NEW AUSTRALIAN SINGLE CELL AND CROP PLANT SOURCES OF HEALTH-ENHANCING LONG-CHAIN OMEGA-3 OILS

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Summary

New sources of omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) are being pursued in a multidisciplinary research effort initiated by CSIRO. Due to a decline in global and national fisheries, alternate sources of these beneficial health-enhancing oils are needed. Australian single cell organisms, both phototrophic microalgae and heterotrophic thraustochytrids, have been isolated and examined for their LC-PUFA profiles, and will be explored as potential commercial sources of LC-PUFA for a range of applications. In the longer term, the project also aims to transfer the genes from such marine microorganisms into important oil seed and crop plants to enable them to produce omega-3 LC-PUFA oils.

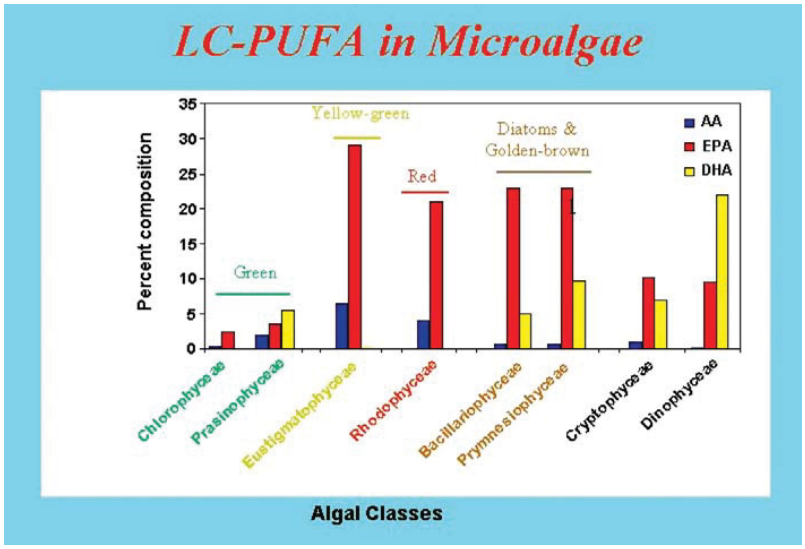


Figure 1. Distribution of long-chain polyunsaturated fatty acids (LC-PUFA) in microalgal classes. Arachidonic acid [AA, 20:6(n-3)], Eicosapentaenoic acid [EPA, 20:5(n-3)] and Docosahexaenoic acid [DHA, 22:6(n-3)].

The health benefits of omega-3 fatty acids are extremely diverse and increasingly being recognized. Nutritional studies continue to demonstrate important benefits from consumption of the omega-3 containing oils. The two main omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) present in fish oil - 20:5ω3, eicosapentaenoic acid (EPA) and 22:6ω3, docosahexaenoic acid (DHA) - are beneficial against various human disorders. It is now well accepted that these fish-derived PUFA decrease the incidence of coronary heart disease (CHD). From this evidence, eating fish two or three times per week results in measurable benefits with respect to parameters examined in CHD studies.

Omega-3 LC-PUFA are also effective in the treatment of inflammatory diseases such as rheumatoid arthritis, and can play a role against hypertension. The biochemical effects of plant and fish-derived oils in some forms of cancer therapy have been examined with promising findings, although further research and trials are required. More recently, a number of studies have also indicated that omega-3 PUFA may be beneficial in infant nutrition and development and against various mental disorders such as schizophrenia, attention deficit hyperactive disorder (ADHD) and Alzheimer's disease. Research in these areas is on-going.

Against this background of an increasing understanding of the immense health benefits of the omega-3 oils, there is a need for new sources of these oils to be obtained. This is because global wild fish stocks are declining. Myers and Worm (2003) used a meta-analytic approach to estimate that large predatory fish biomass today is only about 10% of pre-industrial levels. It is now apparent that global fish stocks will be insufficient to meet human nutritional needs in the future. In an Australian context, recent research has indicated that stocks of up to 60 species (e.g. eastern gemfish, snapper in NSW and WA, southern bluefin tuna and trevally) are already under pressure from environmental degradation and over fishing (Kearney *et al.* 2003). Since mercury accumulation may be a significant problem for pregnant women consuming some species of larger, long-lived fish at the top of the food chain from certain regions (http://www.foodstandards.gov.au, January 2001), additional non-seafood sources of LC-omega-3 PUFA may be nutritionally advantageous.

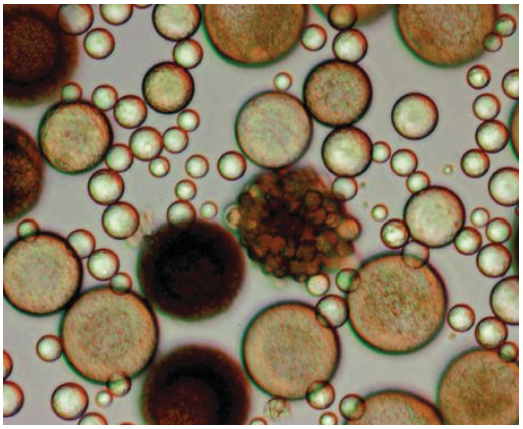


Figure 2. Thraustochytrids, a source of single cell oils, show great morphological diversity from single cells to clusters of cells, complex reticulate forms and motile stages.

