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MINI-REVIEW

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Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid

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Abstract Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid composed of 22 carbon atoms and six double bonds. Because the first double bond, as counted from the methyl terminus, is at position three, DHA belongs to the so-called ω-3 group. In recent years, DHA has attracted much attention because of its beneficial effect on human health. At present, fish oil is the major source of DHA, but alternatively it may be produced by use of microorganisms. Marine microorganisms may contain large quantities of DHA and are considered a potential source of this important fatty acid. Some of these organisms can be grown heterotrophically on organic substrates without light. These processes can be well controlled and DHA with constant quality can be produced all year round. This paper reviews recent advances in the biotechnological production of DHA by marine microorganisms.

Introduction

Lipids (oils and fatty acids) are indispensable for the growth and survival of all organisms. They are important structural components of membranes and, in many organisms, play a crucial role in energy storage. Furthermore, several unsaturated fatty acids can act as precursors for eicosanoids. Natural sources of lipids include plants, animals and microorganisms. Over history, oils from plants (e.g. olive oil, sunflower oil) and animals (e.g. butter, lard) are well known, mostly for their applications in nutrition. Currently, plant oils account for the majority of the natural oils and fats on the world market and account for about 83×10^6 t annually (Gunstone 2001). Plant oils are relatively cheap and are generally considered to be healthier than animal fats, due to their relatively high amounts of unsaturated fatty acids. During the past three decades, however, the interest in some specific animal oils [the long-chain polyunsaturated fatty acids (PUFAs), as present in fish oils] has increased considerably, due to their beneficial health effects.

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Polyunsaturated fatty acids

Long-chain PUFAs are composed of a long hydrocarbon chain (18 or more carbon atoms) and a terminal carboxylate group having two or more double carbon bonds. They are classified according to the position of the first double bond, as counted from the methyl terminus. A so-called ω-3 PUFA has its first double bound at position 3, as counted from the methyl terminus. Other PUFA groups are ω-6, where the first double bond is located six carbons from the methyl terminus, and ω-9, where the first double bond is located nine carbons from the methyl terminus. As a synonym of ω, the symbol *n* is often used to classify PUFAs. Double bonds in PUFAs may also be counted from the carboxylate group and are then represented by the symbol Δ. In Table 1 several ω-3 PUFAs are listed. α-Linolenic acid (18:3 Δ9,12,15), eicosapentaenoic acid (EPA, 20:5 Δ5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6 Δ4,7,10,13,16,19) are the most studied PUFAs within this group. The chemical structures of DHA and EPA are shown in Fig. 1. In living cells, EPA and DHA are normally esterified to form lipid molecules.

Health aspects of ω-3 PUFAs

The position of the double bond in the fatty acids strongly affects the properties of its derivatives. For instance, eicosanoids derived from the ω-6 PUFA arachidonic acid

Table 1 List of ω-3 polyunsaturated fatty acids

Common name	Systematic name (with all double bonds in *cis*-configuration)	Short name
α-Linolenic acid	Δ9, Δ12, Δ15-Octadecatrienoic acid	ω-3 18:3
–	Δ6, Δ9, Δ12, Δ15-Octadecatetraenoic acid	ω-3 18:4
–	Δ8, Δ11, Δ14, Δ17-Eicosatetraenoic acid	ω-3 20:4
Eicosapentaenoic acid	Δ5, Δ8, Δ11, Δ14, Δ17-Eicosapentaenoic acid	ω-3 20:5
–	Δ7, Δ10, Δ13, Δ16, Δ19-Docosapentaenoic acid	ω-3 22:5
Docosahexaenoic acid (DHA)	Δ4, Δ7, Δ10, Δ13, Δ16, Δ19-Docosahexaenoic acid	ω-3 22:6
–	Δ5, Δ8, Δ11, Δ14, Δ17, Δ20-Tetracosahexaenoic acid	ω-3 24:6

