The effect of colour light on production of zooids in 10 strains of the green chlorococcal alga *Scenedesmus obliquus*

Vliv barevných světel na produkci bičíkatých buněk u 10 kmenů zelené chlorokokální řasy *Scenedesmus obliquus*

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Abstract

The effect of lights of different spectral composition on the life cycle of 10 strains of *Scenedesmus(Acutodesmus) obliquus* (Turp.)Kütz. was studied. Subcultures from synchronous mother culture grown under standard conditions were withdrawn at 0, 4, 8, and 16 hours intervals and the dense cultures exposed in nitrogen free medium, temperature 15°C and various intensities of white, blue, red, or green light. Motile cells were observed only at cultures grown under blue or green light. Their number was independent on light intensity in tested span of irradiances (5, 25 and 50 μmol.m^{-2}) that indicates signal role of blue and green light rather than a source of energy. The concentration of motile cells was dependent on developmental stage of cells shifted into the gametogenic conditions. It was proven that production of motile cells is strain specific because they were observed only in the two strains of ten tested.

Introduction

It has been believed for many decades that the chlorococcal green alga *Scenedesmus obliquus* can propagate only vegetatively forming daughter coenobia. Forty years ago, surprisingly, Trainor described motile stage (zoospores which were later recognized as gametes) in the life cycle of this species (TRAINOR 1963). Comprehensive physiological study made by CAIN & TRAINOR (1976) revealed that necessary conditions for gamete production in laboratory are low nitrogen concentration (or nitrogen starvation), low temperature, high density of culture and a low light intensity. The same conditions were effective in inducing gametogenesis in relating algae, i.e. both *Scenedesmus dimorphus* and *Coelastrum microsporum* (TRAINOR & BURG 1965) and in spiny *Scenedesmus (Desmodesmus) armatus* (TRAINOR 1993). Using some additional strains, production of motile cells was later confirmed also in other laboratories (LUKAVSKÝ 1991, HINDÁK & TRAINOR 1995, LUKAVSKÝ & CEPÁK 1998). Because of low yield of gametes (maximally 15%) in comparison with another microalgae (e.g. *Chlamydomonas* is producing 100%) one might propose that we do not have optimal conditions for stimulating gamete production in *Scenedesmus* yet.

Gametogenesis was extensively studied mostly in relative unicellular green alga *Chlamydomonas reinhardtii* and it was shown that it is controlled by a consecutive action of two environmental signals, one chemical, i.e. nitrogen starvation, and another physical, i.e. light (WEISSIG & BECK 1991, BECK & HARING 1996). It was shown that in *Chlamydomonas reinhardtii* (including many higher plants) light plays an important role not simply as a source of energy, but especially as a signal. It was revealed that blue light of 370 and 450 nm was maximally active in inducing of gametes (WEISSIG & BECK 1991). The effect of light of different action spectrum in *Sc. obliquus* has not been studied yet.

The paper investigates the influence of blue, red, white and green lights on the development of *Scenedesmus obliquus* growing in gametogenic conditions. We have found that blue light and in lesser extent also green light is effective in producing motile cells and that the process is strain - specific.

Materials and methods

Experimental organisms and batch culture conditions. 10 strains of *Scenedesmus obliquus* used in experiments are listed in the Table 1. Batch cultures were synchronized by alternating of 16 hours light (400 $\mu mol.m^{-2}$) and 8 hours dark periods. They were grown in the nutrient medium after Zehnder (STAUB 1961) at 28°C and aerated with air containing 3% carbon dioxide (v/v). After three periods, the cultures were synchronous and ready for experiments with colour light. Subpopulations were removed during light period from mother culture at 0, 8, and 16 hours intervals. Cells were washed out, three times, in nitrogen free medium and concentrated (dense population reached about 10^8 cells per ml). Populations were cultivated under white, blue, red and green lights (5, 25 and 50 $\mu mol.m^{-2}$) at 15°C and aerated gently with air containing 3% carbon dioxide (v/v). As a source of colour lights we used the following fluorescent tubes: white light - Osram L36/31-830, blue light - TUNGSRAM 40W F Blue, red light - TUNGSRAM 40W F Red, green light - TUNGSRAM 40W F Green. The experiment was conducted in duplicate.

DAPI staining. Nuclei and chloroplast nucleoids were stained with DAPI (4,6'-diamidino-2-phenylindole) as described earlier (CEPÁK et al. 2002). In short, a drop of fixed sample (in 1% glutaraldehyde) was placed on a slide and a drop of DAPI solution (0,1-1 μg.ml $^{-1}$ of buffer S) was added (KUROIWA & SUZUKI 1980). The preparations were examined with an epifluorescence microscope Olympus BX60 connected with Camera Olympus 5050.

Cell number estimation. Cell concentration was estimated in the Bürker counting chamber (Meopta, the Czech Republic) using a standard procedure.

