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Australian thraustochytrids: Potential production of dietary long-chain omega-3 oils using crude glycerol

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Thraustochytrids can produce high amounts of docosahexaenoic acid (DHA, 22:6ω3). Glycerol, a by-product of biodiesel production from vegetable oil and animal fats, is becoming increasingly available. We investigated the potential of Australian thraustochytrids to use crude glycerol as the main carbon source for production of long-chain (LC, $\geq C_{20}$) omega 3-oils. Crude glycerol content was analysed, and the growth kinetics of eight thraustochytrid strains was examined with crude glycerol. *Aurantiochytrium* sp. strains achieved higher biomass (20 g/L dry cell weight; DCW) and lipid yield (389 mg/g total fatty acids; TFA) compared to *Schizochytrium*, *Thraustochytrium* and *Ulkenia* spp. Impurities in the crude glycerol hindered growth of thraustochytrids, with maximum yield of 9 g/L DCW and 48% DHA TFA at 4-days. Our research shows the potential of heterotrophic thraustochytrids to provide the growing global population with a secure, environmentally sustainable alternative source of health-benefitting LC omega-3 oils for use in feeds and foods.

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1. Introduction

Alternative sources of omega-3 oil production from single cell organisms have been of increasing interest as replacements for fish oil, including microalgae, bacteria, fungi, and yeasts. This interest is due to growing concerns with global food security, health of ocean fish stocks, ecological effects of industrial fishing, and high levels of pollutants in some fish oils (Kralovec, Zhang, Zhang, & Barrow, 2012; Nichols, Glencross, Petrie, & Singh, 2014; Pauly et al., 2002). For example, Martek Biosciences Corporation, which is now part of Royal DSM (Dutch State Mines), has been using *Schizochytrium* sp. (thraustochytrid) and *Crypthecodinium cohnii* (dinoflagellate) to produce DHA-containing oil (Ratledge, 2012).

The dietary consumption of omega-3 long chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA, also termed LC omega-3), in particular DHA (docosahexaenoic acid, 22:6ω3) and EPA

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(eicosapentaenoic acid, 20:5ω3), has many benefits in human health (Horrocks & Yeo, 1999; Tapiero, Ba, Couvreur, & Tew, 2002). Recent studies have shown that the consumption of omega-3 LC-PUFA helps decrease the risk of cardiovascular diseases, neural disorders, arthritis, asthma and skin diseases in humans (Horrocks & Farooqui, 2004; Kris-Etherton, Hecker, & Binkoski, 2004; Takahata, Monobe, Tada, & Weber, 1998). The average Australian diet, along with many other western diets, has been recognised as being deficient in omega-3 LC-PUFA. This deficit highlights the importance of, and need for, increased availability and consumption of the health-benefitting omega-3 LC-PUFA (Meyer et al., 2003).

The use of a crude glycerol stream as a carbon source for a heterotrophic algal production system could not only potentially reduce the greenhouse gas emissions of industries including power plants, but also substantially reduce the cost of commercial production of algal-derived omega-3 oils (Lee Chang, Rye et al., 2014). Pyle, Garcia, and Wen (2008) showed that crude glycerol was a suitable carbon source for thraustochytrid fermentation and a DHA yield of 4.9 g/L with a biomass concentration 22.1 g/L. The crude glycerol-derived *Schizochytrium limacinum* SR-21 (ATCC MYA-1381) biomass had a high level of DHA and a nutritional profile similar to commercial algal biomass, while no heavy metals (such as mercury) were detected, suggesting considerable potential for using crude-glycerol derived algae in omega-3 fortified food or feed (Pyle et al., 2008). A number of studies have been undertaken for a range of thraustochytrids cultivated in industrial waste or low value feedstock, such as bread crumbs (Thyagarajan, Puri, Vongsvivut, & Barrow, 2014), spent yeast from brewery (Ryu, Kim, Kim, Han, & Yang, 2012), empty palm fruit bunches (Hong et al., 2013), and coconut water (Unagul et al., 2007). However, the successful growth and commercial scale up of thraustochytrids using cheap feedstock has been elusive due to a number of factors such as low nutrient content in the feedstock, the presence of possible growth inhibitors in the feedstock, nutrient availability, low productivity, capital cost and strain selection.

Previous studies showed that growth of *Aurantiochytrium* sp. TC 20 was capable of using glycerol as a carbon source. Fortification of the feed with additional nutrients improved the biomass yield from 56 g/L (34% total fatty acids, TFA) to 71 g/L (52% TFA, 14.3 g/L DHA yield, dry cell weight, DCW) at 69 h (Lee Chang et al., 2013). In addition to producing high amounts (>50% TFA) of omega-3 LC-PUFA, such as DHA and EPA for nutraceuticals (Jain, Raghukumar, Sambaiah, Kumon, & Nakahara, 2007; Zuñiga, Ciobanu, Nuñeza, & Stark, 2012), thraustochytrids are also recognised to be capable of producing lipids for potential biofuel application (Lee Chang, Rye et al., 2014). This study examines Australian thraustochytrid strains from eight different chemotaxonomic groups, which have been characterised in detail previously (Lee Chang et al., 2012). The strains were grown in 1 L scale baffled shake flasks for biomass and fatty acid production using crude glycerol as the main carbon sources.

2. Materials and methods

2.1. *Microorganisms*

Thraustochytrid strains (see Table 1) used in this study are part of the Australian National Algae Culture Collection (http://www.csiro.au/ANACC); Genebank accession numbers for all strains are listed in Table 1. Strain isolation information, medium preparation and culturing conditions have been reported previously (Lee Chang et al., 2012).

2.2. *Crude glycerol content*

The crude glycerol was sourced from a local biodiesel plant that was producing biodiesel from vegetable oil and animal fats. Proximate and elemental analyses, including calcium, phosphorus, sulphur, chloride, sodium and hydroxide (as OH^-), of the crude glycerol were performed essentially in accordance with those set out in the Official Methods of Analysis of Association of Official Analytical Chemists International (AOAC International, 2000, 17th Edition), by Allison Laboratories Pty Ltd, Hobart, Tasmania, Australia. The crude protein content was estimated by measuring the Kjeldahl nitrogen content and multiplying the result by 6.25 (Jones, 1941). Moisture content was analysed according to the Dean and Stark (1920) method. The ash content was determined by heating the sample at 625 °C overnight.

The crude glycerol was analysed by the National Measurement Institute, Melbourne, Victoria, Australia (http://www.measurement.gov.au), using gas chromatography (GC) to

Table 1 – List of thraustochytrid strains and their chemotaxonomic and phylogenetic groupings, based on Lee Chang et al. (2012).

TC	Group	CS Number*	Genus	Genebank accession	Colour
2	A	CS-980	*Schizochytrium*	JN675267	Off white
4	B	CS-982	*Thraustochytrium*	JN675271	Orange
9	E	CS-994	*Aurantiochytrium*	JN675249	White
10	D	CS-991	*Ulkenia*	JN675268	Cream
18	H	CS-1012	*Aurantiochytrium*	JN675255	Cream
20	F	CS-997	*Aurantiochytrium*	JN675250	White
30	G	CS-1011	*Aurantiochytrium*	JN675265	Pale orange
33	C	CS-984	*Thraustochytrium*	JN675251	White

* CS Number is assigned for strains held in the Australian National Algae Culture Collection (ANACC) and hereafter strains will be referenced by their sample code – TC.

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