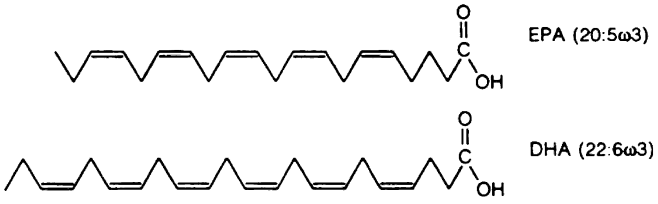

Fig. 1 Schematic representation of docosahexaenoic acid (*DHA*; ω-3 22:6) and eicosapentaenoic acid (*EPA*; ω-3 20:5)

(20:5 Δ5,8,11,14) have strong inflammatory properties, whereas those produced from eicosapentaenoic acid are anti-inflammatory (Gill and Valivety, 1997).

DHA recently attracted much attention because of its various physiological functions in the human body. DHA is an essential component of cell membranes in some human tissues and, for instance, accounts for over 60% of the total fatty acids in the rod outer segment in the retina (Giusto et al. 2000). Furthermore, DHA is regarded to be essential for the proper visual and neurological development of infants, because of its role as a structural lipid component (Nettleton 1993; Crawford et al. 1997; Das and Fams 2003). As pre-term and young infants are unable to synthesize DHA at a rate fast enough to keep up with the demand from the rapidly growing brain (Crawford 1987), they should obtain these compounds from their diet. In general, breast-feeding serves as a good source of PUFAs (Huisman et al. 1996). However, although it was recommended that all infant formulas include DHA (FAO/WHA Expert Committee 1994), the application of DHA in some infant formulas only started recently.

In addition, DHA reduces or inhibits risk factors involved in various diseases like cardiovascular diseases (Kromann and Green 1980; Kang and Leaf 1996; Nordøy et al. 2001) and has some positive effects on diseases such as hypertension, arthritis, arteriosclerosis and thrombosis (Horrocks and Yeo 1999). Although the optimal intake of PUFAs has not yet been established, there is some consensus that the PUFA intake should be at least 3% of the total lipid intake (Gill and Valivety 1997). Studies suggest that, while total fat levels in the typical Western diet are too high, the intake of long-chain ω-3 PUFA is too low (Newton 1998). At present, most consumed PUFAs originate from plant oils and belong to the ω-6 group. In order to redress the fatty acid balance generally seen as optimal for human health, an increase in ω-3 PUFA consumption and a reduction in ω-6 PUFAs is needed. The British Nutrition Foundation (1992) recommends a ω-6 to ω-3 PUFA ratio between 5:1 and 3:1.

Current sources of DHA

The main sources of ω-3 PUFAs, including DHA and EPA, are fatty fish species, such as herring, mackerel, sardine and salmon (Gunstone 1996). The quality of the fish oil, however, is variable and depends on fish species, season and location of catching sites. The application of fish oil PUFAs in foods, for inclusion in infant formulas, or for pharmaceutical applications, may have some disadvantages because of contamination of the fish oil by environmental pollution and problems associated with the typical fishy smell and unpleasant taste. Furthermore, as marine fish oil is a complex mixture of fatty acids with varying lengths and degrees of unsaturation, expensive DHA purification may be required before application.

At present, fish oil production amounts to about 1.1×10^6 t annually (Gunstone 2001), of which 70% is utilized for the production of fish feed for farmed fish (Tuominen and Esmark 2003). The demands for ω-3 PUFAs are rapidly increasing, due to a rapid increase in aquaculture and applications in food and pharmacy. It is therefore expected that, within 10 years, the production of PUFAs from current sources will become inadequate for supplying the expanding market. In order to meet the expected rise in demand and to circumvent the detrimental aspects of fish oils, alternative production processes for PUFAs are currently being developed. These include the development of techniques for refining fish oils (Yamamura and Shimomura 1997) and the exploitation of microbial PUFA sources (Barclay et al. 1994; Kyle 1996; Ratledge 2001; de Swaaf 2003) which may offer a sustainable production of ω-3 PUFAs.

