Journal of Applied Phycology **12:** 567–575, 2000.

Cell wall structure of the agarophytes *Gracilaria tikvahiae* and *G. cornea* (Rhodophyta) and penetration by the epiphyte *Ulva lactuca* (Chlorophyta)

C.J. Dawes*, B.W. Teasdale & M. Friedlander[1]
[1]*Department of Biology, University of South Florida, Tampa, Florida 33620 USA*
[2]*Israel Oceanographic and Limnological Research Institute, Haifa 31080, Israel*

(*Author for correspondence; e-mail cdawes@chuma1.cas.usf.edu)

Received 23 March 2000; revised 8 April 2000; accepted 9 April 2000

Key words: Gracilaria cornea, Gracilaria tikvahiae, Ulva lactuca, epiphyte attachment, red algal cell wall, algal cuticle, decklamelle

Abstract

Use of light, transmission, and scanning electron microscopes revealed that the epidermal cell wall of the red algal agarophytes *Gracilaria tikvahiae* McLachlan and *G. cornea* J. Agardh consists of a decklamelle and outer and inner wall layers. The two species differed, with *G. cornea* having a significantly thicker outer wall and a more diffuse decklamelle. After induction, the zooids of *Ulva lactuca* would attach to glass slides and the two species of *Gracilaria* via an adhesion pad. Within a few days, 3–5 celled germlings penetrated the decklamelle and outer wall layer of both basiphytes. By the time the epiphyte germlings reached the 15 celled stage, they had penetrated the inner wall layer. The differences in epidermal cell wall construction between the two basiphytes may play a role in the ability of zooids of *U. lactuca* to attach in nature where epiphytization of *G. cornea* is infrequent.

Introduction

The 'space race' between seaweeds is evident by the number of macroalgae and microalgae that occur as epiphytes. Competition between seaweed basiphytes and their epiphytes has been shown under natural (Arrontes, 1990) and culture conditions (Friedlander & Ben-Amotz, 1991; Svirski et al., 1993). Further, studies have shown that removal of *Ulva lactuca* from species of *Gracilaria* is difficult to impossible (Friedlander, 1992; Bushmann & Kuschel, 1988). Algal basiphytes can exhibit a variety of defenses against attachment (Ducker & Knox, 1984), including production of a mucilaginous covering, rapid growth (e.g. annuals), sloughing of outer cell walls, allelopathy or the release of toxic chemicals such as phenolics (Davis et al., 1989), and by having ephemeral or annual life histories. For example, the production of hydrogen peroxide by *Gracilaria conferta* is in reaction to bacterial degradation of its cell wall (Weinberger et al., 1999).

A review of how algal spores settle, attach and grow on seaweeds indicates that there is little known how epiphytes attach (Fletcher & Callow, 1992). In contrast, the outer wall structure and chemical composition of epidermal cells of red algae has been elucidated (for review see Craigie, 1990) including species of *Gracilaria* (Verdus et al., 1985; Kling et al., 1989).

With the development of seaweed mariculture, interest has expanded to possible interactions due to the detrimental effects of epiphytes on seaweeds (Friedlander, 1992). A number of studies, that have examined ways to control of epiphytes, have focused on the green alga *Ulva lactuca* and the cultivation of *Gracilaria* spp. (Fletcher, 1995). These include *G. conferta* (Friedlander & Ben-Amotz, 1991; Friedlander, 1992; Friedlander et al., 1991, 1996; Svirski et al., 1993), *G. verrucosa* (Zvyagintesv & Kozmenko, 1995), and *G. chilensis* (Buschmann & Kuschel, 1988; Buschmann & Gomez, 1993; Pickering et al., 1993).

The present study examines the structural interaction between the epiphyte and two species of *Gracilaria* that differ in levels of epiphytization. It is proposed that the green alga penetrates the wall of the basiphyte, forms a resistant attachment, and that differences in wall structure exist in the epiphyte-resistant *G. cornea* and epiphytized *G tikvahiae*.

Materials and methods

Collection and culture

Gracilaria tikvahiae and *Ulva lactuca* were atached on jetties in 0.2 to 0.5 m adjacent to the Skyway Bridge at the mouth of Tampa Bay (27° 35′ 05″ N, 82° 37′ 03″ W). *Gracilaria cornea* was attached on limestone in 0.5 to 1.0 m on the south side of Pigeon Key (24° 42′ 12″ N, 81° 09′ 18″ W) and Bahia Honda Key (24° 39′ 12″ N, 81° 16′ 51″ W) in the Florida Keys (Dawes et al., 1999). All plants were rinsed in ambient seawater and transported in coolers to the laboratory within 12 h. In the laboratory, all plants were cleaned and 1 to 3 cm long branches placed in culture dishes in growth chambers using a 12-h photoperiod and 200 μmol photon m^{-2} s^{-1}, in 24 °C. The medium consisted of 30 to 32 ppt salinity filtered, autoclaved seawater. The *f/2* culture medium of Guillard was modified to *f/4* (Guillard & Ryther, 1962) and added in a 24-h pulse once a week.