Single cell oils (SCO)

It is not widely recognized that marine fish do not actually produce these essential omega-3 LC-PUFA. Rather, the LC-PUFA

MARA-1009

are biosynthesised by single cell microbes (microalgae, select bacteria, and other microheterotrophs) and then are passed up the food chain. SCO sources of LC-PUFA are still considered by some to be too expensive, although it is expected that further research and development can bring production costs down (Blackburn *et al.* 2003).

Over the past decade, our group has profiled the lipid composition of a range of Australian microalgae and other microorganisms that are housed in the CSIRO Collection of Living Microalgae (based in Hobart; http://www.marine.csiro.au/microalgae) (e.g. Volkman *et al.* 1989; Blackburn *et al.* 2003). Considerable diversity in LC-PUFA profiles is apparent across the range of classes of phototrophic algae (Table 1, Figure 1). In general, green algae either do not contain or have only very low levels of EPA and DHA; diatoms contain elevated EPA, as do eustigmatophytes; dinoflagellates contain DHA and other C_{18} PUFA; and red algae can contain elevated proportions of 20:4ω6, arachidonic acid (AA). This diversity in LC-PUFA is potentially valuable and there is local and overseas interest in the production of these light-requiring algae in photobioreactors for high purity LC-PUFA. However, the economics of photobioreactor production are presently not attractive. Research into optimising microalgal production in photobioreactors is occurring in CSIRO, at Marine Research (CMR) and at Manufacturing Infrastructure and Technology (CMIT), and includes collaboration with the University of Florence, Italy (Blackburn *et al.* 2003).

Recently new strains of Australian thraustochytrids have been isolated (Figure 2). Thruastochytrids are a group of single cell organisms that produce both high oil and LC-PUFA content. They were initially thought to be primitive fungi, although more recently have been assigned to the subclass Thraustochytridae (Chromista, Heterokonta), which aligns them more closely with other heterokont algae (e.g. diatoms and brown algae). Under culture, thraustochytrids can achieve considerably higher biomass yield (>20 g/L) than other microalgae. In addition, thraustochytrids can be grown in fermenters with an organic carbon source and therefore represent a highly attractive, renewable and contaminant-free, source of omega-3 oils.

Table 1. Distribution of selected PUFA and LC-PUFA in microalgae and other groups, and areas of application

Group	Genus / Species	PUFA	Application
Eustigmatophytes Diatoms	*Nannochloropsis* *Chaetoceros*	EPA	Aquaculture
Dinoflagellates Thraustochytrids	*Crypthecodinium cohnii* *Schizochytrium*	DHA	Aquaculture, health supplements, infant formula
Red algae Thraustochytrids Fungi	*Phorphyridium* undescribed species *Mortiella*	AA	Aquaculture, infant formula Pharmaceutical industry (precursor to prostaglandins)
Blue green algae	*Spirulina*	GLA	health supplements

Abbreviations: γ–linolenic acid, GLA, 18:3ω6; 20:5ω3, eicosapentaenoic acid, EPA, 20:5ω3; docosahexaenoic acid, DHA, 22:6ω3; arachidonic acid, AA, 20:4ω6.

Representative fatty acid profiles for selected Australian thraustochytrids recently isolated and maintained by CSIRO are shown in Table 2. Strain O is particularly attractive as it contains very high levels of DHA (61%). Other PUFA are present at less than 5% each. High DHA-containing thraustochytrids often also contain high proportions of 22:5ω6, docosapentaenoic acid (DPA(6)), as is observed for strains A, C and H. DPA(6) is only a minor component in our strain O under the culture conditions employed, making this strain particularly interesting. Further work is required to assess the proportion of these two components across a range of culture and other conditions.

Strain A contains both DHA (28%) and EPA (16%) as the main LC-PUFA. The relative levels of these two PUFA is similar to that occurring in salmon and tuna oil, which are increasingly sought for nutritional applications due to their DHA/EPA ratio being >1, a preferred ratio due to the knowledge that DHA and EPA perform different roles in human health. Strains C and H differed from the other strains with AA (10-13%) also being present as a major LC-PUFA. As both DHA and AA are added separately to infant formula, oil from these strains might be attractive in that they may be able to provide both PUFA simultaneously. A number of other LC-PUFA were present in the thraustochytrids including DPA(3) and 22:4ω6 and other components (data not shown).

