



# An overview of systematics, morphology, biodiversity and potential utilisation of Thraustochytrids

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Received: 21 Dec 2019 Accepted: 28 Aug 2020 Published: 10 Sep 2020

Original Article

## Abstract

Thraustochytrids, heterotrophic protists were first reported in 1934 and then on several isolates were reported across the world and their advantageous and harmful properties have been explored and utilised. They are oleaginous saprophytic marine heterokontophytes and are emerging as an alternative oil source in pharmaceutical and aquaculture industries. These are known for their role in marine food web and nutritional recycling and are commercially exploited for their ability to produce abundant nutraceutical fatty acids, particularly long-chain poly unsaturated fatty acids such as docosahexaenoic acid, eicosapentaenoic acid and squalene. This review gives an insight on the available information on taxonomy, morphology, biodiversity and utilisation of Thraustochytrids and also identifies the knowledge gap and understanding in the field of Thraustochytrids and their importance in polyunsaturated fatty acids enriched commercial products.

**Keywords:** Aquaculture feed, DHA, protists, PUFA, systematics, Thraustochytrids

## Introduction

Thraustochytrids are monocentric (only one sporangium) halophilic protists of either saprophytic or parasitic (occasional) nutrition (Rabinowitz *et al.*, 2006; Damare, 2009; Raghukumar and Damare, 2011; Gupta *et al.*, 2013). They are ubiquitous in marine and mangrove habitats and their isolation have been reported 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.*, 1998). Yet abundant, overgrowth of other marine contaminants such as bacteria, yeast and fungi limits its isolation and commercial utilisation (Gupta *et al.*, 2013). Thraustochytrids are euryhaline (0-100 PSU) (Damare, 2009; Jaseera *et al.*, 2019) and their thermal tolerance ranges from 20-30°C and pH from 4 to 9 (Fan *et al.*, 2002). Their characteristic extensive ectoplasmic nets (EN) with highly degrading enzyme profile specifies their primary role in decomposition (Kimura *et al.*, 1999) and act as remineralizers in the marine ecosystem which in turn nourish variety of detritus feeders in that niche and upgrade the ecosystem. They also act as source of dietary docosahexaenoic acid (DHA) in aquatic environment and play a significant role in the microbial loop. DHA distribution from Thraustochytrids occurs through mesoplanktons to higher trophic levels like fishes and crustaceans (Raghukumar

and Damare, 2011). These organisms are considered as one of the potential alternative sources of poly unsaturated fatty acids (PUFA) for both commercial and industrial applications (Leano *et al.*, 2003).

In the early centuries when they were first reported, researchers focussed on their abundance and ecological role in marine ecosystem (Gaertner, 1968). During the course of discovery, their ability to accumulate PUFA and its importance in PUFA market lead researchers to think about their exploitation as an alternative oil source (Bajpai *et al.*, 1991; Singh and Ward, 2005). Now with the advanced scientific techniques, the recent investigations are aimed to elucidate the biosynthetic pathways involved in fatty acid metabolism in Thraustochytrids (Huang *et al.*, 2008; Lippmeier *et al.*, 2009; Nagano *et al.*, 2011).

### Systematics and phylogeny

Formerly Thraustochytrids were documented as fungi, but in recent years with the expansion of molecular phylogeny they are allocated to the family Thraustochytridae of the group Labyrinthulomycetes, (Chromista, Heterokonta), bringing them more close to the heterokont algae (eg. brown algae and diatoms). Previously, Thraustochytrids were identified by morphological, cultural and developmental characteristics (formation of sorus, ectoplasmic net and zoospores). But with the advent of molecular technologies such as 18S rRNA gene amplification and sequencing enabled isolation and identification of several new species (Rabinowitz, 2006). Mo *et al.* (2002) developed three sets of internal primers for conserved regions of Thraustochytrid 18S rDNA for phylogenetic identification. Several terms are used to delineate them and difficulties still continues to align these organisms taxonomically. Cavalier-smith *et al.* (1994) aligned Thraustochytrids as a different group of heterokonta and concluded that besides structural and phylogenetic similarity helix 47 in V9 region of 18S rRNA, Thraustochytrids are also characterised by the presence of AU base pairs like other heterokonta, whereas a few authors like Leyland *et al.* (2017) strongly contradict the evolutionary relationship of Thraustochytrids with heterokont algae and the usage of microalgae for Thraustochytrids.

Thraustochytrids belong to the Labyrinthulomycetes and their sister groups are, Aplanochytrids and Labyrinthulids. So when describing systematics of Thraustochytrids one cannot eliminate Aplanochytrids and Labyrinthulids. Thraustochytrids are unicellular and zoospore forming protists. The members of the Labyrinthulids are colonial and comprise only single genus *Labyrinthula* Cienk. While Aplanochytrids may be unicellular or colonial and consist only single genus *Aplanochytrium* Bahnweg and Sparrow. (Raghukumar and Damare, 2011).