Induction of swarmers

A number of procedures were used to induce zooid production in *Ulva lactuca*. These included desiccation and reflooding and changes in the photoperiod (Smith, 1947; Christie & Evans, 1962), replacement or removal of the growth media (Lersten & Voth, 1960; Nilsen & Nordby, 1975), and removal of inhibitors by using 1 cm^2 blades and changing the media twice daily (Stratmann et al., 1996). In all procedures, the treated blades were floated over branches of *Gracilaria* so that when the zooids were released after 2 to 3 days, they settled on the red alga. Regardless of the procedure, use of field or cultured plants during spring tides (i.e. period of full moon) resulted in the highest success in swarmer production (Smith, 1947).

Preparation for microscopy

Branches of both species of *Gracilaria* were prepared after 10 to 16 days following exposure to zooids of *Ulva lactuca*. Branches identified with young epiphytes were selected using the dissecting and compund microscopes. Sections of fresh or preserved (5% formaldehyde in seawater) branches were examined under light microscopes with the aid of a 1% aqueous solution of toluidine blue or use of a phase microscope. Sectioning was carried out using a cryotome (Lipshaw, model 1700). Section thickness ranged between 12–20 μm.

Fixation for scanning and transmission electron microscopy followed the techniques of Dawes (1988). Approximately 0.5 cm long branch segments were placed in 10 ml vials with 5% glutaraldehyde (25% reagent grade, stored under nitrogen) in 0.1 M cacodylate buffer with 0.25 M sucrose. After 3 h, the sections were rinsed using a decreasing series of sucrose concentrations in the buffer and then postfixed for 3 h at room temperature with 2% osmium tetroxide in 0.1 M cacodylate buffer as described by Ramus (1969). Dehydration with ethanol and propylene oxide was carried out using the procedure of Crang (1997). Embedding was with a low viscosity (hard version) epoxy resin (Ladd Research Industries) with polymerization occurring at 70 °C for 24 h. Ultrathin sections were cut using Sorvall (model MT-2B) or LKB (model 8800 Ultratome III) ultramicrotomes and glass or diamond knives. Sections were stained for 30 min in uranyl acetate and 20 min in lead citrate and examined with a Hitachi H-500 TEM. Branch segments to be examined with the scanning electron microscope (JEOL JSM-35) were fixed as described and ethanol was the sole dehydrant. Critical point drying was with a Ladd unit and the specimens were coated with gold-paladium using a Pelco Sputter Coater (model 3).

Stereological analysis

Transmission electron micrographs were taken of central, non-oblique ultrathin sections from 5 blocks of tissue all at a standard magnification. The micrographs were scanned with a scanner (Hewlett-Packard, model 4C) at 250 dpi (10 dots mm^{-1}) and the cell wall layers measured using a two-point spatial calibration tool of an image analyzer program (Sigma Scan, Jandel Inc). The data consisted of three width measurements of 5 distinct epidermal cells (decklamelle, outer wall, inner wall) for branches from 7 plants (n = 35 $species^{-1}$). Cell wall thickness was compared within and between the two species using one-way ANOVAs ($p < 0.05$). Pair-wise comparisons were made with the Student-Newman-Keuls Method.

