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

by

Kim Jye Lee Chang B. Biotech. (Hons)

(University of Tasmania)

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**Declaration**

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Kim Jye Lee Chang
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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  hydroweprocessed 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:Y$\omega$Z 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 $\omega$ 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 $\omega$ (e.g. $\omega Z$) or n-$\omega$ (e.g. n minus $\omega$).
Publications

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


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Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate: Kim Jye Lee Chang, School of Plant Science

Author 1: Anthony Koutoulis, UTAS School of Plant Science

Author 2: Susan I. Blackburn, CSIRO Marine and Atmospheric Research

Author 3: Peter D. Nichols, CSIRO Marine and Atmospheric Research

Author 4: Graeme A. Dunstan, CSIRO Marine and Atmospheric Research

Author 5: Maged P. Mansour, CSIRO Marine and Atmospheric Research

Author 6: Guy C.J. Abell, CSIRO Marine and Atmospheric Research

Author 7: Lesley A. Clementson, CSIRO Marine and Atmospheric Research

Author 8: Carol Mancuso Nichols, CSIRO Materials Science and Engineering

Author 9: Geoff Dumsday, CSIRO Materials Science and Engineering

Author 10: Lucas Rye, CSIRO Marine and Atmospheric Research

Author 11: Tim Grant, Life Cycle Strategies

Author details and their roles:

Paper 1, Novel odd-chain polyunsaturated fatty acids in thraustochytrids:
Located in chapter 3. Candidate was the primary author and author 3, author 4 and author 5 contributed to the lipid identification and laboratory assistance. Authors 1, 2, 3, and 4 assisted with refinement and presentation.
Paper 2, Biodiscovery of new Australian thraustochytrids for production of biodiesel and long-chain omega-3 oils: Located in chapter 2. Candidate was the primary author, author 3 and author 4 contributed to the lipid identification, author 6 assisted with DNA extraction and sequencing software, and author 7 contributed to the pigment isolation and analysis. Authors 1, 2, 3, and 4 assisted with refinement and presentation.

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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.

Paper 5, Life-cycle assessment: Heterotrophic cultivation of thraustochytrids for biodiesel production: Located in chapter 6. Candidate was the primary author, author 10 and author 11 contributed to the idea, LCA software operation and development. Authors 1, 2, 3, and 4 assisted with refinement and presentation.
We the undersigned agree with the above stated “proportion of work undertaken” for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:  
Anthony Koutoulis  
Supervisor  
School of Plant Science  
University of Tasmania

René Vaillancourt  
Head of School  
School of Plant Science  
University of Tasmania

Susan I. Blackburn  
Supervisor  
Marine and Atmospheric Research  
CSIRO

Peter D. Nichols  
Supervisor  
Marine and Atmospheric Research  
CSIRO

Graeme A. Dunstan  
Supervisor  
Marine and Atmospheric Research  
CSIRO

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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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