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Note

An optimised protocol to prepare *Phaeocystis globosa* morphotypes for scanning electron microscopy observation

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ABSTRACT

A preparation protocol for scanning electron microscopy (SEM) was adapted and tested to the observation of *Phaeocystis globosa* morphotypes. For extra colonial cells, critical point drying (CPD) gave satisfactory results while for intra colonial cells, preservation with Lugol's iodine and/or glutaraldehyde followed by air-drying appeared as the most suitable method.

Phaeocystis is a widespread cosmopolitan genus of marine phytoplankton (Schoemann et al., 2005 for a review) driving global geochemical cycles (Verity et al., 2007 for a review) and ecosystem functioning (Nejstgaard et al., 2007 for a review). It has a polymorphic life cycle, where free-living flagellated cells alternate with gelatinous colonies reaching up to several cm (Riegman and Van Boeckel, 1996; Chen et al., 2002). Three *Phaeocystis* species are known to form large blooms of gel-like colonies namely *P. antartica*, *P. pouchetii* in cold waters and *P. globosa* in temperate coastal waters (Lancelot et al., 1998). Based on field and batch culture observations, four distinct cell types characterizing the life cycle of *Phaeocystis* were recorded (Rousseau et al., 2007 for a review): (i) two types of small haploid flagellates (3–6 µm) observed before colony formation, with one producing star-forming filaments and the other lacking filaments and stars; (ii) a diploid colonial cell (4–8 µm) embedded in the mucilaginous matrix formed by colonies and (iii) a large diploid flagellate (4–9 µm) appearing after colony disruption. However, many aspects of this complex life cycle such as morphotypes number and succession are still under debate. It therefore encourages target studies on *Phaeocystis* morphology (Verity et al., 2007), particularly field investigations since most of the studies carried out so far are based on batch culture observations. Conventional methods for SEM analysis of phytoplankton involve dehydration using either chemical compounds (e.g. osmium tetraoxide) or freeze drying (Hasle and Syvertsen, 1997). Among the latter, critical point drying (CPD) gives the most

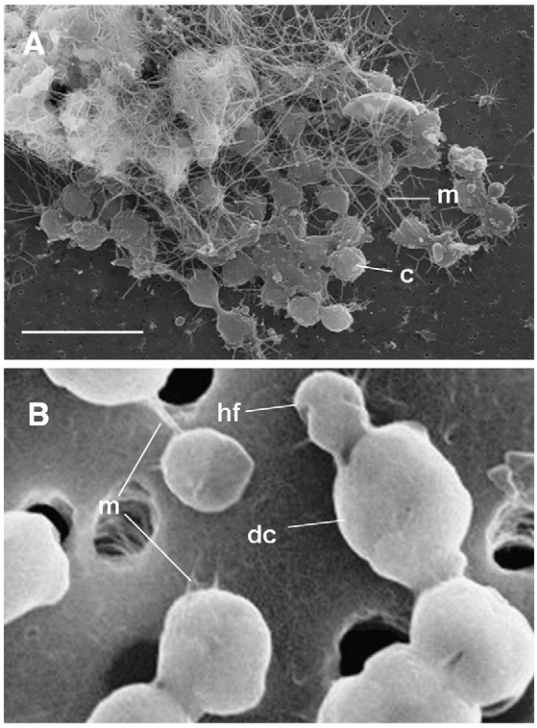

Fig. 1. SEM micrographs of two *Phaeocystis globosa* colonies: A) the first one was prepared with critical point drying. Due to the collapsing effect of the matrix, the colony membrane disappeared and mucous filaments were retracted binding cells together. Cell volume shrunk by 75% and cell shape was drastically distorted; B) the second one was prepared with 10% Lugol/glutaraldehyde and air-drying. Cell shape was well preserved and two types of cells were visible: *c* = cell; dc = diploid colonial cell; hf = haploid flagellate; *m* = mucus; scale bar = 5 µm.

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reliable results (Cohen et al., 1968) and is also successfully applied to the observation of *Phaeocystis* free-living cells outside colonies in culture (Vaulot et al., 1994; Antajan et al., 2004). While drying procedures allow a good preservation of ultrastructural details, first tests of CPD on *P. globosa* colonies showed an important alteration of the mucilaginous matrix and cell shrinkage (Fig. 1A). An alternative method is therefore needed to study in details cells inside colonies. To answer this question, we improved and adapted the existing SEM methodology to the *Phaeocystis* species, *P. globosa*. Two methods for SEM preparation were applied according to the two *P. globosa* cell types encountered during its life cycle: (i) extra colonial cells corresponding to morphotypes observed outside mucilaginous colonies and (ii) intra colonial cells corresponding to morphotypes located inside the colonial matrix. The first method carried out corresponds to the CPD and was applied to extra-colonial cells. The second method, based on fixation followed by air-drying of intracolonial cells needed improvement. Thirty samples were analysed and originated from a coastal survey carried out in spring 2006 in the eastern English Channel (50°46′N, 1°34′ E). Surface seawater was collected at 1 m depth (from 250 to 1000 mL) and samples were slightly prefixed on board with a solution comprising 50:50 of acid Lugol's iodine (Throndsen, 1978) and glutaraldehyde (Baker, Normapur; 37%) at 2% final concentration (v/v).

