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Short communications

Propyl gallate and butylated hydroxytoluene influence the accumulation of saturated fatty acids, omega-3 fatty acid and carotenoids in thraustochytrids

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ABSTRACT

Schizochytrium sp. S31 was shown to have potential for production of the functional food ingredients docosahexaenoic acid (DHA) and astaxanthin, with coproduction of biodiesel. Biomass and lipid levels were greater with glycerol than with glucose as carbon source. Addition of propyl gallate or butylated hydroxytoluene to the media resulted in increased biomass and lipid levels, with propyl gallate being the more effective of the two antioxidants. Medium supplementation with propyl gallate at 0.03% and glycerol as the carbon source resulted in enhanced biomass productivity (28.50 g L^{-1}), lipid accumulation (24.87 g L^{-1}) and astaxanthin levels (452.26 μg L^{-1}).

1. Introduction

Marine microorganisms including thraustochytrids have been explored for their omega-3 fatty acid productivity, particularly with regard to docosahexaenoic acid (DHA) (Burja, Radianingtyas, Windust, & Barrow, 2006; Gupta, Barrow, & Puri, 2012). DHA is a high value product having neutraceutical, functional food and pharmaceutical applications (Curtis, Harwood, Dent, & Caterson, 2004; Huangfu et al., 2013; Linko & Hayakawa,

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1996). Thraustochytrids can accumulate up to 80% of their dry cell weight as oil (Zuñiga, Ciobanu, Nuñeza, & Stark, 2012). Some researchers have explored thraustochytrids as producers for β-carotene and astaxanthin (Aki et al., 2003; Armenta, Burja, Radianingtyas, & Barrow, 2006; Quilodrán, Hinzpeter, Hormazabal, Quiroz, & Shene, 2010). Thraustochytrids are also reported to produce commercially important sterols such as squalene, cholesterol and stigmasterol (Gupta et al., 2012). Therefore, thraustochytrids may be useful for the concurrent production of biodiesel and high value co-products such DHA, carotenoids and sterols, assuming that DHA can be cost-effectively separated from the biodiesel-like fatty acids. Thraustochytrids are able to efficiently utilize a broad spectrum of carbon sources, and so can potentially be fermented using low cost carbon sources (Gupta et al., 2012; Johnson & Wen, 2009; Pyle, Garcia, & Wen, 2008; Quilodrán, Hinzpeter, Quiroz, & Shene, 2009).

Propyl gallate, a well-known antioxidant used in the food industry as a preservative, is also reported to block pyruvate transport into mitochondria and is also a non-competitive inhibitor of Δ5 and Δ6 desaturase enzymes, potentially changing the fatty acid profile in microorganisms including thrautochytrids (Eler, Peralta, & Bracht, 2009; Kawashima, Akimoto, Shirasaka, & Shimizu, 1996). Therefore, we chose to study of effects of propyl gallate, and the antioxidant butylated hydroxytoluene (BHT), on thraustochytrids fermentation to determine the impact on DHA productivity, using the strain designated *Schizochytrium* sp. S31 (Chang, Luo, Gu, Wu, & Wang, 2013).

2. Materials and methods

2.1. Reagents and chemicals

Medium constituents such as D-glucose, glycerol, yeast extract (Sigma-Aldrich, St. Louis, MO, USA) and sea salt (Instant Ocean, Blacksburg, VA, USA) was used in fermentation. Propyl gallate and BHT (Sigma-Aldrich) were used as a growth modulator. The solvents and chemicals used in this study were either analytical or HPLC or GC grade. Methanol, ethyl acetate, acetone, chloroform, hexane (Honeywell, Morristown, NJ, USA or Fischer, Waltham, MA, USA) were used in fatty acid methyl ester (FAME) and carotenoid analysis. Methyl nonadecanoate, BHT, potassium bicarbonate (Sigma-Aldrich) were used in FAME preparation. Carotenoid standards such as astaxanthin, zeaxanthin, canthaxanthin, res/meso-astaxanthin, beta-cryptoxanthin, and echinenone were procured from CaroteNature (Ostermundigen, Switzerland) whereas β-carotene was from Sigma-Aldrich. These standards were used to identify and quantify the carotenoids present in *Schizochytrium* sp. S31 (ATCC 20888).

2.2. Culture maintenance and biomass production

Schizochytrium sp. S31 (ATCC 20888) was procured from American Type Culture Collection (ATCC Rockville, MD, USA) and maintained on GYP agar medium consisting of glucose (0.5%, w/v), yeast extract (0.2%, w/v), peptone (0.2%, w/v), agar (1%, w/v), seawater (50%, v/v) at 25 °C and sub-cultured each 25 days. *Schizochytrium* sp. S31 was inoculated in seed medium containing glucose (3%, w/v), yeast extract (1%, w/v) and artificial seawater (50%, v/v) at 25 °C for 48 h with shaking at 150 rpm. After 48 h, 5% (v/v) culture was inoculated into production medium having glucose or glycerol and cultivated at 25 °C, 150 rpm. After 192 h, biomass was harvested and washed twice with water before freeze drying for 48 h. Freeze dried biomass was stored at –20 °C for subsequent work.

2.3. Effect of propyl gallate (PG) on growth profile, lipid accumulation, lipid composition and carotenoid content

Different concentrations of propyl gallate, at 0.01, 0.03, or 0.05% (w/v) were added in production medium having glycerol and cultivated at 25 °C, 150 rpm. OD_{600nm} and dry cell weight was measured at the interval of 24 h. Freeze dried biomass was used for lipid and carotenoid extraction in this work. All the experiments were carried out in duplicates and values are expressed as mean ±SD.

2.4. Effect of C/N ratio on dry cell weight, lipid accumulation, lipid composition and carotenoid content

Different C/N ratios of 13, 26, 39, 52, and 65, along with 0.3% (w/v) propyl gallate, were used in the production medium. Glycerol at 3, 6, 9, 12, or 15% (w/v) and yeast extract (1%, w/v) were used as carbon and nitrogen source, respectively. Culture parameters were as described in section 2.2.

2.5. Lipid extraction and FAME analysis

Lipid extraction and FAMEs analysis were performed as previously described (Gupta, Vongsvivut, Barrow, & Puri, 2012).

2.6. Carotenoid extraction and quantification

Twenty five milligram of freeze dried biomass were suspended into 1 ml of 3M HCl and this was incubated at 30 °C for 40 min. Acid was removed by centrifugation at 4000 rpm for 15 min, followed by twice washing with distilled water to remove traces of acid. One milliliter of acetone was added to the pellet and this was vortexed for 3 min. The orange colored supernatant was harvested by centrifugation at 2800 *g* for 15 min. Extraction with acetone was repeated twice for the complete extraction of carotenoids. The acetone extract was stored at –20 °C in the dark to avoid degradation, for use in RP-HPLC analysis.

2.7. RP-HPLC carotenoid analysis

Carotenoids were analyzed following the protocol described by Armenta et al. (2006).

3. Results and discussion

3.1. Effect of carbon sources and propyl gallate/BHT on Schizochytrium sp. S31 cultivation

Glycerol (a low cost carbon material) appears to be a better carbon source, as compared to glucose, for biomass production