Labyrinthulids were first included under Myxomycota (slime mold) by Cienkowski (1867) and Sparrow (1936) classified Thraustochytrids under Chytrid fungi. Later, they were categorised under oomycetan fungi because of heterokont nature of zoospores. Aplanochytrids formerly known as Labyrinthuloides were also included in Thraustochytrids as they have globose sporangium and ectoplasmic network (EN). Later Perkins (1973a) designated *Labyrinthuloides* as a new genus of Labyrinthulid with a different locomotion. Porter (1990) assigned both Labyrinthulids and Thraustochytrids in to heterokont algae based on the ultrastructure of zoospore flagella and molecular phylogeny. Based on the data from 18S rRNA gene sequences, two group were later categorised under the families Labyrinthulidae and Thraustochytridae, under Class: Labyrinthulea, Subphylum: Labyrinthista, Phylum: Heterokonta, Kingdom: Chromista (Cavalier-Smith *et al.*, 1994). Dick (2001) revised Labyrinthulomycetes classification and kept it under the Phylum Heterokonta and Class Labyrinthista of Kingdom Straminipila. He classified Thraustochytrids under the family Thraustochytriaceae of order Thraustochytriales, and Labyrinthulids under the family Labyrinthulaceae of order Labyrinthulales. Straminipila include brown algae, Chryophytes, Xanthophytes, Diatoms etc. Based on small subunit (SSU) ribosomal sequences, Leander and Porter (2001) showed that genus *Aplanochytrium* was separate from Labyrinthulids and Labyrinthulomycetes comprises of three distinct clade Thraustochytrids, Labyrinthulids and Aplanochytrids. Labyrinthulids include only single genus *Labyrinthula* Cienk, Aplanochytrids include *Aplanochytrium* (Bahnweg and Sparrow, 1974). Dick (2001) categorized Thraustochytrids in 5 different genera based on cellular morphology and life cycle, which include *Thraustochytrium* Sparrow, *Japonochytrium* Kobayasi et M. Okubo, *Althornia* E.B.G. Jones et Alderman, *Schizochytrium* S. Goldst et Belsky, *Ulkenia* A. Gaertn. Later Yokoyama and Honda (2007) rearranged the genus *Schizochytrium* S. Goldst. et Belsky based on morphological, fatty acid and carotenoid profile and 18S rRNA gene phylogeny into *Schizochytrium*, *Aurantiochytrium*, *Oblongichytrium*. Yokoyama *et al.* (2007) amended *Ulkenia* into *Botryochytrium*, *Parietichytrium* and *Sicyoidochytrium*. Half of all species reported in the Thraustochytridae family (15 species) belong to the genus *Thraustochytrium* (Mo *et al.*, 2002).

Based on morphological, biochemical and phylogenetic markers, nine genera were recognized within the Thraustochytrid family such as *Thraustochytrium* (Sparrow, 1936), *Japonochytrium* (Kobayashi and Ookubo, 1953), *Schizochytrium* (Goldstein and Belsky, 1964), *Ulkenia* (Gaertner, 1977), *Aurantiochytrium* (Yokoyama and Honda, 2007), *Sicyoidochytrium*, *Parietichytrium*, *Botryochytrium* (Yokoyama *et al.*, 2007), and *Monorhizochytrium* (Doi and Honda, 2017). Two very closely related Thraustochytrids *Oblongichytrium* (Yokoyama and Honda, 2007) and *Althornia* (Jones and Alderman, 1971) have been removed from the sensu stricto family (Anderson and Cavalier-Smith, 2012), but are still considered as belonging to the Thraustochytrid group.

Besides molecular phylogeny, Haung *et al.* (2003), and Yokoyama and Honda (2007) developed PUFA profile as a tool to distinguish each monophyletic groups in Thraustochytrids. Huang *et al.* (2003) screened seven strains of marine protists from seawater of Japan and Fiji in relation to their 18S rRNA genes and PUFA profiles and based on this he categorized Thraustochytrids into 5 groups. Group A accumulate n-6 docosapentaenoic acid (DPA, C22:5, n-6) in addition to DHA. Group B comprised of strain accumulating DPA and eicosapentaenoic acid (EPA, C20:5, n-3). EPA accumulating strains were assigned to group C. Accumulation of arachidonic acid (AA, C20:4, n-6) besides DPA as well as EPA made these strains to allocate in group D. Presence of n-6 docosatetraenoic acid (DTA, C22:4, n-6;) in addition to oleic acid (C18:1, n-9) as a major unsaturated fatty acid with a lower DHA content were assigned in group E. Strain with same PUFA profile formed same phylogenetic block and hence it could be used for grouping of Thraustochytrid strains. Carmona *et al.* (2003); Yamaoka *et al.* (2004) and Yokoyama and Honda (2007) revealed that each strains in Thraustochytrids show different carotenoid profile and can be used as biochemical marker. Recently Nishitani and Yoshida (2018) could successfully develop mitochondrial cytochrome C oxidase subunit 1 (COI) primer and suggested as a molecular tool to identify *Aurantiochytrium* and similar genera.

