Algal biomass rich in Omega-3 fatty acid for feed applications

A/ Prof Munish Puri (FRSC) CCB, Deakin University, Australia &
Deputy Director CMBD, Flinders University, Australia

Plan

Introduction to

- CCB and FU (addressing Food security and sustainability, Novel foods)
- Bioactives and emerging markets
- Lipid as SFA, MUFA and PUFA (Omega-3 FA)

Microbial lipid production

- Screening of best lipid producing strains
- C-source optimisation for economising lipid production
- Co-product extraction (offset production cost)

Moving ahead: a biorefinery approach

- pretreatment
- producing algal feed
- Evaluating feed for producing nutritive food

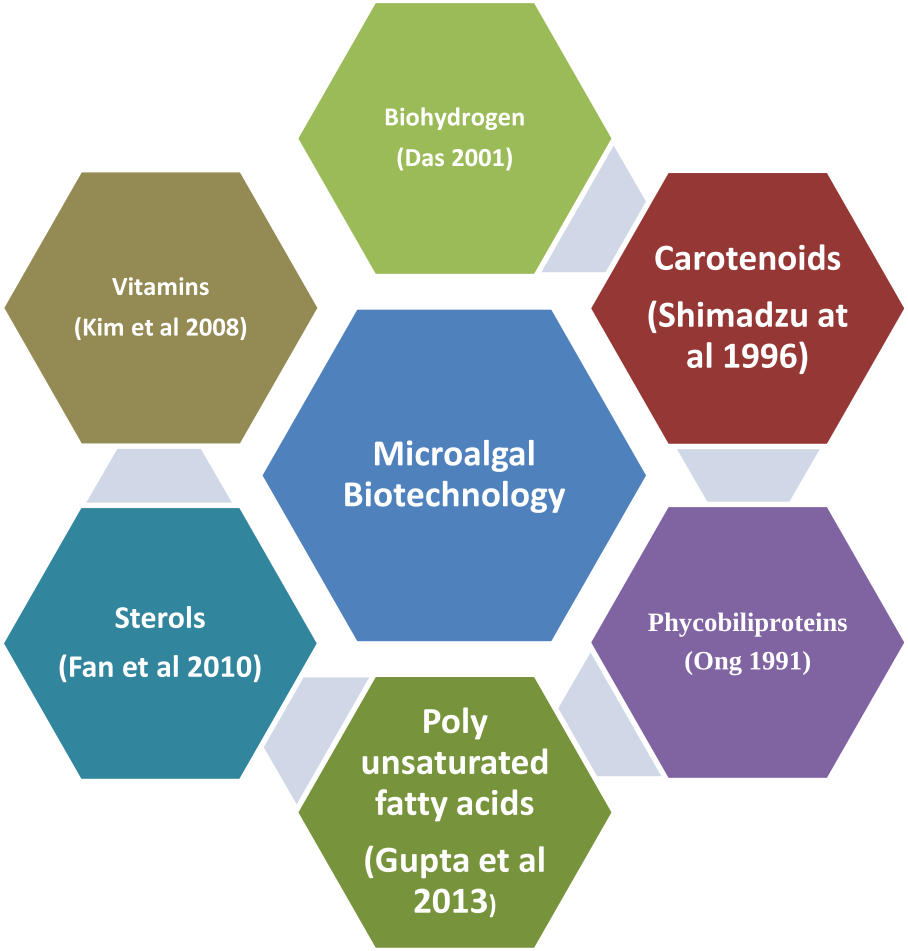
The Australian opportunity

Australia has 3rd largest exclusive economic zone (EES) 10, 148, 250 Km^2 that covers some of the diverse regions and pristine waters that are well managed

"This could be the Answer for who will feed masses "

Marine ecosystem- Natural source of products with economic significance

Puri M (Ed) Food Bioactives; Downstream processing and Biotechnology applications (Springer, 2017).



Puri et al., Trends in Biotechnology 30 (2012) 37-44.

Bioactives are metabolites synthesized by plants for self defence and other purposes and have the potential to be used by humans for variety of applications

Recommendations and availability

- The National Health and Medical Research Council (NHMRC) in 2006, Suggested Dietary Targets for omega-3s

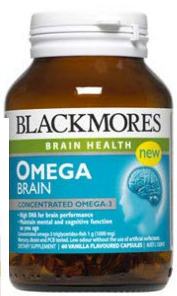
  430mg/day for women

  610mg/day for men

- The Cancer Council Australia
- National Heart Foundation Australia
- The World Health Organization (WHO)

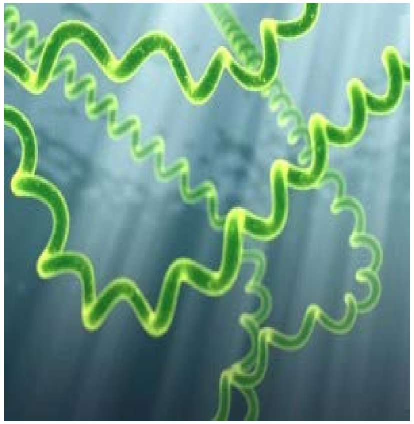
} Recommended Omega-3 in diet

- Fish Oil is considered as major source for DHA and EPA production, but in the recent years, Industrial interest is moved on microalgae, as the process is eco-friendly and <u>economical</u>.

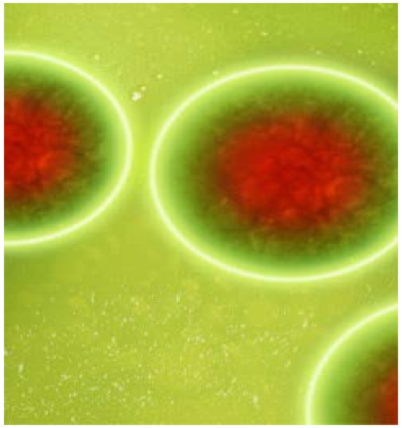


Microalgae

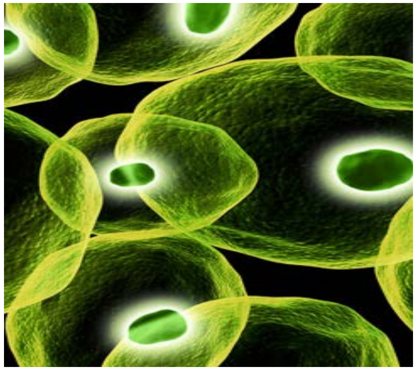
- **A**re microscopic, typically found in freshwater and marine systems living in both the water column and sediments.
- They are unicellular species which exist individually, or in chains or groups.
- The growth of microalgae is extremely fast compared with terrestrial plants, and the biomass can be doubled within 24 h.
- The production of microalgae does not compete with land, water and human food production.
- Microalgae cultivation is not seasonal like oil crop production.
- Microalgae can convert CO_2 into biomass, and may reduce the CO_2 concentration in the atmosphere.
- The biofuels produced from microalgae do not contain sulphur, and are non-toxic and highly biodegradable.



