Review

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Mining terpenoids production and biosynthetic pathway in Thraustochytrids

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Abstract

Terpenoids are major bioactive compounds produced by microalgae and other eukaryotic microorganisms. Mining metabolic potential of marine microalgae for commercial production of terpenoids suggest thraustochytrids as one of the promising cell factories. The identification of potential thraustochytrid strains and relevant laboratory scale bioprocesses has been pursued largely. Further investigations in the improvement of terpenoids biosynthesis expect relevant molecular mechanisms to be understood directing metabolic engineering of the pathways. In this review, fermentative and mechanistic studies to identify key enzymes and pathways that are associated to terpenoids biosynthesis in thraustochytrids are discussed. Exploration of biosynthesis mechanisms in other model organisms facilitated identification of potential molecular targets for engineering terpenoids biosynthetic pathway in thraustochytrids. In addition, the preliminary genetic manipulation and *in silico* analysis in this review provides a platform for system-level metabolic engineering towards thraustochytrid strains improvement. Overall, the review contributes comprehensive information to allow better terpenoids productivity in thraustochytrids.

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1 Introduction

Thraustochytrids were first discovered in 1936 and identified as unicellular fungus-like marine protists (Sparrow, 1936), which were later classified into the class Labyrinthulomycetes and order Thraustochytrida (Chromista, Heterokonta). The order Thraustochytrida is composed of *Aurantiochytrium*, *Thraustochytrium*, *Botryochytrium*, *Parietichytrium*, *Aplanochytrium*, *Labyrinthuloides*, *Oblongichytrium*, *Sicyoidochytrium*, *Japonochytrium*, *Ulkenia*, and *Schizochytrium*. They have wide geographic distribution as marine microheterotrophs, and are spherical, unicellular, or colonial characterized by the ectoplasmic network secreted through organelle sagenogenetosome (bothrosome) (Raghukumar & Damare, 2011). Although closely related taxonomically to the eukaryotic stramenopiles (heterokonts) lineage, thraustochytrids are phylogenetically close to the heterokont algae (e.g., diatoms and brown algae) (Cavalier-Smith et al., 1994), and cited as microalgae especially in commercial outlook (Byreddy et al., 2016; Fan & Chen, 2007; Hong et al., 2013a; Hong et al., 2013b; Jiang et al., 2004; Manikan et al., 2015; Yue & Jiang, 2009). So far, thraustochytrids are broadly included in the highly heterogeneous group of microalgae which have adapted uniquely to a wide range of habitats and have enormous biotechnological potential (Fan & Chen, 2007; Gimpel et al., 2015; Olaizola, 2003; Pulz & Gross, 2004; Raja et al., 2008).

The development of marine navigation and microscopic examination has largely facilitated the mining of novel thraustochytrids from various ecological niches and identification of thraustochytrids species from ocean sediments, water column and mangrove forests of different oceanic provinces (Collado-Mercado et al., 2010; Damare & Raghukumar, 2008; Liu et al., 2014; Raghukumar & Damare, 2011; Singh et al., 2014). Major nutrition preferences of thraustochytrids isolated from different environments are mostly decided by their excretion system to produce various hydrolytic enzymes to break down oligomeric

substrates in the environment, and therefore dictated the diversified living style of thraustochytrid strains (Devasia & Muraleedharan, 2012; Kanchana et al., 2011; Naoki N, 2011; Raghukumar & Damare, 2011; Yousuke T, 2009). Highly adaptive life style of thraustochytrids sparkled research interest to probe their intracellular metabolism and to explore the biotechnological potential of thraustochytrids to produce value-added bioactive compounds, mostly the polyunsaturated fatty acids (PUFAs) (Lee Chang et al., 2011; Yamasaki et al., 2006). Since 2000, massive research efforts were dedicated in fermentative trials to select appropriate thraustochytrid strains and develop proper culture conditions to obtain high PUFAs yields. For instance, *Schizochytrium* sp. LU310 has been thoroughly investigated in its capacity to produce high yield PUFAs using various carbon and nitrogen feeding strategies. *Schizochytrium* sp. strain KH105 was grown in 3 liter culture to achieve a 3.4 g/L yield using glucose and distillery waste water as crude carbon and nitrogen sources. In 35 L culture volume, *Schizochytrium* sp. HX-308 achieved a 17.7 g/L yield using glucose and yeast extract as carbon and nitrogen sources. These findings suggest that thraustochytrids possess molecular machineries to achieve sound carbon utilization efficiency, and like PUFAs biosynthetic pathway, a promising intracellular terpenoids production pathway is speculated. Interestingly, mining metabolic potential of thraustochytrids by preliminary fermentative assays showed that a good variety of terpenoids (primarily carotenoids and squalene) was produced intracellularly, suggesting the presence of the complicated molecular biosynthetic machineries to assemble these bioactive nutraceuticals (Aasen et al., 2016). This encouraged efforts to be devoted into elucidating the biotechnological potential of thraustochytrids to produce terpenoids at industrial scales.

Carotenoids are an important group of terpenoids with anti-oxidative properties that are produced universally by microorganisms and higher plants, conferring protection of unsaturated substances such as PUFAs by reacting with oxygen species to form epoxides

(Guerin et al., 2003). In recent years, marine microalgae, in particular, have attained greater attention in the production of carotenoids (Henríquez et al., 2016; Varela et al., 2015). Carotenoids display broad-scope bioactivity including anti-inflammatory, antibacterial, immunomodulatory, photoprotective, neuroprotective, anticancerogenic and antiatherogenic properties (Higuera-Ciapara et al., 2006). Major carotenoids derived from seafood (such as shrimp and lobster) are astaxanthin, the 3,3′-dihydroxylated and 4,4′-diketolated derivative of β-carotene (Lorenz & Cysewski, 2000). In photosynthetic microorganisms (*Phaeodactylum tricornutum* for instance), major carotenoids (diadinoxanthin and violaxathin) are synthesized for quenching excessive photons through epoxidation *via* diadinoxanthin cycle (Kuczynska et al., 2015). The fact, that different marine organisms accumulate sheer diversified carotenoids, suggests the necessity to study individual carotenoid assemblage machinery present in different species and the regulatory mechanisms that dictates the biosynthetic efficiencies of these carotenoids. At current time, satisfactory microbial production of carotenoids is still scarce, compared to the production efficiency of synthetic carotenoids, which possess a considerable global market share for its low production costs and mature production procedures (Schmidt et al., 2011). Even though the synthetic carotenoids seem to be the most economical and sustainable, it still cannot satisfy the market demand as a human supplement. Also, synthetic carotenoids possess different stereochemistry properties that affect their stability, while natural ones are esterified preventing them from oxidation (Schmidt et al., 2011). Currently, R&D of microbial source carotenoids requires immense biotechnological advancements to significantly reduce the production costs. A rough calculation suggested that pilot scale production of carotenoids using microbial biomass would require a single cell productivity of 4 mg/g dry biomass in a 1500-liter tank with 6 g/liter cell density (Schmidt et al., 2011). Nevertheless, carotenoids from marine microalgae have a strong position in the market.

Cases include using β-carotene from *Dunaliella* as vitamin A precursor, astaxanthin from *Haematococcus* in aquaculture, lutein, zeaxantin, and canthaxantin for pharmaceutical purposes (Pulz & Gross, 2004).

