9. Production of polyunsaturated fatty acids by Australian thraustochytrids: Aquaculture applications

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Interest in the nutritional importance of polyunsaturated fatty acids (PUFAs) has increased markedly during the past decade. As PUFAs are necessary constituents of cell membranes and of many cell-signalling systems, deficiencies in dietary PUFAs may lead to poor growth, low vitality and/or disease. The essentiality of PUFAs as dietary components for marine finfish and crustacean larvae has been amply demonstrated (e.g. Sorgeloos and Leger, 1992).

PUFAs are generally classified into two main groups: the omega-6 (ω6 or *n*-6) and omega-3 (ω3 or *n*-3) series. Of the *n*-6 PUFAs, arachidonic acid [AA; 20:4 (*n*-6)] is of particular importance, as it is a major precursor of many prostaglandins and eicosanoids. Eicosapentaenoic acid [EPA; 20:5 (*n*-3)] and docosahexaenoic acid [DHA; 22:6 (*n*-3)] are two *n*-3 PUFAs which are currently receiving much attention and have been termed 'essential' fatty acids.

At present, selected fish oils and microalgal species are the main industrial sources of PUFAs. However, supplies of fish oil may be unreliable due to the failure or variability of various fisheries. There is concern that insufficient fish oil will be available in the future to meet the expected growth in world demand for *n*-3 oils (Tacon, 1995).

Phototrophic microalgae are also used to provide PUFAs for aquaculture operations (e.g. Volkman *et al.,* 1989). However, the *de novo* synthesis of *n*-3 and *n*-6 PUFAs by heterotrophic microorganisms may provide a cheaper and easier means of producing PUFA-rich biomass and oils. Microheterotrophs do not require all of the elements necessary for the culture of autotrophs (e.g. light, carbon dioxide), and are seen by some as a

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potential alternative to traditional commercial sources of PUFAs. Certain bacteria have been shown to produce EPA and DHA (Nichols *et al*., 1993). These PUFA-producing bacteria also have been successfully used as a means to enrich rotifers (*Brachionus plicatilis*) with these fatty acids (e.g. Lewis *et al*., 1998b).

Another potential source of *n*-3 oils is the little-studied group of microheterotrophs called thraustochytrids. Thraustochytrids are common marine microheterotrophs that feed as saprobes or occasionally as parasites. Thraustochytrids have a wide geographic distribution, with strains isolated from Antarctica, the North Sea, India, Micronesia, Japan and Australia (reviewed by Lewis *et al*., 1999).

Several recent studies have catalogued the ability of some thraustochytrid strains to produce:

- a high biomass in culture,
- a high proportion of lipid as part of this biomass,
- a high proportion of PUFAs in the lipid.

Most reports concerning the production of PUFAs by thraustochytrids have dealt almost exclusively with DHA production, as this compound is the most abundant PUFA produced by many of the strains of thraustochytrids reported to date.

Data available in the scientific literature demonstrate the large variation in biomass, lipid and maximum DHA yields obtained for different thraustochytrid strains. For example, *Schizochytrium aggregatum* produced a biomass of 0.9 g L^{-1} after 10 days (Vazhappilly and Chen, 1998), while a biomass of 48 g L^{-1} after 4 days was achieved using *Schizochytrium* sp. SR21 (Yaguchi *et al*., 1997). Perhaps more importantly, PUFA production by a single strain (*T. roseum* ATCC 28210) cultured under different conditions also showed marked differences. For this strain, a batch-fed flask culture yielded 2100 mg L^{-1} of DHA (Singh and Ward, 1996), as compared to an unsupplemented flask culture, which yielded 650 mg L^{-1} of DHA (Li and Ward, 1994).

Although production of DHA has been the main focus of recent attention, it is evident that some thraustochytrid strains also produce other PUFAs. Lewis *et al.* (1998a) isolated a number of thraustochytrid strains with a range of different PUFA profiles, including one strain (ACEM E) which produced AA to 30% of the total fatty acids (TFA), with no other PUFA exceeding 10% TFA (Figure 9.1).

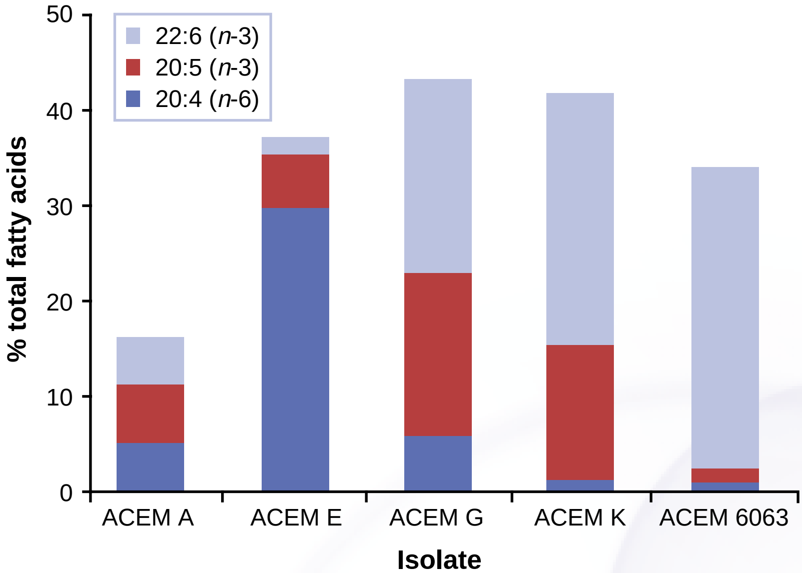

Figure 9.1. *PUFAs in new Australian thraustochytrids (expressed as percentage of total fatty acids; after Lewis et al. 1998a).*

Given the diversity of PUFA profiles seen for thraustochytrids examined to date, we feel there is great potential to use these organisms (singly or in combination) to produce live or manufactured feeds containing PUFA profiles tailored to the specific PUFA requirements of aquaculture species.

