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New Australian thraustochytrids: A renewable source of Biofuels, Omega-¬¨‚Ä†‚ÄövÑv™3 oils and other bioproducts

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New Australian thraustochytrids: A Renewable Source of Biofuels, Omega-3 Oils and other Bioproducts

by

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the requirements of the degree of
Doctor of Philosophy
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Declaration

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Kim Jye Lee Chang
August 2013

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Abbreviations

The following abbreviations have been used in this thesis:

15:0	pentadecanoic acid
16:0	palmitic acid
17:0	heptadecanoic acid
18S rRNA	18S ribosomal RNA gene
AA	arachidonic acid (20:4ω6)
ANACC	Australian National Algae Culture Collection
AQIS	Australian Quarantine and Inspection Service
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
DCW	Dry cell weight
DHA	docosahexaenoic acid (22:6ω3)
DMOX	4,4-dimethyloxazoline
DPA-3	docosapentaenoic acid (ω3)
DPA-6	docosapentaenoic acid (ω6)
EPA	eicosapentaenoic acid (20:5ω3)
EPS	exopolysaccharides
ERoEI	energy returned on energy invested
FAME	fatty acid methyl ester
FAS	fatty acid synthase
FID	flame ionization detector
GC	gas chromatography
GC-MS	gas chromatography- mass spectrometry
Glu	glucose
Gly	glycerol
HC	hydrocarbon
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HRD	hydroprocessed renewable biodiesel
LCA	Life-cycle assessment

LC-PUFA	long chain ($\geq C_{20}$) polyunsaturated fatty acid
MeOH	methanol
MUFA	monounsaturated fatty acid/s
Nutr	nutrients
OC-FA	odd-chain fatty acids
PCR	polymerase chain reactions
PKS	polyketide synthase
PUFA	polyunsaturated fatty acid/s
SD	standard deviation
SFA	saturated fatty acid
SPI	septum-programmable injector
TAG	triacylglycerols
TC	thraustochytrids collection
TFA	total fatty acid/s
Tr	trace
X:YωZ	This was adopted for the naming of fatty acids, where X refers to the number of carbon atoms in the molecule, Y refers to the number of double bonds in the molecule, and Z indicates the carbon position of the first double bond from the terminal methyl end (CH3) of the molecule. The latter is generally referred to as omega Z (e.g. ωZ) or n-Z (e.g. n minus Z).

Publications

The following peer-reviewed publications have been either wholly or partially derived from work associated with this thesis:

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Kim Jye Lee Chang, Geoff Dumsday, Peter D. Nichols, Graeme A. Dunstan, Susan I. Blackburn and Anthony Koutoulis, **High cell density cultivation of a novel *Aurantiochytrium* sp. strain TC 20 in a fed-batch system using glycerol to produce feedstock for biodiesel and omega-3 oils,** Applied Microbiology and Biotechnology (2013) 97:6907–6918

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Paper 3, Comparison of thraustochytrids *Aurantiochytrium* sp., *Schizochytrium* sp., *Thraustochytrium* sp. and *Ulkenia* sp. for production of biodiesel, long-chain omega-3 oils and exopolysaccharides: *Located in chapter 4. Candidate was the primary author, author 3 and author 4 contributed to the lipid identification, and with author 8 contributed to the EPS isolation and characterisation. Authors 1, 2, 3, and 4 assisted with refinement and presentation.*

Paper 4, High cell density cultivation of a novel *Aurantiochytrium* sp. strain TC 20 in a fed-batch system using glycerol to produce feedstock for biodiesel and omega-3 oils: *Located in chapter 5. Candidate was the primary author, author 3 and author 4 contributed to the lipid identification, and with author 9 contributed to the experimental design and Bioreactor operation. Authors 1, 2, 3, and 4 assisted with refinement and presentation.*

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Abstract

The potential of biofuel production from microalgae is of intense interest globally owing to growing concern with rising crude oil prices and future availability. In addition to producing lipids for potential biofuel application, thraustochytrids are capable of forming other high-value bioproducts, such as proteins, enzymes, omega-3 polyunsaturated fatty acids (PUFA), carotenoid pigments and exopolysaccharides (EPS). The co-production of high-value bioproducts during biofuel production is desirable when it adds greater value to the production process and improves process economics.

Thirty-six new thraustochytrids have been isolated from the southeast coast of Tasmania and far north Queensland. They were separated into eight chemotaxonomic groups (A – H) based on fatty acid and sterol composition, with the groups clustered closely with four different genera (*Aurantiochytrium*, *Schizochytrium*, *Thraustochytrium* and *Ulkenia*) based on 18S rDNA molecular identification. In an initial screening study, some strains produced > 60 % docosahexaenoic acid (DHA) under unoptimized culture conditions. *Aurantiochytrium* sp. strains (groups G and H) contained 15:0 (pentadecanoic acid) at between 20 – 30 % of the total fatty acids (TFA) and 16:0 (palmitic acid) in the range of 7 – 15 % TFA, suggesting these strains could be potential candidates for biodiesel production. β,β-Carotene, canthaxanthin and astaxanthin were identified in pigmented strains. Part of the process to scale up is to select the best performing strain based on growth and biochemical characteristics. In the subsequent trials, eight thraustochytrid strains from the different chemotaxonomic groups (A – H) were compared in 1 L scale baffled shake flasks for the synthesis of EPS, in addition to biomass yield and fatty acid profiles. The crude chemical characterization of the EPS, which were released into the culture media by these strains, was performed as an initial step in

determining the potential for biotechnological application of these biomaterials. *Aurantiochytrium* sp. strain TC 20 had the highest biomass production (18.5 g/L) and oil yield (7.5 g/L) after 9 days of growth in 4 % w/v glucose basal media at 20 °C, with 0.18 g/L EPS extracted from the supernatant. The maximum yield of EPS was observed in *Schizochytrium* sp. strain TC 02 (0.3 g/L). High biomass producing strains that also had high lipid and high EPS yield may be better candidates for commercial production of biofuels and other bioproducts. The next phase was to optimize biomass in 2 L bioreactors. The growth of *Aurantiochytrium* sp. TC 20 was also investigated using glycerol as a carbon source. Glycerol is becoming increasingly available, because it is a by-product of biofuel production from vegetable oil and animal fats. Fortification of the feed with additional nutrients improved the biomass yield from 56 g/L (34 % total fatty acids) to 71 g/L (52 % total fatty acids, cell dry weight) at 69 h.

A life-cycle assessment, from the upstream biomass production to the direct emission of biodiesel combustion, was applied to assess the energy balance and the potential environmental impacts of this heterotrophic microalgal-derived biodiesel. The scenario analysis of a virtual production facility, modeled on experimental yield data, demonstrated that cultivation of heterotrophic microalgae for the production of biodiesel is comparable in terms of greenhouse gas emissions and energy usage to production of petroleum diesel. The LCA identified that improvements in cultivation conditions, in particular the bioreactor energy inputs and microalgae yield, will be critical in developing a sustainable production system. This study demonstrates the potential of heterotrophic cultivation of newly isolated endemic thraustochytrids to provide Australia's transportation fleet with a secure, environmentally sustainable alternative fuel feedstock, and co-production of high value bioproducts that can provide additional revenue to benefit the economics of biofuel production.

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