Like carotenoids, squalene (triterpene) is a natural terpenoid of multiple commercial utilities that was originally isolated from shark liver oil (hence its name). Plant source squalene (primarily in vegetable oils) is identified in amaranth seed, rice bran, wheat germ, and olives. All plants and animals produce squalene as a biochemical intermediate or precursor compound to biosynthesize steroid hormones in eukaryotic cells, plants, animals and human (Bloch, 1983). Squalene has wide applications ranging from cosmetic industry to medical and pharmaceutical sector, owing to its broad functionalities. Besides, squalene is found to have radioprotective and cardioprotective effects. As of now, the liver oil of deep sea shark has been the major commercial source of squalene. However, serious concerns on protection of marine wildlife pose a big question on its availability. Although plants and yeasts are capable of squalene biosynthesis, recent reports of some thraustochytrid strains have shown very promising and high production yields (Hoang et al., 2014; Chen et al., 2010). This encourages the exploration of new biosynthetic machinery and organisms to sustain squalene production at commercial scale. Among the reported microbes (Xu et al., 2016), marine microorganisms mainly the thraustochytrid *Aurantiochytrium* strains have high potential for commercial production (Aasen et al., 2016).

During the course of mining efficient terpenoids producers, a few thraustochytrid strains with excellent terpenoids productivity were identified. This inspired us to go deeper into the main findings of the reports on carotenoids and squalene production in the light of present knowledge of terpenoids biosynthesis in thraustochytrids. The molecular biosynthetic mechanisms and relevant regulatory mechanisms were reviewed to further probe into the metabolic potential of the promising thraustochytrids strains, and to realize culture conditions

that allow strain optimization and higher productivity. In this review, we present a summary over the biosynthetic potential of thraustochytrids to produce carotenoids and squalene with a focus on fermentative and mechanistic studies in carotenoid and squalene biosynthesis and their pathway regulation and control. The first section presents fermentative studies on thraustochytrids to produce terpenoids under designated culture conditions. The second section includes the proposed mechanisms along with the characterization of key enzymes involved in terpenoids biosynthesis. The third section deals with the regulatory aspects of terpenoids biosynthetic pathways. The fourth section investigates the reports on genetic manipulations and tools with some preliminary *in silico* analysis of published thraustochytrids genome concerning strain improvement. Overall, we expect this review to provide essential information for strategic manipulation of biosynthetic machinery in thraustochytrids, and to determine the sequential processing schemes of these terpenoids to obtain higher production efficiency.

2 Strain isolation, culture conditions and terpenoids production

For the wide distribution of thraustochytrids in different oceanic provinces, isolation from their natural habitats remains the prerequisite for laboratory culture to obtain the value-added bioactive metabolites. Pine pollen baiting technique has been developed to allow isolation of individual thraustochytrid species from various circumstances regardless of their natural habitats (Gupta et al., 2013b). In sequential laboratory cultivation of the isolated strains, systematic efforts have been made to choose appropriate culture conditions for accumulating higher yield terpenoids. These efforts include, but not limited to, mining novel thraustochytrids strains from various ecological niches and the optimization of culture conditions for existing terpenoids producing strains (**Table 1**). In relatively limited fermentation studies of thraustochytrids, impressive yields were obtained. For instance,

culturing *Schizochytrium mangrovei* PQ6 in culture medium with 90 g/L glucose, 10 g/L yeast extract, and 17.5 g/L artificial seawater (ASW) at 28 °C in the bioreactor gave the best squalene yield of 1.019 g/L (Hoang et al., 2014). A similar yield was also achieved with *Aurantiochytrium* sp. 18W-13a (Nakazawa et al., 2014). Culturing thraustochytrids strains to achieve sound squalene productivity, particularly in comparison with the well-developed *Saccharomyces cerevisiae* as terpenoids source strain gave the impetus to continuous mining and obtain thraustochytrids with higher terpenoids yield. It is also noticeable that as *Aurantiochytrium* sp. 18W-13a gave satisfactory yields in squalene, it also produced carotenoids simultaneously, shown by its orange color appearance in the colonies (Nakazawa et al., 2014).

These facts suggested vast potential of thraustochytrids to be developed into hyper terpenoids producing strains. Mining high carotenogenic thraustochytrid strains also suggested major advantage in gaining better productivity to be achievable in well-chosen thraustochytrid strains. For instance, *Schizochytrium* sp. strain KH105 gave a high astaxanthin yield of 7.7 mg/L by a 5-day culture with *Shochu* distillery wastewater as nutrition source. Similar yields were also achieved for *Aurantiochytrium* sp. KH105 in which a 3.6 mg/L/day for xanthophylls was reported. Both *Schizochytrium* sp. KH105 and *Aurantiochytrium* sp. KH105 have been developed into commercial strains. Besides these strains, new carotenogenic thraustochytrids lineages were also discovered from various ecological niches. Although no impressive yields were obtained in these newly discovered thraustochytrids, experimental evidence showed that they were capable of biosynthesizing a greater variety of carotenoids including β, β-carotene, canthaxanthin and β-carotene (Burja et al., 2006; Lee Chang et al., 2012; Quilodrán et al., 2009). The advantages of gaining high productivity in the commercialized thraustochytrids strains may be combined with the

advantages of creating a good variety of carotenoids in newly isolated thraustochytrids to see an improved yield for all these compounds.

Together with the discovery of individual thraustochytrid strains that are capable of producing either high yield in single carotenoid or good variety of potential carotenoids, systematic medium optimization has also been intensively investigated to increase carotenoid production yield in thraustochytrid strain *Schizochytrium* sp. KH 105 (Aki et al., 2003). The carbon over nitrogen ratio was found to be the single most important factor to affect carotenoid production in this strain. Yields for astaxanthin and cantaxanthin reached 10 mg/g and 6.1 mg/g (dry weight), respectively, when the strain KH 105 was cultured in a medium with a carbon over nitrogen ratio of about 33. Increased glucose concentration in culture medium favored astaxanthin accumulation while disfavored β–carotene accumulation. Reducing concentration of nitrogen source further improved astaxanthin yield. To examine the chance of obtaining high yield carotenoid production using mixed carbon and nitrogen source, a fermentation study was carried out using *Shochu* distillery wastewater. Astaxanthin was the major product identified out of total carotenoids (7.7 mg/L) (Yamasaki et al., 2006). To systematically investigate key medium component that affects carotenoid productivity, a response surface methodology was developed for strain *Aurantiochytrium* sp. KH105. Carbon sources that were involved in this study included active charcoal and citric acid in which active charcoal was found to facilitate astaxanthin accumulation better than citric acid (Iwasaka et al., 2013).

To investigate metabolic potential for squalene production, systematic studies were performed for *Aurantiochytrium* sp. BR-MP4-A1 (Chen et al., 2010; Li et al., 2009). The best squalene yield was achieved for BR-MP4-A1 in a highly mixed culture medium that contains 3% glucose, 0.2% yeast extract in certain strength artificial sea water supplied with multiple trace element solutions. Among the most influential nutrient factor, the type of nitrogen

source is one parameter of easy manipulation. This was manifested in a fermentation study of *Aurantiochytrium* sp. BR-MP4-A1 in which monosodium glutamate, yeast extract and tryptone were found to enhance cell growth and squalene production.