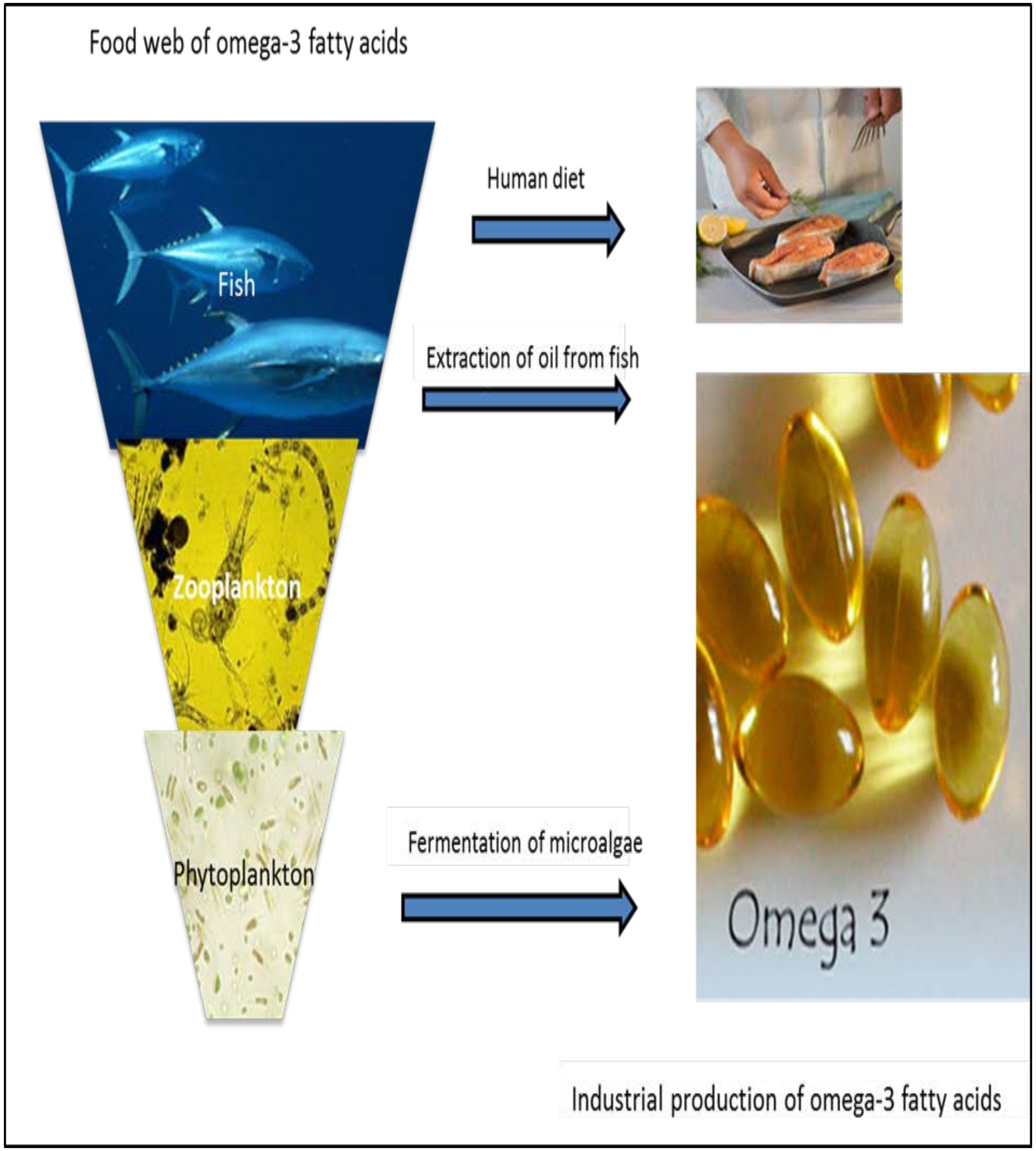
Spirulina



H. pluvialis (Red green)



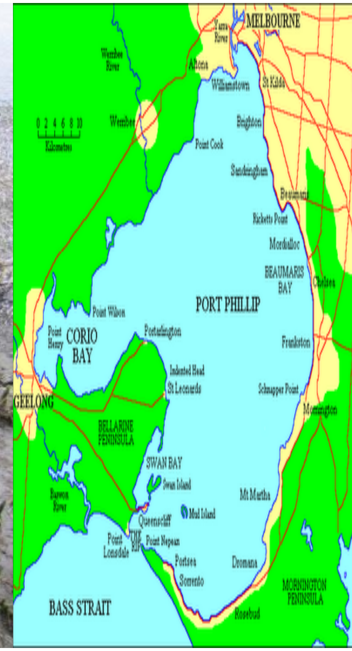
Why Thraustochytrids?



- Thraustochytrids can accumulate 50% of their dry weight as lipids, of which more than 25% is normally DHA (Ward OP, Singh A. Process Biochemistry. 2005;40:3627-52)

- *Crypthecodinium cohnii*, in the production of docosahexaenoic acid (DHA) by Martek Biosciences.
- *Mortierella alpina*, in the production of arachidonic acid, (ARA) by Martek Biosciences.
- *Schizochytrium* sp, in the production of docosahexaenoic acid (DHA) by omega Tech Inc., Boulder, Colorado (now owned by Martek).
- *Ulkenia* sp, in the production of docosahexaenoic acid (DHA) by Nutrinova GmbH, Frankfurt, Germany.

