

The Biotechnological Potential of Thraustochytrids

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Abstract: Thraustochytrids are common marine microheterotrophs, taxonomically aligned with heterokont algae. Recent studies have shown that some thraustochytrid strains can be cultured to produce high biomass, containing substantial amounts of lipid rich in polyunsaturated fatty acid (PUFA). It is also evident that cell yield and PUFA production by some thraustochytrid strains can be varied by manipulation of physical and chemical parameters of the culture. At present, fish oils and cultured phototrophic microalgae are the main commercial sources of PUFA. The possible decline of commercial fish stocks and the relatively complex technology required to commercially produce microalgae have prompted research into possible alternative sources of PUFA. The culture of thraustochytrids and other PUFA-producing microheterotrophs is seen as one such alternative. Indeed, several thraustochytrid-based products are already on the market, and research into further applications is continuing. Many fish and microalgal oils currently available have relatively complex PUFA profiles, increasing the cost of preparation of high-purity PUFA oils. In contrast, some of the thraustochytrids examined to date have simpler PUFA profiles. If these or other strains can be grown in sufficient quantities and at an appropriate cost, the use of thraustochytrid-derived oils may decrease the high expense currently involved with producing high-purity microbial oils. As more is learned about the health and nutritional benefits of PUFA, demand for PUFA-rich products is expected to increase. Results to date suggest that thraustochytrids could form an important part in the supply of such products.

Key words: Thraustochytrids, polyunsaturated fatty acid, biomass, commercial culture, microheterotroph

INTRODUCTION

Thraustochytrids are common marine microheterotrophs that feed as saprobes or occasionally as parasites (Porter, 1990). Thraustochytrids have a wide geographic distribution, with strains isolated from Antarctica (Bahnweg and Sparrow, 1974), the North Sea (Raghukumar and Gaertner, 1980), India (Raghukumar, 1988), Micronesia (Honda et al., 1998), Japan (Naganuma et al., 1998), and Australia

(Lewis et al., 1998a). Originally thought to be primitive fungi, thraustochytrids have more recently been assigned to the subclass Thraustochytridae (Chromista, Heterokonta), aligning them more closely with the heterokont algae (e.g., brown algae and diatoms) (Cavalier-Smith et al., 1994).

Following an early description of thraustochytrids (Sparrow, 1936), little research concerning this group of organisms occurred until a number of descriptive and ecological studies in the 1960s (e.g., Goldstein, 1963; Gaertner, 1968). Findlay et al. (1986) discussed the use of fatty acids as biochemical indicators for thraustochytrids within mangrove detrital systems. Since then, several studies (which

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will be referred to later in this article) have catalogued the ability of some strains of thraustochytrid to produce (1) a relatively high biomass in culture, (2) a high proportion of lipid as part of this biomass, and (3) a high proportion of polyunsaturated fatty acids (PUFAs) in the lipid.

Interest in the nutritional importance of PUFA has increased markedly during the past decade. As PUFAs are necessary constituents of cell membranes and of many cell signaling systems, deficiencies in PUFA can be associated with defects in cellular function, which may lead to disease. A number of studies have further demonstrated that PUFAs are essential dietary components for humans (reviewed by Simopoulos, 1989; Takahata et al., 1998) and also in aquaculture operations for marine finfish and crustacean larvae (Sorgeloos and Leger, 1992; Castell et al., 1994; D'Abramo, 1997).

Generally, PUFAs are classified into two main groups: the omega-6 ($\omega 6$ or n-6) and omega-3 ($\omega 3$ 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)], two n-3 PUFAs that are currently receiving much attention, have been termed "essential" fatty acids. The n-3 PUFAs are known to decrease the incidence of coronary heart disease, stroke, and rheumatoid arthritis (Kinsella, 1987). Evidence on the benefits and risks of n-3 PUFAs for human health has recently been reviewed by Takahata et al. (1998). DHA is essential for normal development of neural tissue in infants, especially in the eyes and brain. The possible role of these PUFAs against other disorders (e.g., asthma, dyslexia, depression, and some forms of cancer) is also becoming increasingly recognized, although further research is necessary (Simopoulos, 1989; Takahata et al., 1998).

