Halophilic benthic diatom
Amphora coffeaeformis—A potent biomarker for lipid and biomedical application

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Biomarkers offer a powerful means to study life and its interaction with the environment. The evolutionary record of eukaryotic phytoplankton has been studied through fossils and molecular biomarkers, such as lipids and nucleic acids. Algal taxonomic groups containing carotenoids serve as age diagnostic biomarkers associated to photosynthetic pigments or fatty acids. Diatoms are known to thrive even in hypersaline conditions which are restricted to higher salinity. Results of this study have revealed the presence of a specific highly branched isoprenoid (HBI) compound, squalene (0.004 g) in the halophilic benthic diatom Amphora coffeaeformis which is considered to be the marker for hyper salinity. It also corresponds to the biomarker of oxidation processes.

Keywords: Hypersaline, Highly branched isoprenoids (HBI), Squalene

Diatoms are unicellular eukaryotic algae that entered the fossil record 150 million years ago. They are found in both freshwater, brackish water and marine ecosystem and also in benthic sediment regions. Diatoms have a unique structural cell wall which contains the hydrated silica wall made up of glass. They are widely used for climate and environmental studies although their use is strongly hampered by the fact that silica cell walls are prone to dissolution. Among the microalgae, diatoms are found to be predominant in ocean ecosystem which involves in CO2 sequestration to mitigate the fossil fuel gases present in the atmosphere.

Petroleum is one of the hydrocarbons which are currently being exploited and there is an urgent need for an alternate resource. Currently, diatoms are responsible for a large fraction of the organic carbon buried on continental margins and are major contributors to nascent petroleum reserves. Diatom nobs in tertiary opal shales were reported by Tolman (1926). These nobs were apparently secreted primarily by diatoms including the hydrocarbons and other organic matter found in them. Occurrences of early historical diatoms have been reported in the late Cretaceous period and they were aggregated as dead diatomite beds in the fossil record. Diatoms are enriched with oils, fatty acids, sterols and metabolites. Geologists claim that much crude oil comes from diatoms.

Biomarkers are considered to be the fingerprints of the nature. Unsaturated alkenes with one to four double bonds are typically used as biomarkers for microalgae. A biomarker has to be unique to its original material and resistant to degradation. Due to rapid developments in analytical techniques and instruments over the last decennia, molecular biomarkers are now commonly applied by both petroleum geochemists and biogeochemists but there is still a large demand for new, well defined biomarkers.

Lipids have relatively high resistance to decomposition over long time compared to other major components of life; lipids are suitable for use as biomarkers. Molecular fossils that are stable under geological conditions are found to have originated from biological lipids. The source of the algae can often be traced from analysis of lipids that act as biomarkers, and are stable over several million years in the petroleum reservoir. The important criterion for biodiesel feedstock is the suitability of fatty acid profile. The purpose of this investigation is to find out the hydrocarbon compounds present in the diatom Amphora coffeaeformis and their significance in relation to the geochemical and biological applications as biomarkers.

Materials and Methods

Diatom culture

Diatom samples were collected from salt pan located at Mimisal (Lat. 9° 51’ 48” N Long. 79° 7’ 15” E), Pudukottai district, Tamil Nadu, India. They were morphologically observed under electron microscope and identified as Amphora coffeaeformis (Fig. 1). The isolated diatoms were cultivated in the laboratory with TMRL medium. The seawater used
The growth medium was sterile filtered, enriched with the full set of nutrients for the growth and kept at 25°C in a thermostatically controlled room, illuminated with white florescence lamps with 12:12 h of light/dark regime. The temperature and salinity was maintained in the range of 23-25°C and 28-30 ppt. The culture was harvested during the exponential phase of diatom on 15th day and made to settle down. The collected wet diatom biomass was dried in hot air oven for 8 h at 60°C. The diatom samples were acid digested to remove the organic content for FESEM study.

Hydrocarbon analysis by GC-MS
Two grams of dried diatom biomass was homogenized with hexane. The hexane layer was used for GC-MS analysis to detect the hydrocarbons. GC-MS analysis was performed using GC Clarus 500 Perkin Elmer mass detector with Elite-5MS column (30 × 0.25 mm × 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1mL/min. The injection port was maintained at 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass scanning range was from 45-450 (m/z).

Identification of components
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST), US Department of Commerce. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test diatom were ascertained.

Results and Discussion
The chromatogram obtained by the hexane fraction is shown in Fig. 2. The area of the peak, molecular weight and molecular formula are presented in Table 1.

Marine ecosystem is considered to be a complex environment which favours the growth of diverse organisms. Diatoms are the microscopic primary producers which represents the base of food chain in the aquatic environment. Based on the field and microscopic studies of sediments and diatom blooms, concluded that diatoms directly produce hydrocarbon oils. Biomarkers are a group of compounds which are primarily the hydrocarbons, widely found in oils, rock extracts, sediment extracts, and recent soil extracts. Marine diatoms serve as a basis for all the essential biomarker. The basic line of reasoning of geologists in attributing crude oil to diatoms is that they comprise the bulk of the ocean phytoplankton, so they must be a major source of the oil. Lipids are considered essential in many
vital processes such as cell survival, proliferation, interaction and apoptosis. Hence, to investigate the hydrocarbon producing nature of marine diatom, the *Amphora coffeaeformis* was isolated from the salt pan (Fig. 1) and tested for its biomarker components.

Results from the GC-MS revealed the occurrence of alkenes and alkylene group of unsaturated hydrocarbons in the diatom (Table 1, Fig. 2). Certain species of diatoms are capable of synthesizing unusual C25 and C30 highly branched isoprenoid (HBI) alkenes. Squalene content in the sample was found to be 0.004 g, and has a higher molecular weight. C13H26 group of compounds in the GC-MS were identified as antimicrobial agents. Due to the oil forming chemical nature of alkenes they are also called as olefins. Alkenes are another class of highly branched isoprenoids (HBI) which are used as biomarkers. HBI are thought to reflect contributions from microalgae and have been recently identified in both benthic and pelagic diatoms.

The compound squalene originally belongs to the triterpenes which are believed to be the forerunner of hydrocarbons. Squalene is a polyunsaturated hydrocarbon liquid (C30H50) which is a commercial medicinal compound found to originate in marine sharks has now been reported to be present in the marine diatoms. It is one of the prime and stable isoprenoids. Squalene is a triterpenoid found in both plants and animals and the precursor to a class of compounds called steroids. In human and animals, squalene supplementation in their diets reduces cholesterol and triglyceride levels. It is found to be common in diatom blooms where it may be used to control buoyancy. High abundance of squalene in oils and rocks from hypersaline environment led to suggest it to be a marker for hyper salinity. The detail of benthic diatoms provides an interesting window into the geological biomarker and the roles of lipids and hydrocarbons.

**Conclusion**

Diatoms serve to be multifunctional which may possibly be used for various ecological and biotechnological applications. Importantly, marine diatoms could be used as age-diagnostic biomarkers for biomedical applications especially the presence of highly branched isoprenoid squalene, which have been proven to hold numeral biomedical property. Also, according to the geological records, marine diatoms are known to be excellent hydrocarbon oil accumulators, hence could be explored for biofuel production.

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