Screening for thraustochytrids

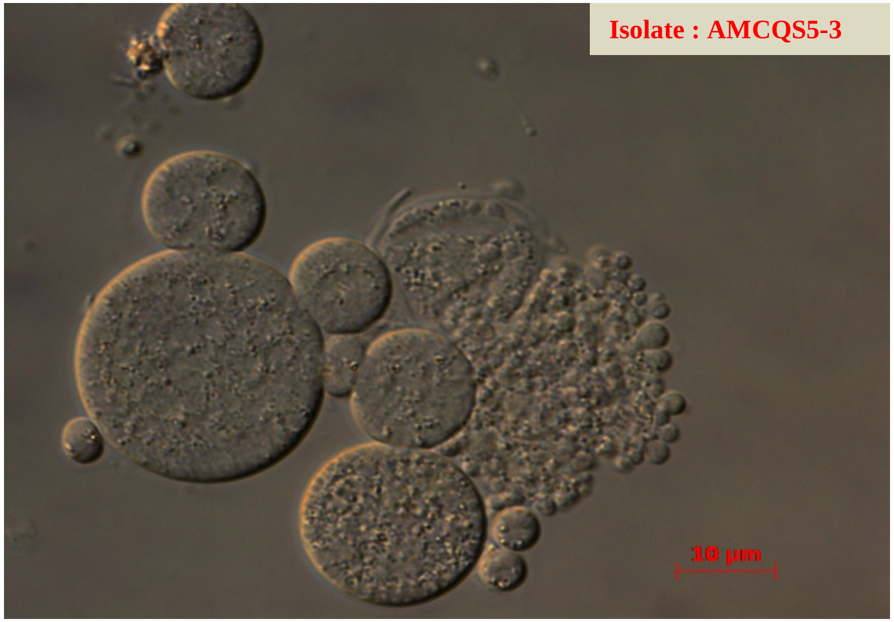


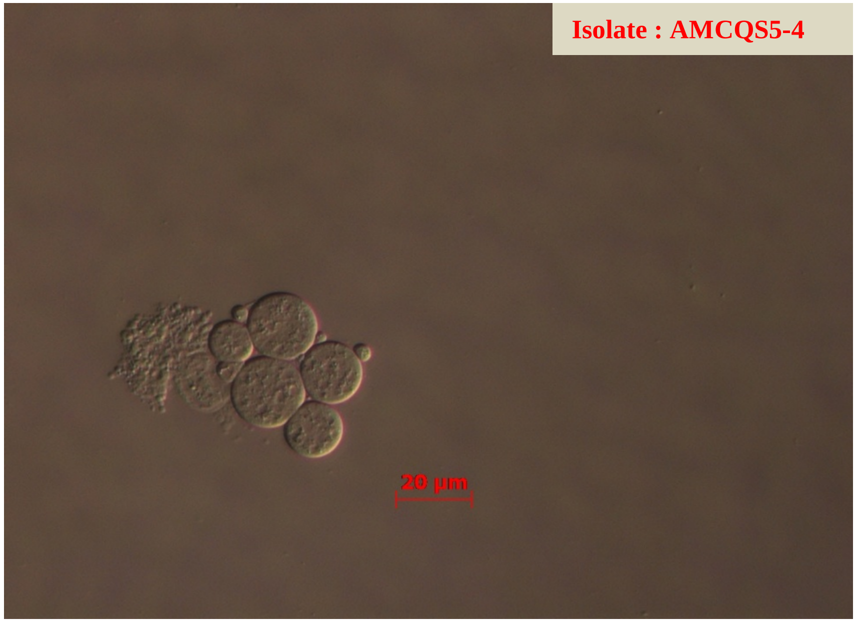
Barwon Heads, Victoria, Australia (November 2012)

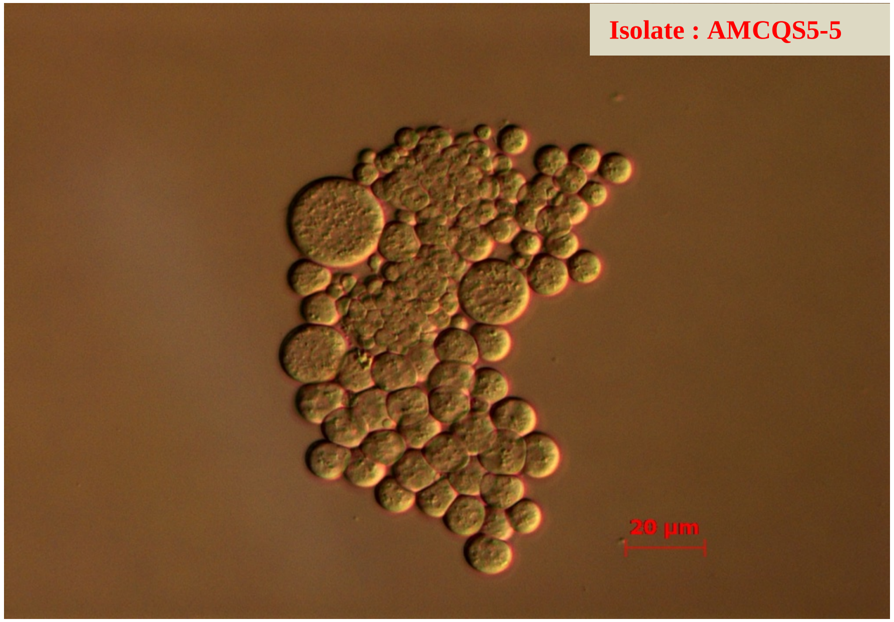
Zuari-mandovi mangrove complex, Goa, India (March 2013)

