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Intra- and interspecies variations in intercellular concentrations and compositions of alkene and alkenone in Haptophyte

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Haptophyte algae are one of the major primary producers in the ocean. Long-chain alkenones, unsaturated linear methyl and ethyl C_{37}-C_{40} ketones, are synthesized by few species of haptophyte algae (*Emiliania huxleyi*, *Gephylocapsa oceanica*, *Isochrysis garbana* and *Chrysotila lamellosa*). Alkenones have frequently been used for estimating the paleotemperature in geological samples, since the number of double bonds change in response to the growing temperature. Along with alkenones, these haptophyte species also produce polyunsaturated long-chain alkenes. Long-chain alkenes are more susceptible to diagenetic process (i.e. photochemical and bacterial degradations), hence are regarded less important to apply in geological past. Therefore, compositions and distributions of long-chain alkenes among its producers have not been systematically examined. Recently, algal biomass is expected to be a new energy resource. We look for the use of haptophyte algae for biorefinery, based on their high rate of reproduction, high content of long-chain lipids, and some other positive features. Alkenes gain importance in this context because of its chemical nature as hydrocarbon. In this study, we analyzed alkene and alkenones in the haptophyte algae in order to gain suite of concentration and compositional data covering wide range of Haptophyte algae.

More than 50 strains of haptophyte algae were obtained from stock culture of Shiraiwa's laboratory and the other culture collections (e.g. NIES, NCMA, RCC). These strain were grown at 17^{o}C & 20^{o}C for 10-21days. Extraction and separation of lipids were performed based on Sawada and Shiraiwa (2004). After extraction, the lipids were separated by silica gel column, and fraction 1, 2 and 3 (hexane, hexane / ethyl acetate (95/5 v/v), hexane / ethyl acetate (9/1 v/v)) were analyzed by gas chromatography with flame ion detector (GC-FID) and gas chromatography / mass spectrometer (GC/MS).

Alkenes (C_{29}-C_{38}) and alkenones with alkenoates (C_{37}-C_{40}) were detected from strains of four species: *E. huxleyi*, *G. oceanica*, *I. garbana* and *C. lamellosa*. Other species lack all of these compounds. The total concentrations of alkenones and alkenes were 0.02-1.96 pg/cell (0.09-11.1 ug/ml) and 0.001-0.57 pg/cell (0.01-1.58 ug/ml), respectively. Both intercellular concentrations and compositions of these compounds showed significant differences between strains. A majority of strains mainly contain C_{31} and C_{33} alkenes, while some others contain C_{37} and C_{38} alkenes in significant proportion. Furthermore, some strains of *E. huxleyi* contained significant amount of C_{29} alkadienes. Rieley et al. (1998, Lipids 33, 617-625) reported that C_{37} and C_{38} alkenes have *trans* double bonds resemble to those of C_{37} and C_{38} alkenones while C_{31} and C_{33} alkenes have *cis* double bonds, suggesting distinct biosynthetic pathway for these two groups of alkenes. However, consistent occurrence of alkenes and alkenones highlights close biochemical relationship between these two groups of compounds, as well as importance of the four alkenone producing species as potential hydrocarbon resource.

Keywords: Haptophyte, alkenone, alkene, biorefinery