Phylogenetic analysis of high terpenoids-producing strains, listed in **Table 1,** was done to infer the evolutionary relatedness to facilitate the isolation of new high terpenoids producers by genetic approaches. To find the phylogenetic relationships among these high terpenoids producing strains, 18S rRNA sequence of each candidate strain was retrieved from NCBI database and was aligned to draw the phylogenetic tree (**Fig. 1**). *Schizochytrium* sp. and *Aurantiochytrium* sp. were phylogenetically close, and *Thraustochytrium* sp. was distantly related to these two genera. Despite that these genera are phylogenetically apart, they all possess high terpenoids productivity. Overall, the review of preliminary studies in terpenoids biosynthesis suggest the presence of diversified intracellular carbon assimilation strategies that allow thraustochytrid cells to make use of different carbon sources, inspiring further discussion in molecular mechanisms of how different carbon sources contribute to the biosynthetic pathways of terpenoids.

3 Molecular mechanisms of biosynthesis

While carotenoid biosynthetic mechanisms in higher eukaryotes are well characterized (Farré et al., 2010; Botella-Pavía et al., 2004), there is generally a lack of mechanistic understanding in microalgae specifically thraustochytrids. The reported pathways in microalgae mostly based on higher eukaryotic cells have been reviewed elsewhere (Varela et al., 2015; Paniagua-Michel et al., 2012; Takaichi 2011; Lohr 2009). In higher plants, plastidial 2-C-methyl-D-erythritol-4-phosphate pathway and cytosolic Mevalonate (MVA) pathways are present for early conversions in carotenoid biosynthesis, unlike microalgae (chlorophytes) which do not possess the cytosolic MVA pathway.

Carotenoids are formed from two common precursors, i.e. isopentenyl-pyrophosphate and dimethylallyl pyrophosphate (Kuczynska et al., 2015; Stauber & Jeffrey, 1988) which are acetyl-CoA derivatives. The conversion of dimethylallyl pyrophosphate (DMAPP) to geranylgeranyl pyrophosphate (GPP) is catalyzed by geranylgeranyl pyrophosphate (GGPP) synthase followed by condensation of two molecules of geranylgeranyl pyrophosphate to phytoene (first uncoloured carotenoid) by phytoene synthase, and then through intermediate lycopene (first coloured carotenoid) eventually to β-carotene by lycopene β-cyclase (Varela et al., 2015). In microalgae (chlorophytes), it was shown that lycopene undergoes splitting into two branches forming α-carotene (catalyzed by lycopene ε-cyclase and lycopene β-cyclase) and β-carotene (catalyzed by lycopene β-cyclase), and the relative activities of these two enzymes determine the proportion of α-carotene and β-carotene (Varela et al., 2015; Lohr 2009). Further probing the sequential reaction steps suggest the presence of diversified mechanisms from β-carotene to designated carotenoids that require elucidation of the distinctive catalytic mechanisms. A case for example was a proposed molecular mechanism for β-carotene to astaxanthin conversion. One proposal suggested that ketolase (*crt*W) and hydroxylase (*crt*Z) were responsible for the sequential ketolation and hydroxylation reactions in thraustochytrid *Schizochytrium* KH105 (Aki et al., 2003), while single P450 (*crt*S) was claimed to be responsible for both ketolation and hydroxylation reactions in *Phaffia* yeast (**Fig. 2**) (Ojima et al., 2006).

In the aforementioned section, the addition of different carbon sources in culture medium was shown to diversify terpenoids accumulation efficiency. Dissecting the underlying mechanisms to explain these effects pave the way for rationale design of appropriate culture condition allowing better accumulation efficiency in carotenoids. An appropriate candidate for discussion in the first place would be glycerol, that was used as major carbon source in a number of studies that aimed at improving yields for both

terpenoids and unsaturated fatty acids (Chang et al., 2013b; Gupta et al., 2013a; Lee Chang et al., 2013). To explain how glycerol contributes to the biosynthesis of these secondary metabolites, a transcriptomic study using *Schizochytrium* sp. S056 was performed. Results suggested transcription level of a few genes occurring in both glycolysis pathway and in acyl-CoA biosynthetic pathway was significantly up-regulated. These genes included *bkdA1* gene coding for 2-oxoisovalerate dehydrogenase (E1 component alpha subunit gene), *pyk* gene coding for pyruvate kinase, and *gapA* gene coding for glyceraldehyde-3-phosphate dehydrogenase (Chen et al., 2016). Both *pyk* and *gapA* are genes within glycolytic pathway catalyzing two irreversible reactions that synthesize key intermediates (phosphoenolpyruvate and glycerate-3-phosphate). This preliminary study indicated that glycerol promoted the biosynthesis of secondary metabolites in thraustochytrids primarily through enhancing the glycolytic activity and the production of NADH.

In a comparative assay, mechanistic details for carotenoid biosynthesis in *Xanthophyllomyces dendrorhous* in culture medium supplemented with glucose (as fermentative carbon source) and succinate (as non-fermentative carbon source) were examined in a transcriptomic study (Martinez-Moya et al., 2015). Succinate medium was shown to better facilitate astaxanthin production than glucose medium. Probing expression profiles of enzymes in related pathways suggested succinate culture promoted higher activity of phosphomevalonate kinase, diphosphomevalonate decarboxylase, and astaxanthin synthase. These are key synthases that are directly involved in carotenoid biosynthesis. Another key synthase in carotenoid biosynthetic pathway was ATP-citrate synthase (ACS), which provided cytosol acetyl-CoA during the course of carotenogenesis. Comparison of transcriptomic profiles between the two culture conditions suggested that genes which contribute to carotenoid biosynthesis occurred in both local biosynthetic pathway and in a global metabolic network.

In plants and microalgae, proteins of carotenoid biosynthesis are normally synthesized in a precursor with cleavable N-terminal translocation sequences (Lohr 2005). This further complicates the interaction mechanisms and makes the carotenoid biosynthesis unique. With the current knowledge of various proposed mechanisms that are spread across literature, it is infantile to frame a tangible evidence of the functional terpenoids biosynthetic pathway that exists in thraustochytrids. Therefore, comparative mechanistic studies involving microalgae cultures and model eukaryotic microorganisms through genetic approaches are warranted to assemble and unravel the complete putative mechanism of terpenoids biosynthesis in thraustochytrids.

4 Regulation of biosynthetic pathways and efficiency

Underpinning the regulatory aspects of terpenoids biosynthesis is a fundamental step to re-direct metabolic pathways for enhancing productivity, which remain elusive for thraustochytrids. Here we discuss and highlight the major factors that show regulatory role in the terpenoids biosynthesis (**Fig. 3**).

4.1 Carbon over nitrogen ratio and lipid hormone signal

While the supplementation of different carbon sources in culture medium resulted in a diversified intracellular transcriptomic profile, the change in carbon over nitrogen ratio ratio primarily dictated terpenoids accumulation rate. The effect of nitrogen limitation on carotenoid biosynthesis exemplified the contribution of a high carbon over nitrogen ratio in improving the production of carotenoids in thraustochytrids (Aki et al., 2003). It has been suggested that nitrogen deprivation led to the activation of AMP deaminase (consuming AMP to produce IMP) and reduction in the activity of NAD^+-isocitrate dehydrogenase, therefore allowing the accumulation of citrate in mitochondria. Citrate could be exported outside of

mitochondria to be converted into acetyl-CoA by ATP-citrate lyase to serve as the only source of cytosolic acetyl-CoA for the biosynthesis of terpenoids (Chang et al., 2013a; Chang et al., 2013b; Hynes & Murray, 2010; Lee Chang et al., 2013; Papanikolaou & Aggelis, 2011). Similar effects were also observed for carotenoids production in several other marine eukaryotes, and for squalene production in thraustochytrids (Chen et al., 2010; Flores-Cotera et al., 2001; Sun et al., 2016).