For the observation of extra colonial cells (≥3 µm), 25 to 50 mL of prefixed samples were filtered through 0.8 µm polycarbonate filters (Isopore, Millipore) previously soaked with a 0.01% (w/v) poly-L-lysine solution (Sigma-Aldrich, Normapur), thus allowing a better adhesion of cells to the filter (Antajan et al., 2004). The filters were then rinsed with ultrapure water (Millipore) to eliminate salt crystals. Dehydration was carried out by transferring the specimens through a graded absolute ethanol series (30, 50, 70, 80, 90 and 100%) and cells were then critical-point dried in liquid CO_2 using a BAL-TEC CPD 030 critical-point drying apparatus. For intra-colonial cells, we followed a fixation procedure recommended by Chrétiennot-Dinet (pers. comm.) based on high concentration (10% v/v) of a solution mixing Lugol and glutaraldehyde added in equal proportion (50:50). This fixative treatment was added for 10 min to 25 to 50 mL of prefixed samples. Samples were then filtered through 3-µm polycarbonate filters (Isopore Millipore) to preserve and concentrate colonies and their intra colonial cells. This filtration step also permitted to eliminate a large proportion of small (<3 µm) extra colonial cells which could produce visual noise on SEM photographs. Samples were then rinsed with ultrapure water and air-dried under a laminar flow hood. Before SEM observations, all dried filters were mounted onto SEM stubs and coated with gold-palladium in a sputter-cotter (Polaron SC7620). Micrographs were taken with a SEM LEO 438VP (tungsten filament). The microscope was equipped for imaging with a secondary electron

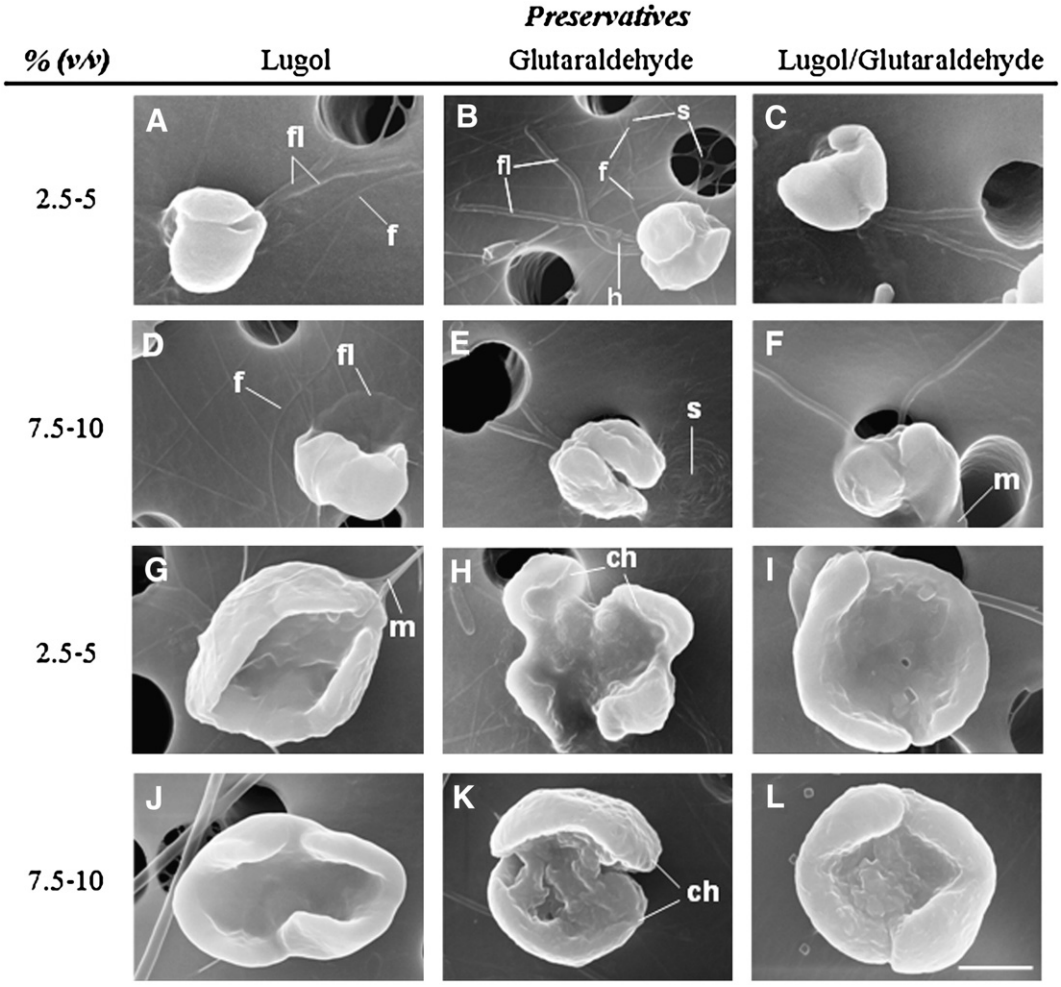

Fig. 2. SEM micrographs of haploid flagellates (A–F) and diploid colonial cells (G–L) belonging to colonies prepared for observation with different preservatives: Lugol, glutaraldehyde and a mixing of Lugol/glutaraldehyde at low concentrations (2.5–5% v/v) corresponding to A–C and G–I micrographs; and at high concentrations (7.5–10% v/v) corresponding to D–F and J–L micrographs. Micrographs presented here were also representative of the dominant cell morphology (>80%); ch = chloroplast; fl = flagella; f = filament; h = haptonema; m = mucus; s: star; scale bar = 2 µm.