As the importance of the presence and proportions of various PUFAs in the diet of both man and beast becomes better understood, the value of these dietary components to a range of industries also increases. At present, selected fish oils and microalgal species are the main industrial sources of PUFA. However, fish oil sources may be unreliable because of the failure or variability of some 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; Ward, 1995). Phototrophic microalgae are also used to provide PUFA for aquaculture operations (Volkman et al., 1989), with additional applica-

tion in the production of nutraceuticals (dietary supplements).

In comparison, the de novo synthesis of n-3 and n-6 PUFAs by thraustochytrids and other heterotrophic microorganisms may provide an easier and less expensive means of producing PUFA-rich biomass and oils. In recent years, interest in the use of microheterotrophs as a source of PUFA has increased (Ratledge, 1993). Microheterotrophs do not require some of the elements necessary for the culture of autotrophs (e.g., light, carbon dioxide), and some see them as a potential alternative to traditional commercial sources of PUFA. Arachidonic acid has been produced in quantity by some fungi (Sajbodor et al., 1990; Gandhi and Weete, 1991). Certain bacteria have been shown to produce EPA and DHA (Nichols et al., 1993; Jøstensen and Landfald, 1997). The recognized need in aquaculture for alternative sources of PUFA for feeding both larvae and adults has seen PUFA-producing bacteria successfully demonstrated as a means to enrich rotifers (*Brachionus plicatilis*, a live-feed organism for finfish larvae) with these fatty acids (Watanabe et al., 1992; Nichols et al., 1996; Lewis et al., 1998b).

An increasing body of research into microheterotrophic PUFA production has concentrated on the thraustochytrids. This review is a brief synthesis of that work.

PUFA PRODUCTION BY THRAUSTOCHYTRIDS

The importance of DHA in human and animal nutrition has received a great deal of research attention during the past decade (Simopoulos, 1989; Takahata et al., 1998). This has prompted many studies into alternative sources of PUFA to focus on this particular fatty acid. Most reports concerning the production of PUFA by thraustochytrids have dealt almost exclusively with DHA production (Table 1), as this compound is often the most abundant PUFA produced by strains of thraustochytrids reported to date.

Data presented in Table 1 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

Table 1. Docosahexaenoic Acid (DHA) Production by Thraustochytrids

Organism	Culture Conditions				Biomass (g/L)	Total Lipid (% dw)	DHA Production			Reference
	Age (d)	Temp (°C)	Vessel	Other			(% TFA)	(mg/g)	(mg/L)	
<i>Schizochytrium</i> sp SR21	2.5	28	Fermenter	pH. 4	21	50	35	224	4,700	Nakahara et al. (1996)
<i>Schizochytrium</i> sp SR21	4		Fermenter 300 rpm	—	48	77	36	277	13,300	Yaguchi et al. (1997)
<i>S. limacinum</i> SR21	5	25	Flask	—	38	37	33	110	4,200	Yokochi et al. (1998)
<i>S. aggregatum</i> ATCC 28209	10	25	Flask 200 rpm	Dark	0.9	—	1.7	30	0.4	Vazhappilly and Chen (1998)
<i>Thraustochytrium</i> <i>aureum</i> ATCC 34304	6	25	Flask 300 rpm	Light	3.8	16.5	49	70	270	Bajpai et al. (1991a)
<i>T. aureum</i> ATCC 34304	6	25	Flask 300 rpm	Light	4.9	20.3	51	104	511	Bajpai et al. (1991b)
<i>T. aureum</i> ATCC 34304	2.5	25	Flask	Light	5.7	8.1	40	—	—	Iida et al. (1996)
<i>T. aureum</i> ATCC 28211	6	25	Flask 200 rpm	Dark	0.8	—	3.7	50	4.0	Vazhappilly and Chen (1998)
<i>T. roseum</i> ATCC 28210	5	25	Flask 250 rpm	Light	7.6	18.2	50	87	650	Li and Ward (1994)
<i>T. roseum</i> ATCC 28210	12	25	Flask 200 rpm	Fed Batch	17.1	25	49	115	2,100	Singh and Ward (1996)
<i>Thraustochytrium</i> sp. ATCC 20892	4	25	Flask 200 rpm	—	2.7	7.3	35	25	68	Singh et al. (1996)
<i>Thraustochytrium</i> sp. ATCC 26185	6	28	Flask 120 rpm	Light	7.5	32	25	—	—	Weete et al. (1997)

