ULTRASTRUCTURE OF THE ADHESION AND MOVEMENT OF THE TETRASPORES OF *GELIDIUM* LAMOUR. *(GELIDIACEAE, RHODOPHYTA)*

by

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Resumen

ECHEGARAY, J. & J.A. SEOANE CAMBA (1996). Ultraestructura de la adhesión y movimiento de las tetrásporas de Gelidium Lamour. (Gelidiaceae, Rhodophyta). *Anales Jard. Bot. Madrid* 54: 55-60 (en inglés).

Se describe morfológicamente la parte más externa de la pared celular de las tetrásporas de *Gelidium crinale* (Turner) J.V. Lamour. y *G. spathulatum* (Kütz.) Bornet y se relaciona esa mofología con los movimientos y desplazamientos realizados por ellas cuando están adheridas a un substrato. También se describen el mecanismo de adhesión y las transformaciones que sufre este mecanismo a través del tiempo. La pared celular presenta una red de fibrillas incluidas en abundante mucílago. Las deformaciones que sufren las esporas demuestran que la pared celular y su estructura son relativamente elásticas.

Palabras clave: *Rhodophyta, Gelidiaceae, Gelidium*, tetrásporas, pared celular, movimiento de las esporas.

Abstract

ECHEGARAY, J. & J.A. SEOANE CAMBA (1996). Ultrastructure of the adhesion and movement of the tetraspores of Gelidium Lamour. (Gelidiaceae, Rhodophyta). *Anales Jard. Bot. Madrid* 54: 55-60.

The outer part of the tetraspore cell wall in *Gelidium crinale* (Turner) J.V. Lamour. and *G. spathulatum* (Kütz.) Bornet is morphologically described in relation to the movements and displacement of these spores when they settle on a substratum. We also describe the mechanism of adhesion and the transformations undergone by this mechanism over time. The cell wall shows a network of fibrillar threads embedded in abundant mucilage. The deformations that tetraspores undergo show that the cell wall is relatively elastic.

Key words: *Rhodophyta, Gelidiaceae, Gelidium*, tetraspores, cell wall, spore movement.

INTRODUCTION

Various studies on the adhesion of spores of benthic seaweeds to the substrate deal only partially with the subject. LINSKENS (1966) observed that the spores of many species of seaweeds have a preference for a certain degree of substrate roughness. COON & *al.* (1972) pointed out that the adhesion behaviour of nonmotile spores of seaweeds depends, to a great extent, on their physical characteristics and their interaction with seawater. CHAMBERLAIN & EVANS (1981), working with carpospores and tetraspores of *Ceramium*, indicated that spore adhesion is resistant to a range of enzymes and chemical agents of known specificity.

According to several authors (CHEMIN,

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1937; BOILLOT, 1963), spores of *Gelidium* are liberated virtually without a cell wall and shows movements of an amoeboid nature that stop after a few hours. The cessation of movement is accompanied by the formation of an external cell wall that allows attachment to the substrate.

In this study we attempt to characterize the behaviour of *Gelidium* spores and determine the mechanism of adhesion to the substrate in relation to the activity of the outer part of the cell wall.

MATERIAL AND METHODS

All spores were obtained from plants of *Gelidium crinale* (Turner) J.V. Lamour. and *G. spathulatum* (Kütz.) Bornet collected from the Catalonian coasts of Garraf and Blanes respectively, and cultivated under laboratory conditions. In all cases, the stichidia and substrates for spore collection were on the bottom of Petri dishes containing filtered and pasteurized sea water, and were left overnight at 17-22 ºC in the dark.

For scanning electron microscope (SEM) studies, we placed about 15 stichidia on coverglasses of 10 mm diameter, or on thin Formvar film prepared at 0.25 % in chloroform and placed on gold grids of 3 mm diameter. The collected spores were fixed for 2 h in 2.5 % glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2. Spores were then washed in 0.15 M sodium cacodylatye buffer. The samples, both in the fixing solution and in the buffer, were kept at 4 ºC for subsequent treatment. Spores collected on the coverglasses were dehydrated through an ethanol series and dried in a critical-point drier, and the spores collected on Formvar film were cryodesiccated at high vacuum using liquid nitrogen at –169 ºC or freon-22 cooled with liquid nitrogen. The spores, on both kinds of substrate, were coated with gold and examined with a Jeol JSM-840, a Cambridge Stereoscan-120 or a Stereoscan S-4 microscope.

For transmission electron microscope (TEM) study, the stichidia were placed on substrates of araldite resin. The substrates were prepared in silicone rubber moulds and polymerized at 60 ºC. The collected spores were fixed for 2 h in 2.5 glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2. The spores were then washed in 0.15 M sodium cacodylate buffer, post-fixed in 1% OsO_4, dehydrated through an ethanol series and embedded in the same resin. The sections were double-stained with 2% uranyl acetate and Reynolds' lead citrate. Observations were made with a Philips 301 microscope.

