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Promising Polyunsaturated Oils from Marine Haptophyta

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Abstract

Energy and Environment play a key role for the sustainability and survival of a society. At present these are the two major concerns in current world. Rapidly increasing energy demands and decreasing healthy environmental conditions pose a serious threat to future generations of humanity. The quest to find clues for prediction of future environment from past information and search for alternate energy sources has already begun. In this regard, the photosynthetic marine micro algae and the organic compounds produce by them are considered as the potential targets to address the environmental challenges. Poly-unsaturated long chain ketones (PULCAs) are such compounds produced by few species of marine Haptophyta. These compounds are identified to be unique lipids which are considered as potential biofuel precursors. The PULCAs are generally called as alkenones and are comprised of more than 40% of total lipid content in haptophyte species like *E.huxley* and *Gephyrocapsa*. Current paper provides a brief overview on these compounds.

Keywords: PULCAs; Haptophyta; Bio-fuel; Biomarkers; Alkenones

Introduction

Due to over use of natural oil reserves there is a significant increase in CO_2 levels as well as simultaneous decrease in the climatic conditions and energy reserves. This situation is leading towards energy crisis and global warming. In order to prevent further damage, the scientific community is searching for alternate solutions from the photosynthetic organisms. Life on earth has proliferated from simple single celled microbes in oceans to multi-cellular complex beings on land, water and air. Out of which marine photosynthetic microbes lie in the primary line of evolution. Marine phytoplankton evolved through a various symbiotic processes to thrive in response to environmental conditions and contributed to global bio-geochemical cycles [1].

Till date a wide range of algal species were discovered and are used as standard models for several research purposes in food, fuel and pharma industries. Some of them are of significant importance to scientific community because of their unique biochemical mechanisms and carbon containing products [2-4]. Especially, certain lipids like fatty acids, sterols, alcohols, Oxylipins etc., produced by these photosynthetic marine microbes like Cyanobacteria and diatoms are very useful in geophysical, food, biofuel and pharma industries. Such lipid deposition from plants and algae in the layers of earth from the past million years triggered formation of crude oil reserves. However, these are being depleted rapidly. Currently there is a high demand to explore renewable and environment friendly energy sources to prevent increasing CO_2 levels and global temperature. Therefore photosynthetic micro algal lipids are extensively studied for this purpose.

Alkenones are one such unique lipid molecules produced by marine haptophyta. These lipids are widely considered as potential subjects for addressing environmental challenges. The haptophytes that produce alkenones are widely distributed in the open oceans and are considered to be dominant species that can survive through diverse nutrient fluctuations [5-7]. These organisms have been thrived in earth's waters over a million years through adverse environmental conditions. These alkenone producing organisms played a key role in contributing to earth's early biosphere through oxygen production, carbon and sulfur cycling [3,4,8]. The alkenones, produced and deposited into sea sediments, by these organisms are also identified to have significant paleo-climate information [5]. In addition, they are also assumed to be potential biofuel precursors. Various studies on alkenones have been carried out till date to elucidate their applications. This article is aimed to give a brief over view on the alkenone research with regard to their biological role and biofuel potential.

Poly-Unsaturated Long Chain Ketones (Pulcas)

Alkenones are unique neutral lipid molecules first discovered in the marine sediments [9,10]. The characteristic features of these unique lipids are very long carbon chains (C_{36}-C_{40}), ~(2 to 4)-*trans* double bonds and a methyl or ethyl ketone functional group at one end as shown in figure 1 [10,11]. Alkenones along with other related compounds like alkenes and alkenoates are collectively referred to as polyunsaturated long chain alkenones (PULCAs). Only few marine haptophytes namely *Emiliania, Gephyrocapsa, Isochrysis, Tisochrysis and Chrysotila* are known to produce these components [5,6,12].

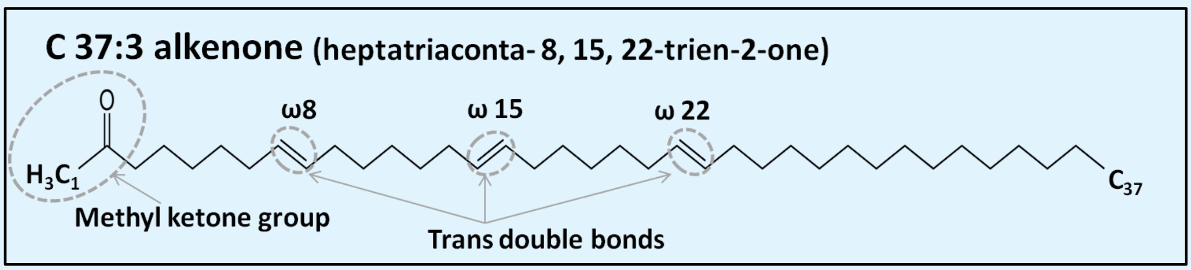

Figure 1: Figure showing structure of $C_{37:3}$ alkenone with its *trans*-double bonds and a methyl ketone group.