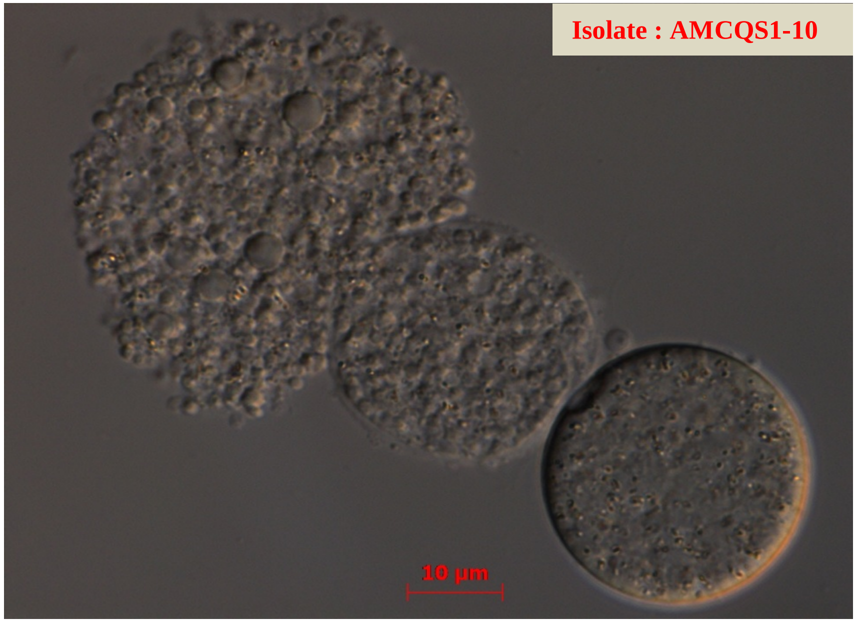
Conducted by Gupta A

Few Isolates

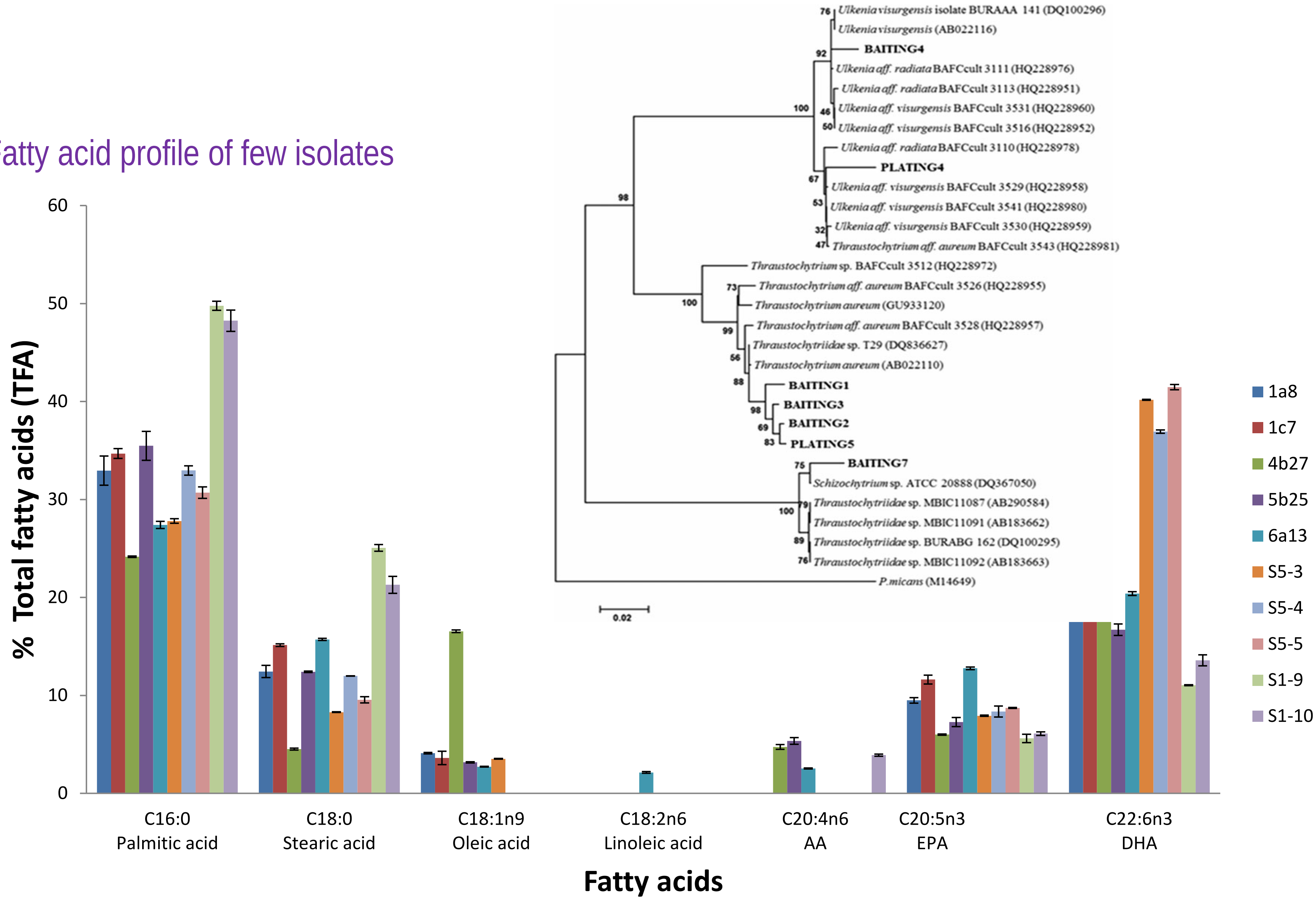




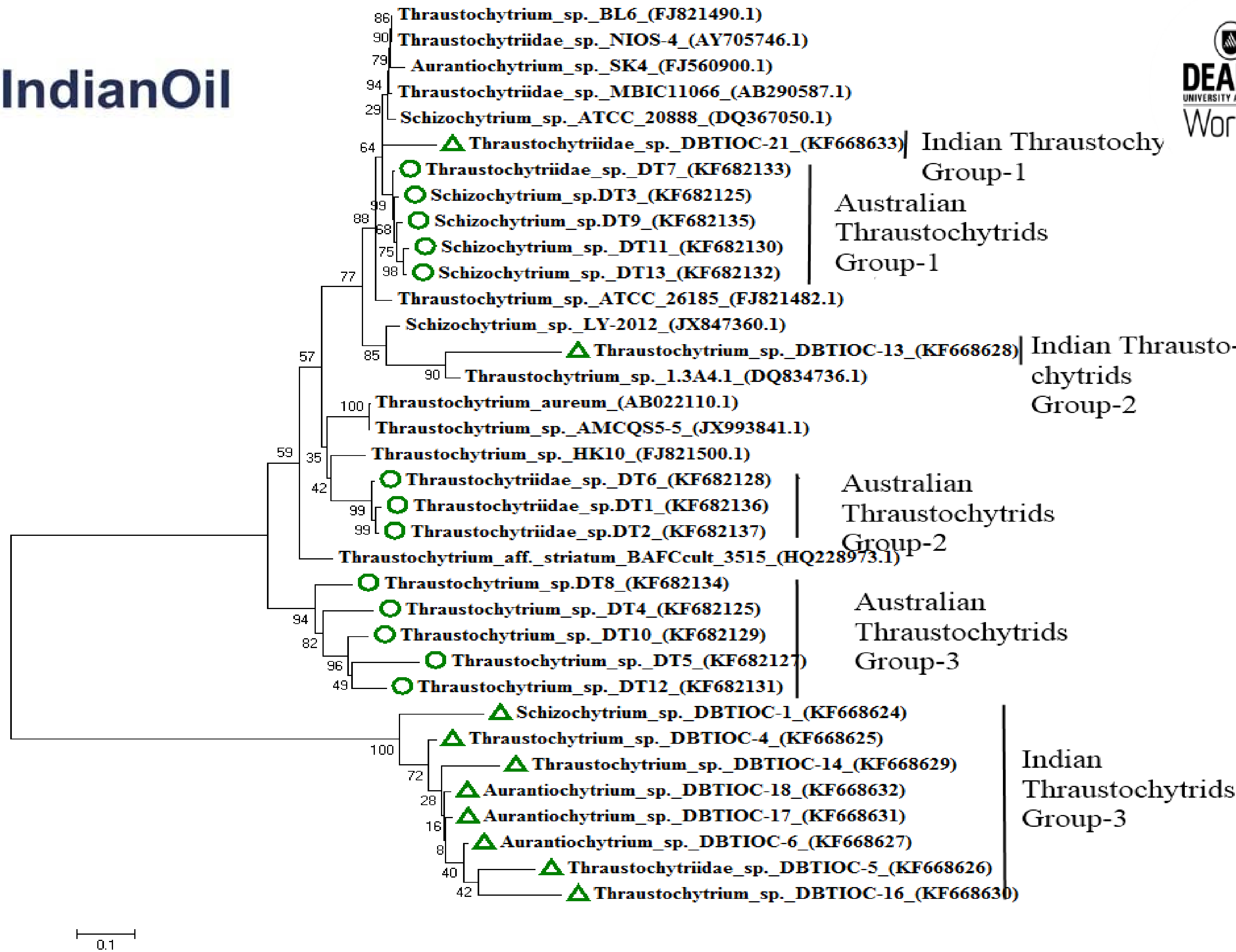




Fatty acid profile of few isolates



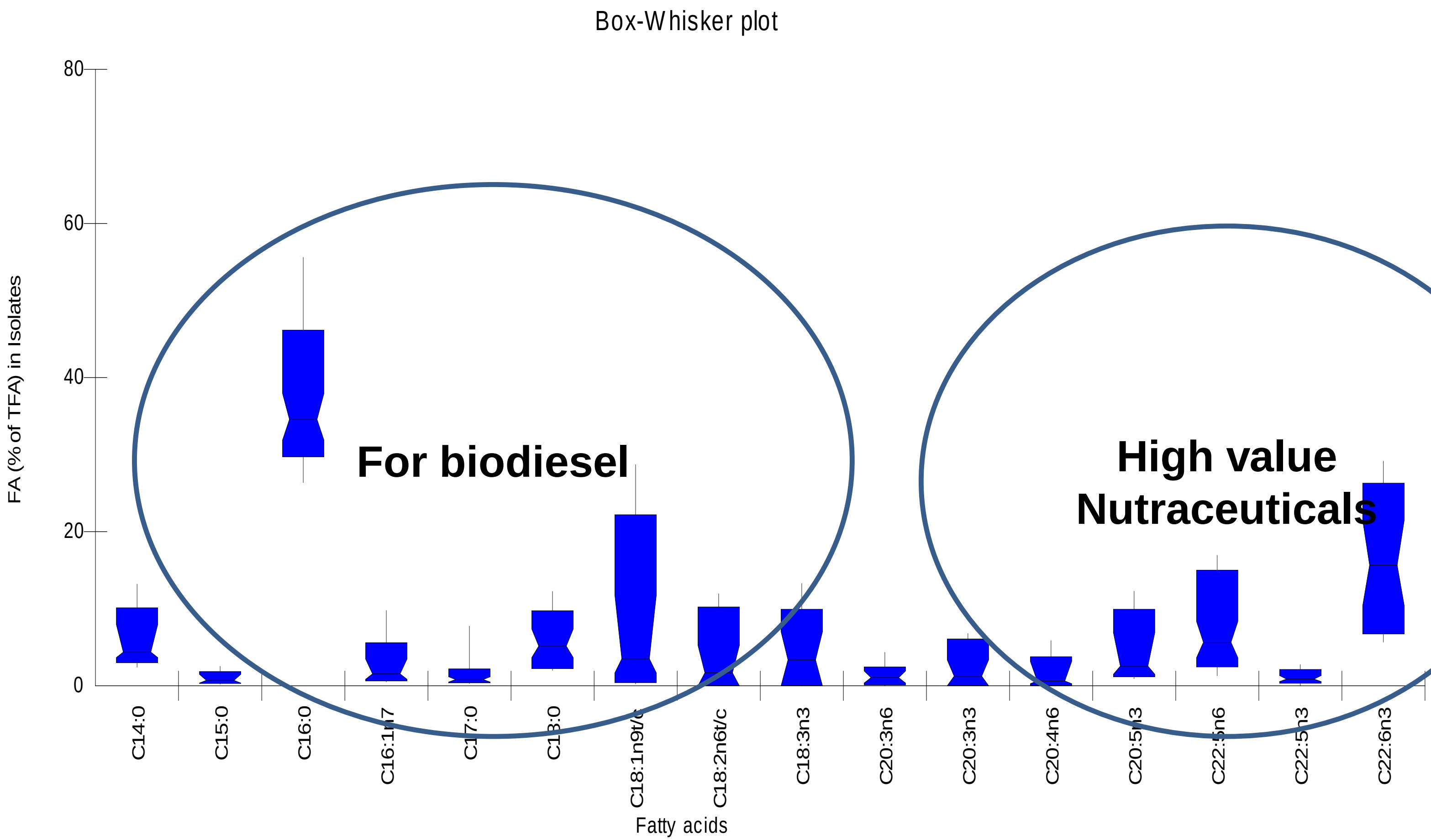
Gupta A et al. Journal of Industrial Microbiology and Biotechnology 2014 (online published)