For light microscope (LM) study, the stichidia were placed directly on glass microscope slides.

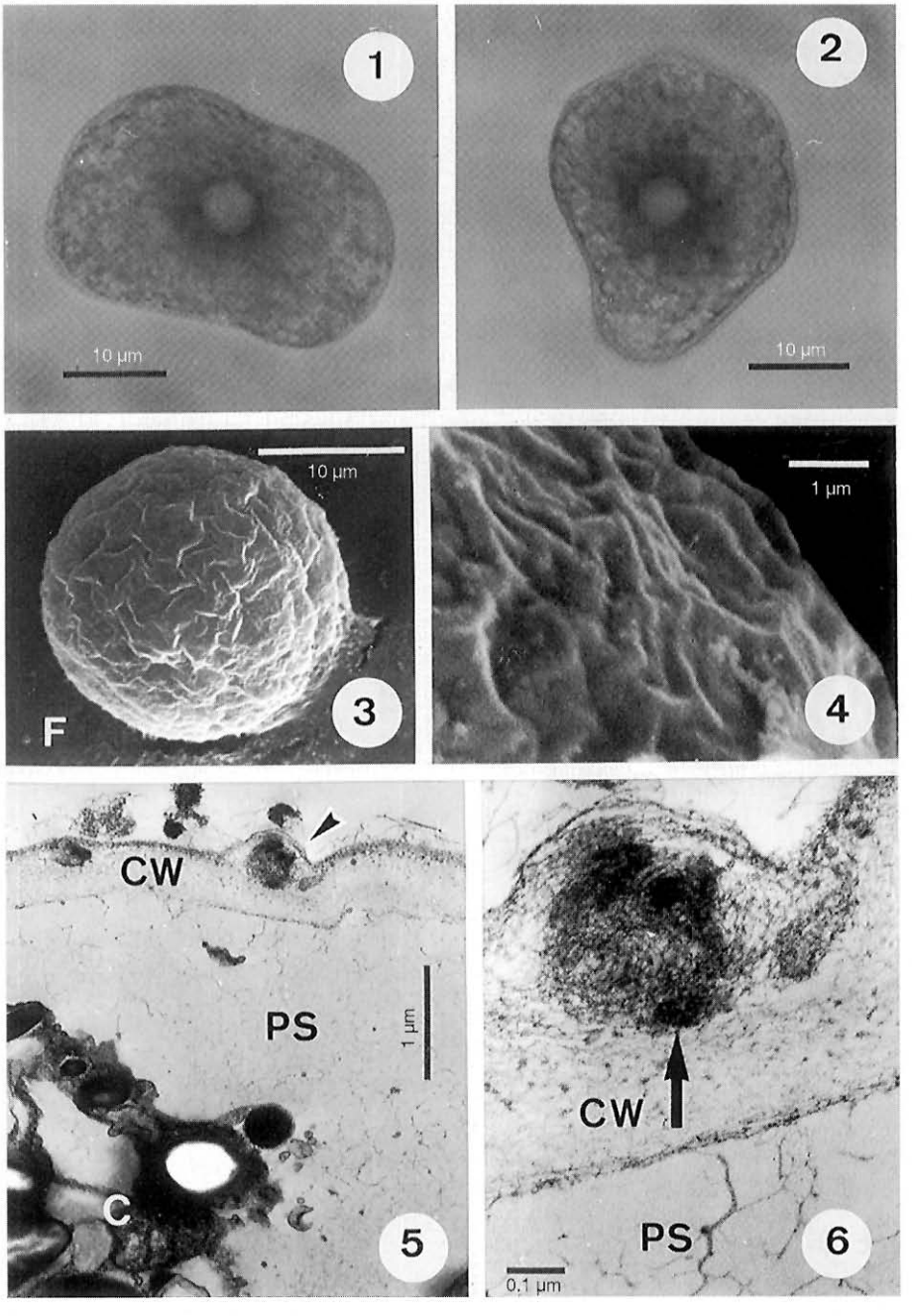
OBSERVATIONS

At liberation, the behaviour, form, and cell wall structure of the tetraspores of both species of *Gelidium* studied here are similar. They appear globose or spherical when examined with light microscopy, and this is more evident with SEM (fig. 3).

Seconds after spore liberation, the spherical shape of these tetraspores can change as a result of amoeboid movement that allows them to move over the substrate (figs. 1, 2). In addition, they swivel, supporting themselves on a single point, and then raise themselves toward the cover slip. It is important to continue tracking them since they readily move out of the plane of focus. The temporary extensions of the cytoplasm are characterised by their relatively rapid movement. Ramified extensions have never been observed.