Microbial production of PUFAs

As a source of oil or, in more general terms, lipids, microorganisms are less well known than plants and animals. Microbial oil or single-cell oil (SCO) production is a relatively new concept, first proposed in the twentieth century (Ratledge 2001). In SCO processes, microorganisms that are able to produce the desired oil are cultivated in a bioreactor.

As the prices for most bulk plant oils are relatively low and animal fats are even cheaper, it is likely that processes for the microbial production of oils should focus on high-value-added products that cannot in the near future be produced in bulk quantities by plants. Earlier attempts to commercially produce SCOs failed because of economics (Davies 1992; Nakahara et al. 1992; du Preez et al. 1995; Ratledge 2001), but the SCOs concept has now yielded several successes with regard to PUFAs and industrial interest is increasing (Barclay et al. 1994; Kyle 1996, 1997; Ratledge et al. 2001b; de Swaaf 2003).

Based on their percentage of PUFAs, oleaginous marine microorganisms such as microalgae or marine fungi may be interesting alternatives for fish oils (see Table 2). At present, the contribution of microbial PUFAs to the oil industry is nearly negligible, but there are several reasons to increase their use in the near future:

1. Properly selected microbial PUFAs may have a high selling price.
2. Microbial sources can supply oils with a high PUFA content.
3. The oxidative stability of microbial lipids in general is high, compared with fish oils.
4. PUFAs can be produced from sustainable raw materials.
5. Furthermore, knowledge on the biochemical pathways and genetics may provide tools for the development of new, interesting production systems or products.

Microorganisms, in particular the marine algae and fungi, are thought to be the primary producers of ω-3 PUFAs in the marine food chain. Although marine fish and mammals appear to have some capacity for de novo biosynthesis of ω-3 PUFAs, the majority of the PUFAs in their body originates from their diet (Ackman et al. 1964).

Microorganisms capable of producing PUFAs above C_{20} compounds include lower fungi, bacteria and marine microalgae (Bajpai et al. 1991; Kendrick and Ratledge 1992; Gunstone et al. 1994; Kyle 1996, 1997; Vazhappily and Chen 1998; Ratledge 2001; de Swaaf 2003). Bacteria, however, are probably not suitable as PUFA producers, as they do not accumulate high amounts of triacylglycerols and may contain unusual fatty acids and lipids not found in other systems (Ratledge 2001).

Oleaginous microorganisms could provide an economically feasible source of PUFAs, provided that most of the PUFAs occur in triacylglycerols which is the preferred form to take lipids within the diet (Kendrick and Ratledge 1992). Furthermore, microorganisms preferably contain one specific PUFA, rather than a mixture of various PUFAs. This gives the microbial oils an additional value as compared with fish oils, which contain mixtures of PUFAs. The development of a microbial PUFA production process requires the selection of the proper microorganism and optimized cultivation techniques (Ratwan 1991). The exploitation of marine microorganisms for the production of DHA is discussed in the next section.

DHA production by marine algae and fungi

Currently, the production of DHA by marine microorganisms is the subject of intensive research and increasing commercial attention (Barclay et al. 1994; Kyle 1996, 1997; Ratledge 2001; de Swaaf 2003). In order to select appropriate strains for DHA production, important parameters to be considered include the specific growth rate, the biomass production under optimal culture conditions, the total lipid content and the DHA proportion of the lipid. Furthermore, for downstream processing, it is important to know whether DHA is present as part of the membrane

Table 2 Percentage of fatty acids with 14 or more carbon atoms within the lipids of selected marine microorganisms. *H* Heterotrophic growth, *P* phototrophic growth