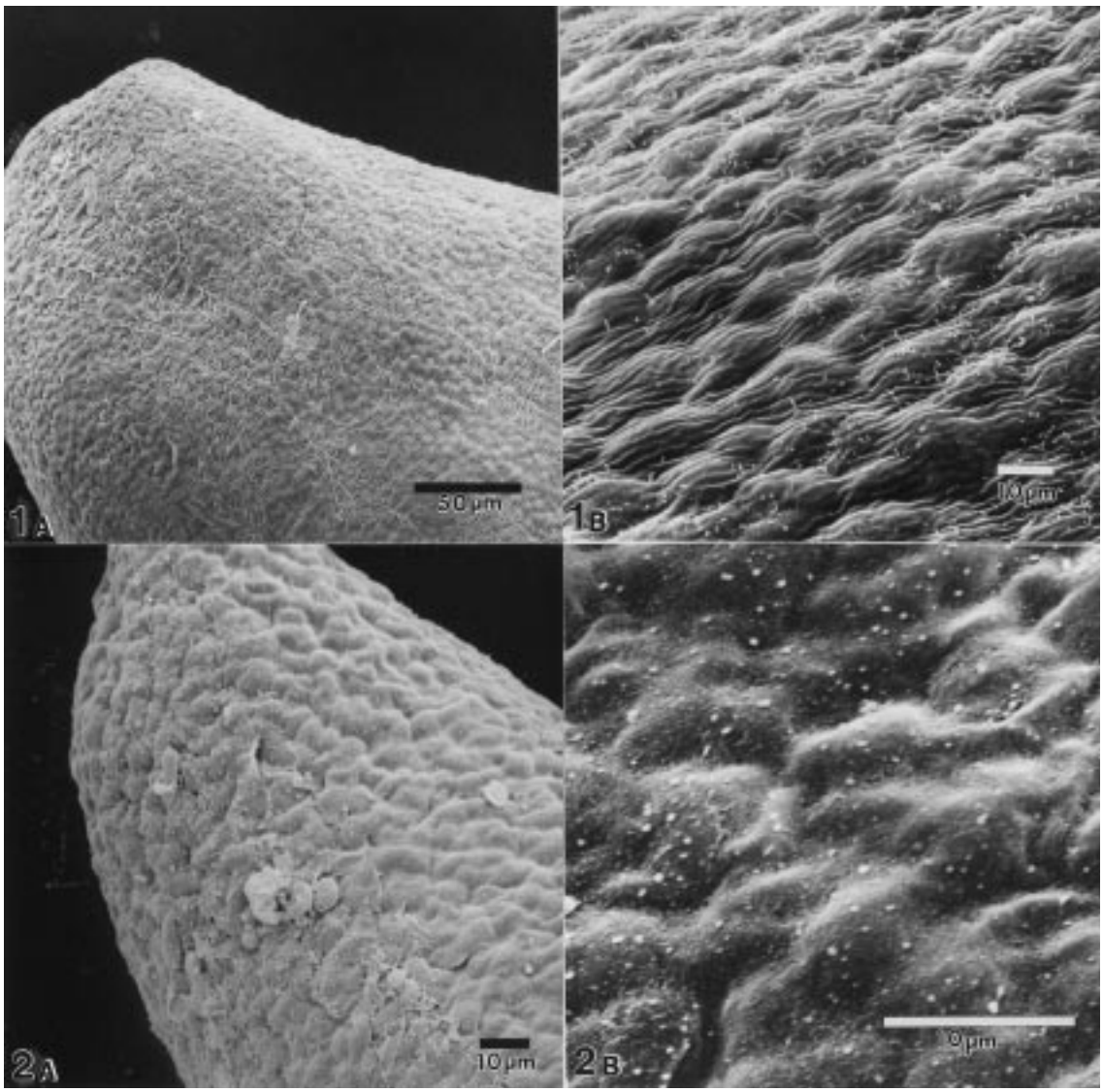

Figure 1. Scanning electron micrographs of young branches of cultured *Gracilaria cornea.* A branch tip (Figure 1A). Mature region 3 to 4 cm below the tip showing the individual epidermal cells underneath the covering layer (Figure 1B). The surface lacks bacterial or algal epiphytes.

Figure 2. Scanning electron micrographs of a young branch of wild *Gracilaria tikvahiae*. A branch tip (Figure 2A). Mature region 3 to 4 cm below tip of branch shows the individual epidermal cells underneath the covering layer (Figure 2B). Bacteria and debris occur on the surface.

Results

Cell wall

Using the scanning electron microscope, branches of *G. cornea* (Figure 1A, 1B) and *Gracilaria tikvahiae* (Figure 2A, 2B) show a continuous covering. Tips of *G. tikvahiae* (Figure 2A) showed significant folding probably reflecting some shrinkage of the meristematic tissue during critical point drying due to surface tension. In contrast, the branch tips of *G. cornea* (Figure 1A), prepared the same was as *G. tikvahiae*, did not show surface tension.

Frozen sections of *Gracilaria cornea* (Figure 3A, unstained) and *G. tikvahiae* (Figure 3B, stained with toluidine blue) showed that the decklamelle and outer wall (arrow) are continuous when viewed with the light microscope. Using the transmission electron microscope, ultrathin sections of the epidermal cells of both species (Figure 4A, 4B) revealed a decklamelle, a uniform outer wall, and an inner cell wall. The

