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Steroid analysis in culture samples of Parmales: Search for Parmales biomarker

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Palmales is picoplankton that has siliceous tests, and may be closely related to diatom, which is a main important primary producer in the Cenozoic ocean. There have been no reports for siliceous fossil of Palmales. It is known to well preserve siliceous diatom fossil in ancient sediment, and however, such fossil is frequantly lost through its dissolution by diagenesis during post-deposition. Therefore, very small siliceous tests of Palmales must be easily dissolved by diagenesis, and it cannot evaluate the timing of first appearance and reconstruct productivity of Palmales by using its siliceous fossil. Thus, we clarified the Palmales biomarkers and their compositions, and these biomarkers are used as molecular fossils for giving understanding evolution processes and historical variations of productivity of this alga. In the present study, we try to search lipid biomarkers, especially steroid, of the Palmales, and to give understanding for taxonomic variability for steroid composition and concentration.

We use culture strains of *Triparma laevis*, *Triparma laevis f. longispina* and *Triparma strigata* for analysis of lipid biomarker. Wet culture samples were extracted with methanol/ dichloromethane, and the extracts were fractionated by silica gel chromatography. Polar fraction was silylated by BSTFA before analyses using GC/MS (Sawada and Shiraiwa, 2004, Phytochem. 65, 1299).

We can identify $C_{21:6}$ n-alkene, $C_{20:5}$ and $C_{22:6}$ n-alkenoic acids as well as C_{27}-C_{29} sterols as Palmales biomarkers. These lipids have been detected from diatom cultures as reported previously (e.g. Rampen et al., 2010, Limnol. Oceanogr. 55, 91). In particular, T. laevis strain is found to be characterized by overwhelmingly abundance of C_{29} beta-sitosterol. However, C_{28} sterol as ostreasterol is more abundant rather than C_{29} sterols in *T. strigata*. These results indicate that there is possibly interspecies variability in sterol composition within *Tripalma* genus. In addition, we can detect a number of unknown polar compounds with higher molecular weight. These unknown compounds may have potential as specific Tripalma biomarkers.

Keywords: Parmales, biomarker, culture, steroid, evolution of diatom, chemotaxonomy