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Enzymatic studies of the extracellular mucilage of two aquatic hyphomycetes, *Lemonniera aquatica* and *Mycocentrospora filiformis*

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The effect of three carbohydrate-digesting enzymes, β-glucuronidase, lyticase and α-mannosidase, and three proteolytic enzymes, α-chymotrypsin, papain and pronase E, on the strength of conidial attachment of *Lemonniera aquatica* and *Mycocentrospora filiformis* was determined using the LH-Fowler Cell Adhesion Measurement Module. Carbohydrate-digesting enzyme treatments showed significant differences in number of attached and detached conidia versus control samples; little or no effect was observed for the proteolytic enzymes. Scanning and transmission electron microscopy showed different degrees of mucilage digestion by the carbohydrate-digesting enzymes on the germ hyphae, hyphae subtending appressoria, and appressoria of the two species. The loss of mucilage integrity and decrease in mucilage thickness were more pronounced on the hyphal sheaths than on the appressorial sheaths. Lyticase caused the most severe damage to the mucilage and cytoplasm of both fungi, particularly *L. aquatica*. β-Glucuronidase and α-mannosidase exhibited more effective mucilage digestion on *M. filiformis* than on *L. aquatica*. Results indicate that the mucilage of the two species is mainly polysaccharide, containing more β-1,3-glucans than β-glucuronide and α-mannosyl residues. Variability of mucilage composition exists between these species and also between different structures of the same fungus.

Key Words——appressorium; aquatic hyphomycetes; mucilage composition; strength of attachment; ultrastructure.

Extracellular mucilages have been demonstrated to be important for fungal spore adhesion (Latge et al., 1986; Epstein et al., 1987; Hyde et al., 1989; Jones and Epstein, 1990; Read, 1990; Boucias and Pendland, 1991). Chemical composition of these fungal extracellular mucilages varies and this may influence the physicochemical properties of the cell surface. Three chemical groups of fungal mucilages are recognized: glycoprotein, carbohydrate, and protein alone (Jones, 1994). The chemical composition of adhesives can be inferred by using enzyme digestion. Enzymes modify the chemical structure of mucilages and hence the adhesive ability of the fungi, and the effect can be measured by appropriate adhesion assays.

The Radial Flow Chamber was designed to measure the critical shear stress for bacterial adhesion on an inert surface and to study fundamental aspects of cell adhesion (Fowler and McKay, 1980; Duddridge et al., 1982). The equipment was then modified and called the LH-Fowler Cell Adhesion Measurement Module (CAMM) (Fowler, 1988). This module has been used previously to study attachment of fungal spores (Hyde et al., 1989; Read, 1990), algal spores (Gunn et al., 1984) and diatoms (Pyne et al., 1984). The conditions of water flow inside the chamber are designed to simulate the natural physical stresses upon species of aquatic micro-organisms, and in this research the module was used to study the attachment of aquatic hyphomycetes.

Attachment of the saprobic aquatic hyphomycetes *Lemonniera aquatica* de Wild. and *Mycocentrospora filiformis* (Petersen) Iqbal has been well studied (Read, 1990; Read et al., 1991; Read et al., 1992a,b,c; Au, 1993; Au et al., 1996) and mucilage has been found associated with all stages of conidial attachment: initial contact, germination, germ tube, hypha and appressorium attachment. Since information on the chemistry of the mucilage is limited (Read, 1990), the results presented in this paper are those from a study intended to characterize the mucilages associated with the germ hyphae, hyphae subtending appressoria and appressoria of *L. aquatica* and *M. filiformis* by indirect enzymatic studies using the LH-Fowler-CAMM. Scanning and transmission electron microscopy were used to observe the extent of mucilage digestion on different structures of the germinated conidia after enzyme treatment.

Materials and Methods

Fungal cultures and preparation of conidial suspension *Lemonniera aquatica* and *M. filiformis* were isolated from

submerged leaf litter from streams in Hampshire, England and in Pokfulam, Hong Kong, respectively. Isolates were subcultured on 2% (w/v) malt extract agar (MEA). Conidial suspensions of *L. aquatica* and *M. filiformis* were prepared by submerging two 9 mm diam discs from a 3–4 wk old MEA culture in 50 ml of sterilized aerated distilled water for 3–4 d at 20°C (ca. $2–4 \times 10^4$ conidia/ml).

Enzyme preparation Three carbohydrate-digesting enzymes, β-glucuronidase from *Helix pomatia* (EC 3.2.1.31), lyticase from *Arthrobacter luteus*, and α-mannosidase from Jack bean (EC 3.2.1.24), and three proteolytic enzymes, papain from papaya latex (EC 3.4.22.2), pronase E from *Streptomyces griseus* XXV (EC 3.4.24.31), and β-chymotrypsin from bovine pancreas type IV-S (EC 3.4.21.1) (not used for *M. filiformis*), were studied for their effect on the strength of attachment using the LH-Fowler CAMM. All enzymes were supplied by Sigma. Their specificity and the corresponding buffers used are listed in Table 1. All glasswares and distilled water used for the enzyme and buffer preparations were sterile.