Addition of specific hormone-like compounds also aided in the biosynthesis of terpenoids. Canonical cases, for example, include lipid hormone methyl jasmonate and terbinafine. Methyl jasmonate displayed an enhancing effect in terpenoids biosynthesis in *Schizochytrium mangrovei* (Yue & Jiang, 2009). Addition of methyl jasmonate in a final concentration of 0.1 mM led to the rapid accumulation of squalene up to 1.6-fold high compared to untreated group at 48 h. However, extended methyl jasmonate treatment triggered cholesterol accumulation and reduced squalene content, suggesting methyl jasmonate to be a global regulator to sterol biosynthetic pathway. Interestingly, the color of *S. mangrovei* cells with methyl jasmonate treatment turned yellowish at late culture stage with methyl jasmonate treatment, suggesting that methyl jasmonate treatment also facilitated the intracellular production of carotenoids. Thus, methyl jasmonate treatment likely enhances biosynthetic efficiency of precursors for the biosynthesis of squalene and carotenoids. Apart from methyl jasmonate, terbinafine, a pathway-specific inhibitor of squalene epoxidase, has also been used for enhancing the production of squalene in *Aurantiochytrium mangrovei* (Fan et al. 2010) and *Chlamydomonas reinhardtii* (Kajikawa et al., 2015).

4.2 Cellular respiration and oxidative stress

Cellular respiration represents one of the culture parameters to be modulated to enhance cell division and terpenoids accumulation efficiency. Although accelerating cellular

respiration facilitates cell division rate, it does not favor carotenoids accumulation in individual cells. This requires a reasonable maintenance of cellular respiration strength and single cell carotenoids yield to achieve good total carotenoids productivity. Propyl gallate has been a frequently used chemical in blocking key reactions in glycolytic pathways and reducing cellular respiratory strength (Eler et al., 2009). Use of propyl gallate reduced the need for pyruvate to maintain regular generation of ATP for cellular activities via TCA cycle and allowed more pyruvate to be converted into acetyl-CoA for terpenoids biosynthesis. However, higher doses of propyl gallate deprived cellular production of ATP and led to cell death and had a negative impact on the production of carotenoids (Nakagawa et al., 1996; Nakagawa & Tayama, 1998). In practice, application of 0.03 mM propyl gallate enhanced the production of antioxidants in *Schizochytrium* sp. S31 to give astaxanthin yield of 452.26 μg/L at stationary phase of fermentation (Singh et al., 2015).

Increased concentration of dissolved oxygen (DO) also favored carotenoids accumulation. Intracellular Reactive oxygen species was closely associated with the dissolved oxygen concentration and was endogenously generated during cellular respiration and β-oxidation (Caffarri et al., 2001; Croce R, 2000). At excessive reactive oxygen species (ROS), transcriptomic study suggested the up-regulation of transcription level in several cellular redox- and stress-related proteins (monooxygenase, cytochrome P450, phosphoglucomutase and glyderaldehyde-3-phosphate dehydrogenase), indicating that they played roles in damping ROS damage through enhancing carotenogenesis (Martinez-Moya et al., 2015). For that reason, insertion mutants of *Vitreoscilla stercoraria* hemoglobin (*VHb*) gene of *Aurantiochytrium* sp. strains MP4 and SK4 displayed a nearly ten-fold increase in astaxanthin yield (Suen et al., 2014). By contrast, high DO reduced intracellular squalene accumulation significantly, suggesting that further molecular details are needed to understand how dissolved oxygen modulation affects carotenoid biosynthetic pathway. It is therefore

very likely that pathway-specific enzymes at late stage of carotenoids assemblage are swiftly responsive toward DO fluctuation while those enzymes in squalene biosynthetic pathway may not.

4.3 Light induction

To facilitate the biosynthesis of designated carotenoids in heterotrophic protists, the protection mechanism requires light induction with appropriate wavelengths. As seen in *Schizochytrium* sp. DZAM when subjected to light irradiation treatment, it can accumulate different carotenoids under different wavelengths (Oclarit & Belarmino, 2009). When strain DZAM was exposed to red LED light, a considerable increase in β-carotene content was obtained (from 2.20 mg/g to 10.78 mg/g). Further increment (to 12.92 mg/g) occurred when UV light was applied. UV irradiation is a promising method to enhance carotenoids production, as substantiated in a recent report which shows that UV-C radiation stimulates the production of carotenoids in *Dunaliella. salina* (24 h) and *H. pluvialis* (48 h), with 50 mJ/cm^2 of total carotenoids and β-carotene in *D. salina* and 30 mJ/cm^2 of total astaxanthin in *H. pluvialis* (Sharma et al., 2015).

Similar study was performed for *Thraustochytrium* sp. CHN-1 with light treatment including super bright blue, red and near-red LED light sources (Yamaoka et al., 2004). Best biomass accumulation was obtained with fluorescent LEDs treatment while the highest yield for carotenoids was obtained under blue LEDs. Random mutants of thraustochytrids also possessed different light responses compared to the wild-type strain. For instance, NTG induction mutant of *Thraustochytrium* CHN-1 gave the highest astaxanthin yield under 5k lux light illumination while *Thraustochytrium* sp. CHN-1 appeared orange with 1.5k lux light illumination (Marvelisa et al., 2003). Like other microalgae, light induction seems to play an important role in carotenoids biosynthesis in thraustochytrids, and perhaps represents a novel,

cost-effective, time-saving, and convenient strategy for regulating carotenoids production in thraustochytrids strains.

4.4 Carbon repartition and competing pathways

Mutual association between biosynthesis of fatty acids and terpenoids has been established to occur *via* same precursor compound acetyl-CoA while contributing to different end products designated to be fatty acids and terpenoids (**Fig. 4**). Interfering fatty acids biosynthesis seems to be an important regulation to allow better production of carotenoids. Lipid accumulation profiles and carotenoids accumulation profiles during different culture periods of *Schizochytrium* sp. HX-308 (Ren et al., 2014) showed a dramatic decrease in total carotenoids content after 24 h, which was resumed at the end of the exponential phase. Time-course analysis in gene expression profile during each growth periods suggested that the catalytic activity of acetyl-CoA carboxylase gradually reduced at the end of exponential phase when carotenogenesis began. This reduction enforced more acetyl-CoA to be partitioned into carotenoid biosynthetic pathway, suggesting that management over carbon partition between fatty acid biosynthetic pathway and terpenoid biosynthetic pathway can be another regulatory mechanism (Martinez-Moya et al., 2011). Additionally, fatty acid biosynthetic efficiency is also shown to be negatively related to squalene biosynthesis (Ren et al., 2015). On the contrary, in *Haematococcus pluvialis*, astaxanthin and fatty acid biosynthesis occur in a stoichiometric fashion (Schoefs et al., 2001), and dissection of this interaction mechanism showed that this inter-dependance is coordinated at the metabolite level rather than at the transcriptional level (Chen et al., 2015) A model of astaxanthin biosynthesis in *H. pluvialis* describing astaxanthin esterification and accumulation in the endoplasmic reticulum, wherein certain diacylglycerol acyltransferases catalyzes the astaxanthin esterification has been proposed (Chen et al., 2015).