The alkenone composition, distribution and double bond number varies depending on algal species, habitat and the growth conditions like temperature, salinity and nutrient availability. For example, alkenones with two to three double bonds and C_{37}-C_{39} carbon chain length are most commonly identified in *Emiliania* and *Gephyrocapsa* thriving in marine and lacustrine environments [5,9-15]. While, C_{35}-C_{36}, $C_{38:4}$-$C_{37:4}$, and C_{40}-C_{41} type alkenones were also produced by these haptophytes and specifically identified in sediments of black sea, cold waters, sulfate-rich lakes and hyper-saline environments [16-20]. The trans-unsaturation of these molecules is identified to be linearly proportional to temperatures and therefore they are used as proxies to estimate ancient sea surface temperatures, SST [21-23]. These compounds are mainly useful in geophysical studies to estimate paleo temperatures and paleo CO_2 [24-26]. Because of such wide distribution, unique structural features and applications, alkenones are considered useful macromolecular components in haptophytes.

Besides being widely accepted as biomarkers for temperature and CO_2, alkenones are also assumed to be potential biofuel precursors. Several studies on the characteristics of alkenones speculated their biological role as neutral lipids, buoyancy regulators or structural backbones [10,27-31]. Their role as storage lipids like triacylglycerol (TAG) is highly under debate and yet to be explored. It was also observed that, TAGs are comparably produced in very low amounts compared to alkenones in haptophytes like *E.huxleyi* and *Gephyrocapsa* [6,32-34]. Alkenone unsaturation being temperature dependent and their role as storage lipids was speculated to have evolved through natural selection [30]. Furthermore, the mechanisms involved in their biosynthesis, trans unsaturation were also explored and identified to be downstream of fatty acid biosynthesis and elongation pathways [36-39]. In the process of unraveling their biosynthesis mechanisms, identification of the localization of alkenones was also studied. The abundance of alkenone composition from membrane fractions was found in endoplasmic reticulum and coccolith producing compartments, while fluroscence and nutrient/light stress studies showed present of nutral lipids in chloroplasts and lipid vesicles [40-41]. Despite such extensive studies on characteristics and applications of alkenones, their biological role and complete mechanism of synthesis are still unknown.

Among all alkenone producing haptophytes, *E.huxleyi* is the major producer of alkenones and a dominant marine coccolithophore [9,10,33,42]. Most studies are carried mainly using *E.huxleyi.* Studies on carbon flow in alkenone producing haptophytes showed that 15-20% of the carbon is incorporated in to lipids among which 63-75% is in the form of neutral lipids [27]. The carbon content is around 48-64% under nutrient replete conditions/ normal conditions and tends to increase under nitrate or phosphate limitations [29,30,41,43]. Recently, nitrate limited experiments of Bakku et al. 2017 [44] also showed alkenones as major C storage pools under N- limitation in *E. huxleyi* as a significant increase in %organic carbon (15% Ca. to 27% Ca.) was observed upon transferring the cells from N-replete to N-limited conditions.

Initial studies on lipid class composition of 8 isolates of *E. huxleyi* showed that neutral lipid composition can vary from 38.3% to 67.3% of total lipid content during log to stationary phases [33]. Alkenones in some strains of *E.huxleyi* and *Gephyrocapsa* are known to occupy nearly 50-60% of the total lipid content and can increase upto 73-77% under stressed conditions [33,44,45]. In *Isochrysis sp.* though comparatively less, nearly 14% w/w of biodiesel are found to be alkenones [46]. Additional studies on lipid bodies of haptophytes like *Tisochrysis lutea* indicated that nearly 70-74% of its lipid body is also composed of alkenones [42]. Furthermore it was observed that using techniques like pyrolysis or butenolysis alkenone can be converted into short chain hydrocarbons like n-alkanes and jet-fuels [48-50]. Also, alkenones are considered to be stable molecules against photo oxidation due to embedded *trans* double bond geometry [36]. Having less unsaturation number, resistant to photo oxidation and absence of glycerol backbone are the advantages of these molecules over TAGs. However, alkenones cannot be used as a direct biofuels due their high boiling points (>60˚ C) compared to TAGS [46,50,51]. Therefore, though the use of alkenones as biofuels could be advantageous technical difficulties in conversion of alkenoenes into industry level biofuels still persist. Identification of alkenone biosynthesis pathways and subsequent regulation towards production of short chain hydrocarbons could be an alternative way for efficient biofuel synthesis.

Conclusion

Renewable organic compounds that can serve as multipurpose resources are very useful for research and industry. Especially lipids that can serve as both biomarkers and biofuel molecules are rare and valuable sources for environmental studies. The PULCAs like Alkenones fit in this category perfectly. Alkenones are already well known for their applications in paleothermometry and paleo-CO_2 analysis. On the other hand their potential to be used as biofuels is yet to be explored fully. At the moment though their biosynthesis mechanism is not known, their role as major storage lipids is confirmed. Despite their high melting points, their accumulation in haptophyte strains like *E.huxleyi* is very convincing for modeling towards large scale production. Future research on revealing alkenone biosynthesis pathways could help in directing towards synthesis of jet-fuel range hydrocarbons. In addition, cultivation of such haptophytes in open oceans could be the next step towards biofuel production.

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