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Manipulation of fatty acid and antioxidant profiles of the microalgae *Schizochytrium* sp. through flaxseed oil supplementation

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ABSTRACT

Due largely to their unique and diverse metabolic profile, microalgae have embedded themselves in a plethora of commercial applications encompassing nutrition, cosmetics and biofuel production. The present research demonstrates the ability to improve the nutritional properties of Schizochytrium sp. through direct medium supplementation with flaxseed oil. Increasing supplemental concentrations, to a maximum of 1% *v/v*, reflected a 2.8-fold improvement in dry cell weight, yielding a maximum 17.1 ± 1.3 g/L. Cultures with higher biomass yields also demonstrated increased glucose utilisation, which may have influenced the final fatty acid profiles of each culture. At inclusion levels above 0.05%, total fatty acid amounts reduced in a near linear fashion, potentially in response to a glucose deficient environment. Supplementation of just 0.05% flaxseed oil resulted in the incorporation of exogenous C18 fatty acids into the algal biomass, as well as significantly increasing docosahexaenoic acid (DHA) titres to 100.3 ± 4 mg/g algae ($p \leq 0.05$). At the same inclusion rate, the antioxidant capacity and phenolic content of algal biomass were significantly enhanced, particularly in the methanolic faction ($p \leq 0.001$). The inclusion of essential linoleic and α-linolenic fatty acids with improved levels of DHA and enhanced antioxidant status demonstrate the suitability of medium supplementation to improve the nutritional value of microalgae.

1. Introduction

The inherent applicability of microalgae in a variety of applications is primarily due to their unique and diverse metabolic profiles. Commercial applications encompass biofuel production, cosmetics, fertilisers and pollution control, but it is their ability to synthesise bioactive components such as essential polyunsaturated fatty acids and antioxidants which makes them ideal for utilisation in both human and animal nutrition [1–3]. While there is an abundance of research detailing the beneficial properties associated with macroalgae [4,5], microalgae represent an attractive commercial resource due to the synthesis of a wide spectrum of metabolites, cultivability, growth rates and potential biomass yields [2,6,7].

Omega-3 polyunsaturated fatty acids (PUFAs), in particular docosahexaenoic acid (DHA), have garnered increasing attention over recent years due to the beneficial effects on both human and animal health. Primarily due to chain length and degree of unsaturation, these fatty acids act as important structural components and are closely associated with neural, cardiovascular and reproductive conditions [8]. Additionally, incorporation of DHA into the cellular matrix, particularly through dietary means, has demonstrated a direct influence on membrane fluidity, lipid and hormone synthesis and the induction of components associated with both inflammation resolving and mediating functions [9].

While the primary commercial source of DHA remains to be fish oil, growing concerns with respect to increasing market price, inconsistent quality, lipid peroxidation, palatability and most importantly, sustainability, has meant that alternative, sustainable sources of DHA will be of critical importance to meet the growing demand for human and animal nutrition going forward [10,11]. Alternative sources of omega-3 PUFAs have been reviewed previously [12,13], with a variety of oleaginous bacteria, yeast and fungi recognised for their ability to synthesise such fatty acids. However, the greatest limiting factors to their commercial development typically lie in achieving commercially viable biomass levels, total DHA yields and appropriate fatty acid compositions.

Given its position in the marine food chain and particularly from a nutritional perspective, the algal synthesis of DHA has been identified as a potentially viable alternative to fish oil incorporation in aquaculture diets [14]. While relatively few wild-type microalgae are known to produce DHA in commercially viable concentrations, within the family Thraustochytriaceae, certain marine microalgae have demonstrated the ability to generate appreciable amounts, such as *Thraustochytrium* spp. [15], *Ulkenia* spp. [16] and *Schizochytrium* spp. [17,18]. Sustainability, ease of cultivation and a wealth of additional endogenous bioactive components have made such strains attractive and novel commodities.

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Improvements in PUFA yields through alterations of the fermentation process have been previously demonstrated in oleaginous algae, with a variety of supplementary components and culture parameters impacting culture performance [19–21].

The ability of specific antioxidants to protect against lipid peroxidation makes the association between fatty acid and antioxidant biosynthesis particularly relevant in oleaginous algae. The presence of endogenous antioxidants such as carotenoids and phenolic compounds in algae is well documented [22–24]. However, such studies typically look to evaluate and quantify the antioxidant capacity of algal strains, while the heterotrophic growth model of the present study facilitated attempts to improve overall antioxidant activity of *Schizochytrium* sp. through direct medium supplementation.

The primary objective of the present study was to manipulate the fatty acid composition of *Schizochytrium* sp., while improving total DHA yields. In conjunction with this, emphasis was also put on improving the overall antioxidant capacity of the algal biomass. Concentrations of flaxseed oil were supplemented to the fermentation medium and subsequently evaluated for influences on growth, fatty acid accumulation and the antioxidant potential of *Schizochytrium* sp.

2. Materials and methods

2.1. Microalgal strain and storage

The *Schizochytrium* sp. SP1 strain utilised in this study was donated by Alltech Inc., USA. *Schizochytrium* sp. was maintained in cryovial stocks in a medium containing (g/L); glucose, 40; yeast extract, 10 and sea salts, 4. Medium pH was adjusted to 6.2 and sterilised by autoclaving at 105 °C for 30 min. Cryovials were made up to a final 35% *v/v* glycerol concentration and stored at −75 °C.