marked differences. For this strain, a fed-batch flask culture yielded DHA at 2100 mg L⁻¹ (Singh and Ward, 1996), as compared with an unsupplemented flask culture, which yielded DHA at 650 mg L⁻¹ (Li and Ward, 1994).

It is beyond the scope of this review to explore in detail the many physicochemical manipulations of culture conditions that have been used to influence PUFA production by different thraustochytrid strains. However, Table 2 gives an indication of how variations in culture conditions can influence the biomass and amount of PUFA produced by various thraustochytrid strains. It is apparent from these results that changes to culture conditions do not have uniform effects on PUFA production by different thraustochytrid strains. Optimization and manipulation of culture conditions to produce the amounts and types of PUFA required for specific applications are definitely areas that

will require extensive research for each strain taken toward commercial production.

Although production of DHA has been the main focus of recent attention, it is evident that some thraustochytrid strains also produce other PUFAs. Yokochi et al. (1997) suggested the fatty acid profiles of DHA-producing thraustochytrids could be used to classify them into six separate categories: namely, DPA (22:5 n-6)/DHA; EPA/DHA; EPA/DPA/DHA; AA/EPA/DHA; LA (18:2 n-6)/AA/DPA/DHA; and LA/AA/EPA/DHA. Lewis et al. (1998a) isolated a number of thraustochytrid strains with a range of different PUFA profiles, including one strain (ACEM E) that produced AA to 30% of total fatty acids (TFA), with no other PUFA exceeding 10% TFA (Figure 1).

Given the diversity of PUFA profiles seen for thraustochytrids examined to date, we think it is likely that other,

Table 2. Some Factors Shown to Affect Cell Yield and PUFA Production by Thraustochytrids

Organism	Culture Vessel	Culture Conditions That Resulted in Changes When Varied	Reference
<i>Schizochytrium</i> sp SR21	Fermenter	Medium composition Impeller speed (300 rpm; 500 rpm) Impeller shape (propeller-shaped, turbine-shaped) Culture age (62–125 h)	Yaguchi et al. (1997)
<i>S. limacinum</i> SR21	Flask	Medium composition Incubation temperature (10–35°C) Seawater concentration (0%–200% seawater)	Yokochi et al. (1998)
<i>T. aureum</i> ATCC 34304	Flask Fermenter	Medium composition Incubation temperature (15°–35°C) Initial medium pH (4–9) Illumination (light; dark) Inoculum age (24–72 h) Culture vessel type (shake flask; fermenter) Culture age (0–12 d)	Bajpai et al. (1991b)
<i>T. aureum</i> ATCC 34304	Flask Fermenter	Medium composition Salinity (0%–200% seawater) Vitamins Culture vessel type (shake flask; fermenter)	Iida et al. (1996)
<i>T. roseum</i> ATCC 28210	Flask	Medium composition Incubation temperature (5°–37°C) Initial medium pH (4–9) Culture age (0–7 d)	Li and Ward (1994)
<i>T. roseum</i> ATCC 28210	Flask	Medium composition Batch feeding (4–12 d after inoculation) Culture age (2–8 d)	Singh and Ward (1996)
<i>Thraustochytrium</i> sp. ATCC 20892	Flask	Medium composition Incubation temperature (15°–35°C) Incubation temperature shift (initial 25°C; final 15°C) Culture age (1–7 d)	Singh et al. (1996)

more unusual PUFAs will also be discovered from this group of organisms.

THE MARKET FOR PUFA

It is anticipated that the market in which thraustochytrid-based oils could have the most impact will be that currently occupied by PUFA-rich oils derived from marine fish. As such, some consideration of this market is warranted. Market data included in this section have been obtained from published papers, when available, and also from industry association and company Web sites and from market research information provided by Australian industry (Clover Corporation Pty Ltd, New South Wales, Australia).