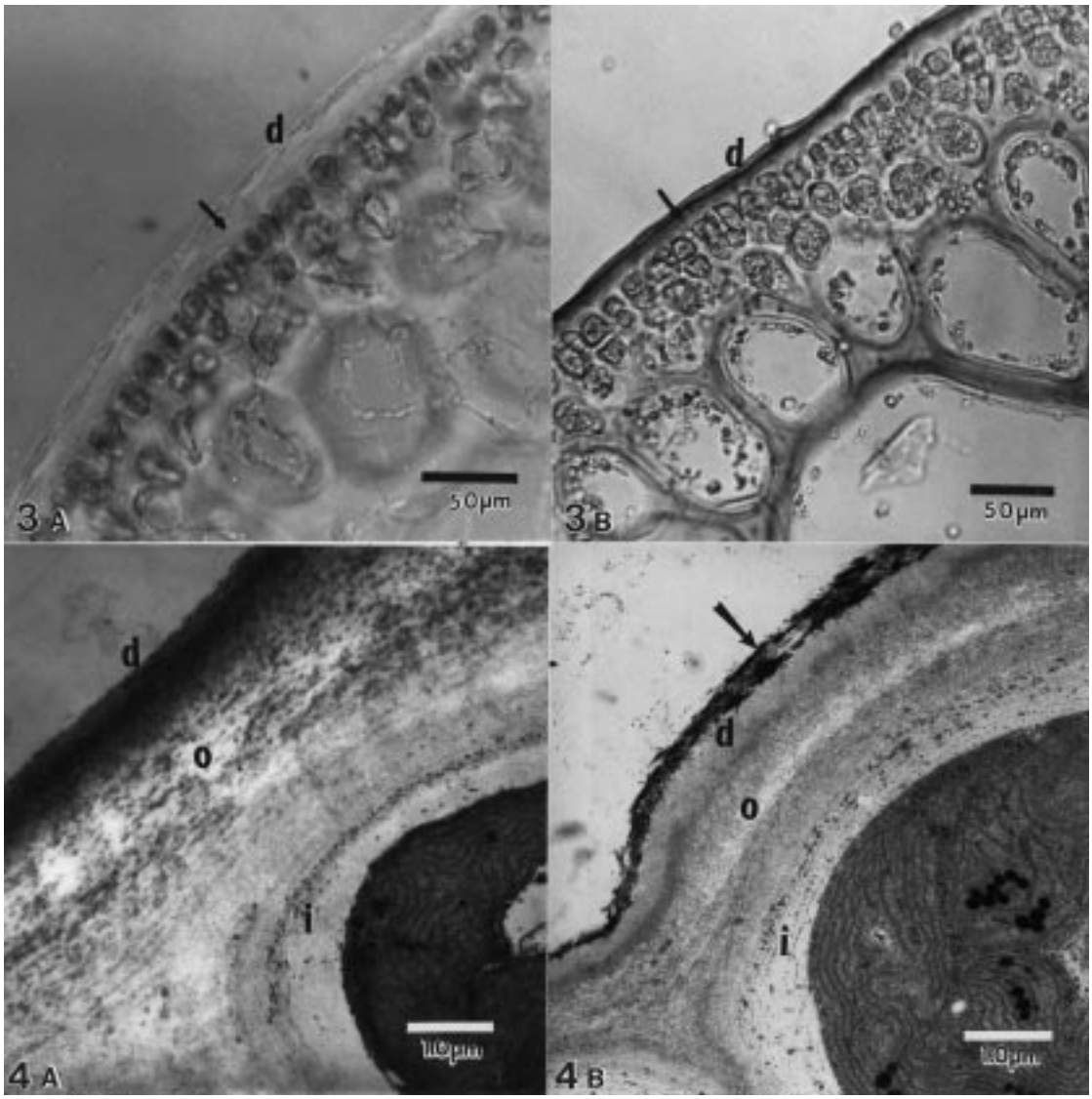

Figure 3. Light micrographs of branch sections showing the wall of the epidermal cells consisting of a continuous decklamelle (d) and outer wall (arrows) over the epidermal cells. A) *Gracilaria cornea*; B) *G. tikvahiae*.

Figure 4. Ultrathin sections showing the construction of the outer epidermal wall of A) *Gracilaria cornea* and B) *G. tikvahiae*. Each epidermal cell has a distinct inner wall (i) that is covered by the outer wall (o) and the decklamelle (d). Debris is evident on the decklamelle of field-collected thalli of *G. tikvahiae* (Figure 4B, arrow).

Table 1. Thickness (μm) of the outer epidermal cell wall of *Gracilaria cornea* (Gc) and *G. tikvahiae* (Gt) as measured 2 cm behind the growing branch tip and using transmission electron micrographs. See text for descriptions of wall layers. n = 35; ± 1 S.D.

Species	Thickness (μm)			
	Decklamelle	Outer wall	Inner wall	Total
G. cornea	0.32 ± 0.10	3.38 ± 0.66	1.21± 0.07	4.92 ± 0.26
G. tikvahiae	0.14 ± 0.03	1.09 ± 0.36	0.64 ±0.17	1.87 ± 0.50

decklamelle, outer, and inner walls of *G. cornea* were significantly thicker than in *G. tikvahiae* (Table 1). Measurements of standard transmission electron micrographs of ultrathhin sections 2 cm behind the apical tip of a branch showed the mean wall thickness to be 4.92 μm for *G. cornea* and 1.87 μm for *G. tikvahiae* (Table 1). The decklamelle is dense and granular in appearance, 0.3 to 0.5 μm thick in mature branches, and distinct from the outer wall of *G. tikvahiae* (Figure 4B). Because field collected plants were used, the branches of *G. tikvahiae* had epiphytes and some sediment (Figure 4B, arrow). Unlike *G. tikvahiae*, the decklamelle of *G. cornea* was more diffuse, being electron dense at the surface and grading into the outer wall (Figure 4A). In both species, the outer wall was continuous with the middle lamellae, while the inner wall surrounded the individual epidermal cells (Figure 4A, 4B).

Epiphyte attachment

Using the scanning electron microscope, the adhesion pad of *Ulva lactuca* was evident for germlings (3 to 5 celled stage) attached to glass slides (Figure 5A, arrow) and to the decklamelle of *Gracilaria cornea* (Figure 5B). The germlings penetrated the decklamelle, which formed a rim (Figure 5B) around their bases. The adhesion pad and decklamelle rim were also visible if the germlings were removed (Figure 5C). In some instances, the removal of a germling resulted in loss of the decklamelle leaving a pit on the surface of the basiphyte (Figure 5C). On occasion, the adhesion pad remained on the surface of the decklamelle as seen on an older (15 celled) filament of *U. lactuca* on *G. tikvahiae* (Figure 5D, arrow), but more commonly the epiphyte had penetrated the decklamellae by the time the germlings were 5 cells long (Figure 5E).

Using the transmission electron microscope, ultrathin sections of *Gracilaria tikvahiae* showed *Ulva lactuca* attached to the decklamelle (Figure 6A), which formed a rim around the epiphyte's base (arrow). In the inital stages of attachment (Figure 6A), basal cells of filaments of *U. lactuca* penetrated the decklamelle but not the outer wall layer. A similar stage is evident in the scanning electron micrographs (e.g Figure 5B). With continued growth, the epiphyte penetrated the outer and inner cell wall layers of the epidermal cells in *G. tikvahiae* (Figure 6B). Attachment and penetration by the epiphyte into *G. cornea* is similar. Initially, the epiphyte penetrated the decklamelle (Figure 7A, arrow) and then its rhizoids (arrows) reached the outer wall (Figure 7B).

