# Lipid biomarker analysis of culture samples of genus *Tetraparma* of the Parmales (Bolidophyceae)

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Parmales 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 Parmales. It is known to well preserve siliceous diatom fossil in ancient sediment, and however, such fossil is frequently lost through its dissolution by diagenesis after deposition. Therefore, very small siliceous tests of Parmales must be easily dissolved by diagenesis, and it cannot evaluate the timing of first appearance and reconstruct productivity of Parmales by using its siliceous fossil. Thus, we clarified the Parmales 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. We have reported the lipid biomarkers from the isolated culture strains of genus *Triparma* (Kanou et al., 2013). In the present study, we analyzed the lipid biomarkers, especially steroids, of newly isolated genus *Tetraparma*, and to give understanding for their taxonomic variability for steroid compositions and concentrations.

We use culture strains of genus *Tetraparma*, *Tetraparma gracilis* and *Tetraparma pelagica*, and no species name, Scaly parma, for analysis of the lipid biomarkers. 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).

We can identify C21:6 *n*-alkene, C20:5 and C22:6 *n*-alkenoic acids as well as C27-C29 sterols in all samples of genus *Tetraparma* and scaly Parma. These lipids have been detected from diatom and genus *Triparma* cultures as reported previously (e.g. Rampen et al., 2010, Kanou et al., 2013). In particular, *Te. gracilis* and *Te. pelagica* strains is found to be characterized by overwhelmingly abundance of C29 stigmasterol. However, the scaly parma have abundant C29 $\beta$-sitosterol rather than C29 stigmasterol, which agree with the results of genus *Triparma* previously. The steroid compositions of genus *Tetraparma* are significantly different from the other Parmales. These results clearly indicate that there is possibly interspecies variability in sterol compositions within Parmales (Bolidophyceae).

The genus *Tetraparma* and scaly parma have been found to be more primitive parma by genomic analysis (Ban et al., 2019). Their lipid biomarkers may give understandings for the relationship between these primitive Parmales and diatom, and have potentials for chemotaxonomic indicators of siliceous microalgae.

Keywords: Parmales, biomarker, chemotaxonomy, evolution of siliceous algae, steroid

 