Gupta A et al. Biochemical Engineering Journal 2014, 78, 11-17.



The phylogenetic tree for Thraustochytrids isolated from Indian and Australian marine sites (triangle-Indian Thraustochytrids, circle-Australian Thraustochytrids).

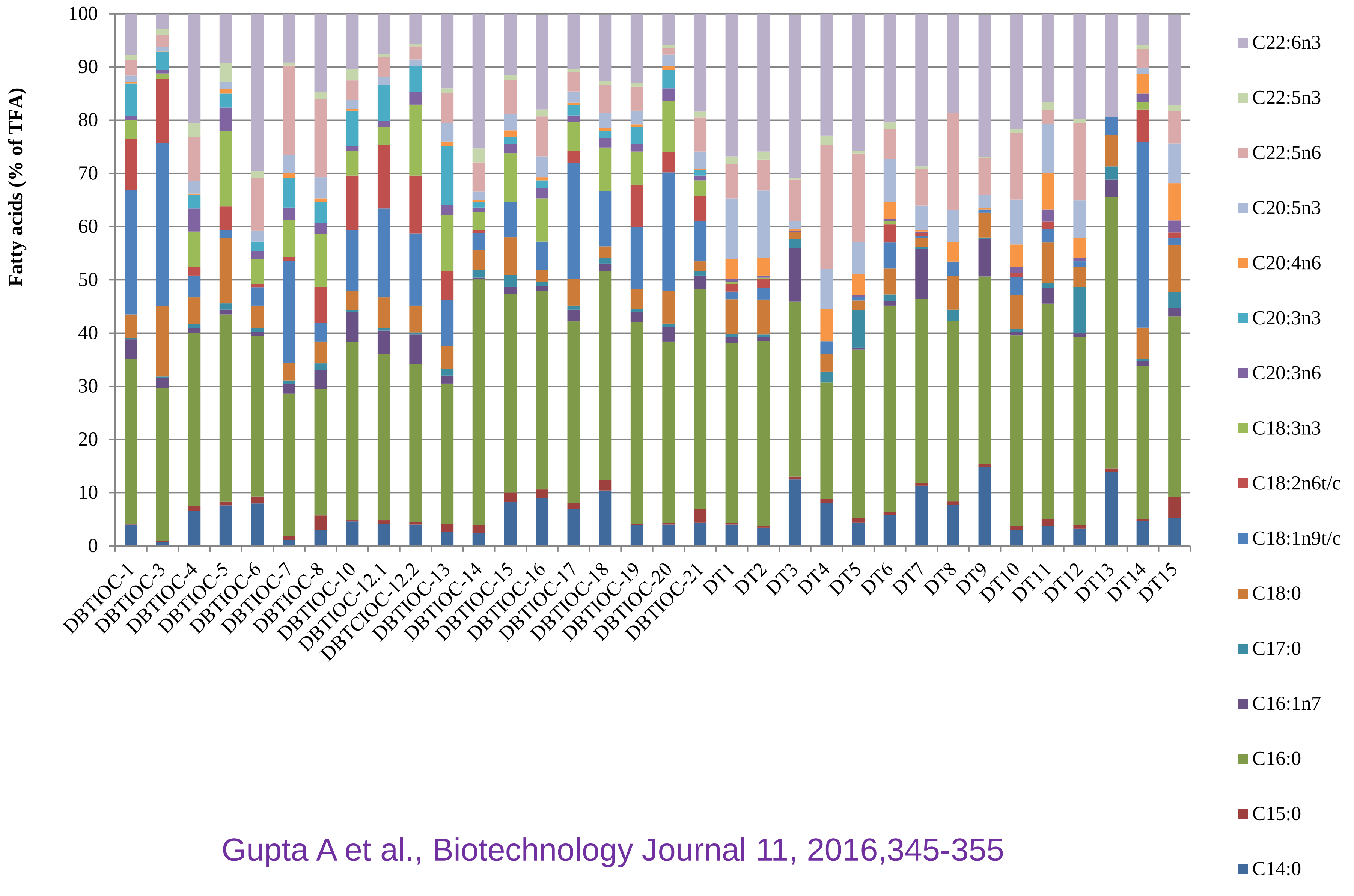
- Total 36 Thraustochytrid strains were isolated (16 from Australia*, 20 from India)