Enzymatic studies of conidial attachment by the LH-Fowler cell adhesion measurement module (CAMM) The operating principle of the CAMM is based on a defined hydrodynamic environment to establish a known shear stress across a test surface in the radial flow chamber. The hydrodynamic shear forces in the chamber are established between two stationary parallel discs (10 cm in diam) set 1 mm apart. One disc is made from perspex, onto which conidia had been settled, and the other is stainless steel. A water flow enters from the inlet at the centre of the stainless steel disc and flows radially at decreasing velocity between the discs to the outer edges. The surface shear stress (T,in Nm^{-2}) produced by the laminar water flow on the perspex at radius r is calculated by the formula (Fowler, 1988):

$T = 3Q\mu/\pi rh^2$ where:

Q = volumetric flow rate (m^3s^{-1})

μ = dynamic viscosity, 1.002×10^{-3} Nsm^{-2} for H_2O

r = radius (mm)

h = disc separation, 1×10^{-3}m

Conidia in suspension (20 ml) were settled on the perspex disc for 12 h at 20°C, by which time, germ hyphae were formed and appressoria had developed (Au, 1993). The settled conidia were then incubated in either enzyme solution, heat-denatured enzyme solution (boiled for 30 min) or the corresponding buffer for 3, 6, 12 and 24 h (1 and 2 h were also studied for lyticase). Excess water on the perspex disc was removed by tilting before the addition of the test solution (25 ml) directly onto the surface.

At the end of the incubation period, the disc was subjected to a shear force of 3.2 Nm^{-2}, which corresponds to a laminar water flow of 3 L min^{-1} at a 15 mm radius. Ten fields were marked 15 mm from the centre on the reverse of the perspex disc. The number of conidia on each of the 10 marked fields was counted before and after 15 min of the shear force treatment. The percentage conidia remaining attached was obtained by averaging (±S.E.M.) the value calculated for each field. A Chi-square test (2×2 contingency table) was employed to analyse whether the numbers of attached and detached conidia in the treated sample were significantly different from its control at $P < 0.05$.

Scanning electron microscopy Based on the results from the strength of attachment studies, suitable concentrations of the carbohydrate-digesting enzymes, 1.0% β-glucuronidase, 0.2% lyticase, and 1.0% α-mannosidase, were selected for SEM study in order to visualize the extent of mucilage digestion by the enzymes. Conidia were settled on Thermanox coverslips (Bio-Rad,

Table 1. Carbohydrate-digesting enzymes and proteolytic enzymes used in the enzymatic studies, enzyme specificity, and buffer/pH.

Enzymes	Specificity	Buffer/pH
Carbohydrate-digesting enzyme		
1% β-glucuronidase	releases terminal glucuronide units linked through the C-1 by a β-configuration	0.01 M sodium acetate, pH 4.5
0.2% lyticase	β-1,3-glucanase, hydrolyzes glucose polymers having β-1,3 glycosidic linkages contains a protease	0.01 M Tris–HCl, pH 7.5
1% α-mannosidase	hydrolyzes α-D-mannosyl residues from terminal non-reducing position of polysaccharides cleaves mannose residues linked through an α-glycosidic bond	0.01 M sodium acetate, pH 4.5
Proteolytic enzyme		
0.2% α-chymotrypsin	endopeptidase, hydrolyzes the carboxyl side of aromatic amino acid e.g. tyrosine, tryptophan, phenylalanine	0.01 M Tris–HCl, pH 7.5
0.2% papain	hydrolyzes peptides, amides, esters especially at bonds involving basic amino acid or leucine or glycine	0.01 M Tris–HCl, pH 6.3
0.2% pronase E	non-selective, broad cleavage of the peptide chains of glycoproteins and proteins	0.01 M Tris–HCl, pH 7.5

UK) for 12 h before incubation in either enzyme solution, denatured enzyme solution or corresponding buffer. At the end of the incubation period (3 and 24 h for β-glucuronidase and α-mannosidase; 1 and 6 h for lyticase) the Thermanox coverslips with attached conidia were fixed by immersion in 2% (w/v) aqueous osmium tetroxide for 12 h at 4°C. Fixed material was washed in distilled water and then dehydrated through a graded ethanol series to acetone. Dehydrated samples were critical point dried in a Ladd Critical Point Dryer (Ladd, USA). Dried samples were mounted on aluminium stubs and gold/palladium-coated in a Bio-Rad E5400 diode sputter coater before being examined at 20 kV in a Cambridge Stereoscan 150 scanning electron microscope.

