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Research Article

MICROBIAL PRODUCTION OF DOCOSAHEXAENOIC ACID (Ω-3 PUFA) AND THEIR ROLE IN HUMAN HEALTH

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ABSTRACT

Production of polyunsaturated fatty acids (PUFAs) has attracted researchers for their various biomedical advantages and formulation approaches used to systemically produce maximum quantity of PUFAs. Docosahexaenoic acid (DHA) is a ω-3 polyunsaturated fatty acid consisting of long hydrocarbon chain of 22 carbon and 6 double bonds. DHA has been traditionally obtained from fish oils but number of problems with fish oils including highly saturated fatty acid content, odor, taste, stability and a complicated purification process. Alternatively, some oleaginous microorganisms have the ability to synthesize long-chain polyunsaturated fatty acid (PUFA). In recent years, DHA has attracted much attention because of its beneficial effect on human health. This article deals with the innovations pertaining to production and nutritional benefits of DHA.

Keywords: Polyunsaturated fatty acids (PUFAs), Docosahexaenoic acid (DHA), Hydrocarbon chain.

INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur such as ω-3 Polyunsaturated fatty acids (ω -3 PUFAs) are a group of fatty acids containing two or more double bonds, of which the last double bond is located at the third carbon atom from the methyl terminal. Docosahexaenoic acid (DHA, 22:6) is a particularly important ω -3 PUFA, with a 22-carbon chain and six double bonds.

It has been reported that DHA is an essential nutrient during early human development [1,2]**.** Thraustochytrids are a group of non-photosynthetic, heterotrophic, marine fungoid protists. It uses nitrogen for their growth and accumulates the lipid by using carbon source. They have been characterized by the presence of a seaenogenetosome, an ectoplasmic net, a cell wall with non cellulosic scales, and a life cycle consisting of vegetative cells, zoosporangium and zoospores. In their classification history, the taxonomic criteria were mainly based on morphological characteristics. However, their evolutionary relationship [3] and taxonomy [4] were not well understood. Some species of the thraustochytrid produce significant quantities of docosahexaenoic acid (DHA).

DHA, along with other long chain (LC-, those fatty acids with carbon chain lengths greater than 18) PUFAs, have been identified as important dietary compounds for humans. *Schizochytrium* species belongs to the order thraustochytrid and is a spherical unicellular microorganism as shown in fig. 1.1. Microbial oil or single cell oil (SCO) production is a relatively new concept, first proposed in the twentieth century [5].Microorganisms capable of producing polyunsaturated fatty acid (PUFA) above C_{20} compounds include lower fungi, bacteria and marine micro algae [6,7,8]. 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[5]. This review briefly summarizes present applications and emerging areas in biotechnology of thraustochytrids, besides suggesting future directions for research. Fish oil is principally the sole conventional source of DHA commercially available; however, its low level of DHA makes it difficult to obtain in purified form.

Some species that belong to the genus Thraustochytrium (Labyrinturomycota) have a high content of DHA [9] as well as a low content of polyunsaturated fatty acids structurally similar to DHA, and were expected to be sources for microbial DHA production [10]. Furthermore microorganisms preferable contain one specific PUFA, rather than a mixture of PUFA. The development of a microbial PUFA production process requires the selection of the proper microorganism and optimized cultivation techniques [11]. In our laboratory we produced DHA exclusively for this purpose from microorganism: *Schizochytrium* using different type of fermentation parameters *viz*. production of DHA by glucose fed batch, acetic acid fed batch, sodium acetate fed batch etc. [12].

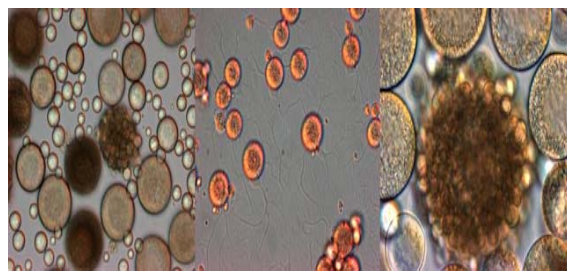

Fig. 1: Isolated Cells of *Schizochytrium* showing 40 x, 10x & 100x Microscopic view of numerous lipid bodies

Nutritional Benefits

DHA recently attracted much attention because of its various physiological functions in the human body. It 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 [13].

DHA and eicosapentaenoic acid (EPA, 20:5), are implicated in early neural and retinal development that's why essential for the proper visual and neurological development of infants, Furthermore, they are used against the prevention of arteriosclerosis and coronary heart disease, alleviation of inflammation, and retarding the growth of tumor cells. It has been shown to have beneficial effects on preventing human cardiovascular diseases, cancer, schizophrenia, and Alzheimer's disease [14,15,16].