Organism	14:0	14:1	15:0	16:0	16:1	18:0	18:1	18:2	18:3	18:4	ω-6 20:4	ω-3 20:5	ω-6 22:5	ω-3 22:6
Thraustochytrium aureum[a] (H)	3			8			16	2	2		3			52
Schizochytrium sp.[b] (H)	4		3	55		1							6	30
Crypthecodinium cohnii[c] (H)	17			17	1	2	10							44
Amphidinium carterae[d] (H)	8	30		15	5	3	5	6	17			4	4	2
Isochrysis galbana[e] (P)	12			10	11	1	3	2		11		25		11
Skeletonema costatum[f] (P)	17			17	11		2	1		6		41		7
Amphidinium sp.[g] (P)	5			27		18	17	2	2			8		17
Pavlova lutheri[h] (P)	14			11	10	–	3	–		4		12		7

[a] Singh and Ward (1996)
[b] Yokochi et al. (1998)
[c] de Swaaf et al. (1999)
[d] Vazhappilly and Chen (1998)
[e] Molina Grima et al. (1993)
[f] Servel et al. (1994)
[g] Viso and Marty (1993)
[h] Meireles et al. (2002)

structure, e.g. in phospholipids, or as part of triacylglycerols in the cytosol and whether or not other PUFAs are present. So far, of the >30,000 defined species of microalgae, only a limited number have been analyzed to determine their lipid composition and fatty acid profiles (Cohen et al. 1995). Examples of marine DHA producers are presented in Table 2 and include both phototrophic and heterotrophic strains. For commercial DHA production, several systems may be considered. The oldest and simplest systems for cultivation of phototrophic algae are open ponds. These cultivation systems are dependent on the weather and climate. The product quantity and quality of separate batches is therefore variable. The processes are time-consuming, due to the low specific growth rates of algae; and the available light limits the biomass concentrations attainable. Due to contamination with bacteria and predation by protozoa, phototrophic cultivation in open ponds is only feasible when suitable selective environments can be used (e.g. high salinity). Furthermore, due to the low biomass concentrations, harvesting costs are relatively high (Barclay et al. 1994). In closed photobioreactors with sunlight or lamps as light sources, the environmental parameters can be better controlled, allowing for higher biomass concentrations and a reduced contamination risk. Scale-up of the process is however limited by the ability to effectively introduce enough light (Pulz 2001). In general, the costs of algal production in mass culture in such fermentors are high. Alternatively, heterotrophic microalgae growing on reduced carbon sources have been considered for the production of specialty SCOs (Barclay 1991; Kyle 1994, 1996; Mukherjee 1999). In heterotrophic cultures: (1) optimal and axenic conditions can be maintained (Chen 1996), (2) oil production can be carried out throughout the year as there is no seasonal or climatic dependence, (3) the process can be controlled and product quality guaranties can be given, (4) high cell densities, over 100 g dry weight l^{-1}, can be achieved (de Swaaf et al. 2003a) and (5) the technology to deal with heterotrophic fermentation is widely available.

For DHA production by heterotrophic marine microorganisms, however, several challenges must also be faced.

1. So far, only a limited number of heterotrophic species are available.
2. Due to the required rich media and the relatively low growth rates of marine microorganisms, the risk of contamination is an issue.
3. The production costs per kilogram DHA should be well below market prices.
4. Legislation and safety items need to be considered.

DHA production by heterotrophic marine microorganisms

In Table 2, fatty acid profiles of several heterotrophic and phototrophic marine microorganisms are shown. *Schizochytrium* spp (Barclay et al. 1994) and *Crypthecodinium cohnii* (Kyle 1994, 1996; Radledge et al. 2001a, 2001b; de Swaaf 2003) are currently used in commercial processes for the heterotrophic production of DHA.

Schizochytrium sp., a traustrochytrid, is an alga-like microorganism which can contain over 70% of its weight as lipids and have a DHA content equal to 35% of the total fatty acids. Over 90% of the lipids are neutral lipids (Yaguchi et al. 1997). About a decade ago, Omega Tech (recently acquired by Martek Biosciences, Columbia, Md., USA) developed a process in which a *Schizochytrium* strain grew to cell densities of 20 g l^{-1} in 48 h. The biomass contained 10% of their weight as ω-3 PUFA (Barclay 1991). This process forms the basis for the commercial cultivation protocol with a *Schizochytrium* strain currently used at Martek Biosciences. The details of this protocol have not been published.