## Morphology

Thraustochytrids have its own distinct morphology and cell wall composition (Damare, 2009). Thraustochytrids were first defined by Sparrow (1936) which are characterized by ovular or spherical sporangia with unilateral immotile EN. The vegetative cells of Thraustochytrids contain single nucleus, single dictyosome (stack of Golgi apparatus), centrioles associated with nuclear pockets, and numerous mitochondria. Associated with the nucleus, paranuclear bodies are present as convoluted smooth endoplasmic reticulum which enclose ribosome free cytoplasm (Moss, 1986). Granular cytoplasm sometimes filled with lipid inclusions are also a distinguishing feature of Thraustochytrids (Azevedo and Corral, 1997). Thraustochytrids possess multi-layered cell wall and is composed of sulphated galactose (Damare, 2009). Vegetative cells are globose to subglobose measuring 4 to 20  $\mu\text{m}$  in diameter. Most Thraustochytrids reproduce by zoospore formation and closely resemble zoosporic fungi. The mode of zoospore formation varies between genera and is the major taxonomic criterion. The zoospores possess a long anterior whiplash flagellum and a short posterior tinsel type flagellum (hence hetero-kont). Tripartite tubular hairs (TTH) cover the anterior flagellum. TTH consist of three parts, a cone like base, a tubular shaft, and two diverging terminal fibers of unequal length (Dick, 2001; Damare, 2009). Thraustochytrids also possess ectoplasmic net elements (EN), which are the extensions of plasma membrane that are associated with sagenogenosomes organelle (Bothrosome). The EN serve

them to absorb nutrients and also to attach on substratum. It also acts as track for the movement (Raghukumar, 2002; Raghukumar and Damare, 2011). The EN can penetrate the sporopollenin layer (a polymer that is highly resistant to microbial breakdown) of Pine pollen. This property has been operated for isolation of the organism from wild and termed as 'pollen baiting' (Raghukumar and Damare, 2011; Gupta *et al.*, 2013). Baiting with *Artemia* larvae is also a standard method for their isolation (Raghukumar and Damare, 2011).

The peculiar features of eleven genera of Thraustochytrids are described in the following pages.

## *Thraustochytrium*

It is characterised by one or several proliferation body. Reproduction occurs via zoospore formation from parent cell and are released either by partial or complete disintegration of the sporangial wall. In non-proliferous form entire sporangia cleaves in to spore. In mono-proliferous form a large portion of cytoplasm do not divide with a wall deposited outside. After zoospore release the part become zoosporangium and release zoospores. In multiproliferous form proliferation body remain and develop to form secondary sporangium. It may also form amoeboid structure which later forms sporangium (Dick, 2001; Raghukumar, 1992).

## *Japonochytrium*

This genus is characterised by a subsporangial dilatation of the EN at the base of the thallus called apophysis and liberates its zoospores through an apical pore in the sporangial wall. *Japonochytrium* shows siltmilarity with non-proliferous form of *Thraustochytrium*. The only isolate presently available is *Japonochytrium* sp. ATCC® 28207 but this is now supposed to belong to *Ulkenia* (Moss, 1986; Yokoyama *et al.*, 2007).

## *Schizochytrium*

Genus *Schizochytrium* shows large pale-yellow colonies. They are globose with thin wall and well developed EN. These groups are characterised by successive bi-partitioning of thallus to form zoospores or each cells may develop into zoosporangia which produce up to 64 reniform to ovoid zoospores. Presence of about 20% of ARA in the PUFA profile is a biochemical marker for *Schizochytrium* (Goldstein and Belsky, 1964; Moss, 1986; Yokoyama and Honda, 2007).

## *Aurantiochytrium*

They are orange pigmented small colonies due to the high production of carotenoids including astaxanthin, phoenicoxanthin,

canthaxanthin and  $\beta$ -carotene. Vegetative cells are generally dispersed as single cells. Like *Schizochytrium* they are also characterised by thin-walled globose thallus, continuous binary division and zoospore formation. The EN is not very well developed. Presence of an amoeboid cell prior to zoospore release is also identification characteristics. Their biochemical marker includes low level of arachidonic acids and all aforementioned carotenoids (Honda *et al.*, 1998; Raghukumar, 1988; Yokoyama and Honda, 2007).