Discussion

Species of *Ulva* and *Enteromorpha* are considered to be holo-epiphytes (Evans, 1981), although they may be difficult or impossible to remove from some species of *Gracilaria* (Buschmann & Gomez, 1993; Friedlander et al., 1996). In contrast, other species (e.g. *G. cornea*) seem to avoid or have low levels of epiphytism under natural and mariculture conditions. The present study demonstrates that *U. lactuca* can be an amphi-epiphyte (Ducker & Konx, 1984) of *G. tikvahiae* and *G. cornea*, even though the two species differ in wall construction. Penetration of the cell wall by the epiphyte helps explain the difficulty in removing *U. lactuca* from tank cultured seaweeds.

Gracilaria tikvahiae and *G. cornea* have cell walls that are similar to that shown in ultrathin sections for *G. verrucosa* (Verdus et al., 1985; Kling et al., 1989; Mariani et al., 1990) and *G. dura* (Delivopoulos et al., 1989). The covering layers and cell wall of epidermal cells of both species consists of a continous decklamelle and an outer wall that was continuous with the midddle lamellae as well as an inner wall that surrounds each cell. Thus, this study supports the description of Mariani et al. (1990) with the inner wall being the primary wall. Further, a fibrillar layer was evident in the cell wall of the two *Gracilaria* species that probably consists of cellulose microfibrils as well as agar (Bellanger et al., 1990; Dawes et al., 1961).

The term decklamelle that Brand (1901) called the wall covering of *Cladophora* is more appropriate than cuticle because it contains protein: 80% in *Porphyra umbilicalis* (Hanic & Craigie, 1969), 38 to 44% in *Chondrus crispus* (Craigie et al., 1992), and 50% in *Iridaea cordata* (Gerwick & Lang, 1977). Although the decklamelle of *Gracilaria* species has not been analyzed, it is clearly visible in ultrathin sections of the two species studied and in *G. verrucosa* (Verdus et al., 1985; Kling et al., 1989; Mariani et al., 1990). Further, the decklamelle of *G. tikvahiae* differs from that of *G. cornea*, the former appearing dense with a distinct boundary at the outer wall, while the latter appears more diffuse and grades into the outer wall. Based on field and mariculture observations, *G. cornea* is known to have a low level of epiphytes compard with other species (e.g *G. conferta*, *G. tikvahiae*). The significantly thick outer wall and more structur-

