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Enhanced biomass and lipid production by supplement of myo-inositol with oceanic microalga *Dunaliella salina*

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ABSTRACT

We investigated the effects of inositols, which are well-known plant growth-promoting agents, on the growth of the oceanic microalga *Dunaliella salina*. Of the four inositol derivatives tested (*myo*-inositol, *scyllo*-inositol, D-*chiro*-inositol, and L-*chiro*-inositol), *myo*-inositol (MI) showed the greatest growth-promoting effect in a concentration-dependent manner. The yield of biomass from the alga cultured with 500 mg L^{-1} of MI was 1.48-times that of the control culture. No significant effect of MI on the total carotenoid content was observed, but neutral lipid content was significantly increased, 1.34-times greater than the control. MI also influenced the fatty acid methyl ester composition, with the levels of linoleic, linolenic, and linolelaidic acids significantly higher than those of the control culture. To the best of our knowledge, this is the first demonstration that MI promotes the growth of a marine microalga. Our results suggest that MI has potential for enhancing the efficiency of biofuel production by *D. salina* through growth promotion and increasing lipid productivity.

Abbreviations: CN, cetane number; DI, D-*chiro*-inositol; DU, degree of unsaturation; ESM, Erd-Schreiber's modified; FAME, fatty acid methyl ester; LI, L-*chiro*-inositol; LCSF, long-chain saturated factor; MI, *myo*-inositol; NLC, neutral lipid content; SI, *scyllo*-inositol; TCC, total carotenoid content.

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1. Introduction

Microalgae are attractive sources of biomass for industrial applications, and many species are capable of providing renewable fuels such as methane produced from anaerobic degradation of microalgal biomass [1,2] and biodiesel obtained by transesterification of microalgal lipids [1]. With the exception of a few species, microalgae can generally double their biomass within 24 h by asexual reproduction. In addition, they can accumulate lipids, a promising biodiesel source, up to a maximum of 90% of their dry weight [2,3]. Moreover, microalgae can significantly absorb CO_2 gases and accumulate valuable co-products by photosynthesis using wastewater-containing nutrients, and do not require as much arable land as do agricultural crops [1,4,5]. These merits suggest that microalgae can be promising resources for renewable and sustainable fuel production. Fatty acid methyl esters (FAMEs) derived from oils are known biodiesels that can be easily obtained from non-edible microalgae [4]. The FAME composition can affect the quality of biodiesel, including its ignition quality, cold-filter plugging point, oxidative stability, viscosity, and lubricity [4,6]. In recent years, microalgal strains with high growth rates and lipid contents have been screened and applied to obtain a high yield of biodiesel at the laboratory-scale and for pilot plant-scale cultivation [4].

The unicellular marine green alga *Dunaliella salina* is well known for its important role in primary production in most hypersaline environments [7]. Since this alga was found to provide one of the richest natural sources of β-carotene as a constituent of its useful carotenoids, *D. salina* has become an increasingly attractive biomass source, capable of yielding multiple value streams [8]. In addition, *D. salina* has the ability to survive and accumulate high lipids and triacylglycerides under high salt concentrations [9]. These properties of *D. salina* make it particularly well suited for industrial-scale culture in open-air oceanic environments to generate biomass for biofuels and other biomaterials [9]. For more cost-effective biomass production, biotechnology-based genetic and metabolic engineering approaches need to be applied [10]. Toward this end, research has been conducted to develop safe and biologically acceptable strategies for improving biomass yield and oil accumulation in microalgae [11].

Improvements in yield and productivity can also be achieved through optimization of growth media and conditions. In this regard, inositols have been highlighted as potentially valuable media supplements for microalgae culture. Inositols are naturally occurring six-fold alcohols of cyclohexane, with the formula $C_6H_{12}O_6$, and play a significant role as structural elements of many secondary messengers in eukaryotic cells, especially *myo*-inositol (MI) [12]. Because MI is involved in the phosphatidylinositol signaling pathway that takes part in auxin transport, cell wall biosynthesis, phytic acid biosynthesis, and the production of stress-related molecules, it is considered an important factor for normal plant growth and development [13]. This finding suggests that MI acts as a growth-promoting agent in photosynthetic organisms, including microalgae. In this study, we investigated the effects of MI on *D. salina* growth, total carotenoid content (TCC), neutral lipid content (NLC), and fatty acid methyl ester (FAME) composition.

2. Materials and methods

2.1. Algal source and culture conditions

D. salina CCAP 19/20 was obtained from Yeungnam University, Korea and has been maintained in the Dunaliella Culture Collection at Brooklyn College (DCCBC). For identification and confirmation of the algal strain, rDNA sequence analysis was conducted. Microalgal DNA was extracted with the DNeasy® Blood & Tissue Kit (Qiagen, The Netherlands) [14], and the 18S rRNA gene was amplified using two eukaryotic primers (Ecol-7F, 5′-ACC TGG TTG ATC CTG CCA G-3′ and Ecol-1534R, 5′-TGA TCC TTC YGC AGG TTC AC-3′) and PCR pre-mix (AccuPower®; Bioneer, Korea) [15]. The PCR program consisted of an initial denaturation at 94 °C for 10 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min, with a final extension at 72 °C for 5 min. The PCR products were sequenced by SolGent Co., Korea. The similarity of the 18S rRNA gene sequences to those of microalgae was verified using the BLAST search program [16].

The strain was cultured and maintained in Erd-Schreiber's modified (ESM) medium, which contains 120 mg $NaNO_3$, 5 mg K_2HPO_4, 0.1 mg vitamin B_1, 0.01 mg vitamin B_{12}, 0.001 mg biotin, 0.26 mg EDTA-Fe^{3+}, 0.33 mg EDTA-Mn^2, and 1 g Tris(hydroxymethyl)aminomethane in 1 L artificial seawater [17]. The pH and salinity of the medium were adjusted to 8.0 and 34 PSU, respectively. The medium was sterilized at 121 °C for 15 min. The algal strain was maintained at 26 °C in a plant chamber (KG-8407; Vision, Korea) under 200 μmol m^{-2} s^{-1} of cool-white fluorescence illumination, on a 12 h light/dark cycle.

2.2. Experimental procedures

MI, *scyllo*-inositol (SI), D-*chiro*-inositol (DI), and L-*chiro*-inositol (LI) were purchased from Sigma–Aldrich. These inositol derivatives were dissolved in sterilized ESM media and filtered through a 0.2-μm membrane filter. The initial cell density was adjusted to 3×10^4 cm^{-3} in a 24-well plate, the cells were cultured in 2 mL of medium containing each inositol derivative at concentrations in the range of 0–500 mg L^{-1}, and grown under the above-described conditions. Algal cell densities were determined daily by using a hemocytometer, and the growth conditions were monitored at 680 nm every day by using a microplate reader (Synergy HT; Biotek, USA). The growth rate (divisions day^{-1}) was calculated with the following equations [18]:

$$K' = \ln \frac{(N_2/N_1)}{(t_2 - t_1)}$$

$$\text{Division day}^{-1} = \frac{K'}{\ln 2}$$

where N_1 is the cell density at day 1 (t_1) and N_2 is the cell density at day 10 (t_2). Biomass was measured as described by Zhu and Lee [19]. In brief, a 10 mL of *D. salina* culture was filtered through a pre-weighed 0.7-μm pore-size glass microfiber filter (GF/F, Whatman), and then dried at 80 °C. After