### *Oblongichytrium*

Colonies of *Oblongichytrium* are large and pale yellow and their form and shape are similar to *Schizochytrium*. They are also spore formers and release when the sporangia are transferred from agar to a broth medium. Zoospores are narrow, elliptical to oblong in shape. They have a well-developed EN and undergo continuous binary division like *Schizochytrium*, but their biochemical marker is characterized by a high DPA  $\omega$ 3:DPA  $\omega$ 6 ratio and little production of ARA (Yokoyama and Honda, 2007).

### *Ulkenia*

Genus *Ulkenia* grows as small colonies on agar plate. They are characterized by an under-developed EN, and thallus with various size and shape varying from subglobose or globose to pear shaped. Strains of *Ulkenia* also exhibit repeated binary divisions and transformation of mature cell into amoeboid cells prior to zoospore formation. The amoeboid cells are large globose in shape and divide directly to form zoospores and discharge its content (Gaertner, 1977; Moss, 1980; Dick, 2001; Yokoyama *et al.*, 2007). It is also characterised by the formation of naked protoplasts and is released through the opening formed by a partial dissolution of the cell wall, and are motile through active amoeboid movements. The cell wall remains after the release of the protoplast. (Yokoyama *et al.*, 2007). The naked protoplast is either a uninucleated limax cell or multinucleated (Moss, 1980, 1986; Raghukumar, 1982). The protoplasts are round, and divide to form spores. The spores are non-flagellated at the time of release that develop flagella (About 30 min after) at a later stage (Yokoyama *et al.*, 2007).

### *Sicyoidochytrium*

The genus *Sicyoidochytrium* shows small colonies and undeveloped, unbranched EN similar to *Ulkenia* and a comparable life cycle. The uninucleated naked protoplast shows active motility (Dick, 2001; Moss, 1986). The protoplast begins to form zoospores after undergoing a few divisions, which then develop into a zoosporangium. The peculiar feature of this genus is that some cells still remains attached to each other showing a dumbbell-like organization even after the release of zoospores

which later complete their division becoming zoospores with heterokont flagella that can swim away (Yokoyama *et al.*, 2007).

### *Botryochytrium*

Their colonies are comparatively larger and possess fully developed EN. (Yokoyama *et al.*, 2007). They are characterised by the presence of a botryose (grape-shaped) zoosporangium, a structure developed by the protoplast. Immediately after the release of multinucleate protoplast (Moss, 1986) the cell wall disappears and the protoplast develops into a botryose zoosporangium by cleavage of early stage zoospores from a centripetal division. The appearance of star shaped zoosporangium before zoospore formation, dumbbell shaped spores and fatty acid profile showing high relative level of DPA-6 are some other characteristics of *Botryochytrium* (Yokoyama *et al.*, 2007).

### *Parietichytrium*

The life cycle of *Parietichytrium sarkarianum* is similar to *Botryochytrium radiatum* Colonies are large with well-developed ectoplasmic nets. Releases protoplast and the cell wall remains even after the release of protoplast and zoospores are fully divided at the time of discharge. Protoplasts develop botryose by a centripetal division, then form star-shaped before zoospore release. Relatively high levels of DTA in fatty acid profile is also a distinct feature of these isolates (Yokoyama *et al.*, 2007).

### *Althornia*

Cells of *Althornia crouchii* lack EN and its associated SAG (Jones and Alderman, 1971). Their cellwall consist of scales and also produce zoospore in the same way as *Thraustochytrium* spp. (Moss, 1986). The genus was proposed based solely on morphological factors and not by molecular means.

### *Monorhizochytrium*

Genus *Monorhizochytrium* is a variant strain of *Thraustochytrium globosum*, NBRC112723 and a recent addition to the Thraustochyrid group. This genus has been proposed by Doi and Honda (2017). Only limited characteristic features of this genus is described explaining only partial morphological features and not ultrastructure of this organism and biochemical features like PUFA and carotenoid content. This organism follow non proliferous life cycle, that is the young cells directly mature into zoosporangium.

### *Biodiversity*

Thraustochytrids are omnipresent in aquatic ecosystem and some of the most common habitats are decaying mangrove

leaves, decomposing algae, and faecal pellets of marine invertebrates (Leander *et al.*, 2004). Marine detritus and marine aggregates formed by coagulation of transparent extracellular polysaccharides (TEP) support its growth (Raghukumar and Damare, 2011). Thraustochytrids are abundant in habitat which holds detritus, mangroves, salt marshes and river effluents. Any decomposing waste materials support the growth of Thraustochytrids (Raghukumar, 2002). These microbes may live as parasites or commensal or mutualistic in edible invertebrates. Thraustochytrids are pathogens and a major peril while developing marine invertebrate cell culture (Rabinowitz, 2006). Scharer *et al.* (2007) described a new species of Thraustochytrid-*Thraustochytrium caudivorum* as parasite in a marine free-living flatworm *Macrostomum lignano* causing dissolution of posterior part of the worm.

