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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 $\alpha$-D mannose, $\alpha$-D glucose, $\beta$-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140 species, some of which are important sources of bacteriological agar and agarose (Bailey, J.C. & D.W. Freshwater, unpublished).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 $\mu$mol 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 $\mu$g $ml^{-1}$ of Calcofluor White M2R for 15 min (Kim & Fritz, 1993).

### *Probing with FITC-lectins*

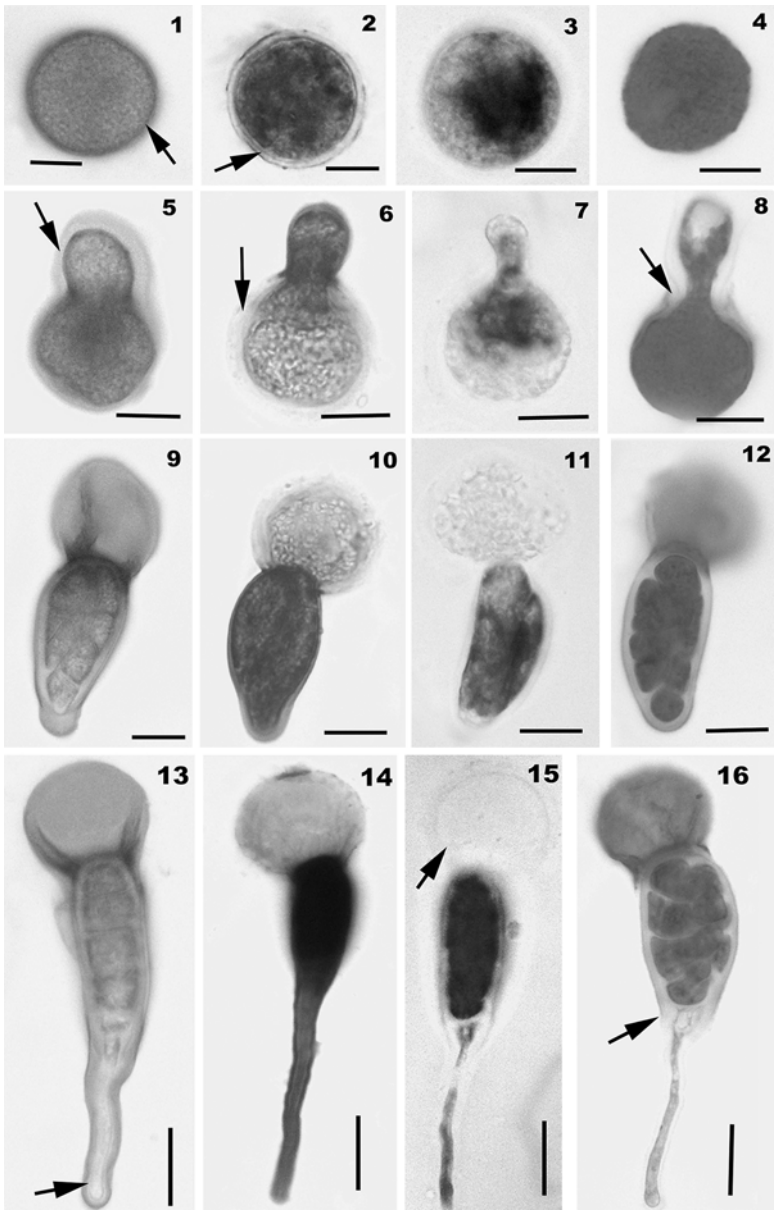
Spores were incubated with 100 $\mu$g $ml^{-1}$ of FITC-lectins in 0.6 M of sorbitol with 10 mM of $CaCl_2$ diluted in distilled water for 1 h and washed with deionized water. The control was made with FITC-lectin, 0.2 M of glucose, 0.4 M of sorbitol and 10 mM of $CaCl_2$ for 15 min. (Apple et al., 1996). 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 $\mu$m 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

