Isolation and transmission electron microscopy of symbiotic *Chlorella* with PV membrane from *Paramecium bursaria*

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By Percoll density gradient centrifugation, symbiotic *Chlorella* cells were isolated from *Paramecium bursaria* with their surrounding peri-algal vacuole (PV) membranes. Integrity of PV and purity of *Chlorella* cells with PV membrane were monitored by transmission electron microscopy after *Chlorella* cells were isolated using different homogenization media. The best result, in which >80% of the isolated *Chlorella* cells retained the PV membrane without notable contamination with other cytoplasmic components, was achieved when isolation was performed with 200 mM sucrose, 1 mM EDTA–KOH and 10 mM HEPES–KOH (pH 7.5). Addition of K^+ was favorable in yielding higher amounts of *Chlorella* with PV membrane, but shrinkage of *Chlorella* cytoplasm was inevitably observed. Inclusion of Mg^{2+} in the isolation medium was effective in preserving nuclei of paramecium cells, but adhesion of cytoplasmic debris to PV membrane became pronounced. It was also found that the PV membrane was extremely vulnerable to even low levels of mechanical stress such as pelleting down onto the bottom of a glass centrifugation tube. Therefore, *Chlorella* cells were settled on a cushion of 80% Percoll. Isolation of PV membrane-bearing *Chlorella* cells will allow analysis of membrane proteins of the PV membrane and of metabolic activities of *Chlorella* with PV membrane *in vitro*.