The cell wall has a network of fibrillar threads embedded in abundant mucilage (figs. 5, 6, 7, 9).

From the TEM observations we determined that two complementary phenomena occur during the adhesion of these spores. The spore adheres strongly to the substrate first by the action of the fibrillar structures, and secondly by the action of the mucilage that covers these structures (figs. 7, 9). All this is shown by the traces left by the fibrillar threads and by the mucilage left at points of adhesion (figs. 8, 10) after the spore has moved on. Towards the periphery of the adhesion point the deposition

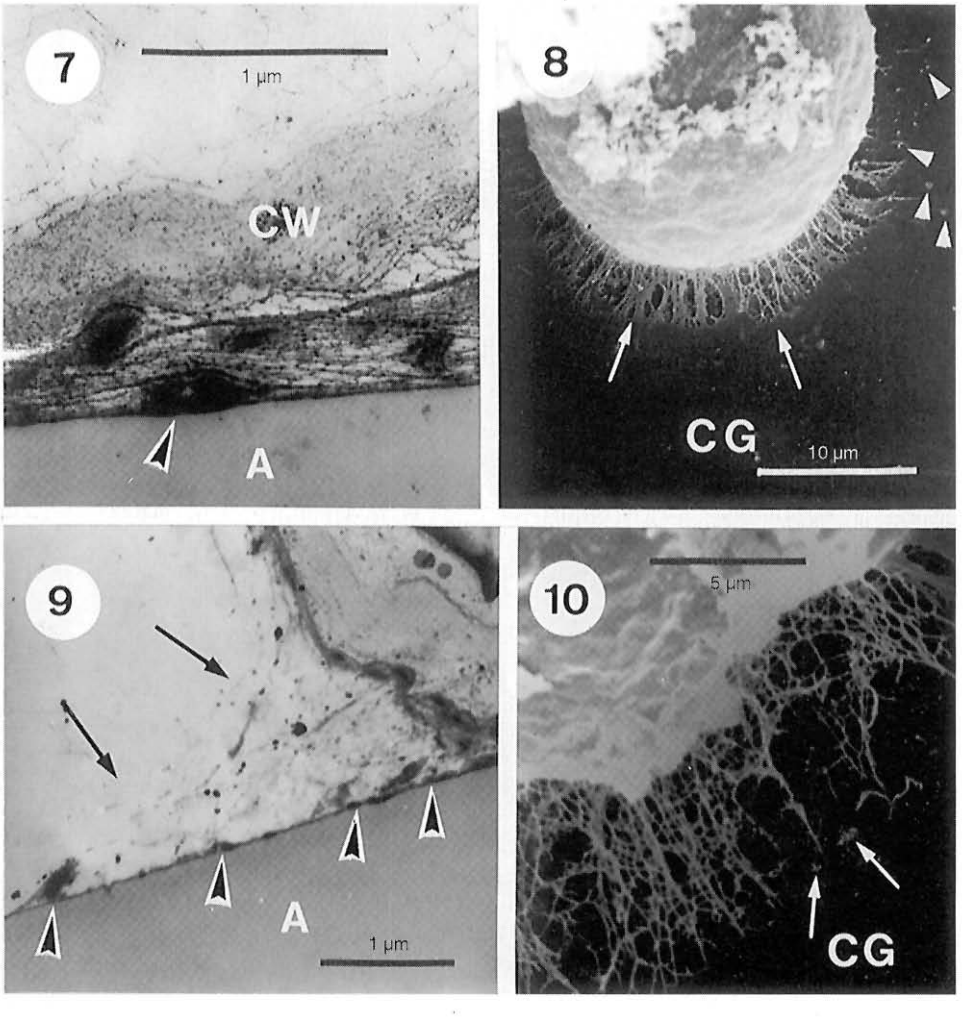


Figs. 1-6.–Spores of *Gelidium crinale:* 1-2, light micrographs showing the same spore at two different times during movement; 3, SEM view of spore without alcohol treatment, cryodesiccated with liquid nitrogen; 4, SEM detail of the spore surface; 5, TEM view of germinating spore showing the cell wall with electron-dense elements embedded in mucilage (arrowheads). Note the displacement of cytoplasm leaving a periplasmic space; 6, detail from figure 5, showing the fibrillar structure of the electron-dense elements (arrow). (F = formvar film; CW = cell wall; C = cytoplasm; PS = periplasmic space.)

of mucilage is greater (fig. 9). This results in the formation of a discoid surface of adhesion, which can reach the size of about the diameter of the spore (fig. 8), affixing it to a smooth substrate. The extent of this adhesive disk depends on the characteristics of the substrate itself. The displacement procedure can cause progressive cracking and ripping in the mucilage (fig. 8) to such an extent that it shrinks (fig. 10) and largely disappears, leaving traces on the substrate.