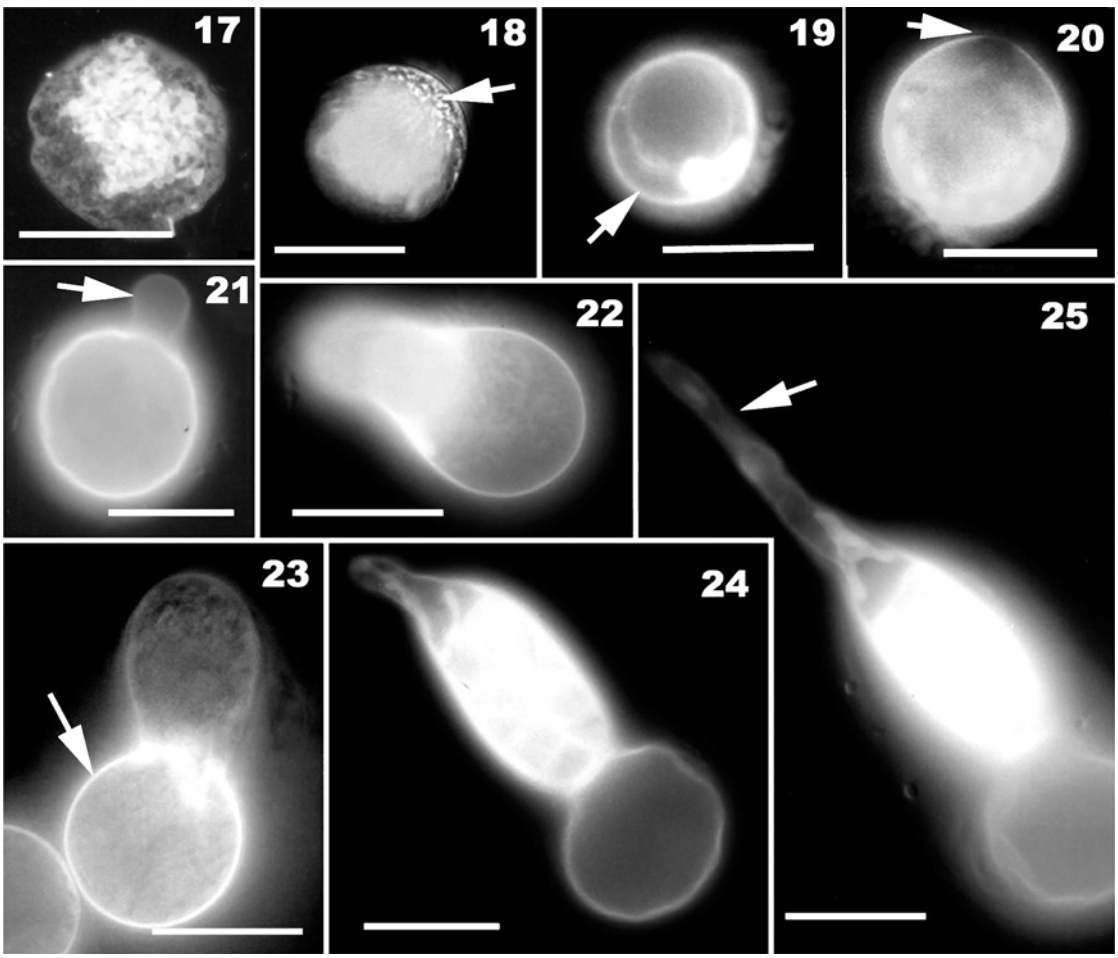



*Figures 1–16*. Initial germination phases of tetraspores of *Gelidium floridanum* under light microscopy. *Figures 1–4*. Histochemical reactions showing the mucilage and the cell wall stained with AB, AT-O CBB and PAS, demonstrating the presence of neutral and acid polysaccharides. Note the positive reaction to AB (arrow) and its metachromasia with AT-O (arrow). *Figure 5*. First stage in the formation of the germ tube; the layer of mucilage stained with AB is pointed (arrow). *Figure 6*. Elongation of the germ tube showing the migration of the protoplasm stained with AT-O and a metachromatic halo over the body of the spore (arrow). *Figure 7*. Developing germ tube stained with CBB. *Figure 8*. Cell surface stained with PAS showing a rupture of the wall (arrow). *Figures 9–12*. Tetrasporelings showing the cellularization of the germ tube stained with AB, AT-O, CBB and PAS. Scale bar = 10 $\mu$m. Figures 13–16 Tetrasporelings of *Gelidium floridanum* bearing rhizoids. *Figure 13*. Tetrasporelings stained with AB showing strong positive reaction around the entire body, including the rhizoid (arrow). *Figure 14*. Tetrasporeling stained with AT-O. *Figure 15*. Tetrasporeling stained with CBB showing the negative reaction on spore body (arrow). *Figure 16*. Tetrasporeling stained with PAS: observe that mucilage is weakly positive (arrow). Scale bar = 10 $\mu$m.

amount of neutral polysaccharides in the rhizoidal cell (Figure 16).