Thraustochytrids are rarely seen in association with living marine phytoplanktons and algae. They grow in the living cells of diatoms. The technique of isolation of Thraustochytrids from algal surface was developed by Vishniac (1956). Low density of Thraustochytrids on the living algal surface is mainly due to the antimicrobial compounds released from these plants. Due to these reasons Thraustochytrids cannot act as parasitic/mutualistic in or on living marine plants (Raghukumar, 2002). Their abundance is also very low during the early stages of decomposition and their number increase rapidly during the later period of decomposition (Raghukumar, 2002). Thraustochytrids colonise only after bacterial colonization (Raghukumar and Damare, 2011). They are also found in association with marine animals. Examples for such relations are Thraustochytrids with hepatopancreatic cells of crabs and oyster and adductor muscles of oysters. Thraustochytrids may also act as parasites in marine animals and mostly seen in mollusc (Perkins, 1973b; Raghukumar, 2002). Polglase (1980) reported skin ulceration in an octopus *Eledone cirrhosa* and its associate causative agent as *Ulkenia amoebiodaea*. Mc Lean and Porter (1982) showed *Schizochytrium* causing yellow spot disease in *Tritonia diomedea*.

Raghukumar and Schaumann (1993) detected AfDD (Acriflavin direct detection) technique to enumerate Thraustochytrid cells in coastal and oceanic water column based on the presence of sulphated polysaccharides in the cell wall of these cells. This method relies on the principle that acriflavin stains acid sulphated polysaccharides red and the nucleus yellow green. Since cell walls of Thraustochytrids contain acidic polysaccharides their cell wall can be stained with this fluorescent microscope using blue excitation. This invention directed many studies related to the significance of biomass and productivity of Labyrinthulomycetes in water column (Raghukumar and Damare, 2011). Kimura *et al.* (1999) studied the abundance of Thraustochytrids in oceanic water column in Japan. Oligotrophic condition during

the pre-monsoon period, limits the extent of Thraustochytrids from the water during winter from November to February in the Arabian Sea (Raghukumar, 2011).

## Utilisation

**Source of hydrolytic enzymes:** Thraustochytrids are utilised for various commercial application. Their potential to produce variety of hydrolytic exoenzymes such as proteases, lipases, cellulases, amylases, xylanases, gelatinase, urease, phosphatase, chitinase and  $\alpha$ -glucosidase are significantly explored (Raghukumar *et al.*, 1994; Bremer and Talbot, 1995; Damare, 2009; Taoka *et al.*, 2009; Nagano *et al.*, 2011; Raghukumar and Damare, 2011). They can break down lignocellulosic substrates (Gupta *et al.*, 2013). They are well addressed for various bioactive compounds of pharmaceutical interest (Mo *et al.*, 2002). Kanchana *et al.* (2011) observed lipase activity in the genera *Thraustochytrium* sp. with optimum activity at alkaline pH and established that the lipase production is salt dependant. Gupta *et al.* (2016) reported that Australian Thraustochytrids are more potent enzyme producer than Indian Thraustochytrids and detected the presence of enzymes like alkaline phosphatase, leucine acrylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, valine acrylamidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase, esterases and lipases. Priyanka *et al.* (2017) also established the ability of Thraustochytrids to produce multiple degradative enzymes. The presence of enzymes indicates their role in mineral recycling and upgrading marine environment.

**Source of carotenoids:** Thraustochytrids are a promising source of carotenoids such as  $\beta$ -carotene, xanthophylls, astaxanthin, canthaxanthin etc. (Aki *et al.*, 2003). The strain *Aurantiochytrium* possess pigments such as  $\beta$ -carotene, echinenone, canthaxanthin, phoenicoxanthin, and astaxanthin and gives an orange colour to the colony. *Schizochytrium* produce only  $\beta$ -carotene and hence pale yellow in colour. The strain *Oblongichytrium* possesses canthaxanthin and  $\beta$ -carotene while *Japonochytrium* contain only astaxanthin and  $\beta$ -carotene and *Thraustochytrium* possess all the pigments mentioned above (Yokoyama and Honda, 2007). Carotenoids are important group of antioxidants and scavenge harmful free oxygen radicals. *Dunaliella* and *Haematococcus* were the industrially utilised microalgal genera for carotenoids production. In aquaculture industries carotenoids are mainly used to improve coloration in cultured ornamental fishes. Atienza *et al.* (2012) utilised two Thraustochytrid strains (*Thraustochytrium* sp. SB04 and *Schizochytrium* sp. SB11) to explore carotenoid production potential and their use as alternative feed in *Oreochromis niloticus* (Nile tilapia). Thraustochytrids can be cultured in medium containing glycerol as source of carbon instead of glucose for fatty acid and carotenoid production and there by effectively converting industrial waste material in to pharmaceutically important product could be achieved (Gupta *et al.*, 2013)