Box-Whisker plot for clustering of different isolates



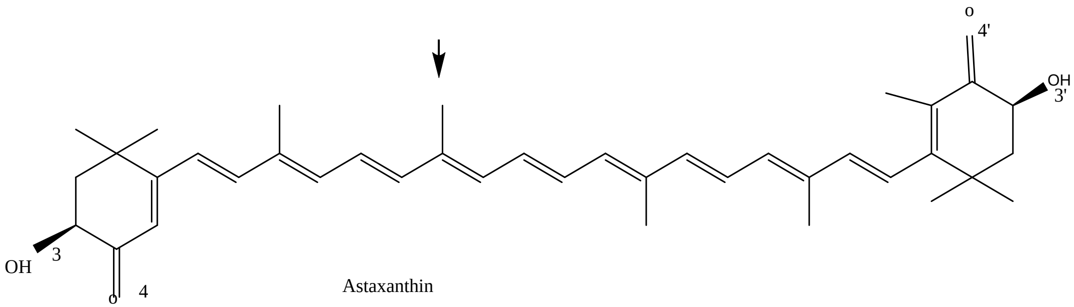
* Courtesy Singh, Adarsha, Avinesh and Tam

Fatty acid profiles of Australian and Indian Thraustochytrid strains



Gupta A et al., Biotechnology Journal 11, 2016,345-355

Astaxanthin

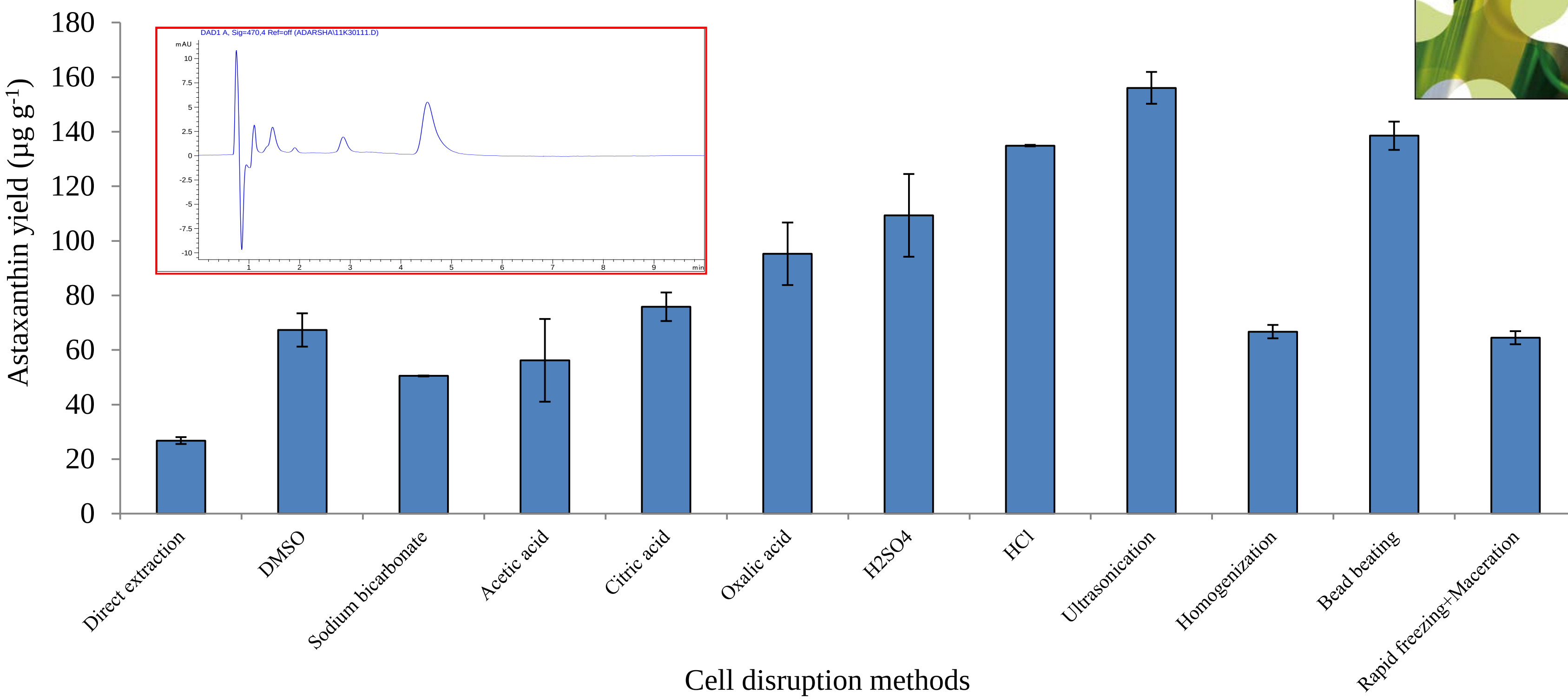


- Global astaxanthin market value : US $ 1.1 billion (Market publishers, 2015)
- Synthetic astaxanthin : $2000/kg
- Natural astaxanthin : $7000/kg

"Market growth rates for natural astaxanthin are so high that production lags behind demand and prices are skyrocketing" Ulrich Marz (BCC research analyst, July 2015)

Sources : Plant (eg., *Adonis aestivalis* (Linda et al 2008)
Fungi (eg., *Xanthophyllomyces dendrorhous* Jacobson et al 1999)
Algae (eg., *Haematococcus pluvialis* (Yves and Benoit 2010),
Thraustochytrids (Aki et al 2003, Gupta et al 2013)
Bacteria (eg., *Paracoccus carotinifaciens sp*. Akira et al 1999)

Understanding response surface optimisation to the modeling of “Astaxanthin” extraction from a novel strain *Thraustochytrium* sp. S7



Highlights

- Orange color pigmented strain *Thraustochytrium* S7 was isolated from New Zealand waters.
- Efficient utilization of glycerol produced significant amount of cell dry biomass.
- Ultrasonication aided cell disruption resulted in the highest astaxanthin yield.
- Response surface optimisation resulted in significant decrease in lysis time (30 min to 10 mins).

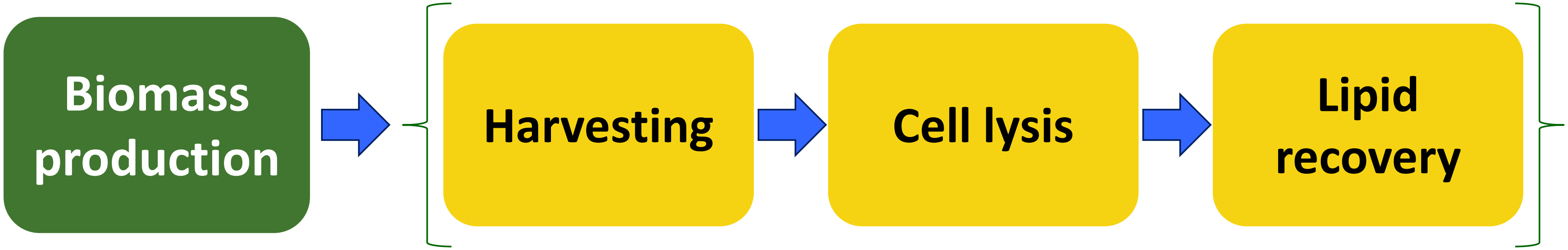
Singh D et al. Algal Research 11 (2015) 113-120
Singh D et al. Algal Research 15 (2016) 202-209

