An easy method for light microscopic distinguishing of xanthophycean and eustigmatophycean strains

Snadná metoda rozlišení kmenů tříd Xanthophyceae a Eustigmatophyceae ve světelném mikroskopu

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Abstract

The paper presents a method of selective fuchsin-based cell wall staining distinguishing xanthophycean and eustigmatophycean species. The members of Xanthophyceae exhibit distinct positive reaction, whereas eustigmatophycean strains remain unstained. The method could be useful in the distinguishing of living populations of the species of both classes.

Introduction

The algal class Eustigmatophyceae was established by HIBBERD & LEEDALE (1971, 1972). Eustigmatophyceae are characterized by the complex of some unique structural features of both vegetative cells and zoospores, in which they differ from Xanthophyceae, as well as from other algal groups (HIBBERD, 1990). As visible in LM, main features distinguishing Eustigmatophyceae from Xanthophyceae are the presence of prominent reddish globule and polyhedral pyrenoid in vegetative eustigmatophycean cells, and the extraplastidial stigma of eustigmatophycean zoospores. However, there are some eustigmatophycean species apparently lacking these prominent features. Some species do not possess the polyhedral pyrenoid (e.g. *Pseudocharaciopsis ovalis, Monodopsis subterranea, Nannochlopsis spp.* (ANDERSEN et al., 1998; NEUSTUPA & NĚMCOVÁ, 2002). All members of the family *Monodopsidaceae* do not produce zoospores. In addition, there are distinct stages in the life cycle of eustigmatophycean species when the reddish globule, as well as the polyhedral pyrenoid, are not visible in the vegetative cells (NEUSTUPA & NĚMCOVÁ 2002). There is also a considerable similarity in the shape of vegetative cells between some eustigmatophycean and xanthophycean genera (*Eustigmatos*/ *Pleurochloris*, *Pseudocharaciopsis*/*Characiopsis, Pseudellipsoidion*/ *Ellipsoidion*). Since the members of both groups often share similar ecology (soil and aerophytic biotopes), the correct distinguishing between members of

both groups is frequently required by algologists studying algae of terrestrial localities.

This paper presents an easy method for distinguishing between the members of Eustigmatophyceae and Xanthophyceae. The method is based on the staining of cell walls of living cells with simplified Schiff´s reagent. PRÁT (1947) used this method for the first time. In his study, he examined the reaction of algae from all main taxonomic groups was. He could not explain the striking difference in the staining of several coccal and filamentous species classified into the Xanthophyceae at that time. Most xanthophycean strains examined in his study showed prominent positive reaction. However, in several strains, the cell wall remained entirely unstained. Surprisingly, all the strains with negative reaction were later reclassified into the Eustigmatophyceae (HIBBERD & LEEDALE, 1971, 1972; HIBBERD, 1981).

Material and methods

The investigated strains were taken from The Culture Collection of Algae of Charles University in Prague (CAUP) (PUNČOCHÁŘOVÁ, 1990). Eustigmatophycean and xanthophycean strains recently isolated from various terrestrial localities were also examined. The strains were cultivated on BBM–agar in the temperature of 15°C, and under the illumination of 5000 lx (light source Tungsram 36W F33, cool white).

The reagent was prepared according to the recipe described in PRÁT (1947): 0,05 g of basic fuchsin, 10 ml of distilled water, 1 ml of molar HCl, 0,06 g of $K_2S_2O_5$.

A sample of an algal culture was placed on the objectslide together with a drop of the reagent. In most cases, positive reaction was visible even by naked eye as a violet coloration of the culture. After approximately 10 minutes, the intensity of the coloration remained stable. If no reaction was observed in 30 minutes, no staining appeared later. The cultures were examined with the light microscope Olympus BX 51 using direct illumination and Nomarski differential contrast.

The intensity of the reaction was characterised with semiquantitative scale: 0 – no reaction, 1 – low reaction of only a few cells, 2 – positive reaction of most cells, 3 – distinct positive reaction of all cells.

Results and discussion

In accordance with PRÁT (1947), the cell wall of the tested xanthophycean strains showed a distinct positive reaction with the violet-coloured cell walls. On the other hand, cell walls of eustigmatophycean strains exhibited no reaction (Tab. 1).

The strain CAUP D 901 designated as xanthophycean *Monodus sp.* exhibited negative reaction. As the eustigmatophycean nature of several *Monodus*-like species has already been documented and those species have been reclassified into the eustigmatophycean genus *Monodopsis* (HIBBERD, 1981; SANTOS & LEEDALE, 1995), the correct classification of this strain into the Eustigmatophyceae is hypothesised.

The method of cell wall staining with Schiff reagent provides quick information on possible eustigmatophycean character in investigated strains classified traditionally into the Xanthophyceae. It could be used as the first choice method before the conducting of ultrastructural, biochemical, or molecular investigation of perspective strains.

However, the mechanism of the fuchsin-induced selective cell wall staining remains unclear. The staining of the cell wall material cannot be attributed to some particular chemical component because of non-selectivity of the reaction of biological materials with fuchsin-based reagents (NĚMCOVÁ, 2000).

Acknowledgements

The work has been supported by the grant no. 134/2000/B-Bio of the Grant Agency of Charles University.

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Table 1: List of tested strains.

Species	Class	Reaction
Botrydiopsis intercedens (D301)	X	2
Botrydiopsis intercedens, izol. NEUSTUPA	X	3
Bumilleriopsis filiformis (D101)	X	3
Ophiocytium maius (D702)	X	2
Xanthonema solidum (D201)	X	3
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X. hormidioides, isol. NEUSTUPA	X	2
Tribonema vulgare (D501)	X	3
Tribonema minus (D502)	X	3
Heterococcus fuornensis – isol. NEUSTUPA	X	2
Monodus sp. (D 901)	E ??	0
Eustigmatos vischeri (Q101)	E	0-1
E. magnus (Q102)	E	0
E. magnus, isol. NEUSTUPA	E	0
E. polyphem (Q103)	E	0
E. sp., isol. NEUSTUPA	E	0
Pseudellipsoidion edaphicum (Q401)	E	0-1
Pseudocharaciopsis ovalis (Q301)	E	0
P. ovalis (Q302*)*	E	0
Monodopsis subterraneus – isol. NEUSTUPA	E	0
Vischeria sp. (Q201)	E	0