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PROPERTIES AND STRUCTURE OF A BLEACHED PIGMENTLESS MUTANT OF *EUGLENA GRACILIS**

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Introduction

Diverse bleached mutants have been derived from the green flagellate *Euglena gracilis* by physical and chemical agents (see e. g. Kirk 1967, Schiff et al. 1971, Parthier and Neumann 1977). These mutants differ with regard to the presence or absence of a recognizable plastid structure, as well as stigma and carotenoid content (Lefort 1963, Moriber et al. 1963, Rogers et al. 1972, Ben-Shaul et al. 1972, Marčenko 1973, Kivic and Vesk 1974a and 1974b, Heizmann et al. 1976, Kronestedt and Wales 1976, Parthier and Neumann 1977). Most of the investigated mutants still contain more or less expressed plastid structures resembling proplastids of the etiolated wild type *Euglena*. Some thylakoids, plastoglobuli and prolamellar body-like structures have often been observed in such plastids. In some cases very aberrant structures, hardly recognizable as plastids, or even no such structures were found.

The bleached mutants show, in most cases, more or less pronounced carotenogenesis (Dolphin 1970, Marčenko 1973, Gross and Stroz 1975, Parthier and Neumann 1977), limited photoinduced plastid development (Salvador et al. 1972, Marčenko 1973, Parthier and Neumann 1977) and photoinduced plastid enzyme synthesis (Parthier and Neumann 1977, Russell and Draffan 1978). Mutants completely lacking plastids, stigmata, and pigments are comparatively rare.

The properties and ultrastructure of an obviously pigmentless, completely white *Euglena* mutant are examined.

* Dedicated to Professor Zvonimir Devidé on the occasion of his 60th birthday.

Materials and Methods

Two almost identical strains of a white *Euglena* mutant were derived by chance from a carotenoid containing mutant Y-1 (Marčenko 1973) by intense illumination (over 10,000 lux) and subsequent growth in the dark at room temperature (the temperature during illumination was not controlled).

Cells were grown axenically in a rich organic medium (Marčenko 1970), or in the synthetic medium of Cramer and Myers modified by Padilla and James (1964).

Growth rates are expressed as K, the number of doublings per day, according to Hoogenhout and Amesz (1965). Cell counts were made in a haemocytometer.

Preparations for electron microscopy were done as described previously (Marčenko 1973).

Extraction and analysis of pigments: Cells were harvested by centrifugation, washed with distilled water and extracted with acetone. The extract was evaporated in vacuo and the residue transferred to n-hexan. The spectrum was recorded with a Perkin Elmer Mod 137 spectrophotometer.