Potential application of the LC-PUFA-producing thraustochytrids can be considerable and varied. These unique microorganisms can be used in aquaculture to supply essential LC-PUFA to live feeds used for rearing larvae, and as components in aquaculture feeds (e.g. pellets) used for raising juvenile and adult finfish and crustaceans (prawns and lobsters). Just as the LC-PUFA are beneficial for human nutrition and well being, they are also key ingredients for cultured marine and freshwater species. Increasingly fish-derived LC-PUFA oils are being incorporated into food products and in infant formula. It will be possible to also incorporate SCO oils, and in some instances the micro-organisms themselves without first separating the oil. Indeed, the other components of microorganisms such as antioxidants, vitamins and protein can offer a highly nutritious, whole cell package. This is already fundamental to the use of microalgae as feeds in aquaculture. It is also the basis of most of the handful of microalgae that have established a niche in the health supplement market e.g. *Dunaliella* for beta-carotene (produced by Cognis Australia), *Haematococcus* for astaxanthin and *Chlorella* for immune

Table 2. Fatty acid composition (% of total) of new Australian thraustochytrid strains

Fatty acid	Percentage composition Strain A	C	H	O
16:0	18.0	16.4	13.5	22.1
20:4ω6 AA	4.0	10.5	13.4	0.7
20:5ω3 EPA	15.8	7.7	5.2	4.1
22:5ω6 DPA(6)	16.6	9.3	12.7	3.4
22:6ω3 DHA	28.2	21.6	19.2	61.0

system function as well as for general health and well-being (all these are green algae), as is the "super food" *Spirulina* (a blue green alga or cyanobacterium). Other than the thraustochytrids, the only microalga marketed for LC-PUFA is the dinoflagellate *Crypthecodinium cohnii* used for infant nutrition and health supplements (Martek Biosciences), suggesting the opportunity for new microorganism-based "super foods" containing omega-3 oils.

Proposed land plant oils with LC-PUFA

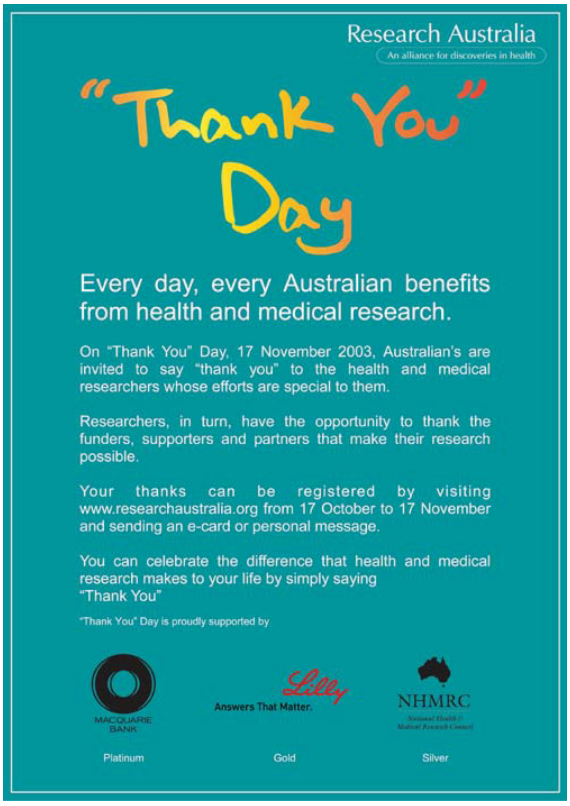
Some land plants have the mechanisms to produce 18:3ω3, α-linolenic acid (ALA), the simplest member of the omega-3 fatty acid family. But land plants do not have the extra enzymes needed to synthesize the longer chain omega-3 fatty acids, i.e. EPA and DHA that are derived from ALA.

Microalgae and other simple plants contain the chain elongation and desaturase enzymes needed to make large amounts of the LC omega-3 PUFA oils, particularly EPA and DHA. Using gene technology, a multidisciplinary research team at CSIRO aims to transfer the genes from marine microorganisms into important oil seed and crop plants to enable them to produce omega-3 LC-PUFA oils - EPA and DHA. Initially transfer of the appropriate genes to model plants will be attempted in order to characterize gene activities. Candidate crop plants for commercial applications will be selected after considering a range of matters and could include canola and flaxseed.

If the project is successful, farmers could in the future be growing oil seed and crop plants that contain the healthy omega-3 LC-PUFA. The technology to develop plant-based omega-3 crops has great potential to provide farmers with a new premium market for their produce. The novel omega-3 technology would also improve the economic and environmental sustainability of the rapidly growing aquaculture industry, by providing plant-derived aquaculture feeds to replace the current unsustainable use of fishmeal and fish oil based feeds.

Team photo: CSIRO Marine Research, Hobart members of the Omega-3 oils project team (left to right from top) - Peter Nichols, Stanley Robert, Mina Brock, Susan Blackburn, Peter Mansour, Bronwyn Innes, Dion Frampton.

Differences may exist in the nutritional properties of oils that may be due to position of the LC-PUFA on triglyceride molecule (termed regiospecificity); therefore we aim to assess these properties during the project to maximize potential health benefits. The project team will also seek novel lipases from insect and other sources, determine and optimise regiospecificity of PUFA-containing oils presently produced and those being designed, and perform laboratory and farm-based feeding trials with a range of animals.



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