Discussion

Despite the existence of a relatively large body of information about ultrastructure of



Figs. 7-10.–Spores of *Gelidium* (figs. 7-9, *G. crinale;* fig. 10, *G. spathulatum):* 7, TEM view of the cell wall of spore attached to araldite substrate. Note the participation of the electron-dense elements in adhesion to the substrate (arrowhead); 8, SEM view of periphery of the attached surface of a spore (arrows). Note the mucilage left on the substrate (arrowheads); 9, TEM view of the same peripheral surface of adhesion (arrow) as that in figure 7. Note the peripheral electron-dense elements (arrowheads) attached to the araldite substrate; 10, SEM view of the adhesive mucilage torn when the spore is separated from the substrate. Note the remnants of adhesive elements still attached to the substrate (arrows). (CW = cell wall; A = substrate of araldite; CG = coverglass.)

tetrasporogenesis there is a paucity of data about ultrastructure of released and attaching tetraspores.

A spherical form is characteristic of all liberated spores of *Gelidium*. Although lacking in locomotive appendages, they can produce a series of movements and displacements within a relatively small area. These displacements are carried out with at least one point of support on the solid surface.

There appear to be different types of movement with differing orientations, which suggests that they can be used for a variety of activities. Thin cytoplasmic projections may act as environmental sensors and are not involved in locomotive movement. There are also lobulate projections that may be accompanied by displacement or, as indicated by CHEMIN (1937), only accompanied by internal changes. It may be significant that at this time, the spores tend to orientate their plastids around the more or less central nucleus, while the organelles involved in secretory activity tend to be localised on the periphery (unpublished observations). However, an intense metabolic activity seems to occur inside the cell. In agreement with ROSENVINGE (1927), we did not observe the excretions mentioned by BOILLOT (1963).

Spore motility has also been observed in other species of Gelidiales, Ceramiales, Nemalionales, Cryptonemiales, Gigartinales and Rhodymeniales (BORNET & THURET, 1867; ROSENVINGE, 1923-24, 1927; CHEMIN, 1927, 1937; BOILLOT, 1971). This suggests that it is a widespread phenomenon, although each group has its own characteristics. According to some of these authors the movements may last several hours, but we have only been able to observe them for relatively short periods, no more than an hour.

The principle that spores are liberated without a cell wall was proposed by ROSENVINGEN (1927) in the case of *Callithamnion*. However, BUFFHAN (1893) stated that "the paraspores have a cell wall even before their liberation". DIXON (1973) pointed out that during tetraspore formation each of the four spores is surrounded by a cell wall that represents a new layer with a different chemical composition to that of the tetrasporangium division wall. CHAMBERLAIN & EVANS (1973) described free tetraspores of *Ceramium* surrounded by a sheath of hyaline mucilage. PUESCHEL & COLE (1985) admit the existence of a thin electron-dense wall surrounding both spore and germ tube, as well as the presence of extracellular materials incorporating fibrils swirled around a focus, as observed in carpospores of *Porphyra variegata*. In the *Gelidium* spores studied here, TEM observations show a cell wall with internal threads of a fibrillar nature which seems to play a important role in the adhesion mechanism. Such threads sometimes correspond with surface ridges which are more or less prominent, depending perhaps on the fixative (SYLVESTER & WAALAND, 1984; RICHARDS & TURNER, 1984). Whether these threads corresponde to the swirled fibrils described by PUESCHEL & COLE (1985) remains to be investigated.

The mechanism of spore adhesion to the substrate is probably complex, and without doubt the surface of the spore is involved in the activity. The fibres may constitute active centres of adhesion, possibly by enzymatic means as is partly demostrated by the numerous marks left by the spore in the course of its displacement. Such fibres must obviously permit a firm adherence, which is especially important considering the exposed habitats colonized by these seaweeds.

Bearing in mind the distinct deformation undergone by these spores in the free state, we may suppose that the cell wall, surrounded by its mesh of fibrillar threads embedded in abundant mucilage, provide it with resistence against the characteristic turbulent strength of the water in habitats where these algae grow, by imparting relative elasticity.

As a result of the above considerations, the orientation and displacement movements that take place in these *Gelidium tetraspores* do not occur before the formation of the cell wall (CHEMIN, 1937; BOILLOT, 1963). Rather, they occur after the cell wall has formed and has affixed to the substrate. Selection

of a definitive location terminates these movements and initiates germination.

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