the complex medium and after dilution plated on agar plates. One part of the plates were illuminated continuously and the other 14 hours daily. The colonies were scored 9 days after plating.

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ZUSAMMENFASSUNG

DER EFFEKT DES BELICHTUNGSREGIMES AUF DAS TEMPERATUR-INDUZIERTE UND SPONTANE AUSBLEICHEN VON *EUGLENA GRACILIS*

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Es wird gezeigt, dass bei kontinuierlich belichteten Kulturen von *Euglena gracilis* der Prozentsatz der sowohl temperaturbedingt als auch spontan entstehenden apochlorotischen Kolonien in bezug auf die periodisch belichteten Kulturen (14 Std Licht: 10 Std Dunkelheit) signifikant höher ist.

SADRŽAJ

UTJECAJ REŽIMA SVJETLOSTI NA SPONTANO I TEMPERATUROM INDUCIRANO IZBLIJEĐIVANJE VRSTE *EUGLENA GRACILIS*

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Ustanovljeno je, da je postotak bezbojnih euglena (bilo nastalih spontano, bilo indukcijom kod povišene temperature) u kontinuirano osvijetljenim kulturama značajno povišen u odnosu na periodički osvijetljene kulture (14 h svjetlosti: 10 h tame).

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