Sensitivity of autotrophic microorganisms to selected groups of biologically-active compounds: Chlorophyll fluorescence study

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INTRODUCTION

In stock cultivations of microalgae and cyanobacteria, much effort to obtain axenic cultures is carried out by several treatments such as *e.g.* exposition to UV light, phenol treatment, and addition of antibiotics to culture medium. Generally, a wide range of antibiotics is used to suppress the growth of fungi and bacteria. In our study, we tested two antibiotics (neomycin, tetracycline) added to the cultivation medium, their effects on growth rate of microalgae and primary processes of photosynthesis. Neomycin and tetracycline act against both gram-positive and gram-negative bacteria. We hypothesized that the addition of the two antibiotics would lead to a better function of photosynthetic apparatus. We also tested the hypothesis that the addition would cause different response of photosynthetic parameters to the time of cultivation. Last but not least, we wanted to evaluate the interspecific differences in photosynthetic parameters in response to neomycin, and tetracycline, respectively.

MATERIAL AND METHODS

Two microalgal species *Heterococcus* sp. (CCALA strain No. 878, *see* References) *Klebsormidium* sp. (CCALA strain No. 859) were used for cultivation on agar plates. The cultivations were performed on two different cultivation media (the Bold basal medium - BBM, and the Zendher medium - Z). To study the effects of antibiotics on the growth rate and photosynthetic performance, two antibiotics (neomycin and tetracycline) were added into the cultivation media. Cultured microalgae (1 ml) were spread plated on BBM and Z agar plates.

After inoculation, the growth of the cultures was monitored by chlorophyll fluorescence (ChlF) using the method of slow Kautsky kinetics supplemented with quenching analysis using a FluorCam HFC-010 fluorometers (Photon systems Instruments, CZ). The following ChlF parameters were recorded in 5 d interval: Steady-state chlorophyll fluorescence (F_S), maximum capacity of photosynthetic processes in photosystem II (F_V/F_M), and effective quantum yield of photosystem II (Φ_{PSII}).

RESULTS AND DISCUSSION

Steady-state chlorophyll fluorescence (F_S) was monitored as a proxy of growth of the cultures. Time courses of F_S revealed that the cultures growth followed typical S-curves (*see* Fig. 1 - right panel). Addition of neomycin had a positive effect on growth of *Heterococcus*sp., and *Klebsormidium*sp. cultures, when cultivated on BBM medium. The

effect was apparent in the last part of the cultivation period, *i.e.* after 30 d of cultivation. The neomycin-induced increase in F_S was found in *Klebsormidium* sp. and *Heterococcus* sp. The effect of tetracycline addition on the growth of the cultures was not significant.

Monitoring of F_V/F_M, and Φ_{PSII} revealed that the primary processes of photosynthesis varied during the cultivation period in the two algal strains. However the effect of the antibiotics addition on F_V/F_M, and Φ_{PSII} was not found statistically significant. In forthcoming studies, other chlorophyll fluorescence parameters should be tested for their sensitivity to algal strain response to different agar medium and/or the addition of antibiotics in a wide range of concentration.

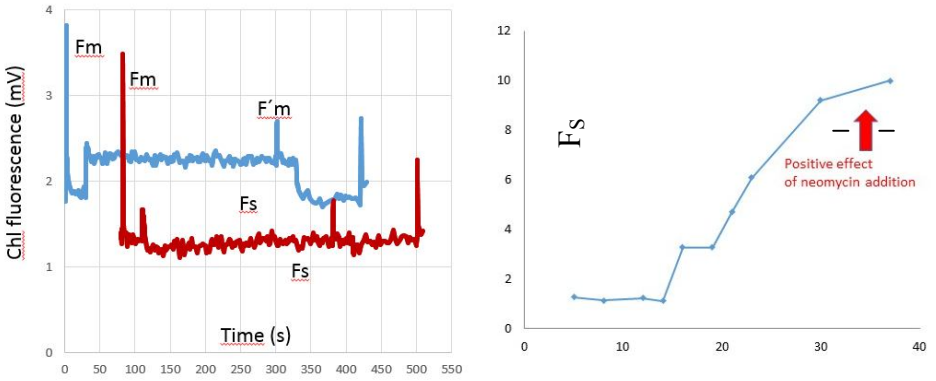

Fig. 1. Kautsky kinetics in *Heterococcus* sp. showing a positive effect of neomycin addition: red - control, blue - addition of neomycin (left). Chlorophyll fluorescence signal (F_S) recorded in *Klebsormidium* sp. grown on the BBM medium with addition of neomycin (right). F_S of untreated control at the end of cultivation is indicated by a line.

The co-action of antibiotics with microalgae used in axenic cultures cultivated on agar plates depends on the bacteria associated with microalgae, their concentration and physiological features. It is essential to check the susceptibility of microalgae before treating them with antibiotics as some microalgae are sensitive to antibiotics at higher concentrations (Han et al. 2016). In our study, for the two tested strains of Antarctic terrestrial microalgae, addition of neomycin could be recommended from the point of view of growth, since the chlorophyll fluorescence parameters reflecting vigor and growth potential of the cultures increased with neomycin addition.

REFERENCES

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Culture Collection of Autotrophic Organisms web page (CCALA): http://ccala.butbn.cas.cz/