Nonetheless, balancing the gene expression and maximizing the metabolic flux towards terpenoids biosynthesis remains a major challenge, and therefore rationally designed genetic systems and metabolic engineering approaches are needed to improve the selective production of terpenoids in thraustochytrids. Studies on targeted induction or constitutive activation of microalgae endogenous genes would warrant an interesting subject for future investigation.

5 Genetic manipulations and *in silico* analysis of terpenoids pathway

The distinctive features of the terpenoids biosynthetic pathway in thraustochytrids suggest the unexplored biosynthetic machinery for terpenoid assembly, and call for further exploration of the genomic resources to identify alternate pathways and specific enzymes for genetic manipulation. A thorough understanding of the terpenoids biosynthetic pathway is essential for its successful engineering. In addition to reverse genetics and synthetic biology application (Hlavova et al., 2015), highly predictive models are crucial to step-up metabolic engineering in microalgae especially thraustochytrids which display notable production of terpenoids among others (Veyel et al., 2014). A recent review on metabolic engineering of carotenoids in eukaryotic microalgae highlighted very few reports that made efforts towards optimization of carotenoids production through metabolic engineering (Gimpel et al., 2015). A plausible reason could be the involvement of cumbersome chloroplast or nuclear transformation systems, because terpenoids metabolic pathway in microalgae mostly occurs in these organelles. In this context, novel nuclear genome editing tools would aid in accurate gene deletion and integration. This will further allow re-routing metabolic networks and achieve anticipated expression levels of transgenes (Gimpel et al., 2015).

Previous reports highlighted a high number of genes that are involved in carotenoid biosynthetic pathways through cloning and sequencing (Cunningham and Gantt 1998).

However, in the recent years, pathway genes have been inferred from genome sequences and identification of orthologs (Lohr et al. 2005). In an effort to predict the function of the unknown members of the polyprenyl transferase subgroup in isoprenoid synthase superfamily, a large-scale collaborative study using genetic data was done that revealed high accuracy of function prediction of newly characterized enzymes (Wallrapp et al., 2013). The study demonstrates the potential to predict function to the complete polyprenyl transferase subgroup of the isoprenoid synthase superfamily computationally, and can thus serve to direct the prediction of gene functions in the terpenoids biosynthetic pathways of thraustochytrids. With this framework, we carried out a preliminary bioinformatics analysis to probe the highly conserved carotenoid biosynthetic route in thraustochytrids, the key enzyme bifunctional (2E,6E)-farnesyl diphosphate synthase relating to the carbon repartition efficiency. This enzyme sometimes also participates in squalene biosynthesis in certain microbial species. An inspection into the reported genomic sequence data of *Aurantiochytrium* sp. T66 provided sequence information regarding this key enzyme, and further amino acid sequence alignment of the enzyme to other reported homologues (*Aurantiochytrium* T66, *Saccharomyces cerevisiae* S288c, *Monosiga brevicollis* MX1, *Saprolegnia diclina* VS20, *Phaeodactylum tricornutum*, and *Nannochloropsis gaditana*) suggested the presence of highly conserved active site amino acids in these homologues. Three highly conserved domains were identified: Domain 1 (YN**GGK*NRG**W), Domain 2: (LGW*IE*LQA*FLV*DD*MD*S*TRRGQPCW), and Domain 3 (QDDYLDC*******GK*GTDIQD). Such information would be helpful to explore into the genetic systems of the homologues and may lead to the rational design of mutagenesis experiments for thraustochytrids strains. In addition, we also analyzed the reported sequences of squalene synthase protein in four *Aurantiochytrium* spp. mined from NCBI Protein database; and discovered highly conserved amino acid residues in the four strains of this

genus. The typical size of the squalene synthase is 387 amino acids with only three variable residues (**Fig. 5**).

In other carotenogenic marine organisms, genetic manipulation of pathway-specific enzymes (e.g., cytochrome P450 monooxygenase, phytoene synthase, isopentenyl-pyrophosphate isomerase and lycopene cyclase) has been reported to improve the production yield of certain carotenoids (Ojima et al., 2006; Ukibe et al., 2009). While mechanistic studies provide molecular targets for engineering in terpenoids biosynthesis, rational mutagenesis allows creating mutants with improved terpenoids biosynthetic efficiency. Representative study in rational mutagenesis design could introduce *Vitreoscilla* hemoglobin gene through site-specific insertion into 18S rRNA gene within two *Aurantiochytrium* species (Suen et al., 2014). Recently, Cheng et al. (Cheng et al., 2016) developed a feasible method to screen *Aurantiochytrium* sp. by a combination of heavy-ion mutagenesis and mutant-preselection by fatty acid synthase (FAS) inhibitors and cold stress for docosahexaenoic acid. Such selection strategy can be tested for screening thraustochytrid strains with high terpenoids production efficiency.

For squalene, genetic modifications in squalene biosynthetic gene cluster with squalene synthase and other related genes have been conducted in model organism *Saccharomyces cerevisiae*. Most studies on thraustochytrids genetic manipulation are limited to *Aurantiochytrium* spp. which restricts the use of other thraustochytrids strains in large scale production of terpenoids. A study by Sakaguchi et al. (Sakaguchi et al., 2012) was the first attempt to establish a versatile transformation system for thraustochytrids. The established system is applicable to both multiple transgene expression and gene targeting, and facilitates the molecular breeding of thraustochytrids for production of squalene-based fuels. However, the development of genetic systems for thraustochytrids require further

advancements to provide effective tools to manipulate the expression level of target genes along the terpenoids biosynthetic pathway.

Besides pathway specific genes, overexpression of glycolysis related genes (glucose-6-phosphate dehydrogenase (*Zwf1*) and an NADH kinase (*Pos5*) in recombinant yeast *Saccharomyces cerevisiae* increased intracellular NADH concentration and enhanced the production of lycopene and β-carotene (Zhao et al., 2015). Manipulation of the biosynthetic activity of squalene pathway also enhanced carotenoid production. In food yeast *Candida utilis*, overexpression of HMG-CoA reductase (in *Saccharomyces cerevisiae*) and the disruption of the squalene synthase gene (*ERG9*) led to the improved carotenoid yield (7.8 mg/g dry biomass) (Shimada et al., 1998). These linkages provided potential manipulation targets in thraustochytrids to attain better production of desired terpenoids.

Squalene biosynthetic pathway in thraustochytrids also initiates with acetyl-CoA and gives sequential intermediates involving squalene synthase that catalyzes the condensation reaction of two farnesyl-pyrophosphate (FPP) molecules to form presqualene-diphosphate (Hong et al., 2013b). The manipulation of squalene synthase has been extensively studied in model *Saccharomyces cerevisiae* to dictate the biosynthesis of squalene and other novel terpenoids *via in vitro* and *in vivo* strategies (Asadollahi et al., 2010; Kajikawa et al., 2015; Katabami et al., 2014; Paradise et al., 2008). Recently, it is revealed that squalene biosynthesis in bacteria follow a different route than eukaryotes and involves three key genes, a report that suggests a new pathway in most bacteria (Pan et al., 2015). This report offers new insights into the evolution of this pathway, and suggests further investigation of squalene biosynthetic genes in thraustochytrids might reveal intriguing new genes that catalyze terpenoids biosynthetic reactions.