**Source of squalene:** Thraustochytrids are also sources of squalene (SQ-2,6,10,15,19,23 hexamethyltetracos-2,6,10,14,18,22-hexaene) a polyunsaturated triterpenic hydrocarbon (C<sub>30</sub>H<sub>50</sub>), which is a key precursor of cholesterol biosynthesis, bile acids, and steroids in plants and animals. Squalene fascinated researchers as it defends oxidative stress, lowers serum cholesterol levels, and suppresses chemically-induced tumors. Squalene is mainly used in cosmetic industries and pharmaceutical sectors. *Aurantiochytrium* was recognised as high SQ producer (Nakazawa *et al.*, 2014; Masato *et al.*, 2017). Chen *et al.* (2010) identified nitrogen constituents as critical components for squalene production in *Aurantiochytrium* sp. Various physico-chemical and culture conditions were optimised for squalene production in *Aurantiochytrium* sp.18W-13a and maximum production of approximately 171 mg/g dry weight and 0.9 g/L at 25 °C, 25-50 ppt salinity and 2-6% glucose concentration could be achieved (Nakazawa *et al.*, 2012).

**Raw material for biodiesel:** Thraustochytrids being an oleaginous organism act as renewable source of biodiesel production (Chang *et al.*, 2014). The available fossil fuel cannot meet the increasing demand of growing global population. Algal biomass comprising carbohydrate, protein and fat are one among several renewable feedstocks for biofuel production and are termed as third generation biofuel. They can be cultivated in nonarable land and thus need not compromise the land for agriculture (Anisha and John, 2011). The ability of fast growth and high lipid content of Thraustochytrids was exploited in producing a feedstock for shorter chain fatty acids suitable for biodiesel (Chang, 2013).

**Source of lipids and PUFAs:** Thraustochytrids have gained much attention in the field of applied biology especially due to their ability to accumulate high amount of lipid with high proportion of poly unsaturated fatty acids (PUFAs) specifically docosahexaenoic acid (DHA) (Yaguchi *et al.*, 1997; Raghukumar, 2008). The two main groups of PUFAs are: the omega-6 (n-6) and omega-3 (n-3) series. Arachidonic acid [AA; 20:4 (n-6)] a n-6 PUFA is a major precursor of many prostaglandins and eicosanoids and two n-3 PUFAs eicosapentaenoic acid [EPA; 20:5 (n-3)] and docosahexaenoic acid [DHA; 22:6 (n-3)], have been termed as "essential" fatty acids as they are synthesised only little in human beings. PUFAs are essential constituents of cell membranes and of many cell signalling cascades. The n-3 PUFAs limit the incidence of coronary heart disease and were observed in Eskimo populations of Greenland who are known to maintain better cardiovascular health. PUFAs also have important role in fighting against other disorders like stroke, rheumatoid arthritis, asthma, dyslexia, depression, and some forms of cancer. DHA is indispensable for normal development of neural tissue in infants, especially in the eyes and brain. PUFAs are significant in aquaculture activities for efficient larval rearing (Lewis *et al.*, 1999).

Total fat in Thraustochytrids account for 10-50% of biomass and 30-70% of which is DHA (Singh and Ward, 2005). The existing commercial source of DHA is fish oil and its demand

impede the growing aquaculture industries. Moreover, fish oils encompass only 7-14% DHA, EPA and other saturated fatty acids and its consumption as food additives is limited due to problem with overfishing, smell and poor oxidative stability. However, oil from alternative source like microalgae are superior to fish oil in all aspects and stability, reduced smell, simple PUFA profile, and easy cultivation (Rabinowitz *et al.*, 2006). Table 2 shows comparison of Thraustochytrids with other conventional oil producers.