2.2. Cultivation conditions

Schizochytrium sp. was cultured to log phase from cryovial stocks and used to inoculate medium supplemented with increasing concentrations of flaxseed oil. Basal medium was designed to promote lipid accumulation, consisting of (g/L); glucose, 50; yeast extract, 10; and sea salts, 4. Concentrations of flaxseed oil (0–1% (*v/v*)) were supplemented to the basal medium and inoculated with a 5% (v/v) volume of log phase starter culture. Triplicate cultures were grown in baffled 250 mL Erlenmeyer flasks at 30 °C for 96 h at 200 rpm. At predefined time points, culture aliquots were aseptically removed for analysis.

2.3. Sample preparation

Biomass from supplemented algal cultures was harvested by centrifugation, triple-washed with deionised water to remove residual culture medium and dried by lyophilisation. Aliquots of culture medium were retained for glucose assessment by HPLC. Biomass yields were determined gravimetrically and expressed as dried cell weights (DCW). Lyophilised biomass was further used for fatty acid and antioxidant analysis.

Antioxidant and phenolic components were fractionated into hexane, methanol and water extracts by employing a modified 3-stage sequential extraction procedure [24,25]. Initially, 50 mg of dried biomass was extracted with 1 mL hexane. Bead beating was carried out for 30 s and the mixture was extracted at 200 rpm at room temperature for 1 h. Following centrifugation at 8000 rpm for 5 min, the supernatant was recovered and the residue was extracted with 1 mL methanol. The procedure outlined above was repeated and following recovery of the methanol fraction, residual biomass was extracted with 1 mL deionised water at 80 °C. Extracts were purged with nitrogen and stored at 4 °C prior to use.

2.4. Fatty acid analysis

Lyophilised algal biomass (100 mg) was suspended in 2.5 mL of 0.5 M methanolic NaOH at 110 °C for 10 min and esterified for a further 45 min in 2.5 mL of 12% (*w/v*) BF_3-methanol. Fatty acid methyl esters (FAMEs) were dissolved in 2.5 mL hexane and washed with 2 mL of saturated NaCl. Following centrifugation, the upper organic layer was collected for analysis by gas chromatography equipped with a flame ionisation detector (FID).

FAME composition was determined by gas chromatography (Agilent 7890) using a capillary column (Agilent DB-23). Injector and detector temperatures were held at 220 and 240 °C, respectively, with column temperature ramping from 170 to 210 °C at 4 °C/min, having been held for an initial 3 min period. Nitrogen was used as the carrier gas, at a flow rate of 2 mL/min. FAMEs were identified by comparison to appropriate standards (Supelco 37 Component FAME Mix) and quantified against chromatogram peak area using behenic acid as an internal standard.

2.5. Trolox equivalent antioxidant capacity (TEAC) assay

Antioxidant capacity was assessed using a modified $ABTS^{\cdot+}$ method [26]. Briefly, a 1:1 mixture of 7 mM ABTS and 2.45 mM potassium persulfate was left to stabilise in the dark for 16 h at room temperature. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm. $ABTS^{\cdot+}$ solution was mixed with appropriately diluted test samples and after 5 min constant shaking at 30 °C, absorbance was determined at 734 nm. Trolox solution (0–300 μM) was used to generate a standard curve. Results were expressed as μmol Trolox equivalents (TE)/g dry weight algae and calculated as a mean value with standard deviations of triplicate samples.

2.6. Determination of total phenolic content

Total phenolic content was determined using a modified Folin–Ciocalteu method [27]. Briefly, 25 μL of extracted sample was mixed with 125 μL Folin reagent (10% (*v/v*) in deionised water). After 4 min, 150 μL saturated sodium carbonate (75 g/L) was added. Solutions were incubated in the dark for 2 h at room temperature, after which time, plates were agitated for 1 min and absorbance was measured at 750 nm. In order to generate a standard curve, gallic acid was used in concentrations from 0 to 100 μg/mL. Results were expressed as mg gallic acid equivalents (GAE)/g dry weight algae and calculated as a mean value with standard deviations of triplicate samples.

2.7. Statistical analysis

Pearson's correlation and regression analysis were carried out to assess the relationship between antioxidant activities and phenolic contents. All statements of significance were calculated by either one- or two-way ANOVA and based on a confidence interval of 95% unless otherwise stated. Data were analysed using the Minitab statistical software package, version 16.0 (Coventry, U.K.).

3. Results and discussion

3.1. Growth characteristics

Dry cell weight findings for *Schizochytrium* sp. cultures supplemented with flaxseed are displayed in Fig. 1. Over the course of the fermentation, maximum yields were achieved after 48–72 h for each treatment. Statistical analysis indicated that both fermentation time and supplemental flaxseed oil had a significant effect on biomass yields ($p \leq 0.001$). Increasing concentrations of flaxseed oil typically resulted in increased dry cell weight, with a maximum of 17.1 ± 1.3 g/L DCW achieved after 72 h with 1% *v/v* flaxseed oil. This increase represented

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