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Enhanced coproduction of astaxanthin and lipids by the green microalga *Chromochloris zofingiensis*: Selected phytohormones as positive stimulators

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ABSTRACT

Phytohormones comprise a variety of trace bioactive compounds that can stimulate cell growth and promote metabolic shifts. In the present work, a two-stage screening strategy was innovatively established to identify positive phytohormones for enhancement of astaxanthin and lipid coproduction in microplate-based cultures of mixotrophic *Chromochloris zofingiensis*. The results showed that auxins were the most efficient stimulators for astaxanthin accumulation. The maximum content of 13.1 mg/g and yield of 89.9 mg/L were obtained using indole propionic acid (10 mg/L) and indoleacetic acid (7.8 mg/L), representing the highest levels of astaxanthin in this microalga reported to date. Total lipids with the highest content (64.5% DW) and productivity (445.7 mg/L/d) were coproduced with astaxanthin using indoleacetic acid. Statistical analysis revealed close relations between phytohormones and astaxanthin and lipid biosynthesis. This study provides a novel original strategy for improving astaxanthin and lipid coproduction in *C. zofingiensis* using the selected phytohormones as positive stimulators.

1. Introduction

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a high-value ketocarotenoid with a broad range of commercial applications in aquaculture feed, functional food, nutraceuticals, cosmetics and pharmaceuticals due to its superhigh antioxidant activities and pigmentation (Ambati et al., 2014). Compared with synthetic astaxanthin consisting of a mixture of three isomers, natural astaxanthin from various microorganism mainly comprising the (3S, 3'S) stereoisomer has stronger antioxidative activity and meets food safety requirements. Thus, natural astaxanthin commands a high price of thousands US dollars per kg in the global market, which is several-fold higher than synthetic astaxanthin (Shah et al., 2016). Currently, *Haematococcus pluvialis* as well as *Xanthophyllomyces dendrorhous* are dominant producers for natural astaxanthin production in industry due to their ability to biosynthesize high levels of astaxanthin. However, *H. pluvialis*-based astaxanthin production has several limitations, such as a long period of cultivation, greater contamination by other microbes and zooplankton, and the highest costs for astaxanthin production (Shah et al., 2016). Regarding *X. dendrorhous*, the primary astaxanthin stereoisomer found is 3R, 3'R; thus, it has recently been sold as a pigment in animal feed.

To overcome these crucial techno-economic challenges, numerous studies have been conducted either on the selection of ideal alternative species with superior characteristics or the utilization of integrated culture strategies for astaxanthin production. In recent years, the green microalga *Chromochloris zofingiensis* has been regarded as the most promising candidate for the economical production of astaxanthin (Liu et al., 2014). In comparison to *H. pluvialis*, *C. zofingiensis* has the ability to accumulate large amounts of astaxanthin and lipids simultaneously under stress conditions, subsequently storing them in lipid droplets in the form of mono- and di-esters with fatty acids. These properties make *C. zofingiensis* capable of meeting commercial demands for multiple bioproducts (lipids, carbohydrates, etc.) in addition to astaxanthin due to its high growth rate in organic trophic mode to achieve high cell densities and carotenoid accumulation. Several breakthrough findings and remarkable progress have been achieved; however, most of them have been focused on the optimization process by nutrient deficiency, high light irradiation and oxidative stress. Nevertheless, astaxanthin productivity by *C. zofingiensis* is still far from the requirements necessary for commercial production.

Phytohormones are a broad-spectrum collection of plant-derived bioactive compounds that have biofunctions in modulating cell reproduction and regulating the biosynthesis of specific metabolites (e.g.,

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