## A novel strategy to promote microalgal growth and lipid productivity by supplementation of biomass derived elicitors

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Introducing biomass-derived additives into microalgae cultivation to increase its yield has been regarded as a more cost-effective and environment-friendly method compared with gene-editing and nutrients supplementation.

A significant promotion of growth and accumulation of metabolites of freshwater microalga *Euglena gracilis* was obtained by adding phytic acid derived from rice bran. Phytic acid concentration of 0.05% (v/v) showed a maximum biomass increase of 1.6-fold compared to the control group<sup>1</sup>). Phytic acid could serve as the sole phosphorus source for the growth of *E. gracilis*, and phytase which catalyzes the hydrolysis of phytic acid was discovered for the first time in *E. gracilis*<sup>2</sup>.

Effects of lignocellulose-related mannitol and xylitol on the growth, photosynthetic pigment content, cell morphology, and metabolites biosynthesis of freshwater microalga *E. gracilis* were investigated<sup>3</sup>). The results revealed that both mannitol and xylitol effectively promoted the growth of *E. gracilis*, and at the optimal dosage of 4 g·L<sup>-1</sup>, the biomass yield was increased by 4.64-fold and 3.18-fold, respectively. Increase of cell aspect ratio was only observed in mannitol treatment groups, indicating that *E. gracilis* had different physiological responses to mannitol and xylitol. The lipid content of *E. gracilis* was significantly promoted by these two sugar alcohols.

At the optimal concentration of 5000 mg·L<sup>-1</sup>, lignosulfonates (LIGNs) could promote the growth of *E. gracilis* up to 1.95 folds, and increase the yield of chlorophyll *a*, chlorophyll *b* and carotenoids by 3.50, 1.59 and 3.48 times, respectively<sup>4</sup>). Cell morphological changes in aspect ratio and size caused by LIGNs were also observed. LIGNs improved the lipid yield since both cell density and lipid content increased, which could contribute to the biofuel production in the future.

Feasibility of three major <u>phenolic</u> compounds from lignin's basic structures (guaiacyl-, hydroxyphenyl- and syringyl- types) for freshwater microalga *E. gracilis* cultivation was evaluated<sup>5)</sup>. The results indicated that *trans*-4-hydroxy-3-methoxycinnamic acid (HMA), 4-hydroxybenzaldehyde (HBA), and syringaldehyde (SRA) could all promote microalgae growth in a phytohormone-like role, and the highest promotion effect was achieved under HMA treatment. HMA at 0.5 g·L<sup>-1</sup> enhanced the cell biomass yield by 2.30 times, while <u>HBA</u> and SRA at the concentration of 0.1 g·L<sup>-1</sup> increased the

yield by 1.30 and 1.21 times, respectively. In addition, increased carotenoids and lipid biosynthesis were also observed under the treatments of phenolic compounds.

Syringic acid (SA) and *p*-coumaric acid (*p*-CA) are two major lignin hydrolysates. The cell growth of microalga *E. gracilis* was increased by 1.63 and 1.93 times at the optimal dosage of  $0.5 \text{ g}\cdot\text{L}^{-1}$  <u>SA</u> and  $0.3 \text{ g}\cdot\text{L}^{-1}p$ -CA, respectively<sup>6</sup>. Moreover, increased chlorophyll *a* content was only observed under *p*-CA treatment, indicating the influences of these compounds on photosynthesis were different. The variation trend of carbohydrate content was different under the treatment of the *p*-CA and SA, demonstrating that different phenolic compounds will determine different biosynthesis was improved by both of these phenolic acid treatments.

The balance between algal growth and the accumulation of metabolites is always the main contradiction in algal biomass production. Hence, the security and effectiveness of regulating microalgal growth and metabolism simultaneously have drawn substantial attention. Since the correspondence between microalgal growth and reactive oxygen species (ROS) level has been confirmed, improving its growth under oxidative stress and promoting biomass accumulation under non-oxidative stress by exogenous mitigators such as various antioxidants would be worth of concern<sup>7</sup>).

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