A related process was developed in Japan by Nagase Biochemical Industries, using a strain named *Schizochytrium* sp. strain SR21 (Nakahara et al. 1996; Yaguchi et al. 1997; Yokochi et al. 1998). In a bioreactor, this strain was able to produce 48.1 g dry cells l^{-1} and 13.3 g DHA l^{-1} in 4 days (Yaguchi et al. 1997). More recently, several traustochytrids were isolated from a variety of biotopes (Bowles et al. 1999; Fan et al. 2001; Huang et al. 2001). These strains produced yields ranging from 1.6 g DHA l^{-1} after 41 h cultivation (Bowles et al 1999) to 2.7 g DHA l^{-1} in about 52 h (Fan et al. 2001). The DHA production rates of different strains and cultivation conditions are presented in Table 3.

In addition to DHA, *Schizochytrium* strains also produce relatively high amounts of ω-6 docosapentaenoic acid (DPA) and some odd fatty acids, such as 15:0

Table 3 DHA productivities of heterotrophic marine microorganisms. *DCW* dry cell weight, *S* Shake-flask cultivation, *B* bioreactor

Strain	Device	Carbon source	Cell concentration (g DCW l^{-1})	DHA concentration (g l^{-1})	DHA productivity (mg l^{-1} h^{-1})	Reference
C. cohnii	B	Glucose	27.7	1.4	19	de Swaaf et al. (1999)
C. cohnii	B	Acetic acid	109.0	19.0	48	de Swaaf et al. (2003a)
C. cohnii	B	Ethanol	83.0	11.7	53	de Swaaf et al. (2003b)
Thraustochytrium strain G13	B	Glucose	14.0	1.6	38	Bowles et al. (1999)
Schizochytrium sp.	S	Glucose	13.3	2.8	53	Fan et al. (2001)
Schizochytrium sp. SR21	S	Glucose	36.0	4.2	35	Yokochi et al. (1998)
Schizochytrium sp. SR21	B	Glucose	48.1	13.3	138	Yaguchi et al. (1997)

(Nakahara et al 1996; Yokochi et al 1998; Ratledge 2001). In mammalian systems, ω-6 DPA cannot be converted to DHA and does not have the same functionality as DHA. Research on whether DPA has positive, negative or neutral health effects is still in progress (Gawrisch and Eldho 2002; Zeller et al. 2002). At present, *Schizochytrium* strains are mainly used as poultry feed additives to give DHA-enriched eggs and as a feed for aquaculture.

The marine dinoflagellate *C. cohnii* can produce high percentages of DHA (25–60%), whereas other PUFAs represent less than 1% of the derived oil (Harrington and Holz 1968; Beach and Holz 1973; de Swaaf et al. 1999; Ratledge et al 2001a; de Swaaf 2003). Starting in the early 1990s, this organism was developed by Martek Biosciences Corp. as a commercial source of oil rich in DHA. Since then, several patents have been filed to protect their process and product (e.g. Kyle 1994; Kyle et al. 1995, 1998). An overall description of the process was provided by Kyle (1996). An axenic culture of *C. cohnii* is cultivated in large-scale bioreactors, while environmental parameters like temperature, pH, air flow, pressure, agitation and dissolved oxygen are well controlled. Although details of the current commercial process have not been published, the organism is most likely cultivated with glucose as the principal carbon source.