Moreover, it has been demonstrated that base-line blood levels of long-chain n-3 fatty acids were inversely related to the risk of sudden death due to cardiovascular disease [17]. The fatty acids can be extracted from algae and used in fortified foods, or the biomass can be used directly as a feed additive in various animal industries such as aquaculture [18] or poultry [19]. In the aquaculture industry, n-3 PUFAs are essential nutrients for cultured marine fish. Currently, the aquaculture industry is experiencing rapid increases in fish oil price due to flat supply and increased global demand for this commodity. In fact, the Food and Agriculture Organization (FAO) of the United Nations predicts that fish oil demand in 2015 will be 145% of historical global production capacity [20].

Numerous experimental studies have indicated the fact that only a low dose of n-3 PUFA (20 mg/kg/day) is necessary to afford protection against cardiac arrhythmia [21]. 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 [22], they should obtain these compounds from their diet. In general, breast-feeding serves as a good source of PUFAs[23].

It has some positive effects on diseases such as hypertension, arthritis, arteriosclerosis and thrombosis [24]. 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 [25].

DHA supplementation of juice at either 50 mg/day or 100 mg/day for 6 weeks was effective in increasing plasma phospholipid DHA contents of children [26]. Moreover supplementation with AA and DHA may be beneficial in reducing the risk of HIV-1 transmission, particularly during the period of breastfeeding [27]. DHA also can act as breast and colon cancer chemopreventive agents [28, 29].

Culture conditions

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. Thraustochytrids vary largely in biomass production, total lipids, and DHA content [30, 31].

Obtaining the maximum possible biomass with a high amount of total lipids in the shortest possible time is the strategy in media development for DHA production. The best time to harvest for DHA varies generally from 4 to 7 days [32,33].

Ashford used initial medium contained (g/l): sea salts (27), glucose (40), proteose peptone (8), yeast extract (5) and MOPS (21). Further cultures were grown in a defined medium modified from Ashford containing (g/l): glucose (40), glutamic acid monosodium salt (4), NaCl (12.5), $MgSO_4{\cdot}7H_2O$ (2.5), KCl (0.5), $CaCl_2$ (0.1), KH_2PO_4 (0.5), unless stated otherwise. This medium also contained trace element solution (5 ml/l) and vitamin solution (1 ml/l). The trace element solution contained (g/l): EDTA disodium salt (6.0), $FeCl_3{\cdot}6H_2O$ (0.29), H_2BO_3 (6.84), $MnCl_2{\cdot}4H_2O$ (0.86), $ZnCl_2$ (0.06), $CoCl_2{\cdot}6H_2O$ (0.026), $NiSO_4{\cdot}6H_2O$ (0.052), $CuSO_4{\cdot}5H_2O$ (0.002), $Na_2MoO_4{\cdot}2H_2O$ (0.005). The vitamin solution contained (mg/l): thiamine (100), biotin (0.5) and cyanocobalamin (0.5) [34].

Glucose was autoclaved separately according to Ganuza and Izquierdo and feeding medium for continuous culture was filter-sterilized (pore size, 0.2 μm). Static-flask cultures (3–7 days, 27°C) were used to inoculate (10% v/v) shake-flask cultures (250 ml, 120 rpm, 27°C) containing 50 ml of defined medium. The medium used in flask cultures was buffered with 1 M MOPS, and pH was adjusted to 7 by addition of KOH before autoclaving (121°C, 15 min). Shake-flask cultures (24 h) were used to inoculate the fermenters (10% v/v) [35]. Yokochi et al, (1998) used basal medium, consisted of 3.0% glucose and 1.0% yeast extract in half the salt concentration of artificial sea water and optimal culture conditions for the specific strain *Schizochytrium limacinum* SR21 have also been investigated.A high total fatty acids content, up to more than 50%, was obtained with corn steep liquor as the nitrogen source and an increase in carbon source concentration led to a high-DHA yield [36].

Although batch and continuous culture are used most frequently in the laboratory and are most useful for experimental work, industrial processes often use a hybrid of the two techniques called fed batch culture. This involves ordinary batch growth until the substrate is fully utilized, followed by regular additions of fresh substrate, which is then rapidly metabolized.The total culture volume may be allowed to increase or portion may be removed as fresh substrate is added.

This type of culture was developed empirically but microbiologists are now initiating to understand the physiology of growth under such conditions and the reasons for its efficiency in terms of product formation, yield and greater control which, may lead to further advances in industrial microbiology.