The absence of a cell wall in unattached spores was evidenced by a negative reaction with calcofluor (Figure 17). After the primary attachment there was a deposition of cellulose starting at one of the poles and gradually covering the entire sporeling (Figures 18 and 19). The germination started through a polar disorganization of the cell wall, evidenced by a reduction of the fluorescence, at the point where the germ tube will later be formed (Figure 20). The cell wall of the spore became thinner (Figures 21–23) and the tube went through a series of cell divisions forming smaller cells with high fluorescence using calcofluor (Figures 24 and 25). The distal cell of the tube elongated and gave rise to the rhizoidal region, which had little cellulose as shown by a weak fluorescence with calcofluor (Figure 25). Table 1 summarizes the results of the histochemical tests.

The lectin reactions are shown in Table 2. Three of the five lectins tested showed a positive reaction. In the non-germinated spores, after incubation with RCA there was a light-green fluorescence of the mucilage indicating the presence of $\beta$-D-galactose as the residue of the terminal sugar in the adhesive substance (Figure 26). When incubated with Con-A FITC, the non-germinated spores fluoresced strongly showing a high concentration of $\alpha$-D-mannose and $\alpha$-D-glucose




*Figures 17–25*. Tetraspores and tetrasporelings of *Gelidium floridanum* in different stages of development stained with calcofluor. *Figure 17*. Non-germinated tetraspore without cell wall. *Figure 18*. Beginning of cell deposition on a pole (arrow). *Figure 19*. Tetraspore surrounded by the cell wall. *Figure 20*. Polar disorganization of the cell wall signalling the start of the germination process (arrow). *Figures 21–23*. Germ tube in varying stages of development covered by a thin cell wall. *Figures 24–25*. Tetrasporelings showing a very thin cell wall on the rhizoids (arrow). Scale bar = 20 $\mu$m.

*Table 1.* Reactions of *G. floridanum* tetraspores to different treatments: + + + strongly positive, ++ positive, + weakly positive; – negative.

| | | | Germinated spore | | |
|---|---|---|---|---|---|
| Reactives | Substances labeled | Spore | Body of the spore | Germ tube | Rhizoid |
| Alcian blue | Acid polysaccharides | + + + | ++ | + | + + + |
| Toluidine blue | Sulphated polysaccharides | ++ | + | + | + |
| Coomassie brilliant blue | Proteins | – | – | – | – |
| PAS | Neutral polysaccharides | – | – | + | – |
| Calcofluor | Cellulose | + | ++ | ++ | + |

*Table 2.* Fluorescence of the mucilage that surrounds the tetraspores of *Gelidium floridanum* with FITC conjugated with some lectins: + + + strongly positive, ++ positive, + weakly positive; – negative.

| Lectin | Specificity | Fluorescence |
|---|---|---|
| Con-A | $\alpha$-D-mannose, $\alpha\alpha\alpha$-D-glucose | + + + |
| RCA | $\beta$-D-galactose | ++ |
| UEA | $\alpha$-L-fucose | – |
| WGA | N-acetyl-$\alpha$ (1, 4)-D-glucosamine | + |
| SBA | $\alpha$ or $\beta$ N-acetylgalactosamine, $\alpha$ or $\beta$-D-galactose | – |

in the mucilage (Figure 27). The mucilage also fluoresced, but only slightly, when incubated with WGA, showing a low concentration of N-acetyl-alpha-(1,4)-D-glucosamine (Figure 28). The other tested lectins (UEA and SBA) did not fluoresce.