Results and discussion

We have found that the colour of light plays an important role in producing zooids in the alga *Scenedesmus obliquus* growing under gametogenic conditions, i.e. nitrogen free medium, low temperature and dense culture (Fig. 1). The most effective was blue light. The maximal amount of zooids was observed after 72 hours of cultivation in nitrogen free medium in the strain TOMASELLI (Fig. 2a). After that period, their concentration continuously declined until they disappeared. It is partly in agreement wit results obtained by another authors (CAIN & TRAINOR 1976). They have found that motile cells also disappeared but their lifetime was shorter (only a few hours) when compared with zooids releasing in our experiment. It can be explained with different strains and culture conditions used. Zooids were released also under green light that was not so effective as blue one (Fig. 2b). In this case concentration of zooids in the strain TOMASELLI reached maximally 5% contrary to blue light when portion of zooids producing cells reached 25% of all cells.

Similar effect of blue and green light was observed in experimentations with intertidal brown alga *Silvetia compressa* (PEARSON et al. 2004). This alga releases gametes from receptacles rapidly upon a dark transfer (following a photosynthesis-dependent period in the light, termed potentiation). During the potentiation period in white light, gametes were not released. However, gametes were released during potentiation in blue light or in low red light/blue light ratios of intensities, but not in red light alone or high-red light/blue-light ratios. In the green alga *Protosiphon botryoides* zoospores production was also induced by green (522 nm) however on contrary to our results inhibited by blue (432-461 nm) light (CARROLL et al. 1970). Our results are in accordance with outcomes that blue light is most effective in production of gametes in flagellate *Chlamydomonas reinhardtii* (WEISSIG & BECK 1991, BECK & HARING 1996).

We have found that the competence to produce zooids in *Scenedesmus obliquus* is a strain specific. We observed the production of motile cells only in two strains of ten tested (Fig. 1). Other strains were not able to do this. We do not confirmed a surmise that freshly isolated strains are able to produce zooids (TRAINOR 1998). Recently, we isolated two strains (CEPÁK 2004/1 from pond at botanical garden in Belfast and CEPÁK 2004/2 from pond at botanical garden in Amsterdam) and zooids production was not monitored contrary to strains PRINGSHEIM and TOMASELLI that were isolated many decades ago.

Another prerequisite for gamete formation is the state of cells (phase of the cell cycle) entering gametogenic conditions. We found that daughter cells and little developed cells (Fig 3 – Oh) as well as mother cells before cytokinesis and dividing cells (Fig.3 – 12h) have not released zooids. The cells in the middle of the cell cycle, i.e. bi- or tetra-nuclear (Fig 3 –4h, 8h), produced the highest amount of flagellated cells. It is in accordance with results obtained in experiments with unicellular green flagellate *Chlamydomonas eugametos* (ZACHLEDER et al. 1991). Using synchronous cultures, motile cells were formed in a larger amount (up to 80%) only during particular interval of the cell cycle, i.e. growth phase. In experimentation with the marine diatom *Thalassiosira weisflogii* was also pointed out that there is a preferential response to induction signals only during one portion of their cell cycle, the early G_1 stage (ARMBRUST et al. 1993). The same conclusion results in the study on *Scenedesmus obliquus* (CEPÁK & LUKAVSKÝ 1998) where aplanospores (zoospores without flagella) were observed (50% of all cells).

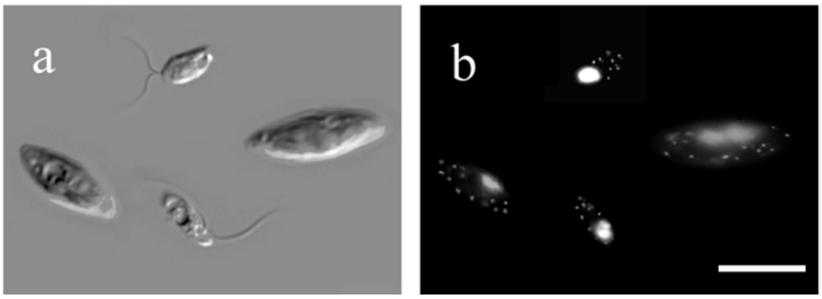

Fig. 1: Microphotographs of vegetative cells and zooids of the alga *Scenedesmus obliquus* (strain PRINGSHEIM/Praha Ac. A 125) in culture grown under blue light in nitrogen free medium and 15°C. a – DIC, b – DAPI stained cells (large spots = nuclei, small spots = chloroplast nucleoids). Bar = 10 μm.