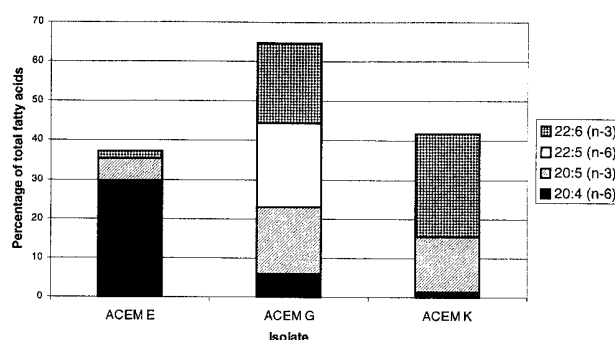


Figure 1. Composition of PUFA in new Australian thraustochytrids, expressed as percentage of total fatty acids; after Lewis et al. (1998a). Key: 22:6 (n-3) indicates docosahexaenoic acid; 22:5 (n-6), docosapentaenoic acid (n-6); 20:5 (n-3), eicosapentaenoic acid; and 20:4 (n-6), arachidonic acid.

The current and potential world market for fish oil products spans a number of sectors from unprocessed, oil-rich biomass for animal feeds, to high-quality food-grade oils for use as food additives and nutraceuticals, and to very-high-purity oils and even individual fatty acids for use in the pharmaceutical industry.

At present, the major commercial source of PUFA-rich oils is fish oil. Annual worldwide production of fish oil has remained stable at approximately 1.3 million tonnes for the past 10 years or so, and is unlikely to rise (Tacon, 1997). The International Fishmeal and Oil Manufacturers Association (www.ifoma.com) estimates that inclusion of fish oil in aquaculture feeds will rise from 380,000 tonnes in 1994 to 582,000 tonnes in 2001 and 1,133,000 tonnes in 2010. This could well result in a worldwide undersupply of fish oil, leading to increased demand for fish oil alternatives.

Fish oil is included in aquaculture feeds as a source of both dietary energy and PUFA (New and Csavas, 1995). Considerable research is occurring worldwide in an effort to find alternatives to fishmeal and fish oil in aquaculture feeds. However, this research is tempered by the obligate dietary requirement of many marine finfish species for long-chain PUFA (LCPUFA: e.g., EPA and DHA). It seems likely that cheap, plant- or animal-derived oils, which often contain low levels of LCPUFA, will be used increasingly as alternative sources of energy in some aquaculture feeds. If such substitution does occur, sufficient LCPUFA to meet the dietary requirements of cultured aquaculture species may be required from other sources. Typically, many cultured marine species require around 1% to 2% wt/wt LCPUFA in their diets (e.g., Rees et al., 1994; Salhi et al., 1994). Pike and Barlow (1999) estimated that marine aquaculture finfish species will require about 2×10^6 tonnes of feed in 2010. These figures point to a potential demand, for these species alone, for at least 20,000 tonnes of LCPUFA per annum.

The imprecise boundaries surrounding the nutraceutical market make estimating the size of this market sector more difficult. Sales of marine supplement oils were in the order of \$55 million in the United States in 1996 (Molyneaux and Chong, 1998), and represented 20% of sales from health food retail outlets. In the United Kingdom, fish oils account for approximately 29% (U.S. \$140 million) of the total annual market for nutraceuticals (Mukherjee, 1999). Analysis of consumer awareness and knowledge indicates n-3 PUFAs rate highly in both market perception and potential market success.

The western European market for infant formula in-

creased from 81,500 tonnes in 1988 to 103,933 tonnes in 1994. There is an increasing trend for infant formula manufacturers to include PUFA-rich oils in their products. Typical inclusion levels of PUFA-rich oils are designed to achieve a final DHA concentration in dry infant formula of 0.1% to 0.2% wt/wt. Extrapolating these figures suggests a potential annual demand in the European infant formula market for up to 100 to 200 tonnes of DHA.

