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# Molecular characterization of bacteria and kleptoplast within *Virgulinella fragilis*

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To understand the survival strategy of *Virgulinella fragilis* in oxygen-depleted and sulfide-enriched environments, molecular phylogenetic analyses and ultrastructural observation (TEM) were conducted. It is thought that existence both of endosymbiotic bacteria and kleptoplasts allow *V. fragilis* to survive in low oxygen environments. However, our observations suggest *V. fragilis* have similar bacteria and kleptoplast that exist both in oxygen-depleted and in oxygenated environmental specimens.

Samples were collected both from Namako-ike Lagoon, Kagoshima, Japan, and Wellington Harbor, New Zealand. The water depth of Namako-ike is 22 m, isolated from surrounding seas by a gravel bar. Lake water is stratified throughout the year, and shows low oxygen concentration (<0.1 mg/l) below a depth of 15m, where a hydrogen sulfide enriched environment is present. Light partially reaches the bottom of the lake due to existence of a discontinuity layer. On the other hand, well-oxygenated sea water (7 mg/l at 19 m) is present in Wellington Harbor. However, in the 1970's, environmental conditions were extremely degraded and an oxygen-depleted and sulfide-enriched environment predominated in the harbor. In this period, *V. fragilis* dominant in this harbor, which is the type locality of *V. fragilis* (Grindell and Collen, 1976). Environmental improvement has occurred since this time; the harbor is now a well-oxygenated environment.

Bacteria and kleptoplasts were determined from both Namako-ike and Wellington specimens. In Namako-ike specimens, a single morphological type of large-sized bacteria exists in host foraminifers. In contrast, two morphological types of bacteria exist in Wellington specimens. One morphotype is large-sized, and shows features similar to Namako-ike specimens. The other morphotype consists of small-sized bacteria. The large-sized bacteria from both locations exist in the cell cortex singularly in vacuoles whereas small-sized bacteria in

Wellington specimens were rapidly digested. In the results of clone analyses for bacterial 16S rDNA, most common were *Desulfobacterium corrodens* (ä-proteobacteria, similarity=94%) in both Namako-ike Lake and Wellington specimens, which we presume to be equivalent to the large-sized bacterium. Other clones from Wellington specimens were uncultured á-proteobacteria (similarity=90%) that we presume to be small-sized bacterium. Kleptoplasts existing inside the foraminiferal cells have a double membrane chloroplast-like structure located close to the foraminiferal cytoplasm, and are not exist inside vacuoles. Kleptoplasts were determined by a central pyrenoid and thylakoid lamellae. These morphological features were thought to be of diatom origin. In Wellington specimens, kleptoplasts had an accumulation of starch-like features. This suggests that some of the kleptoplasts in light conditions have a photosynthesis function. In the results of clone analyses, kleptoplasts were shown to be different diatom species; *Skeletonema pseudocostatum* (similarity=99%) for Namako-ike specimens and *Coscinodiscus radiatus* (similarity=99%) for Wellington specimens. *V. fragilis* obtain diatoms from each environment, and keep them in their cytoplasm.

Specimens from both environments have similar bacterial species and kleptoplast that are kept in the foraminiferal cell. Thus, it is not necessary to keep both endobionts in oxygen-depleted condition; both endobionts may have different behavior in each environment. In light or partially light conditions in shallow waters, we suggest two hypotheses:

1) In oxygen-depleted environment, the occurrence of hydrogen-sulfide due to ä-proteobacteria in the foraminiferal cell cortex is oxidized with kleptoplastic photosynthesis oxygen.
2) In oxygenated environment, bacteria show parasite-like behaviors that utilize organic materials likely caused by host foraminiferal activities or photosynthesized products in kleptoplasts.


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