Thraustochytrids – a promising source of astaxanthin

Astaxanthin production by Thraustochytrids isolated from different environments

Organism	Astaxanthin Yield	Reference
Thraustochytrium sp. CHN-3	2.8 mg/g	Yamaoka 2004
Thraustochytrium sp. S7	150.85 µg/g	Singh et al 2015
Thraustochytrium sp. TC 004	0.112 µg/g	Kim et al 2012
Thraustochytrium sp. ONC-T18	Traces	Armenta et al 2006
Thraustochytrium aggregatum	8.9 µg/mL	Chatdumrong et al 2007
Thraustochytriidae sp. As4-A1	63 µg/mL	Quilodran et al 2010
Ulkenia sp.	0.0234 µg/mL	Kim et al 2012
Schizochytrium sp. KH 105	6.1 µg/mL	Aki et al 2003
Schizochytrium limacinum BR2.1.2	13.1µg/mL	Chatdumrong et al 2006
Schizochytrium limacinum	8.9 µg/mL	Chatdumrong et al 2006

Lipid extraction from Algae

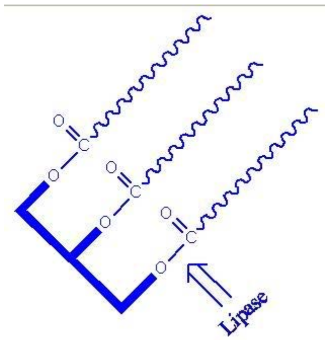


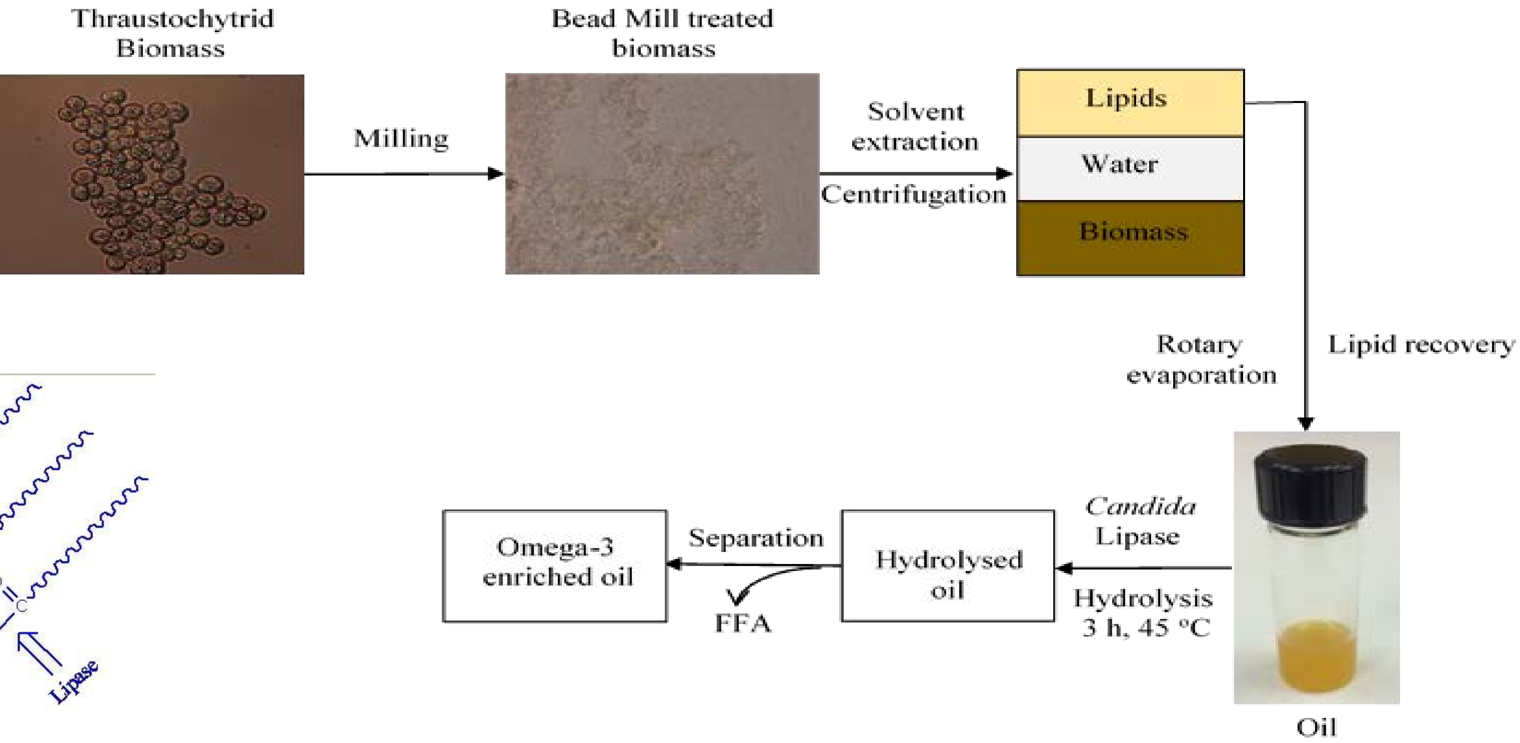
- Oil extraction from algae is a major bottleneck.
- Use of organic solvents alone limits the extraction efficiency.
- Lipid extraction can be improved by switching the solvents and their combinations.
- Using a suitable solvent and cell disruption method will further increase the total lipid yield.

Byreddy, Gupta, Barrow, Puri. Marine Drugs 2015, 13(8), 5111-5127
Byreddy et al. Cell disruption method. Algal Research 2017, 25, 62-67

Lipid recovery: Downstream Processing

- Optimisation of bead milling for lipid recovery
- Enzymatic concentration of Omega-3 FAs from extracted oil





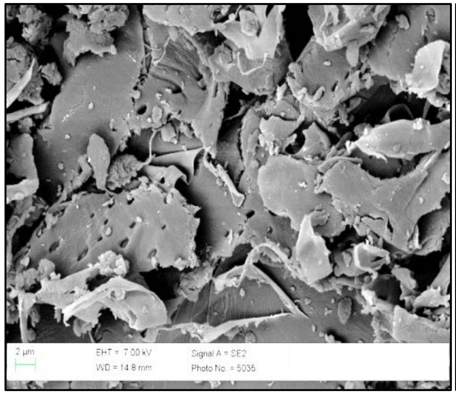
Byreddy, Barrow & Puri. Bioresource Technology 200, 2016, 464-469.
Byreddy et al., Process Biochemistry 2017, 53, 30-35.