Overexpression of squalene synthase in the prokaryotic *E. coli* and the eukaryotic *Xanthophyllomyces dendrorhous* has been reported to give higher yields of both carotenoids

and squalene (Furubayashi et al., 2014; Martinez-Moya et al., 2011). Elucidating catalytic mechanisms of squalene synthase in converting the same substrate FPP into two different products will clearly aid in the rational design of mutagenesis to disable the unspecific activity of squalene synthase to generate phytoene, and to augment its cognate function as squalene synthase. In addition, synthases occurring at early steps of squalene biosynthesis (such as FPP synthase and 3-hydroxy-3-methylglutaryl-CoA reductase) are also identified. Expression level of these and other heterologous sterol synthases in squalene biosynthetic pathway has also been used to improve the biosynthetic efficiencies of squalene and other squalene-derived sterol products in *Saccharomyces cerevisiae* (Szkopińska et al., 2000; Tokuhiro et al., 2009). It is well known that overexpression of squalene synthase in *C. reinhardtii* increases the rate of conversion of ^{14}C-labeled FPP into squalene but do not lead to squalene over-accumulation, instead knockdown of squalene epoxidase leads to significant levels of squalene accumulation without any growth inhibition (Kajikawa et al., 2015). Thus, understanding the functional significance of genes encoding enzymes directly involved in squalene biosynthesis by microalgae would allow strain optimization for enhanced productivity.

A complete list of genes involved in the MVA and sterol biosynthetic pathway has been reported for the marine diatom *Phaeodactylum tricornutum* (Fabris et al., 2014) and *Chlamydomonas reinhardtii* (Lohr 2009). Annotation of individual genes revealed a few novel characteristics of the classical terpenoids biosynthetic pathway in *P. tricornutum*. This includes a putative-intron region of *Arabidopsis* gene as MVA kinase gene, and covalent bonding of isopentenyl-diphosphate d-isomerase to squalene synthase to form a single fusion protein. Nevertheless, *in vitro* and *in vivo* studies of squalene synthase activities in microalgae are scare (Kajikawa et al., 2015; Hong et al., 2013b; Okada et al., 2000), and need further investigations. In addition, the unique features advance an interesting subject of

understanding terpenoids biosynthesis, and also suggest studying key synthases involved in these pathways of thraustochytrids. As more genome sequence data becomes available for thraustochytrids, the genetic manipulation of terpenoids biosynthetic pathway would get easier. Besides, the tools for efficient gene transfer are needed for thraustochytrids strain improvement through metabolic engineering approaches.

6 Summary and perspectives

Terpenoids are natural products that microbes produce with significant bioactivities (Guerin et al., 2003; Higuera-Ciapara et al., 2006; Kawee-ai et al., 2013). The production of terpenoids in microalgae has been extensively reported with special relevance to *Chlamydomonas reinhardtii* wherein metabolic engineering of strains was attempted with little success. Marine microalgae, thraustochytrids in particular, maintain fast duplication rates, easy biomass accumulation and amenable genetic manipulation, holding the promise to be developed into a commercial terpenoids producer. Compared to chemically synthesized terpenoids, natural terpenoids are free of structural concerns and show higher potential bioactivity *in-vivo* (Lorenz & Cysewski, 2000). Besides the discovery of high yield strains, cost-effective production of carotenoids also requires the optimization of culture medium composition and chemical extraction-purification procedures. Experiences in culturing commercial strains of thraustochytrids suggest considerations to be taken in both production costs and the processing schemes. The natural preference of thraustochytrids to use various highly polymeric substances as carbon and nitrogen sources suggest the applicability of the strategic utilization of unprocessed or even waste materials in culturing thraustochytrids. This provides an economic and environmental friendly approach for growing thraustochytrids and harvesting bioactive products from them. Laboratory studies have shown the appropriate use of various carbon and nitrogen sources to affect terpenoids accumulation. Strategic choice of

these nutrition sources required precise information of intracellular carbon repartition patterns to be known. Till now, there is still limited information known to allow an accurate designation of key enzymes to be targeted to ensure the high yield production of desired terpenoids in thraustochytrids. Further discovery of key synthases and other conversion enzymes, along with mechanisms of catalysis is no doubt needed to enhance carotenoid biosynthesis.

Development of molecular engineering approaches, in-situ gene knockout and gene complementation for instance, are useful biotechnology to confer manual control in the expression level of desired genes associated with terpenoids production. Cases have been reported for engineering specific FAS to achieve better yield in desired fatty acids through *in-vitro* and *in-vivo* assays, encouraging similar approaches to be taken to enhance terpenoid production in thraustochytrids. Discovering molecular targets for genetic engineering is therefore of much significance. Comprehensive evaluation in the contribution of related enzymes and pathways to terpenoids biosynthesis has been initiated in thraustochytrids. Transcriptomics studies suggested a few key enzymes to be manipulated to improve their yields. Identification of homologues of key enzymes like FPP synthase and squalene synthase would aid in the development of suitable genetic systems in thraustochytrids. Several thraustochytrids genome sequences are becoming available that will aid computational studies and develop hypotheses for further experimental testing through omics tools and enzyme kinetics. Such strategy will lead to a system-level metabolic engineering for thraustochytrids strain improvement. We expect these studies to be carried out in thraustochytrids to allow better terpenoid production for commercial application in future.

7 Conclusions

Terpenes are important nutraceuticals from a large number of microorganisms and higher plants. Thraustochytrids are emerging heterotrophic protists holding great potential to become an excellent microbial factory to produce terpenes as a complement to traditional producers such as yeast and microalgae. At current stage, the knowledge in the molecular mechanisms that explain the terpene productivity of thraustochytrids is still infantile. A review of both fermentative studies and molecular mechanistic studies from thraustochytrids and other marine microorganisms provided valuable information that would allow future developments of metabolic engineering design and synthetic tools towards thraustochytrids strain improvement and commercial applications**.**

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Conflict of interest

Authors declare that they have no conflicting interests.

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Figure legends

Figure 1 Phylogenetic analysis of high yielding terpenoids-producing thraustochytrids. The tree was built using RAxML (version 8.2.8) after executing 1000 rapid bootstrap inferences and thereafter a thorough ML search. *Phaeodactylum tricornutum* and *Saccharomyces cerevisiae* were included for phylogenetic comparision.

Figure 2 Carotene to astaxanthin conversion in thraustochytrids and *Phaffia rhodozyma*. Two eukaryotic marine protists are included to demonstrate the conversion routes from β-carotene to astaxanthin. Intermediate steps occurring in thraustochytrids features the combined use of ketolase and hydroxylase for the hydroxylation of carbon atoms on the ring. In *Phaffia rhodozyma*, a single P450 monooxygenase is considered responsible for all hydroxylation reactions.