Table 1: List of *Scenedesmus obliquus* strains used in experiments

Strain	Locality	Origin
TOMASELLI/Praha Ac. A 835	*	CCALA
PRINGSHEIM/Praha Ac. A 125	*	CCALA
HINDÁK	*	CCALA
GAFFRON/Gött. 276-6/mut.BISHOP8 X-ray mutant with blocked PSI	river Nil, Egypt	CCALA
GAFFRON/D-3	Germany, pond at town Czygan	CCALA
LHOTSKÝ,O. 1966/7	Czech Republic, Opatovický mlýn	CCALA
GENF 145	*	CCALA
SAG 276-6	*	CCALA
CEPÁK 2004/1	pond at botanical garden, Belfast (North Ireland)	own isolate
CEPÁK 2004/2	pond at botanical garden, Amsterdam (Netherland)	own isolate

* missing information
CCALA – Culture Collection of Algal Laboratory, Třeboň, Czech Republic

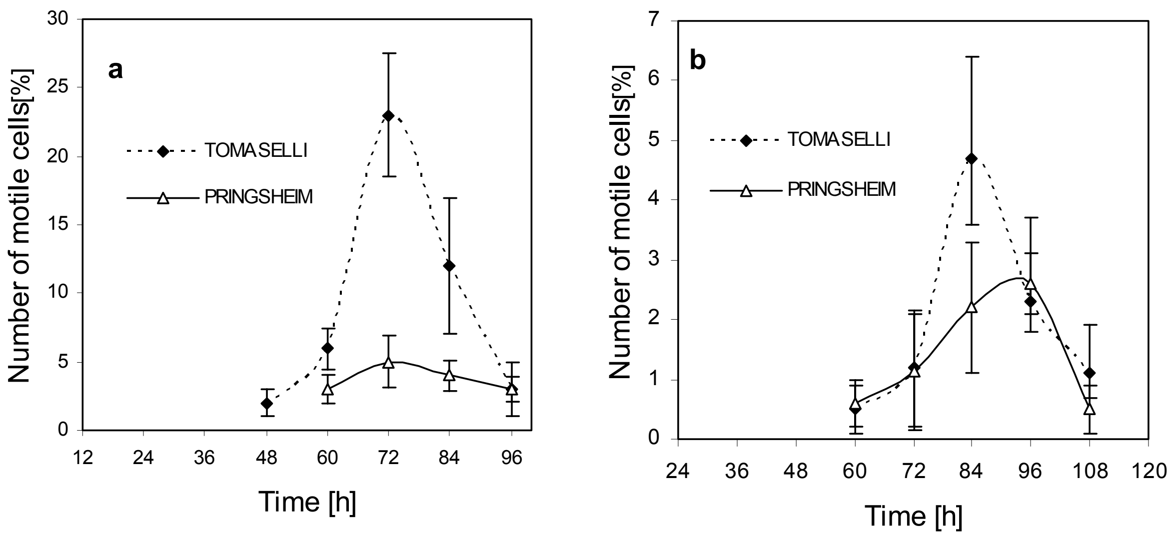

Fig. 2: The effect of light quality on the amount of motile cells in the green alga *Scenedesmus obliquus*. Synchronous populations were cultivated 8 hours under standard conditions and shifted into nitrogen-free medium and 15°C and grown under blue (A) or green (B) light of 10W.m^{-2}.

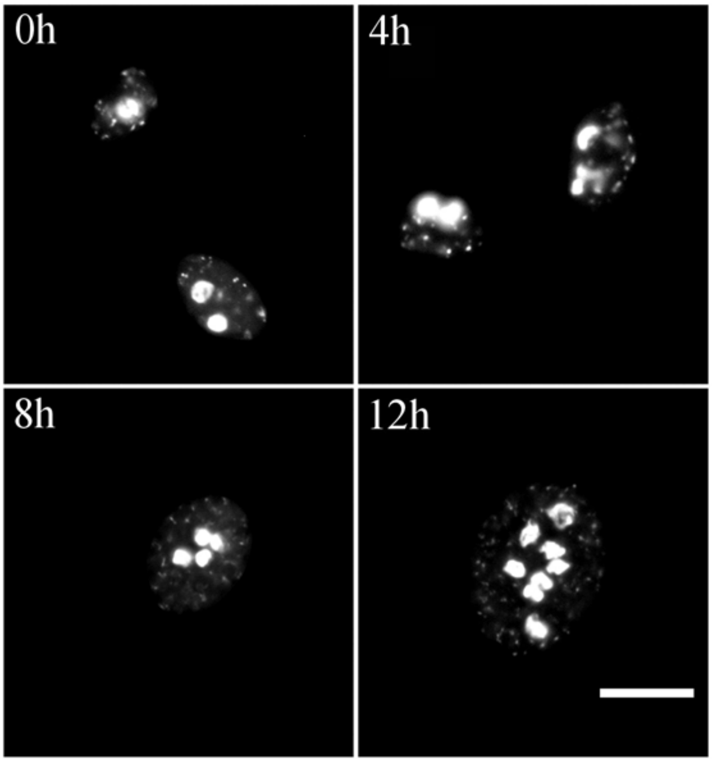

Fig. 3: Fluorescent microphotographs of cells of the alga *Scenedesmus obliquus* strain TOMASELLI staind with a fluorochrom DAPI in various stages of the cell cycle. The age of cells is indicated at the upper left-hand corner. Large spots = nuclei, small spots = chloroplast nucleoids. Bar = 10 μm.

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