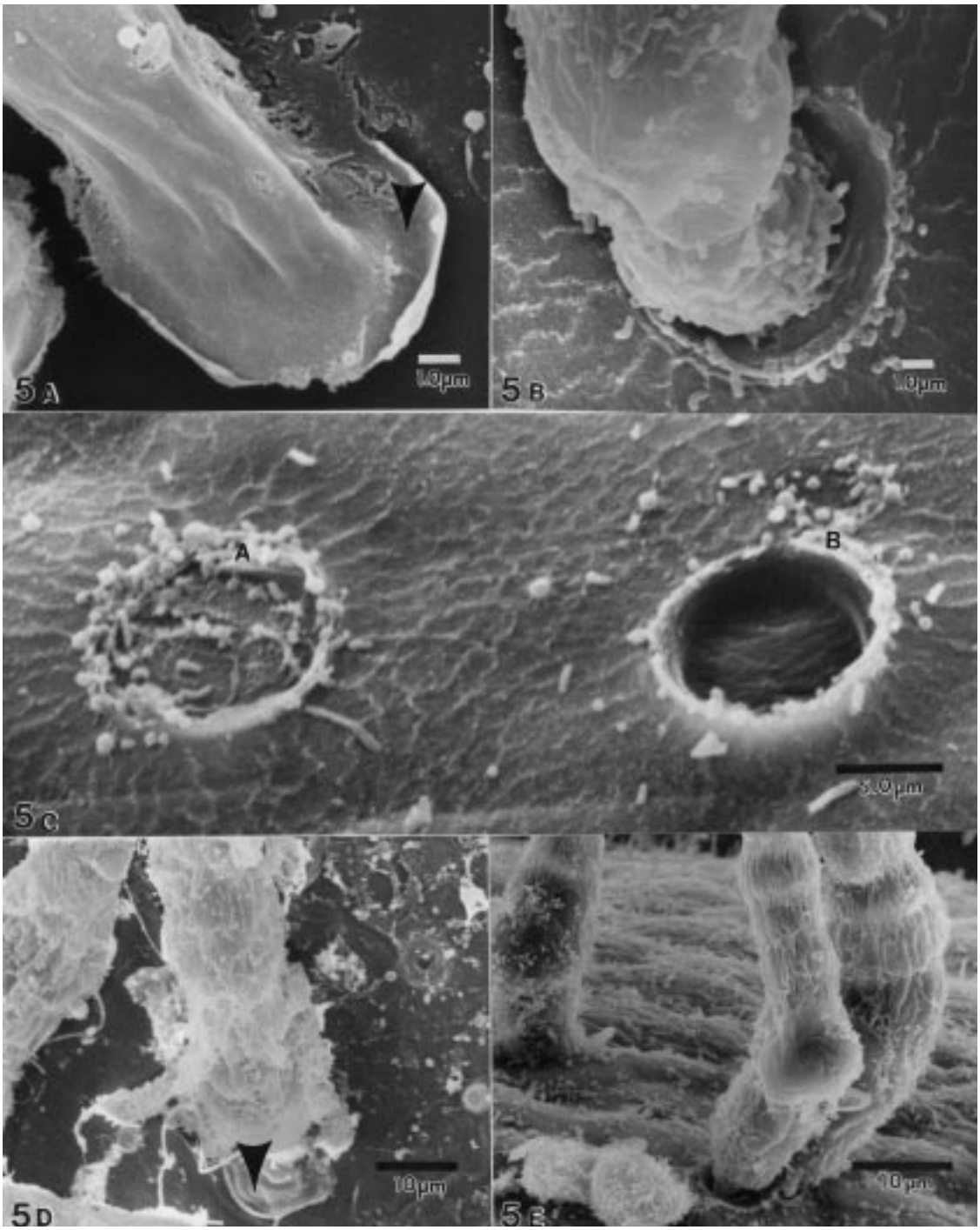

Figure 5. The attachment of *Ulva lactuca* germlings. Two-week old germlings formed an adhesion pad on a glass slide (Figure 5A, arrow), and penetrated the decklamelle of *Gracilaria cornea* (Figure 5B). Bacteria are evident around the attachment sites. If the germlings are removed, their adhesion pads can remain or cause removal of the decklamelle (Figure 5C: A, B respectively). Adhesion pads are also visible at the base of older (15 celled) filaments of *U. lactuca* on *G. tikvahiae* (Figure 5D, arrow). The germlings penetrate the decklamelle as seen with 5-celled filaments of *U. lactuca* on *G. cornea* (Figure 5E).

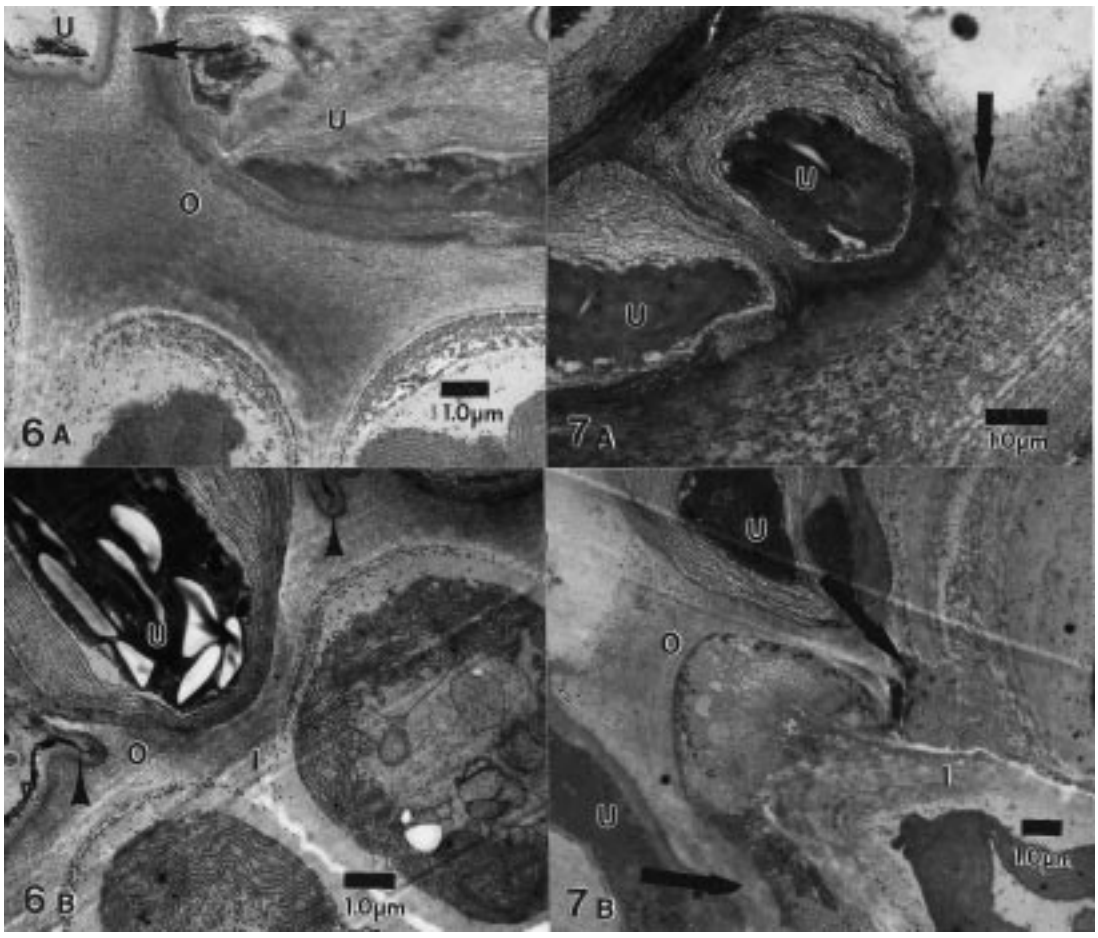

Figure 6. Ultrathin sections showing the penetration of the basal cell of a young filament of *Ulva lactuca* through the decklamelle and outer wall of *Gracilaria tikvahiae*. During attachment, the decklamelle is pushed up forming a rim (arrow) around the adhesion pad of *U. lactuca* (U) and has not penetrated the outer (o) wall (Figure 6A). In later stages, the germling's basal cells (U) penetrate the outer and inner walls (I) with the decklamellar rim also visible (Figure 6B, arrows).