Production of DHA

The large numbers of microalgae contain DHA, only a few species have demonstrated production potentials on an industrial scale [37], in that they can accumulate high oil contents in their biomass, produce a high percentage of total lipids as DHA, and reach high biomass densities in a short time.

The DHA content of total lipids was more or less constant in two Thraustochytrium strains, although the total lipid content varied depending on culture conditions. However, this may not always be the case [38]. Thraustochytrids vary largely in biomass production, total lipids, and DHA content [30].

In another research work, a DHA yield of > 4.0 g/L was obtained in both glucose and glycerol media at 9% and 12% concentrations, respectively, which indicated that

S. limacinum SR21 is a promising algae strain for DHA production. High-yield production culture of *Schizochytrium* strains has also been investigated [33].

The new high cell density fed batch fermentation process developed by the commercial sector splits the overall fermentation process into a biomass density increasing stage and a DHA production stage; resulting in biomass densities of at least 100.0 g dry cell/L in the fermentation broth and at least 20% of their dry cell weight as lipids [45].

When studied the effects of incubation time with glucose fed batch on lipid production, it was found that productivity increases as the incubation time increase.

Recently, some researchers reported that crude glycerol can be used to produce docosahexaenoic acid (DHA, 22:5 n-3) through fermentation of the alga *Schizochytrium limacinum.* Developing

A DHA-containing microalga from biodiesel waste glycerol is an excellent opportunity to provide alternative omega-3 sources [46].

Role of constituents in production of DHA

It was found that instead of taking initial high concentration of glucose, addition of glucose in batches enhances the accumulation of lipid. Although batch and continuous culture are used most frequently in the laboratory and are most useful for experimental work, industrial processes often use a hybrid of the two techniques called fed batch culture. Carbon source is must for any organism to survive and have a great impact on the lipid production, when used in various concentrations and also mode of addition. However, the maximum lipids are produced at the end of the exponential or the early stationary phase. The effect of methanol on algal growth is unknown; this leads some researchers to explore the effects of methanol on DHA production by S. *limacinum*. Denver grown *S. limacinum* in a medium containing pure glycerol (70 g/L) with different concentrations of methanol. Considering the fact that methanol evaporates at 65 °C, the autoclave process (at 121 °C for 15 min) resulted in a significant loss of methanol found the negative effects of methanol on growth and DHA production of *S. limacinum*[47].

The effect of salt concentration on growth was examined by Yokochi in the range from 0 to 200% that of sea water and the little change of cell growth was observed in the range 50%±200%. The strain could even grow in medium without salt, and the cell growth obtained in this medium was almost half that in 50% sea water. Culture at 25 °C resulted in the highest dry cell weight.

Table 1: Tabulation of biomass, lipid, and DHA yields in different thraustochytrids

Organism	Biomass (g L−1)	% lipids in biomass	% DHA in lipids	DHA g L−1	Reference
Thraustochytrium aureum ATCC 34304 (4 days growth)	4.9	20.3	51.0	0.5	[6]
T. aureum ATCC 34304; 69 h growth	5.7	8.0	40.0	–	[39]
Thraustochytrium sp. 20892 (4 days)	6.1	15.2	53.1	0.7 (growth at 25°C for 3 days, followed by 15°C for 1 day	[32]
T. roseum ATCC 28210 (5 days)fed batch culture	17.1	25	50.0	2.1	[38]
Schizochytrium limacinum SR21;4 days in a fermentor	48.0	77.5	35.6	13.3	[33]
Schizochytrium limacinum SR21;5 days	38.0	5.0.0	43.1	4.2	[36]
Thraustochytrium sp. KK17-3 (3 days)	7.1	19.9	52.1	0.3	[40]
Thraustochytrid strain 12B (3 days)	31.0	57.8	43.1	6.8	[41]
Schizochytrium limacinum SR21; 7 days	22.1	-	-	4.9	[42]
S. limacinum OUC88(5 day)	22.11	-	18.45	4.08	[43]
Schizochytrium limacinum SR 21	37.9	-	-	6.56	[44]

CONCLUSION

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. The different constituents of media played an important role for production of desired lipid in any fermentation process. It is concluded that nutrient limitation, usually nitrogen, is the key factor inducing lipid accumulation in oleaginous microorganisms. Moreover, the effect of methanol on growth of DHA production is negative. It has been reported that ethanol is beneficial for the growth of some microalgae producing DHA. Effect of salt concentration in medium shows that 50% salt concentration give better production, in spite of this 25˚C temperature give rise to the good cell growth. A diet rich in ω-3 fatty acid not only provides the healthy fats, but it also lowers the blood level of potentially harmful once, such as cholesterol and possibly, even reversing the effect of excess trans fatty acids.

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