Recently, de Swaaf et al. (1999) gave a detailed description of a process for DHA production by *C. cohnii* ATCC 30772, with glucose as carbon source. In these experiments, the biomass increased from 1.5 g l^{-1} to 27.7 g l^{-1} in 74 h; and the total amounts of lipid and DHA after 91 h were 3.7 g l^{-1} and 1.6 g l^{-1}, respectively. However, compared with glucose, the use of acetic acid and ethanol as carbon sources proved to be much more efficient with respect to DHA production (Ratledge et al 2001a; de Swaaf 2003; de Swaaf et al. 2003a, 2003b). Laboratory-scale, pH-controlled, fed-batch cultivations of *C. cohnii* ATCC 30772, a so-called pH auxostat culture with 50% acetic acid as carbon source, achieved DHA productivities up to 38 mg l^{-1} h^{-1} (Ratledge et al. 2001a; de Swaaf et al 2003a). In comparison with several other *C. cohnii* strains, ATCC 30772 appeared to be the best strain with respect to DHA production (Ratledge et al 2001a). The productivity of DHA by *C. cohnii* ATCC 30772 could be even further increased by the use of pure acetic acid and prolonged cultivation periods (de Swaaf et al. 2003a). This resulted in cultures producing a dry weight of 109 g l^{-1}, 61 g lipid l^{-1} and 19 g DHA l^{-1}. The maximum overall productivities of lipid and DHA were 152 mg l^{-1} h^{-1} and 48 mg l^{-1} h^{-1}, respectively. Vigorous mixing was required to sustain a sufficient oxygen level during these high-cell-density cultivations. This was complicated by culture viscosity, which resulted from the production of viscous extracellular polysaccharide (de Swaaf et al. 2001). The addition of a commercial polysaccharide-hydrolase could decrease the viscosity of the culture and reduce the stirring required (de Swaaf 2003a).

A further improvement was achieved by the development of an ethanol fed-batch protocol. In shake-flask cultures, the specific growth rate was optimal with 5 g ethanol l^{-1}; and growth did not occur without ethanol or with >15 g ethanol l^{-1}. In a fed-batch cultivation of *C. cohnii* with pure ethanol as feed, 83 g dry biomass l^{-1}, 35 g lipid l^{-1} and 11.7 g DHA l^{-1} were produced in 220 h. The overall volumetric productivity of DHA in this process was 53 mg l^{-1} h^{-1}, the highest value reported so far for this alga (de Swaaf et al 2003b; Table 3).

Selection of carbon source for efficient DHA production

In commercial microbial cultivation processes, glucose or other sugars are often used. Glucose is relatively cheap and often readily utilized by heterotrophic microorganisms.

As mentioned above, compared with glucose, the usage of acetic acid and ethanol as carbon sources resulted in far superior lipid and DHA productivities by *C. cohnii*. This may be explained by acetyl-CoA metabolism. The synthesis of fatty acids is a cytosolic process with acetyl-CoA as the basic building block (Ratledge and Evans 1989). After the synthesis of 16:0 or 18:0 fatty acids by the fatty acid synthetase system, elongation and desaturation reactions can lead to various mono-unsaturated fatty acids and PUFAs. Alternatively, PUFAs may be produced by polyketide synthetase (Metz et al. 2001). The routes of supply of cytosolic acetyl-CoA depend both on the carbon source used for growth and on the organism (Ratledge and Evans 1989). Simplified, the main flux of carbon from glucose to cytosolic acetyl-CoA in oleaginous yeasts (and probably in other oleaginous eukaryotes) involves glycolysis, transport of pyruvate into the mitochondrion, conversion of pyruvate into citrate, transport of citrate into the cytosol and cleavage of citrate by ATP: citrate lyase to yield acetyl-CoA (Fig. 2). In theory, acetyl-CoA may be supplied in the cytosol in a more direct way by cultivation of the organism on C_2 compounds, like acetate and ethanol. The conversion of acetate into acetyl-CoA involves a one-step enzymatic reaction catalyzed by the enzyme acetyl-CoA synthetase (Fig. 2). Acetyl-CoA synthetase has been localized in the mitochondrion, microsomes (Klein and Jahnke 1971) and cytosol (Kispal et al. 1991) of *Saccharomyces cerevisiae*. The cytosolic activity of acetyl-CoA synthetase has also been detected in mammals (Knudsen et al. 1992), insects (Storey and Bailey 1978) and plants (Gerbling et al. 1994). The utilization of ethanol by *C. cohnii* could suggest the presence of an alcohol dehydrogenase, which converts ethanol to acetaldehyde, and an acetaldehyde dehydrogenase, which converts acetaldehyde to acetate. As *C. cohnii* is able to use acetate for its DHA production (de Swaaf et al. 2003c), it seems interesting to investigate the applicability of C_2 compounds as carbon sources for lipid accumulation by other oleaginous eukaryotes too.