Figure 7. Ultrathin sections showing the penetration of the basal cell of young filaments of *Ulva lactuca* through the decklamelle and outer wall of *Gracilaria cornea*. Initially, the attachment by the epiphyte (U) is superficial with the basal cells penetrating only the decklamelle (Figure 7A, arrow). In later stages, rhizoids (U, arrows) penetrate the outer wall (o) growing toward the inner (I) wall (Figure 7B).

ally diffuse decklamelle of *G. cornea* probably play a role in epiphyte resistance.

Sporelings of *Ulva lactuca* produce adhesion pads during the initial phase of attachment on glass slides as reported previously for *Ulva* (Bråten, 1975; Callow et al., 1997; Shihira-Ishikawa & Nishijima, 1998) and *Enteromorpha* (Evans & Christie, 1970; Leonardi & Cáceres, 1991). In the present study, the basal cell of *U. lactuca* germlings penetrated the decklamelle and the outer wall of both species within a few days followed by rhizoid development. Penetration by *U. lactuca* appears to result from digestion of the decklamelle and the two wall layers. The penetration of paint surfaces by germlings of *Enteromorpha* spp. (Moss & Woodhead, 1970) demonstrates how effective these green algae are in attachment.

Studies by Weinberger et al. (1999) on *G. conferta* have demonstrated a possible extracellular defense system against cell wall degrading microorganisms. Although under normal conditions *Gracilaria cornea* is not epiphytized, it can be when held in static cultures under induced blades of *Ulva lactuca*. The differences in deckelamellae construction between the two species probably have ecological and physiological bases under natural conditions that are not now evident. However, it appears that epiphyte-free species may have a structural basis that could be useful in mariculture.

Acknowledgements

Mrs Betty Loraamm is thanked for her help throughout the ultrastructural studies. The Binational Agriculture Research Development Foundation provided support for this study.

References

Arrontes J (1990) Composition, distribution on host and seasonality of epiphytes on three intertidal algae. Bot. mar. 33: 205–211.

Bellanger F, Verdus MC, Henocq V, Christiaen D (1990) Determination of the fibrillar part of *Gracilaria verrucosa* (Gracilariales, Rhodophyta) cell wall in order to prepare protoplasts. Hydrobiologia 204/205: 527–531.

Brand F (1901) Ueber einige Verhältnisse des Baues und Wachshums von *Cladophora*. Bei. Bot. Cent. 10: 481–451.

Bråten T (1975) Observations on mechanisms of attachment in the green alga *Ulva mutabilis* Føyn. An ultrastructural and light microscopical study of zygotes and rhizoids. Protoplasma 84: 161–173.

Buschmann, AH, Gomez P (1993) Interaction mechanisms between *Gracilaria chilensis* (Rhodophyta) and epiphytes. Hydrobiologia 260/261: 345–351.

Buschmann AH, Kuschel FA (1988) Cultivo intermareal de *Gracilaria* sp.: Colonizacion de esporas e interacciones con *Ulva lactuca* Biota (Chile) 4: 107–113.

Callow ME, Callow JA, Pickett-Heaps JD, Wetherbee R (1997) Primary adhesion of *Enteromorpha* (Chlorophyta, Ulvales) propagules: Quantitative settlement studies and video microscopy. J. Phycol. 33: 938–947.

Christie AO, Evans LV (1962) Periodicity in the liberation of gametes and zoospores of *Enteromorpha intestinalis* Link. Nature 4811: 193–194.

Craigie JS (1990) Cell walls. In Cole KM, Sheath RG (eds), Biology of the Red Algae, Cambridge University Press, Cambridge, pp. 221–257.

Craigie JS, Correa JA, Gordon ME (1992) Cuticles from *Chondrus crispus* (Rhodophyta). J. Phycol. 28: 777–786.

Crang RFE (1997) Protocol of plant specimen preparation for transmission electron microscopy. Microsc. Today 97: 18–20.

Davis AR, Targett NM, McConell OJ, Young CM (1989) Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and growth. In Scheuer PJ (ed.), Bioorganic Marine Chemistry, Springer, Berlin, pp. 85–114.

Dawes CJ (1988) Introduction to Biological Electron Microscopy: Theory and Techniques. Ladd Research Industries, Publisher, Burlington VT.

Dawes CJ, Orduna-Rojas J, Robledo D (1999) Responses of the tropical red seaweed *Gracilaria cornea* to temperature, salinity and irradiance. J. appl. Phycol. 10: 419–425.

Dawes CJ, Scott FM, Bowler E (1960) Light and electron microscope study of cell walls of brown and red algae. Science 132: 1663–1664.