Already several food and beverage products enriched with DHA or other PUFAs are on the market. Mukherjee (1999) reported the availability of products such as enriched spreads, breads, eggs, and soft drinks in Europe and Japan. Bread enriched with refined tuna oil as a source of LCPUFA is achieving substantial market penetration in Australia. As awareness, by both consumers and regulators, of the importance of adequate levels of PUFA in our diet increases, it can be assumed that demand for a greater range of PUFA-enriched products will increase.

THE POTENTIAL OF THRAUSTOCHYTRIDS

Large-scale culture of thraustochytrids has real potential to be developed further as a commercial source of PUFA. If and where thraustochytrid-derived products are to fit into the market will be determined by our ability to produce, refine, or enrich the oils to meet market specifications.

Thraustochytrids are already being used for commercial production of PUFA-rich products. A *Schizochytrium* strain is the basis for two products marketed for enriching rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) with PUFA, 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 products. It is possible, however, that thraustochytrids will offer some advantage over other oils as sources of PUFA for aquaculture. Many aquaculture species require proportionally more DHA than EPA in their diet (Narciso et al., 1999). The PUFA profiles of many thraustochytrids fit this criterion, while most oils from the fish meal industry contain more EPA than DHA.

Other uses of thraustochytrid oil are being actively explored. Monsanto (www.monsanto.com) is producing *Schizochytrium* sp.-derived oil under a cooperative technology agreement with OmegaTech (www.omegadha.com). This oil is currently being used as a feed ingredient for laying hens to produce DHA-enriched eggs, and is under

investigation for other food applications (Fitch Haumann, 1999).

Ratlidge (1993) stated that an increase in demand for pure PUFA preparations (e.g., oils containing EPA or DHA only) is likely to be the driving force behind any commercial success of microbial oils. Oils derived from fish and microalgae generally have a complex fatty acid (total and polyunsaturated) profile, and do not readily lend themselves to the isolation of high-purity (>98%) fatty acids. Conversely, oils produced by some thraustochytrids have relatively simple fatty acid profiles (e.g., *Schizochytrium limacinum*; Yokochi et al., 1998) and may well be more amenable to cost-effective refinement.

Development of economically viable technologies for the production of microbial PUFA for aquaculture, livestock, and human diets is the subject of intense worldwide research at present. Given the potential for significant economic gain by those funding the research, much of the data and results being generated have not been released to the scientific community. However, selected information is available via the Internet (e.g., www.aquafauna.com; www.omegadha.com; www.zenecal-sm.com; www.monsanto.com).

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:

First is the collection, screening, and maintenance of PUFA-producing strains. Several strains with potential for the commercial production of DHA-rich oils have been isolated already. However, if thraustochytrids are isolated and optimized that produce higher yields, more attractive PUFA profiles, or other less common but sought after PUFAs, then demand for these isolates and compounds may well increase.

Second, efficiency of PUFA production must be optimized. 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 be also considered. Different markets will provide demand for strains that produce high levels of PUFA measured either in terms of biomass (i.e., PUFA production wt/wt cell mass) or volume (i.e., PUFA production wt/vol fermentation medium).

Third, appropriate conditions for long-term storage of microbial cells and their products must be determined. The

form and stability of thraustochytrid biomass and of oils will be major factors in determining the suitability of these products for use as food additives.

Finally, oil extraction and refinement technologies must be developed to meet market demands for cost-effective and safe trophic transfer of PUFA to the target consumers. 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 is, or will soon be, economically feasible. However, the commercial success of value-added oil products, for which the market is willing to pay, is yet to be proved.

In summary, recent research has clearly demonstrated thraustochytrids as either an already usable or potential source of PUFA-containing biomass and oils. Although this short review has concentrated on PUFA production, the high growth rates and biomass already achieved with some thraustochytrid strains suggests that these organisms could have wider use as cell factories for the generation of other products. Further research and development on the PUFA-producing thraustochytrids is necessary to allow increased transfer of this knowledge to the biotechnology and associated industries. Similarly, increased market acceptance of, and demand for, specific omega-3 products, including within a range of nutraceuticals and functional foods, will require increased knowledge of specific nutritional and health benefits of these oils.

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