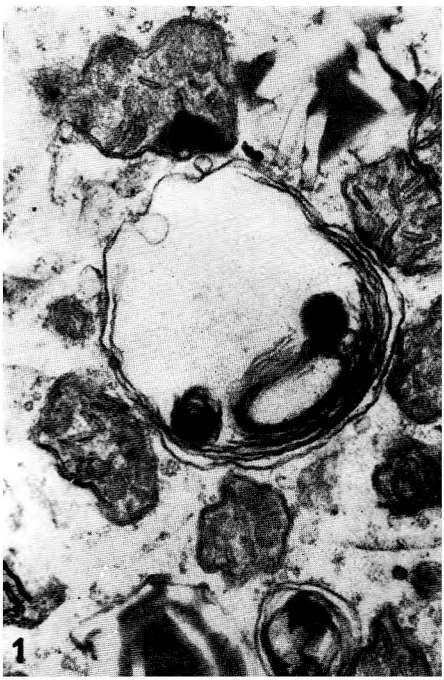
Results

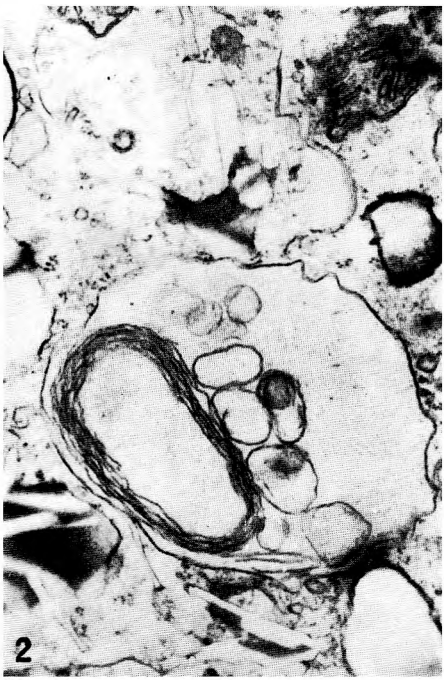
Two completely white strains were obtained as the result of bleaching of the yellow mutant with light of high intensity. These mutants never changed its white colour whether growing in the dark or in the light. They never reverted to the yellow or green wild type. According to the nomenclature of mutants of *Euglena* (Schiff et al. 1971) it could be called WYL. The growth of the two almost identical strains WYL-1 and WYL-2, at different temperatures, compared with the growth of the wild type, is shown in Table I. As shown by counting divisions of individual cells on agar plates the slow growth rate of the white strains can, at least partially, be attributed to some cells dying at the stage of cell division (Marčenko 1980). The growth of the white strains is photoinhibited (Marčenko 1980). Photoinhibition of the growth of some bleached strains of *Euglena* has also been observed by Cook (1968), Mitchell (1971) and Nicolas et al. (1980).

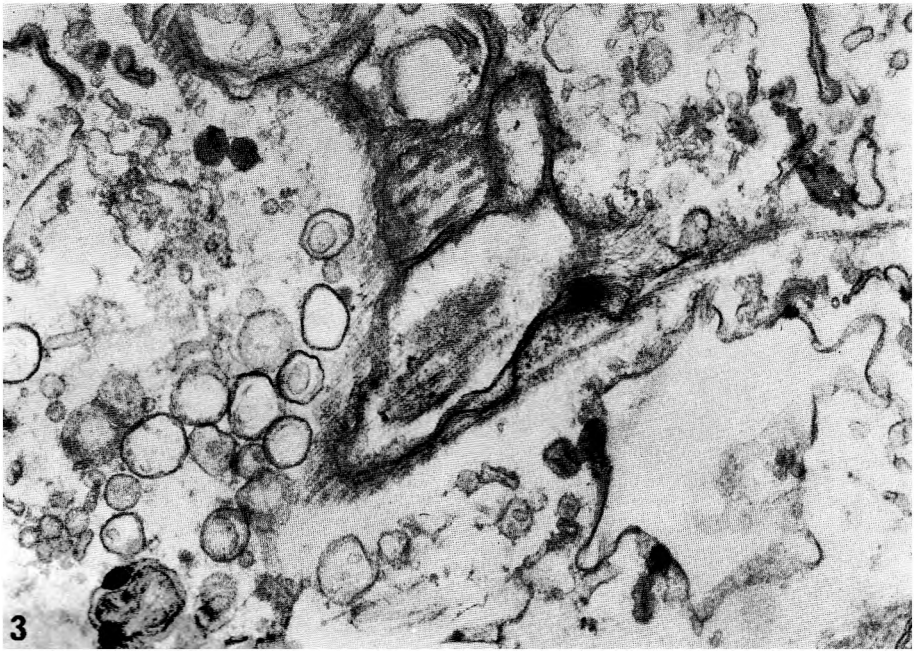
Pigment analysis reveals no traces of chlorophyll or coloured carotenoids. In the short wave region, however, some characteristic peaks appear which are close, but not identical to the absorption maxima of phytoene indicating most probably, the presence of ergosterol (Gross and Stroz 1975).

Figs. 1. and 2. Possible plastid remnants in a pigmentless *Euglena* mutant WYL light-grown. Note the concentric lamellae and membrane-bound non-osmiophilic vesicles (Fig. 2). 24,000 : 1.

Fig. 3. Eyespot-region in WYL mutant, light-grown. Note the membrane-bound vesicles of the stigma remnant in the vicinity of the flagellar region. 32,000 : 1.







Figs. 1—3.

Table I. Growth rates of *Euglena* strains at different temperatures in the dark

Strain	Temperature	Growth constant K (number of doblings per day)	Generation time
Wild type	20 °C	1,36	18h
Wild type	25 °C	1,49	16h
Wild type	30 °C	2,00	12h
WYL-1	20 °C	0,90	27h
WYL-1	25 °C	1,15	20h
WYL-1	30 °C	1,59	15h
WYL-2	20 °C	0,90	27h
WYL-2	25 °C	1,13	21h
WYL-2	30 °C	1,45	16h

Light-microscopic examination reveals the absence of visible stigma in the light- or dark-grown mutant WYL, and no detectable plastids.