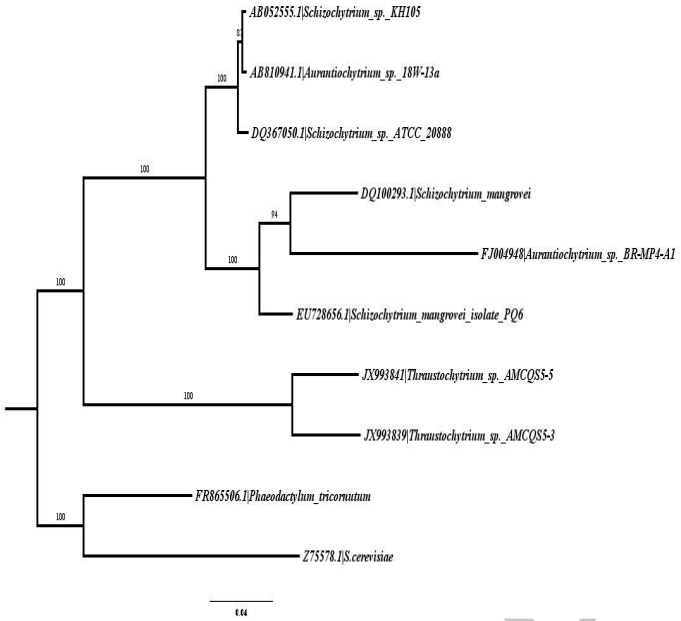
Figure 3 Overview of putative terpenoids biosynthetic pathway and regulations in *Thraustochytrids*. External signals (oxidative stress and medium carbon over nitrogen ratio) dictate the regulation of intracellular formation of terpenes and polyketides. Oxidative stress poses a global regulation mode by working on early conversions during glycolysis and in latter steps towards carotenoid biosynthesis. Medium carbon over nitrogen ratio dictates the enzymatic activity of NAD^+-isocitrate dehydrogenase and intracellular citrate stock, therefore affecting total acetyl-CoA concentration and the subsequent conversions to terpenoids. Putative Mevalonate, Polyketide and MEP/DOXP pathways for terpene biosynthesis are also presented.

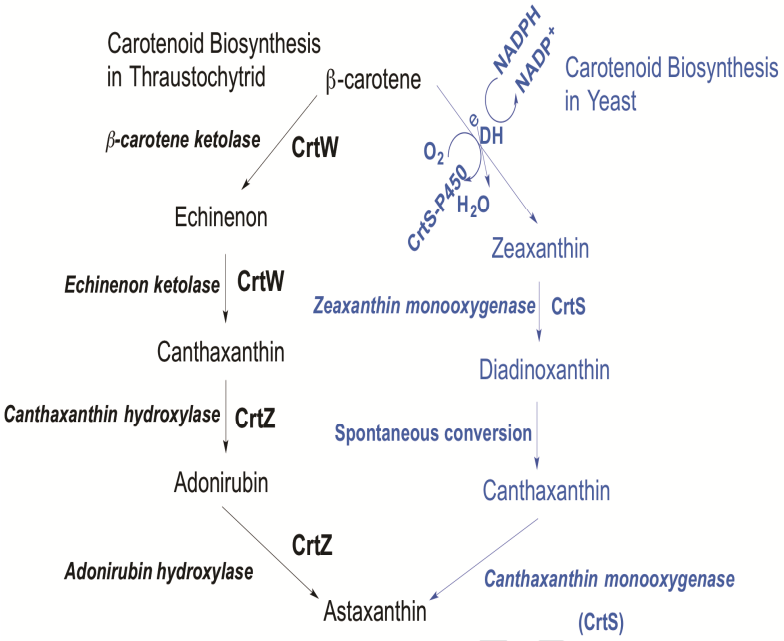
Figure 4 Association between terpenoids and docosahexaenoic acid (DHA) biosynthesis in *Schizochytrium* species. Using acetyl-CoA as initiating building block for both DHA and terpenoids biosynthesis, there are two competing routes, Mevalonate pathway and Polyketide synthase pathway, that are responsible for terpenoids and DHA biosynthesis, respectively.

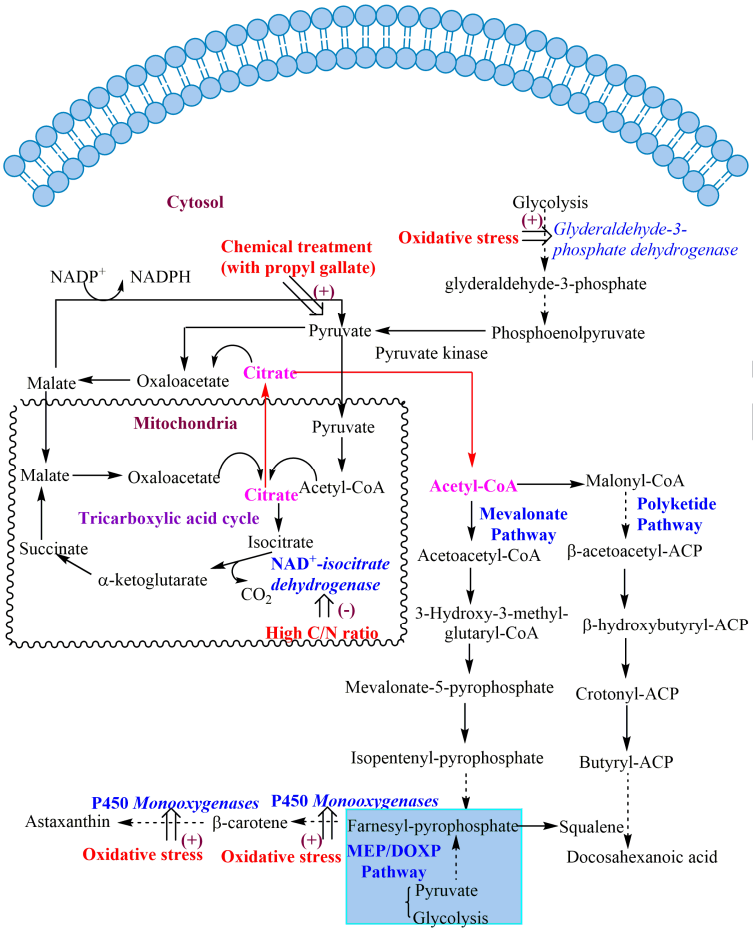
Figure 5 Amino acids sequence alignment for squalene synthase protein reported in four *Aurantiochytrium* strains. Amino acids are colored to reflect different polarities of the amino acid side chains with red color showing non-polar small side chains and aromatic side chains, red pink color showing alkaline side chains, green color showing polar while pH neutral side chains and blue showing acidic side chains. Identical and similar amino acids are denoted with asterisks and dots. The four *Aurantiochytrium* strains are: *A.* sp. KRS101 (AFU06380.1), *A.* sp. TA4 (ALE71467.1), *A.* sp. qe-4 (ALE71466.1), and *A. limacinum* (ABE97915.1).

