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PSII-efficiency, polysaccharide production, and phenotypic plasticity of *Scenedesmus obliquus* in response to changes in metabolic carbon flux

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ABSTRACT

To investigate the response of *Scenedesmus obliquus* to changes in metabolic carbon flux, *S. obliquus* was cultured in medium with different concentrations of glyoxylate over 9 days. Results showed that growth rates were not affected in the lower concentration glyoxylate (0.25 and 0.5 mM). However, growth rate of *S. obliquus* was inhibited in the higher concentration glyoxylate (0.85 and 1.25 mM) during the early phase before recovering at higher densities. Changes in growth rates in different glyoxylate concentrations were in line with changes in F_v/F_m and Φ_{PSII}. Colony formation was observed in *S. obliquus* in the four glyoxylate treatments. As a consequence, the mean number of cells per particle of *S. obliquus* in the glyoxylate treatments were significantly higher than those in the control. The total polysaccharide content of *S. obliquus* cells increased with increased glyoxylate concentrations. The increased glyoxylate-stimulated polysaccharide levels were directly correlated with colony size of *S. obliquus*.

1. Introduction

Scenedesmus, one of the most common genera of freshwater green algae, has been shown to have high phenotypic plasticity (Trainor, 1964; Trainor et al., 1976). Many factors may influence the formation of unicellular or colonial morphology in species of *Scenedesmus* (Lürling and Beekman, 1999), including abiotic and biotic factors (see Lürling and Beekman, 2002 and references therein; Lürling, 2006).

It is known that colony formation in algae is linked to polysaccharide production (De Philippis and Vincenzini, 1998; van Rijssel et al., 2000; Thornton, 2002; Yang et al., 2008), which may be stimulated by different factors. Glyoxylate, a stimulator of carbon metabolism, was reported as a substance with the capability of inhibiting photorespiration and increasing photosynthesis in higher plants (Oliver and Zelitch, 1977) and some cyanobacteria (Bergman, 1980, 1981). An excess of carbon flux in algae, such as *Anabaena cylindrica* (Bergman, 1986) and *Cyanospira capsulate* (De Philippis et al., 1996) occur in response to the addition of glyoxylate, which results in an intracellular accumulation of polysaccharide and a release of soluble extracellular polysaccharide.

As glyoxylate can induce an increase of polysaccharide in some cyanobacteria (Bergman, 1986; De Philippis et al., 1996), and polysaccharides affect the stickiness of the cell surface and contribute to cell aggregation in some algal species (De Philippis and Vincenzini, 1998; van Rijssel et al., 2000; Thornton, 2002; Yang et al., 2008), we hypothesize that: (1) growth

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and Photosystem II efficiency (PSII-efficiency) of algal cultures may change after addition of glyoxylate; (2) addition of glyoxylate will induce polysaccharide production in *Scenedesmus obliquus*; (3) colony formation may occur in *S. obliquus* as a sequence of an increase in the amount of polysaccharide; (4) the level of colony formation may be related to the amount of total polysaccharide. To test these hypotheses, we assessed the effects of glyoxylate on PSII-efficiency, polysaccharide production, and phenotypic plasticity of *S. obliquus*.

2. Materials and methods

2.1. Microorganism and cultivation

S. obliquus (FACHB 416) was obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences. To maintain cultures and for experiments, it was cultured axenically in liquid BG-11 medium (Rippka et al., 1979), at 25 °C under a 12:12 h light:dark cycle at 40 μmol photons m^{-2} s^{-1}, and a pH 6.5–7.0. Based on our preliminary experiments and following the method of Bergman (1980), four glyoxylate (Sigma Fluka; St. Louis, MO, USA) treatments (0, 0.25, 0.5, 0.85, 1.25 mM), were examined over nine days; these were used to assess both the influence of glyoxylate concentration and time on polysaccharide production and its subsequent influence on colony formation. For each treatment, exponential-phase cells were inoculated into 500-mL Erlenmeyer flasks containing 200 mL of liquid medium to a density of 3.38×10^5 cells mL^{-1}, with three replicates per treatment. To reduce effects caused by minor difference in photon irradiance, the flasks were shaken three times each day and rearranged randomly.

2.2. PHYTO-PAM fluorometer analysis

The maximal efficiency of PSII photochemistry was determined as F_v/F_m with a PHYTO-PAM fluorometer (Walz, Germany), where $F_v = (F_m - F_0)$ and F_m and F_0 are the maximal and minimal chlorophyll fluorescence yield, respectively, of a dark-adapted suspension. The effective quantum yield of PSII (Φ_{PSII}) was determined according to the following expression $(F_m' - F_t)/F_m'$ where F_m' is the light-adapted maximum fluorescence and F_t is the fluorescence before a saturating pulse (Genty et al., 1989).

2.3. Polysaccharide contents assay

The cultures in the suspension were sampled on day 1, day 5, and day 9 to quantify total polysaccharide. Samples (10 mL) from the cultures were taken for the extraction of the different polysaccharide fractions. The culture samples were centrifuged at 17 000 g for 20 min at 4 °C. The supernatants were then filtrated through membrane filters (GFC, 47 mm in diameter; Whatman, Maidstone, UK). To remove ions, the filtrates were subjected to dialysis tubing with a 3500-molecular-weight cutoff (Spectrum 132 725) for 72 h against at least five consecutive 1-L changes of de-ionized water. The filtrates were then used to assay the content of soluble extracellular polysaccharide. The pellets were re-suspended in 6 mL of de-ionized water and frozen at −20 °C for 12 h, after which they were thawed, in 85 °C water. This freeze–thaw cycle was repeated three times, and then the samples were sonicated and incubated in 85 °C water for 1 h to extract the bound polysaccharide. After cooling,

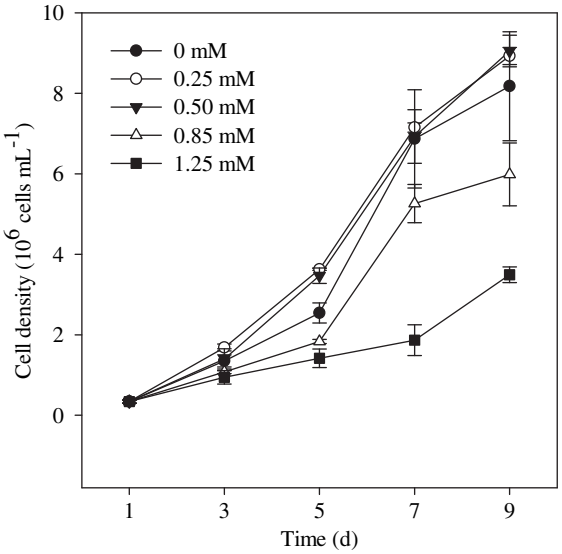

Fig. 1. Changes in cell density of *S. obliquus* in different glyoxylate treatments. Vertical lines represent ±1 SE.