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Spore adhesion and cell wall formation in *Gelidium floridanum* (Rhodophyta, Gelidiales)

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Abstract

The attachment of spores to a substratum is essential for their germination and, therefore, to the completion of the life cycle of the red algae. In most red algae, spores are liberated without a cell wall, within a sheath of mucilage which is responsible for their primary attachment. Utilizing fluorescent-labeled lectins, we identified carbohydrate residues and their locations in the mucilage and cell walls of spores of *Gelidium floridanum*. Cell wall formation and mucilage composition were studied with calcofluor, toluidine blue – O (AT-O), alcian blue (AB) and periodic acid-Schiff (PAS). In the mucilage we identified α-D mannose, α-D glucose, β-D-galactose, N-acetyl-glucosamine and N-acetyl-galactosamine. The first two sugar residues were not found in the cell wall of the germ tube but they were present on the rhizoid's cell wall indicating their importance to substrate adhesion. A cell wall is produced soon after the spore's attachment, beginning with a polar deposition of cellulose and its gradual spread around the spore as indicated by calcofluor. The cell wall matrix was positive to AB and metachromatic to AT-O, indicating acidic polysaccharides, while cellulose microfibrills were positive to PAS. A polar disorganization of the cell wall triggers the process of germination. As spores are the natural form of propagation of *Gelidium*, the understanding of the mechanisms of spore attachment may contribute to the cultivation of this valuable seaweed.

Introduction

The Gelidiales is a comparatively small order of red algae with about 140 species, some of which are important sources of bacteriological agar and agarose (Bailey, J.C. & D.W. Freshwater, unpublished). The order is characterized by a set of attributes, including a triphasic isomorphic life-history, and an intercalary carpogonium, which upon fertilization produces a gonimoblast connected to nutritive cells. The members of the order have agar in their cell walls and the spores germinate following a typical pattern known as "*Gelidium*-type" (Hommersand & Fredericq, 1988).

Spores are the natural form of dispersal in most red algae and their fixation to a substratum is a fundamental process in the development of the adult thallus (Chamberlain & Evans, 1981). Spores are the obvious link connecting the life-history phases of macroalgae, and their attachment is the first signal to triggering the metabolic changes that lead to germination.

Spores in red algae are released without a cell wall and they are surrounded by an optically transparent mucilage which is responsible for the first attachment to the substrate (Avanzini, 1989). This mucilage is composed of glycoproteins (Chamberlain & Evans, 1973; Pueschel, 1979) or sulphated polysaccharides (Ramus, 1974). Red algal polysaccharides have been characterized under light microscopy using different histochemical techniques (e.g. Gordon & McCandless, 1978; Cole et al., 1985; Rascio et al., 1991). However, most studies have been carried out with vegetative cells and not with spores. The mucilage that surrounds tetraspores of *Champia parvula* (C. Agardh)

Harvey reacts positively to sulphated and carboxylated polysaccharides (Apple et al., 1996).

Cytochemical methods based on the property of lectins to interact specifically with mono- and disaccharides have also been utilized to characterize cell wall compounds (Costas et al., 1993; Costas & López-Rodas 1994; Hori et al., 1996). Lectins conjugated to fluorescent dyes have been used to detect carbohydrate residues in mucilage and cell walls of microalgae (von Sengbusch et al., 1982; von Sengbusch & Müller, 1983; Callow, 1985).

In our study, we utilized cytochemical techniques to characterize the polysaccharides that participate in the attachment and cell-wall formation in the initial phases of tetraspore germination of *Gelidium floridanum* W. R. Taylor, an agarophytic alga of commercial importance in Brazil.

Material and methods

Tetrasporophytic specimens of *Gelidium floridanum* were collected at Ponta do Sambaqui, Ilha de Santa Catarina, in November 2003. Branch tips with tetrasporangia were placed on microscope slides in a petri dish with sterile seawater in the dark at 23 °C. After spore release, the branches were removed and the slides were exposed to fluorescent light (40 μmol photons m^{-2} s^{-1}; 14:10 h light: dark photoperiod), and kept at 23 °C. Periodically, slides, with attached spores, were fixed in 2.5% paraformaldehyde in phosphate buffer 0.2 M (pH 7.2), by dropping fixative on the slides that were covered with parafilm, during 5 h at 4 °C. After that, the slides were washed twice in phosphate buffer for 10 min.

Histochemical staining

Periodic acid-Schiff (PAS) was used to identify neutral polysaccharides, and the control consisted of staining the material without pre-treatment with the periodic acid (Gahan, 1984). Toluidine Blue (AT-O) was used to identify acid polysaccharides through a metachromatic reaction (Gordon & McCandless, 1978). Alcian Blue (AB) was used to identify acid polysaccharides according to Ravetto (1964). Coomasie Brilliant Blue was used to identify proteins according to Gahan (1984). To study the cell wall deposition, the spores were incubated in sterilized seawater containing 10 μg ml^{-1} of Calcofluor White M2R for 15 min (Kim & Fritz, 1993).

Probing with FITC-lectins

Spores were incubated with 100 μg ml^{-1} of FITC-lectins in 0.6 M of sorbitol with 10 mM of $CaCl_2$ diluted in distilled water for 1 h and washed with deionized water. The control was made with FITC-lectin, 0.2 M of glucose, 0.4 M of sorbitol and 10 mM of $CaCl_2$ for 15 min. (Apple et al., 1996). The tested lectins were Con- A, RCA, UEA, WGA and SBA. Chemicals were supplied by Sigma (Saint Louis, USA). All preparations were observed with an epifluorescence microscope with the adequate filters for FITC and calcofluor and photographed with Fujichrome ISO 400.

Results

Tetraspores of *Gelidium floridanum* were liberated without a cell wall within a mucilage layer which is responsible for their primary attachment to the substratum. On release, tetraspores were spherical and 26–30 μm in diameter. The mucilage layer was composed of a mixture of acid polysaccharides as indicated by its positive reaction to AB and its violaceous metachromasia with AT-O (Figures 1 and 2). The spore's surface did not react with CBB and PAS, indicating the absence of neutral proteins and polysaccharides in the mucilage layer (Figures 3 and 4).

Soon after attachment, a thin cell wall was produced around the spore. The germination process was characterized by a polar evagination which gradually elongated, being pushed by the migrating cytoplasm, and giving rise to the germ tube. At this stage, a treatment with AB showed the presence of acid polysaccharides in the cell wall of the germ tube (Figure 5). When stained with AT-O, the metachromasia is restricted to a thin layer around the germ tube differing from what was seen in the mucilage surrounding the spore (Figure 6).

Neither the mucilage nor the cell wall reacted with CBB, indicating the absence of proteins in this region (Figure 7). The presence of neutral polysaccharides in the cell wall was shown by a positive reaction to PAS (Figure 8). As the germination proceeded, the tube became divided into several cells, yielding the same reactions as at the beginning of the germination process (Figures 9–12). The distal cell of the tube elongated and gave rise to the first rhizoid (Figures 13–16) which reacted like the other cells, giving, however, a stronger reaction with AB and showing a higher concentration of sulphated polysaccharides (Figure 13). Its weaker reaction with the PAS showed a reduction in the