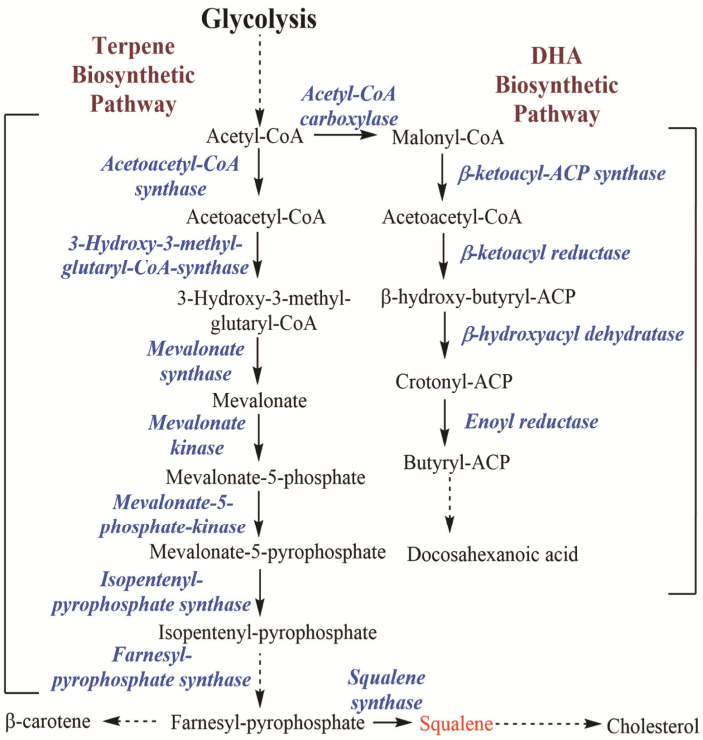
Table 1 Terpenoids production in the reported thraustochytrids strains

Strains	Compounds	Carbon source	Yield	References
Aurantiochytrium sp. KH105	Xanthophyll	Fructose	3.60 mg/L/day	Arafiles et al. 2014
Schizochytrium sp. KH 105 (commercial)	Astaxanthin/ cantaxanthin	Glucose	(10 mg/g)/(6.1 mg/g)	Aki et al. 2003
Thraustochytrids strains from Tasmania (temperate) and Queensland (tropical), Australia	β, β-carotene, canthaxanthin and astaxanthin	Glucose	112 μg/L (astaxanthin)	Lee Chang et al. 2012
Thraustochytrids strains from Puerto Montt coastal zone, Southern Chile	Astaxanthin	Glucose	229±15 μg/L	Quilodrán et al. 2010
Thraustochytrids strains from Atlantic Canada	β-carotene	Glucose	20.23 μg/g	Burja et al. 2006
Schizochytrium sp. KH105 (commercial)	Astaxanthin	Glucose	7.7 mg/L	Yamasaki et al. 2006
Thraustochytrium AMCQ S5-3, *Thraustochytrium* AMCQ S5-5 and *Schizochytrium* ATCC 20888-S31	Astaxanthin	Glucose	50–60 μg/g 50–60 μg/g 20.28 μg/g	Gupta et al. 2013b
Schizochytrium limacinum BR2.1.2 (commercial)	Astaxanthin	Glucose	8.9 mg/L	Chatdumrong et al. 2007
Aurantiochytrium sp. KH105 (commercial)	Astaxanthin	Glucose, charcoal, citric acid	1.1 mg/L	Iwasaka et al. 2013
Schizochytrium mangrovei	Squalene	Glucose	0.162 mg/g	Jiang et al. 2004
Aurantiochytrium sp., BR-MP4-A1	Squalene	Glucose	0.18 mg/g	Li et al. 2009
Aurantiochytrium sp. 18W-13a	Squalene	Glucose	1.0737 g/L	Nakazawa et al. 2014
Schizochytrium mangrovei PQ6	Squalene	Glucose	1.019 g/L	Hoang et al. 2014
Aurantiochytrium sp., BR-MP4-A1	Squalene	Glucose	5.90 mg/L	Chen et al. 2010
Aurantiochytrium mangrovei FB3	Squalene	Glucose	3.1 mg/L	Fan et al. 2010









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gb|ALE71467.1|      MPNKPDAPIRLAVGIFYIVLRALDTVEDDMNLSSFDSYVLEEDKKDVEDARSAAKQRLLC
gb|ALE71466.1|      MPNKPDAPIRLAVGIFYIVLRALDTVEDDMNLSSFDSYVLEEDKKDVEDARSAAKQRLLC
gb|ABE97915.1|      MPNKPDAPIRLAVGIFYIVLRALDTVEDDMNLSSFDSYVLEEDKKDVEDARSAAKQRLLC
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gb|AFU06380.1|      QFAQRLSDSVEGNADKHQPLHGFGEGHERELIENMDAIVYGMSVLPPKLRQVVLDITEEM
gb|ALE71467.1|      QFAQRLSDSVEGNADKHQPLHGFGEGHERELIENMDAIVYGMSVLPPKLRQVVLDITEEM
gb|ALE71466.1|      QFAQRLSDSVEGNADKHQPLHGFGEGHERELIENMDAIVYGMSVLPPKLRQVVLDITEEM
gb|ABE97915.1|      QFAQRLSDSVEGNADKHQPLHGFGEGHERELIENMDAIVYGMSVLPPKLRQVVLDITEEM
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gb|AFU06380.1|      GVGMAGYVSRDLKNGTDDAKDFEQYCHYVAGTVGDGLTRIFASCGYCPADLVSHRELWDA
gb|ALE71467.1|      GVGMAGYVSRDLKNGTDDAKDFEQYCHYVAGTVGDGLTRIFASCGYCPADLVSHRELWDA
gb|ALE71466.1|      GVGMAGYVSRDLKNGTDDAKDFEQYCHYVAGTVGDGLTRIFASCGYCPADLVSHRELWDA
gb|ABE97915.1|      GVGMAGYVSRDLKNGTDDAKDFEQYCHYVAGTVGDGLTRIFASCGYCPADLVSHRELWDA
                    ************************************************************

gb|AFU06380.1|      MGSFLQRTNIIRDYLEDLVDGRAWWPRSVWELYVTKDAEFGRSKSLSRLADSASIEAGHS
gb|ALE71467.1|      MGSFLQRTNIIRDYLEDLVDGRAWWPRSVWELYVTKDAEFGRSKSLSRLADSASIEAGHS
gb|ALE71466.1|      MGSFLQRTNIIRDYLEDLVDGRAWWPRSVWELYVTKDAEFGRSKSLSRLADSASIEAGHS
gb|ABE97915.1|      MGSFLQRTNIIRDYLEDLVDGRAWWPRSVWELYVTKDAEFGRSKSLSRLADSASIEAGLS
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gb|AFU06380.1|      TSCLNHMIADALEMVGSCLSYLEALNDPDVISFCALPQVMAIATLSVCFDNQKVFQGVVK
gb|ALE71467.1|      TSCLNHMIADALEMVGSCLSYLEALNDPDVISFCALPQVMAIATLSVCFDNQKVFQGVVK
gb|ALE71466.1|      TSCLNHMIADALEMVGSCLSYLEALNDPDVISFCALPQVMAIATLSVCFDNQKVFQGVVK
gb|ABE97915.1|      TSCLNHMIADALEMVGSCLSYLEALNDPDVISFCALPQVMAIATLSVRFDNQKVFQGVVK
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gb|ALE71466.1|      IRKGQAARIMLDMSPMEHPTIFELQQNYLSWFARCTREIQAKARRAATRDPQAQRTDNIC
gb|ABE97915.1|      IRKGQAARIMLDMSPMEHPTIFELQQNYLSWFARCAREIQAKARRAATRDPQAQRTDNIC
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gb|ABE97915.1|      GKIIKVVDSKLQCLQESGRPLGQTHSD
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Highlights

1. Thraustochytrids are promising marine microalgae that posses diversified intracellular nutrient assimilation strategies for high terpenoid production.
2. Complete putative mechanism of terpenoid biosynthesis in thraustochytrids strains is still unclear and warrants further research.
3. Discovery of key synthases and pathway enzymes along with mechanisms are needed to enhance terpenoid biosynthesis.
4. Identification of homologues of key enzymes, FPP synthase and squalene synthase, would aid to develop suitable genetic systems in thraustochytrids.