We have fed two strains of our thraustochytrids (ACEM A and ACEM 6063), both separately and mixed with each other or with microalgae, to rotifers. These experiments revealed two noteworthy results:

- Our strains of Australian thraustochytrids can be used to enrich rotifers with PUFAs to levels that are reported to be nutritionally significant for many aquaculture species (i.e. 1–2% w/w dry weight).

- Marked variations in the final PUFA profile of enriched rotifers can be achieved by changing the strain(s) with which rotifers are enriched (Figure 9.2).

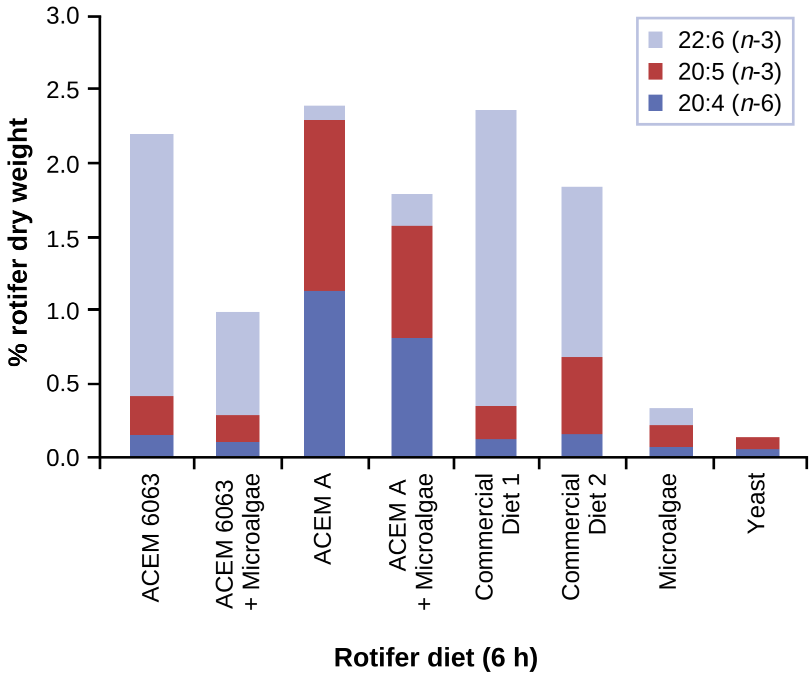

Figure 9.2. *PUFA enrichment of rotifers using Australian thraustochytrids, compared with two commercial PUFA enrichment diets and the microalgae Isochrysis sp. (clone T.ISO) and bakers yeast.*

Thraustochytrids are already being used in the USA for commercial production of PUFA-rich products. A *Schizochytrium* strain is the basis for two products marketed for enriching rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.) with PUFAs, prior to feeding these organisms to cultured finfish larvae (Barclay and Zeller, 1996; www.aquafauna.com; www.sandersbshrimp.com). These products have entered the market in direct competition with microalgal and fish-oil-based products. It is possible, however, that thraustochytrids may offer some advantage over other oils as sources of PUFAs for aquaculture. Many aquaculture species require proportionally more DHA than EPA in their diets (Narciso *et al.*, 1999). The PUFA profiles of many thraustochytrids fit this criterion, while most oils derived from the fish meal industry contain more EPA than DHA.

Thraustochytrids are clearly a new and potentially competitive player in the PUFA market. Considerable work is required before the production of oil from these organisms significantly increases its share of the market for PUFA-rich products. To achieve this aim, the following key stages need to be negotiated:

1. Further isolation, screening and maintenance of PUFA-producing strains: Several strains with potential for the commercial production of DHA-rich oils have been already isolated. However, if thraustochytrids that produce higher PUFA yields and/or more attractive PUFA-profiles can be isolated and optimised, demand for these isolates and compounds may well increase.
2. Optimisation of efficiency of PUFA production: The types and amounts of PUFAs produced by individual strains of thraustochytrids are susceptible to manipulation by varying culture conditions. Enhancement of PUFA profiles using molecular techniques may also be considered. Different markets will provide demand for strains that produce high levels of PUFAs measured either in terms of biomass (i.e. PUFA production w/w cell mass) or volume (i.e. PUFA production w/v fermentation medium).
3. Determination of appropriate conditions for long-term storage of microbial cells and/or their products: The form and stability of thraustochytrid biomass and/or oils will be major factors in determining the suitability of these products for use as food additives.
4. Development of production technologies to meet market demands for cost-effective and safe trophic transfer of PUFAs to the target consumer(s). The bottom line for the biotechnological future of thraustochytrid-oils will be their competitiveness against other PUFA-rich oils. Examples given above indicate that large-scale culture of thraustochytrids for commercial purposes already is, or soon will be, economically feasible.

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