Delivopoulos SG, Pellegrini M, Pellegrini L (1989) Ultrastructure of the developing tip of the thallus in the red alga *Gracilaria dura* (C. Agardh) J. Agardh (Gigartinales, Gracilariaceae). Cytobios 57: 177–184.

Ducker SC, Knox RB (1984) Epiphytism at the cellular level with special references to algal epiphytes. In Linskens H-F, Heslop-Harrison J (eds), Encyclopedia of Plant Physiology 17, Springer, Berlin, pp. 113–133.

Evans LV (1981) Marine algae and fouling: A review, with particular reference to ship-fouling. Bot. mar. 24: 167–171.

Evans LV, Christie AO (1970) Studies on the ship-fouling alga *Enteromorpha*. I. Aspects of the fine-structure and biochemistry of swimming and newly settled zoospores. Ann. Bot. 34: 451–466.

Fletcher RL (1995) Epiphytism and fouling in *Gracilaria* cultivation: An overview. J. appl. Phycol. 7: 225–333.

Fletcher RL, Callow ME (1992) The settlement, attachment and establishment of marine algal spores. Brit. phycol. J. 27: 303–323.

Friedlander M (1992) *Gracilaria conferta* and its epiphytes: the effect of culture conditions on growth. Bot. mar. 35: 423–428.

Friedlander M, Ben-Amotz A (1991) The effect of outdoor culture conditions on growth and epiphytes of *Gracilaria conferta*. Aquat. Bot. 39: 315–333.

Friedlander M, Gonen Y, Kashman Y, Beer S (1996) *Gracilaria conferta* and its epiphytes: 3. Allelopathic inhibition of the red seaweed by *Ulva* cf. *lactuca*. J. appl. Phycol. 8: 21–25.

Friedlander M, Krom MD, Ben-Amotz A (1991) The effect of light and ammonium on growth, epiphytes and chemical constituents of *Gracilaria conferta* in outdoor cultures. Bot. mar. 34: 161–166.

Gerwick WH, Lang NJ (1977) Structural, chemical, and ecological studies on iridescence in *Iridaea* (Rhodophyta). J. Phycol. 13: 121–127.

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt and *Detanula confervacea* (Cleve) Gran. Can. J. Microbiol. 8: 229–239.

Hanic LA, Craigie JS (1969) Studies on the algal cuticle. J. Phycol. 5: 89–102.

Kling R, Pellegrini M, Bodard M (1989) Aspects ultrastructuraux de la différenciation cellulaire dans le point végétativ de la fronde de *Gracilaria verrucosa* (Gigartinales, Rhodophyta). Cryptogam. Algol. 10: 209–234.

Leonardi PI, Cáceres EJ (1991) Ultrastructure of the fresh-water alga *Enteromorpha flexuosa* spp. *pilifera*. I. Germination of biflagellate zoospores (Ulvophyceae, Chlorophyta). Nova Hedwigia 53: 115–126.

Lersten NR, Voth PD (1960) Experimental control of zoid discharge and rhizoid formation in the green alga *Enteromorpha*. Bot. Gaz. 122: 33–45.

Mariani P, Tolomio C, Baldan B, Braghetta P (1990) Cell wall ultrastructure and cation localization in some benthic marine algae. Phycologia 29: 253–262.

Moss B, Woodhead P (1970) The breakdown of paint surfaces by *Enteromorpha* sp. New Phytol. 69: 1025–1027.

Nilsen N, Nordby Ø (1975) A sporulation-inhibiting substance from vegetative thalli of the green alga *Ulva mutabilis* Føyn. Planta 125: 127–139.

Pickering, TD, Gordon ME, Tong LJ (1993) Effect of nutrient pulse concentration and frequency on growth of *Gracilaria chilensis* plants and levels of epiphytic algae. J. appl. Phycol. 5: 525–533.

Ramus J (1969) Pit connection formation in the red alga *Pesudogloiophloea*. J. Phycol. 5: 57–63.

Shihira-Ishikawa I, Nishijima M (1998) Attachment and development of *Ulva* swarmers. Abstract C88. XVIth International Seaweed Symposium Program, Abstracts and Directory. Marine Science Institute, University of the Philippines, Quezon City, Philippines, pp. 59.

Smith GM (1947) On the reproduction of some Pacific coast species of *Ulva*. Am. J. Bot. 55: 4–11.

Stratmann J, Paputsoglu G, Oertel W (1996) Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. J. Phycol. 32: 1009–1021.

Svirski E, Beer S, Friedlander M (1993) *Gracilaria conferta* and its epiphytes: (2) Interrelationship between the red seaweed and *Ulva* cf. *lactuca*. Hydrobiologia 260/261: 391–396.

Verdus MC, Christiaen D, Stadler T, Morvan H (1985) Étude ultrastructurale et cytochimique de la paroi cellulaire chez *Gracilaria verrucosa* (Rhodophyta). Can. J. Bot. 64: 96–101.

Weinberger F, Hoppe H-G, Friedlander M (1999) Oligoagars elicit a physiological response in *Gracilaria conferta*. J. Phycol. 35: 747–755.

Zvyagintsev, A, Kozmenko VB (1995) Fouling of aquacultural installations and epiphyton of *Gracilaria* in Posyeta Bay, Sea of Japan. Russ. J. mar. Biol. (Biologiya Morya) 21: 12–16.