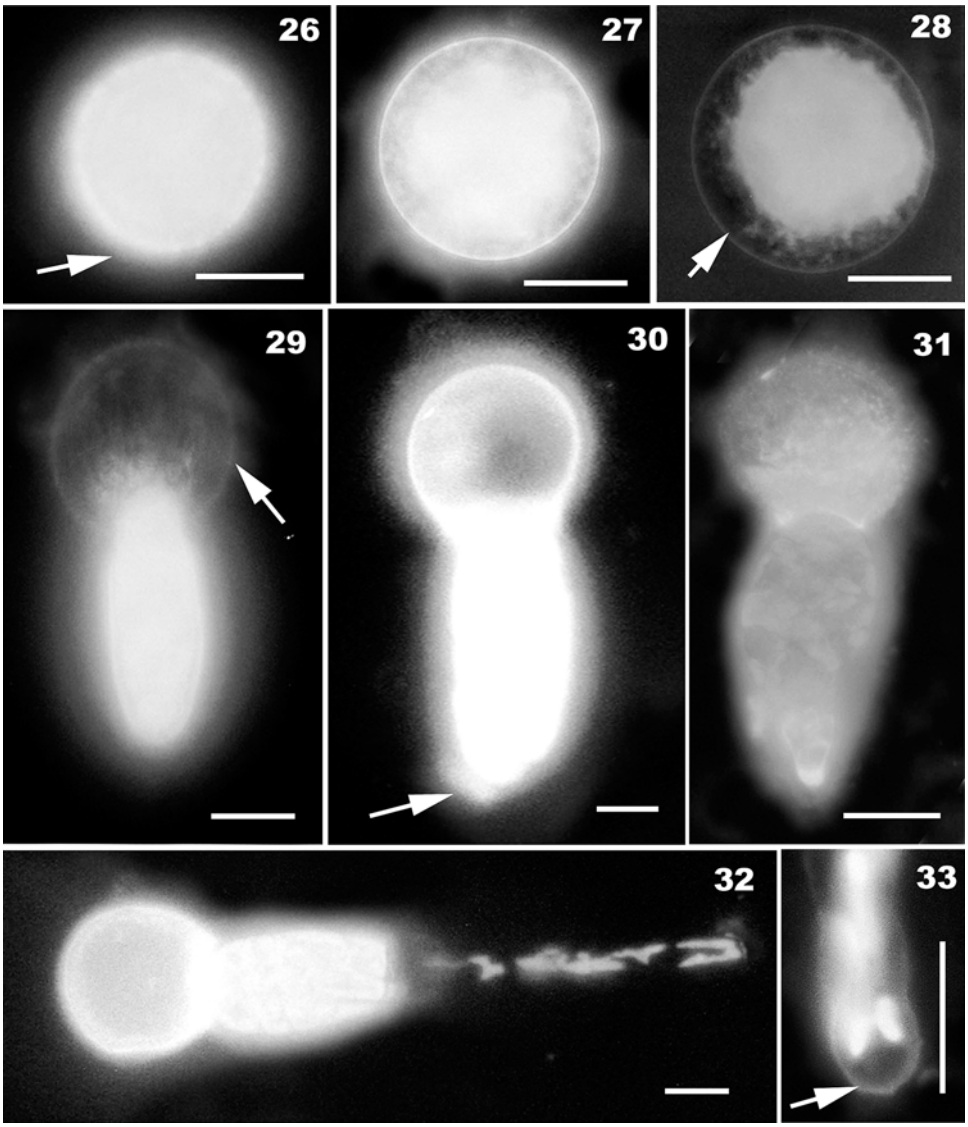
In the cell wall of the germ tube, sugar residues were not detected with the lectins employed. However, this result may have been masked by the intense orange auto-fluorescence of the cells of the germ tube (Figures 29–31). At this stage fluorescence was only observed at the distal pole of the germ tube when incubating with Con-A (Figure 30). Carbohydrate residues labelled by Con-A, absent in the germ tube, were detected in the rhizoidal region (Figures 32 and 33). The presence of $\alpha$-D mannose and $\alpha$-D glucose in the mucilage and in the rhizoid indicate a relation of these carbohydrates with the adhesion mechanisms.

## Discussion

Red algal spores usually do not germinate unless they attach to a substratum. As the spores are liberated without a cell wall, the primary attachment is mediated by the mucilage that surrounds the spores, which is produced during the sporogenesis (Chamberlain & Evans, 1973; Pueschel, 1979). The production of mucilage seems to be associated with the large quantity of dictyosomes that are seen during the ontogeny of the spores. This mucilage has a role in the release of the spores and might also have a role in the protection of the spores until the formation of the cell wall.

