

**CHANGE OF UNSATURATION DEGREE OF ALKENONES DURING
ACCLIMATION TO SALINITY CHANGE IN *ISOCHRYSIS GALBANA* WITH
REFERENCES TO PALEOSALINITY PROXY**

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Long-chain (C₃₇–C₃₉) alkenones, which are derived from Haptophycean algae, especially Gephyrocapsaceae and Isocrysidaceae, have been well used as the proxy for paleotemperatures of sea surface water, as reviewed by Brassell (1993). Recently, it was reported that the relative abundance of 4-unsaturated alkenones (e.g. C_{37:4} alkenone) increased with decreasing salinity, and therefore, the unsaturation ratios and the compositions of the alkenones could be potentially used as a proxy for paleosalinity of ambient waters (Rosell-Mele, 1998; Harada et al., 2003). We have been investigated variations of the unsaturation ratios of the alkenones for culture strains of Haptophycean algae *Emiliania huxleyi* and *Gephyrocapsa oceanica*, which are typical source organisms in marine environment, against the changes of salinity in order to clarify the relationships between alkenone unsaturation ratio and salinity (Sawada et al., 2001). Nevertheless, the 4-unsaturated alkenones could not be detected in both low and high salinity conditions of these algae. Thus, in this study, the variations of the alkenone unsaturation ratios of a culture strain *Isocrysis galbana*, which is generally coastal Haptophycean species, were investigated and the potential for paleosalinity proxy in the 4-unsaturated alkenones was examined.

Isocrysis galbana Parke (strain CCAP927/I) was collected from Port Erin, Isle of Man, UK (Marlowe et al., 1984). This strain was grown at 15°C and 20°C under 35, 32, 27, 20 and 15‰ of salinity for 120–190 hours, and harvested in 48, 96 and 192 hours. Salinity conditions of culture cells were established by dilution of natural seawater and the concentration of medium (IMP medium) kept constant in all cultures. 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 (hexane and 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). The alkenones were detected in Fraction 1&2. Alkenone unsaturation degree (U^k₃₇) was calculated by the equation of Prahl et al. (1988) as follows, U^k₃₇ = [37:2] / ([37:2] + [37:3]).

The time courses of the cell numbers of *I. galbana* indicated that the growth rates in 15‰ and 20‰ of salinity were significantly higher than those in 32‰ and 27‰ of salinity. This result indicated that the *I. galbana* can grow favorably in the lower salinity condition than typical ocean salinity. The relative abundance of C₃₈ ethyl alkenoates (C₃₆ fatty acid ethyl ester) was higher, and 3-unsaturated ethyl alkenoates, which was not common in *E. huxleyi* and *G. oceanica*, could be abundantly identified. Nevertheless, there were no peaks in the mass fragmentgram of m/z 526.5 (molecular ion of C_{37.4} alkenone). The fact clearly indicated that no 4-unsaturated alkenones must be contained in all samples of *I. galbana*, which agreed with the results obtained in *G. oceanica* and *E. huxleyi* as reported in Sawada et al. (2001). This result implies that abundance of 4-unsaturated alkenones in coastal and lacustrine environments is attributed to the occurrence of peculiar species that can synthesized the 4-unsaturated alkenones rather than physiological response against the environmental change as low (or high) salinity condition.

The U^k₃₇ values decreased at the range of 0.2 in 15‰ and 20‰ of salinity in spite of growing sufficiently, while the values were consistently uniform in 35‰, 32‰ and 27‰ of salinity. It was presumed that the U^k₃₇ values in *I. galbana* cell in 15‰ and 20‰ of salinity varied as a result of the acclimation to the lower salinity. In addition, the relationship between U^k₃₇ and growth temperature was established in *I. galbana* strain CCAP927/I at 15°C and 20°C, and was quite different from the U^k₃₇-temperatures relationships of *E. huxleyi* (Prahl et al., 1988; U^k₃₇= 0.034T + 0.039) and other strain (strain CCMP1323) of *I. galbana* (Versteegh et al. 2001; U^k₃₇= 0.00932T + 0.0413). This fact suggests that there are possibly genetic variations for the U^k₃₇ - growth temperature calibration in *I. galbana*.

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