DHA production is greatly influenced by medium composition, incubation temperature, pH, culture age, seawater concentration and speed and shape of impeller in fermenters (Lewis *et al.*, 1999). Apart from these physical and chemical variables, selection of suitable candidate is important to improve the yield. Over past several years, research activities were focussed to optimise various physico-chemical factors to enhance DHA productivity. Table 1 describes production potential of selected Thraustochytrids with reference to their cultural condition. The optimum salinity and temperature required for biomass and fatty acid production is 15-22.5 PSU salinity and 20-25 °C respectively (Leaño *et al.*, 2003). A culture medium composed of 3-5% w/v glucose concentrations in a half-strength seawater (50-60% v/v), at pH 6.0, and incubation temperatures 20-30 °C support growth and maximum yield of biomass and fatty acid in two Thraustochytrids (*Thraustochytrium* sp. SB04; and *Schizochytrium* sp. SB11) isolated from fallen mangrove leaves in Subic Bay, Philippines (Arafiles *et al.*, 2011). Shabala *et al.* (2013) developed a fermentation strategy for cultivating Thraustochytrids in low salt media as low 1-10 mM. This would prevent corrosion of fermenters used for culturing Thraustochytrids. Taha *et al.* (2013) also formulated a medium with low concentration of NaCl to avoid unwanted steel corrosion.

In Thraustochytrids two pathways operate for fatty acid biosynthesis. Standard (elongase-desaturase) pathway and polyketide synthetic pathway. Elongase-desaturase pathway includes two pathways FAS I and FAS II. FAS II comprises individual enzymes rather than a multi domain system which is involved in FAS I. A substrate specific desaturase enzyme is required to catalyse unsaturated fatty acid biosynthesis. In Thraustochytrids besides universal pathway another pathway called polyketide pathway (PKS) also operate to add-on the synthesis of PUFAs (Xie and Wang, 2015). Nagano (2011) studied the genes involved in fatty acid biosynthetic pathway through molecular based approaches in three Thraustochytrid genera *Thraustochytrium*, *Schizochytrium* and *Aurantiochytrium* and he confirmed the presence of elongase and desaturase system in *Thraustochytrium* and *Schizochytrium* and absences in *Aurantiochytrium* which agree an alternate pathway of fatty acid synthesis called polyketide pathway in the latter. Huang *et al.* (2008) elucidated

Table 1. Biomass, lipid and DHA production potential of selected Thraustochytrids with reference to their culture conditions.

| Organism   | Age (days) | Temperature (°C) | Culture condition  | Dry cell weight (g/L) | Total lipid (% DW) | DHA production |       | Reference                     |
|--|------------|------------------|--|-----------------------|--------------------|----------------|-------|-------------------------------|
|  |            |                  |  |                       |                    | % TFA          | (g/L) |                               |
| <i>Aurantiochytrium</i> strain BL10                        | 6          | 26               | 140g/L initial glucose concentration and 10PSU salinity, with feeding of 50g/L glucose after 3.5 day | 59.0 g/L              | 73%                | 29%            |       | Yang <i>et al.</i> (2010)     |
| <i>Thraustochytrium aureum</i> ATCC 34304                  | 6          | 25               | 20g/L glucose<br>2g/L Na-glutamate   | 3.8                   | 16.5               | 70.4           | 0.269 | Bajpai <i>et al.</i> (1991)   |
| <i>Schizochytrium limacinum</i> SR21                       | 4          | 28               | 12g/L glucose 1.4g/L corn steep liquor-3.3g/L ammonium sulfate                                       | 48.0                  | 77                 | 36             | 13.3  | Yaguchi <i>et al.</i> (1997)  |
| <i>Schizochytrium limacinum</i> SR21                       | 2.5        | 28               | 60g/L glucose, 2g/L ammonium sulfate, pH-4,  | 21                    | 50                 | 35             | 4.7   | Nakahara <i>et al.</i> (1996) |
| Thraustochytrid strain 12B                                 | 3          | 28               | 120g/L glucose, 50% seawater   | 31.0                  | 57.8               | 43.1           | 6.8   | Perveen <i>et al.</i> (2006)  |
| <i>Thraustochytrium</i> sp. ( <i>striatum</i> )<br>ONC-T18 | 5          | 25               | 100g/L glucose, 2g/L yeast extract, 8g/L monosodium glutamate  | 28.0                  | 81.7               | 31.5           | 4.6   | Burja <i>et al.</i> (2006)    |
| <i>Schizochytrium</i> KH105                                | 4          | 25               | 50% Shochu distillery waste, 80g/L glucose, 19.5g/L sea salt, pH-7.5                                 | 30.0                  | 43.0               | 25.8           | 3.4   | Yamasaki <i>et al.</i> (2006) |
| <i>Thraustochytrium roseum</i><br>ATCC28210                | 12         | 25               | 25g/L starch, 2g/L yeast extract, with 10g/l glucose feeding after 4 and 6 day                       | 17.1                  | 25                 | 49             | 2.1   | Singh and Ward (2005)         |

Table 2. Comparison of Thraustochytrids with conventional oil producers.