Applications and future aspects

The intake of ω-3 PUFAs via our diet occurs mainly via the consumption of sea food, which is characteristically rich in ω-3 PUFAs. The average intake varies among populations. Intake is high among the Inuit in Greenland (10–14 g day^{-1}), intermediate in countries such as Japan and Norway (1–3 g day^{-1}) and low in most Western populations (<0.5 g day^{-1}; Schmidt et al. 2001). In an expert panel, there was a general agreement that two fish-based meals per week is a healthy dietary habit to obtain sufficient ω-3 PUFAs. In practice, this does not often occur in Western diets (Nordøy et al. 2001).

In order to provide additional omega-3 fatty acids, fish oil capsules are available. Furthermore, PUFAs are included in the diet of livestock to raise the PUFA content of their products. For example, eggs and milk enriched with DHA are on the market (Horrocks and Young 1999). At present, Martek Biosciences Corp. uses *C. cohnii* to manufacture oils that contain high DHA levels for inclusion in infant formulas. Capsules containing DHA are sold as neutraceuticals (Kyle 1996, 1997; http://www.martekbio.com, visited 30 June 2003).

In addition to the use of DHA for health applications, a strong demand for DHA (and other PUFAs) results from the introduction of large-scale marine fish farms. The normal growth and development of several marine fish larvae depend on the supplementation of ω-3 PUFAs in the diet, particularly DHA and eicosapentaenoic acid (Rodríguez et al. 1998). At present, over 10^6 t fish oil are produced, of which over 70% is currently used for aquaculture (Tuominen and Esmark 2003). When these oils contain, on average, 10% DHA, this amount of oil equals over 10^5 t DHA. Based on the best DHA production data described so far (138 mg DHA l^{-1} h^{-1}; Table 3), one bioreactor with a volume of 200 m^3 and operating for 300 production days could produce 200 t DHA annually. These calculations indicate that about 10% of the DHA currently available in fish oils could be replaced with 50 large bioreactors.

The large-scale application of microbial DHA or other PUFAs in human nutrition and animal feeds, however, depends on the quality and the production costs of the oils. The quality of microbial oils can be kept high by proper selection of the microorganism and by well controlled production methods. Due to the high quality and relative high cost price, microbial oil rich in DHA is currently applied mainly in infant formula and in pharmaceutical and neutraceutical products. Calculated on the amount of DHA, a product like Neuromin is currently sold for about € 2,000–3,000 kg^{-1} DHA. It is clear that DHA generates the high added-value in these products.

Compared with microbial oils, oils rich in DHA can be obtained more cheaply from fish oils. In order to deal with the drawbacks and improve the application of fish oils, various companies are developing methods to increase the quality of these fish oils. Therefore, to be or remain competitive and to enlarge the application areas, the production costs of microbial DHA have to decrease.