The strong positive reaction of the mucilage of *G. floridanum* tetraspores with AB pH 1.0 and AT-O indicated an abundance of sulphated polysaccharides. The adhesive nature of sugars conjugated with proteins has been demonstrated in seaweeds (Chamberlain & Evans, 1973; Pueschel, 1979). However, our tests with CBB did not show the presence of proteins in the *G. floridanum* mucilage. Similar results were obtained by Chamberlain and Evans (1973, 1981) with non-liberated spores of *Ceramium* sp. and by Apple et al. (1996) with spores of *Champia parvula* showing that protein, if indeed it is present at all, is not detected by histochemical methods.

Our results with calcofluor white show that the cellulose deposition begins soon after the spores' adhesion to the substratum. Northcote and Pickett-Heaps (1965) have shown that the synthesis of cellulose is mediated by the Golgi bodies, and Villemez et al. (1968) have shown that it occurs on the plasmatic membrane. The presence of sulphated polysaccharides in the spores' mucilage and in the cell wall of the sporelings was shown by the metachromasia with




*Figures 26–33.* Tetraspores and tetrasporelings of *Gelidium floridanum* incubated with lectins conjugated with FITC. *Figure 26*. Non-germinated spore incubated with RCA-FITC showing a light fluorescence in the mucilage. *Figure 27*. Non-germinated spore with Con-A-FITC showing strong fluorescence in the mucilage. *Figure 28*. Another non-germinated spore incubated with WGA-FITC weak fluorescence is seen as a halo over the mucilage (arrow). *Figures 29-31*. Germinated spores incubated with RCA, Con-A and WGA, respectively. Note the fluorescence at the extremity of the germ tube in *Figure 30* with Con-A (arrow). *Figure 32*. Tetrasporeling showing a rhizoid fluorescing with Con-A-FITC. *Figure 33*. Detail of the extremity of the rhizoid fluorescing with Con-A-FITC (arrow). Scale bar = 10 $\mu$m.

AT-0. This is expected in agarophytes, where the presence of agar, a complex polymer with a neutral and a sulphated fraction, is well known. Our results show that sulphated polysaccharides are present in the adhesive mucilage, particularly on the rhizoid as well as in the amorphous matrix of the cell wall. Apple et al. (1996) noted that in *C. parvula,* the composition of the adhesive mucilage on the rhizoids appeared to be different from the mucilage that surrounds the spores.

The labelling of the mucilage by lectins conjugated with fluorescent dyes indicates the presence of specific sugars in the adhesive mucilage of algal and fungal spores (Apple et al., 1996). Apparently lectins have a greater affinity for sugars linked to proteins than for sugars alone (von Sengbusch et al., 1982). This

suggests that the sugars present in the adhesive mucilage of the spores of *G. floridanum* are components of glycoproteins. Although we did not detect any proteins with CBB, they may be present in small amounts or blocked by polysaccharides. Three of the lectins conjugated with FITC complexed with different sugars on the spores' mucilage (Table 1). The presence of $\beta$-D-galactose was detected by FITC-RCA, but only slightly, suggesting that this sugar is not the main component of the mucilage. On the other hand, non-germinated spores fluoresced strongly when incubated with FITC-ConA, showing a high concentration of $\alpha$-D-mannose and $\alpha$-D-glucose in the mucilage, although Con-A is specific for $\alpha$-D-mannose and less so for $\alpha$-D-glucose (von Sengbusch et al., 1982; Walko et al., 1987). The mucilage showed a fluorescence limited to a thin layer when it was incubated with WGA, indicating a low concentration of N-acetyl-$\alpha$-(1, 4)-D-glucosamine. No fluorescence was shown when the spores were incubated with UEA or SBA.

No sugar residues were detected on the germ tube by the tested lectins, although the fluorescence may have been masked by the intense auto-fluorescence of the germ tube cells. However, the presence of $\alpha$-D-mannose and/or $\alpha$-D-glucose was detected by Con-A at the distal region of the rhizoids. The presence of $\alpha$-D mannose and/or $\alpha$-D glucose in the mucilage and in the rhizoids suggests the participation of these sugars in the mechanism of attachment of *G. floridanum* spores.

Our results suggest that: (1) the attachment and germination of the tetraspores of *G. floridanum* depends on the compounds of the extra-cellular matrix formed during the spores' ontogeny; (2) the mucilage that surrounds the spores facilitates its primary interaction with the substratum; (3) the mucilage may have a role in keeping the spores apart from each other, and in protecting them before the formation of the cell wall, functioning as a buffering layer between the naked spore and the substratum, and (4) the same sugars are involved in the adhesion of the naked spores as in adhesion of the rhizoids to the substratum.

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