| Organism         | Fat%  | PUFA% | DHA%  | EPA%  |
|------------------|-------|-------|-------|-------|
| Sardine          | 3.68  | 35.01 | 5.44  | 20.05 |
| Mackarel         | 5     | 30.61 | 3.47  | 18.06 |
| Tuna             | 6-8   | 49.4  | 35.66 | 4.74  |
| Anchovies        | 1.97  | 24.50 | 13.83 | 2.87  |
| Thraustochytrids | 10-50 | 50    | 20-80 | 6-20  |

the enzymatic machinery involved in PKS pathway for DHA production and identified several synthases which included beta-ketoacyl synthase, a beta-ketoacyl reductase, and an enoyl reductase through a sequence tag analysis. Lippmeier (2009) also concluded the existence of two pathways for fatty acid biosynthesis in *Schizochytrium* sp. and *Cryptocodinium cohnii* by using auxotrophic PUFA mutant of these strains. Recently several studies were conducted at the gene level to improve the expression efficiency of promoter and thereby to obtain a better yield (Xie and Wang, 2015).

### DHA supplements in market

Global fish oil market extends from crude oil rich fish trashes to good quality food grade oil. Utilisation of fish oil has reached in every sector ranging from aquaculture to nutraceutical industries. Oil derived from Thraustochytrid sanctified aquaculture industry with ultra-high DHA. Now DHA algal oil is available for use as food and dietary supplements (FDA 2004). Martek is one such company which produce DHA algal oil from *Schizochytrium* which is known to contain approximately 37% of DHA and 16% of EPA (w/w) and secured GRAS (generally recognised as safe) certification (GRN000137) from US- FDA in 2003 when used as direct food ingredient at 1.5gm DHA in a day. Several clinical trial conducted in humans from babies to adult concluded its consumption is

safe, and its source organism is nontoxic and not genetically modified. Monsanto (Missouri, U.S), along with Omega Tech (U.S) is also producing *Schizochytrium* sp. derived oil' to fortify infant formula and to produce DHA enriched eggs by feeding hens. Aquaculture industry a fastest growing food sector also rely on LCPUFA (e.g. EPA and DHA). The fish diet should be incorporated with about 1-2% w/w LCPUFA as a source of energy and PUFA. Enrichment of *Brachionus* sp. and *Artemia* nauplii with freeze-dried cells of Thraustochytrids, resulted in high DHA content and brought about increased growth and survival of many marine fish larvae (Lewis *et al.*, 1999). AlgaMac series from Aquafauna biomarine. Inc is a spray dried *Schizochytrium* that can be used either as live feed and /or for *Artemia* enrichment to nutritionally fortify animals which in turn enhanced survival rate and disease resistance (Bio-Marine, 2000). Thraustochytrids also produce upto 90% monounsaturated fatty acids (MUFAs) such as palmitoleic acid (C16:1), oleic acid (C18:1), eicosenoic acid (C20:1), and erucic acid (C22:1). This significant production of lipid along with fast growth, and high biomass in culture make them potent candidates for bio-fuel production (Fisher *et al.*, 2008; Gupta *et al.*, 2013). The genus *Schizochytrium* has been marketed as Microalgae for aquaculture through Aquafauna Biomarine Inc. (eg. AlgaMac, 2000) and Sanders Brine Shrimp Co. (eg. Docosa Gold). These products have high concentrations of DHA (Barclay and Zeller, 1996), and so are being applied

as alternatives to commercial oil enrichments (eg. Selco) for zooplankton fed to larvae.

### Future prospects

Marine microorganisms represent understudied groups and there is still vast unknown diversity in such hostile environment. Thraustochytrids are known for its ability to produce oil in much higher quantities and exploited in nutraceutical and aquaculture industries. Much research should be contributed in these sector to effectively utilise these organisms in an economic way to replace fish oil. Although these marine organisms were reported for the production of hydrolytic exoenzymes much knowledge about their extraction, purification and industrial exploitations have not been developed. These studies will boost up the wide knowledge on marine enzymes in the water column as well as their ecological role. More techniques must come to evaluate the properties of algal oil and to incorporate them for human consumption. The amendment of genetically altered strains and the advancement of a proficient huge scale development framework for the commercialisation of these supplements from Thraustochytrids would address a noteworthy worldwide need in fatty acid advertise. Much research must be contributed in the area of industrial and domestic waste management and bioremediation as these organisms can produce exoenzymes and can effectively assimilate majority of the carbon source and can convert it into value added products of commercial interest.

### Acknowledgements

The authors are thankful to the Director, ICAR-CMFRI for his generous encouragements and to the Head in Charge, Marine Biotechnology Division, CMFRI for providing necessary facilities. The first author is grateful to the University Grants Commission (UGC), New Delhi, Ministry of Human Resources and Development, Government of India [F.2-5/2009(SA-I)] for the financial support.

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