Electron-microscopic examination of light-grown cells reveals, besides numerous mitochondria, some structures of the size of proplastids (ca 2 μm) with very aberrant lamellae, often concentrically arranged (Fig. 1.). Sometimes these structures contain membrane-bound empty vesicles, lacking osmiophily, of the size of plastoglobuli (Fig. 2). Such bodies reminescent of plastid remnants were not observed in the dark-grown mutant. Non-osmiophilic membrane-bound vesicles of the size of stigma-globules in the vicinity of the flagellar region were also present (Fig. 3). The presence of the paraflagellar body in our mutant could not be confirmed with certainty.

Leff and Krinsky (1967) claimed to have obtained permanently bleached "white« cells of Euglena by the mutagenic action of visible light of high intensity, under aerobic conditions. Bleached strains thus obtained have been considered aplastidic, and lacking plastid DNA. To test whether these cells are possibly identical to the WYL mutant, wild type *Euglena* was subjected to the treatment described, but no completely pigmentless or stigmaless strains were produced (Marčenko, unpublished). Only chlorophyll was lacking, but stigma, carotenoids and some plastid structures were present.

Pringsheims' observation of stigmaless *Euglena*, produced by heat bleaching and subsequent growth in the dark (1951), could not be confirmed in our laboratory. However, their observations were based on light-microscopic examination only, so that the presence of stigmata might possibly have been overlooked. There is also a difference in the strains: theirs was "*bacillaris*" and ours "*Z*".

Discussion

The yellow mutant Y-1 containing distinct plastid structures and carotenoids (Marčenko 1973), when grown in the dark and in the light, can easily be bleached with the same agents used for bleaching the green *Euglena* (Marčenko 1974). The bleached cells can be called "colourless" or rather "pale-yellow" as they also contain stigmata and exhibit light-induced carotenogenesis. The amount of carotenoids in the "pale-yellow" mutants in the dark is negligible, and they appear almost white, which makes them easily distinguishable from the yellow strain.

Most of the bleached mutants derived from wild type green *Euglena* also belong to the category of the "colourless" or "white" strains. The term "white" is rather misleading, as most of these mutants exhibit some plastid structures and some photoinduced carotenogenesis (Dolphin 1970, Kivic and Vesk 1974 a). Some of these "white" strains even exhibit a limited chlorophyll synthesis (e. g. the strain "blanc" of Salvador et al. 1972). Distinct plastid structures and carotenogenesis were found in the W_3BUL mutant (Palisano and Walne 1976, Parthier and Neumann 1977), earlier considered aplastidic. Heizmann et al. (1976) observed in the same mutant some plastid structures only after cycloheximide treatment.

The WYL mutant described in the present paper is, however, completely pigmentless. Thus, it does not resemble any of the bleached mutants examined by Parthier and Neumann (1977) which all contain some carotenoids. In their strains these authors also observed some prolamellar bodies, osmiophilic globuli and even some stacked thylakoids, all of which were absent from our strain. It is more closely like the almost aplastidic strains described, e. g. by Kivic and Vesk (1974 a), and the empty "eye-spot" vesicles resemble that of *Euglena* derived *Astasia longa* (Kivic and Vesk 1974 b). *Astasia longa* is a naturally occurring pigmentless *Euglena* which lacks any plastid structure (Rogers et al. 1972, Kivic and Vesk 1974 a).

It is proposed that the term "white" should be reserved for strains which under no condition develop any visible pigment. The proper characterization of strains becomes particularly important in studies on the correlation of plastid structure and function.

Summary

The structure and growth were examined in a white, completely pigmentless, mutant of *Euglena gracilis*. Although it lacks any pigments, coloured stigma and defined plastids, some non-osmiophilic structures resembling stigma vesicles and, possibly, very aberrant remnants of plastids appear in this strain. The value of the exact characterization of bleached mutants is discussed.

*

I am indebted to Dr. N. Ljubešić for electron microscopy of the specimens.

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SADRŽAJ

SVOJSTVA I STRUKTURA BEZBOJNE MUTANTE VRSTE *EUGLENA GRACILIS*

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Istraživani su rast i ultrastruktura bijele bezbojne mutante vrste *Euglena gracilis*. Iako bez pigmenta, u stanicama postoje ostaci stigme i plastida, koji sadržavaju samo koncentrične lamele i ostatke globula.

Ukazano je na potrebu točnije karakterizacije izblijeđenih mutanti euglena.

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