The scale of cultivation and the volumetric productivity (r_{DHA}) have been identified as the major factors determining the production costs of fermentative DHA production (Sijtsma et al. 1998). Factors that determine r_{DHA} are biomass concentration, the lipid content of the cells, the DHA content of the lipid and the cultivation

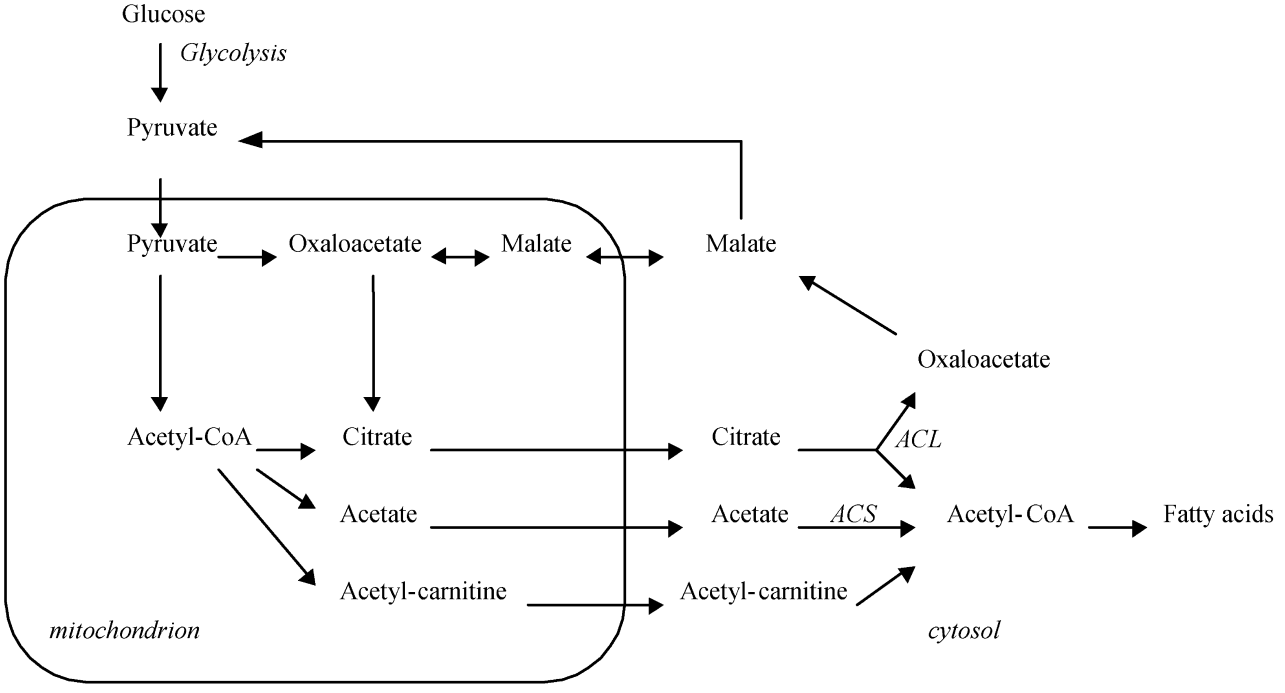

Fig. 2 Acetyl-CoA metabolism in oleaginous yeasts (de Swaaf 2003; modified from Ratledge and Evans 1989). *ACL* ATP:citrate lyase, *ACS* acetyl-CoA synthetase

time. Obviously, a high DHA content of the biomass is also desirable from the viewpoint of product recovery.

Consequently, new and promising processes developed on a laboratory scale, such as e.g. the described ethanol process for *C. cohnii*, need to be scaled-up to an industrially relevant scale (>50 m^3).

For the future, screening many of the yet-unknown marine microorganisms may result in strains that are even better DHA producers than the ones we know now. Once novel strains have been identified, efficient production techniques, also including a detailed knowledge of lipid metabolism and the genes involved, should be developed. In addition, it may be possible to modify several very good microbial oil producers (e.g. the yeast *Cryptococcus curvatus*), which do not yet produce relevant PUFAs, in such a way that they are able to produce the desired PUFAs. Genetic engineering of these organisms may potentially lead to the production of tailor-made oils at lower costs. Therefore, for researchers in many different disciplines, it will be a challenge to explore the potential of marine microorganisms for biotechnological applications within the next years.

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