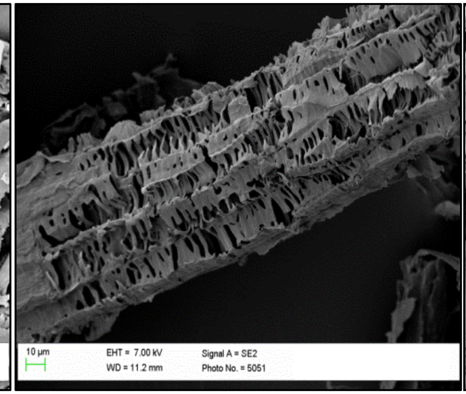
Use of algae biomass rich in PUFAs for food and feed

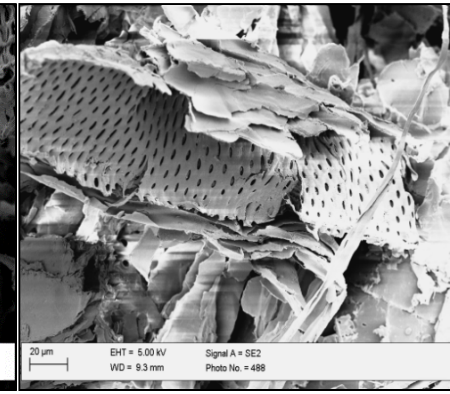
- Increased awareness of Omega-3 fatty acid health benefits has lead to increased fish consumption
- This warrants that fish production should be sustainable
- Currently aquaculture industry used fish meal (FM) and fish oil (FO) to feed cultured fishes
- Efforts have been made to replace FM and FO by plant ingredients (such as soybean meal, cottonseed, rapeseed etc), to reduce the cost of fish feed. But this leads to increase in the price of food crops (Garcia-Ortega et al., 2016)
- Use of microalgal biomass for improving Omega-3 fatty acids content in fish
- Some studies have been conducted, however, are focussed on phototrophic algae biomass based on high production cost

Agriculture biomass: Hemp used for sugar production

SEM images - Effect of pretreatment on hemp



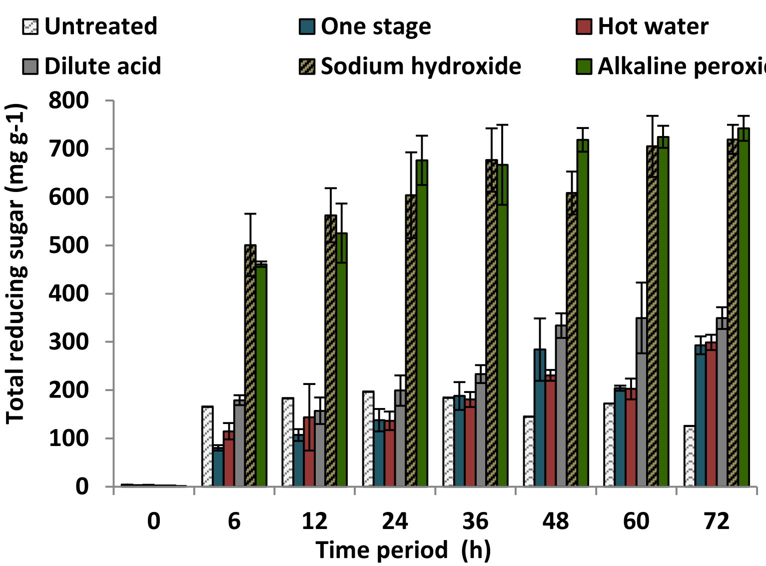




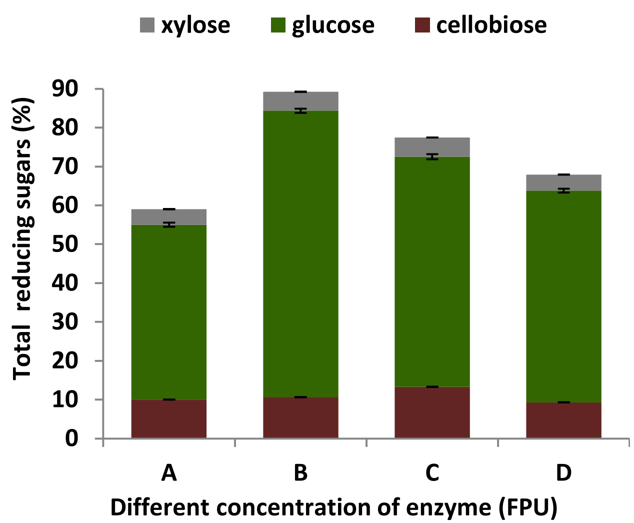
Raw hemp (*Cannabis sativa*)

Pretreated hemp

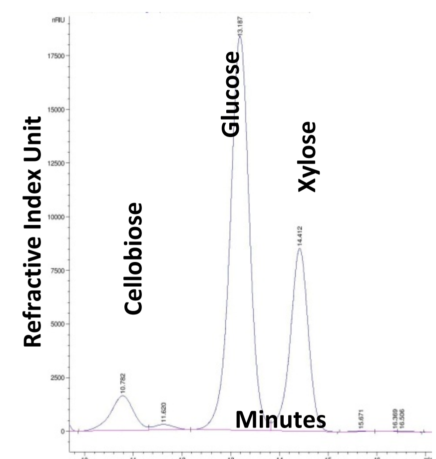
Abraham, Verma, Barrow, Puri. Biotechnology for Biofuels 5 (2014) 215-16.



Fermentable sugars produced from different pretreatments



Reducing sugar produced from different concentration of cellulase



HPLC of reducing sugar hydrolysate from enzymatic hydrolysis

Heterotrophic algae biomass rich in PUFAs for fish feed

- Some studies have been conducted using algal biomass, however, are focussed on phototrophic algae biomass based on high production cost (Sprague et al., 2015;
- Earlier study focused on overall lipid and fatty acid composition
- We investigated a Zf fish model (small size, short generation time and their capacity to produce numerous eggs)
- Three different diets were investigated (control, algal biomass and 50:50)
- This study investigated the distribution of omega-3 LC-PUFAs in the fish body
- Transformation of omega-3 fatty acids to the next generation was measured by its content in eggs

Conclusion

- Renewable biomass (forest and crop residues and food waste resource) as c-source could provide the basis for maintaining and growing industry demand for algal bioactives
- Nanobiotechnology guided innovation brings cost-effectivity
- Extracting and isolating valuable co-products would enhance productivity and profitability of a *biorefinery* set up
- ZF a good model to study distribution of FA.

Acknowledgements

Collaborators

Germany
India
Taiwan
NZ
RMIT, Melb
ANU, Canberra

Group Members

Adarsha Gupta
Reinu Abraham
Tamilselvi, P Ajji
Avinesh, Shailendra
Lovis, M Verma
J Adcock, P Vongsvivut
Prof Barrow's Group
Y Gibert

Funding

CMBD, Flinders University
SRC CCB, Deakin University
DEST, Australia
ARC, Australia

DEAKIN UNIVERSITY
AUSTRALIA NEW ZEALAND MARINE BIOTECHNOLOGY SOCIETY
Flinders UNIVERSITY
इंडियनऑयल IndianOil | IndianOil
Australian Government
Department of Education, Science and Training

I will look forward to your support and interest in joining a CRC bid….

Program objectives are:

P1: Sutainable production of marine Biomass (biproducts)

P2:Marine biorefinery and Bioprocess development

P3: Education & Training, Commercialisation