Transmission electron microscopy Enzyme-treated or control conidia (similar preparation procedures and incubation times were used as described above) were fixed in 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 3 h at room temperature. After thorough washing in buffer followed by distilled water for 30 min, material was post-fixed in 2% (w/v) aqueous osmium tetroxide for 12 h at 4°C. Fixed material was then dehydrated in a graded ethanol series to acetone. Samples were infiltrated and embedded in Möllenhauer's resin (Möllenhauer, 1964). Ultra-thin sections were stained with Reynolds' lead citrate (Reynolds, 1963) and a saturated solution of uranyl acetate in 50% ethanol (40 min each) before examination with a JEOL 100SX transmission electron microscope at 60 kV.

Results

Conidial attachment In the presence of lyticase, detachment of the settled conidia of *L. aquatica* increased rapidly with incubation time (Fig. 1a), and only 13% of settled conidia remained attached after 12 h of incubation. The number of conidia that remained attached was significantly different from that in the controls ($P<0.05$) at all study periods. Both the β-glucuronidase- and α-mannosidase-treated conidia of *L. aquatica* showed no significant difference in number of conidia detached from the control samples except after 24 h of incubation (Fig. 1a).

For *M. filiformis*, after 24 h of incubation in lyticase, only 23% of conidia remained attached (Fig. 1b); the percentage attachment decreased rapidly with incubation time but to a lesser extent compared to *L. aquatica* (Fig. 1a). Both the β-glucuronidase- and α-mannosidase-treated conidia exhibited a moderate decrease in percentage conidial attachment with time, 75–80% of conidia remaining attached after 24 h of incubation. With the exception of α-mannosidase-treated conidia at 3 h, the number of conidia remaining after enzyme treatment was significantly lower than the control ($P<0.05$).

For both *L. aquatica* and *M. filiformis*, the numbers of conidia remaining attached in the proteolytic enzyme-treated samples were, in most cases, not significantly different from the controls ($P<0.05$) (Figs. 2a, b).

Electron microscopy Micrographs are presented to illustrate various stages in the digestion of mucilage in *L. aquatica* and *M. filiformis*. Each of the cytological phenomena shown represents a frequency of observation ≥60% based on the examination made on five randomly chosen block materials (when the same target cytological change was repeatedly observed on the first three replicate block materials, no further sectioning of replicate block materials was required).

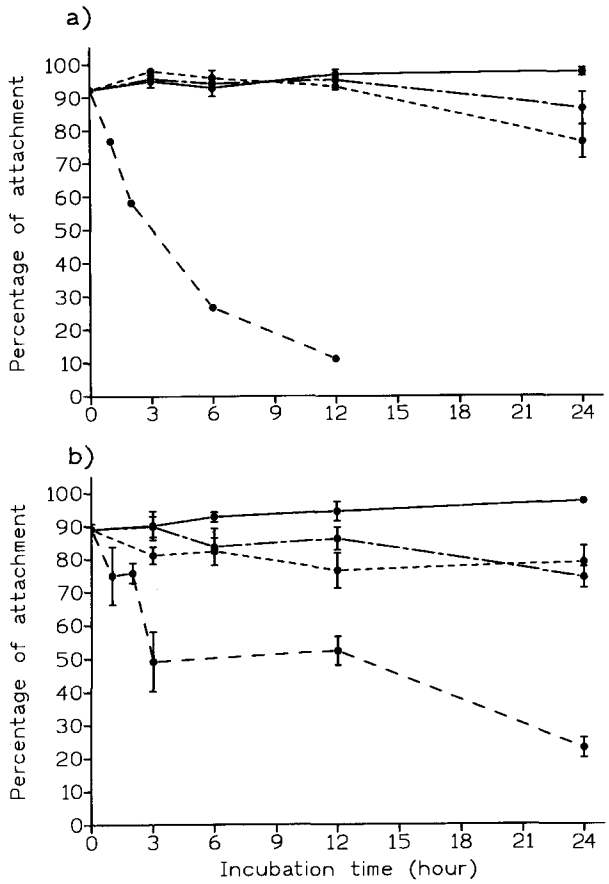


Fig. 1. The effect of up to 24 h of incubation with carbohydrate digesting enzymes on percentage attachment of conidia at 3.2 Nm^{-2} shear force.
1a. *Lemonniera aquatica*; 1b. *Mycocentrospora filiformis*.
(—— Control; ---- β-glucuronidase; – – – lyticase; —·—·— α-mannosidase)

Germinated *Lemonniera aquatica* (Fig. 3) The mucilaginous sheath around the germ hypha incubated in buffer solution (the control) is electron-dense and homogeneous (Fig. 5). Inclusions of similar electron-density to the mucilage are present on the outer surface of the electron-transparent cell wall and lomasome-like (multivesicular) bodies are associated with the convoluted plasma membrane (Fig. 5). Electron-dense inclusions always occur within electron-transparent regions of the cytoplasm associated with the germ hyphal wall (Fig. 6). After 1 h of incubation in lyticase, the sheath surrounding the germ hypha became less distinct, the cell wall was absent, and the cytoplasmic contents had degenerated (Fig. 7). After 6 h of incubation, the cell wall of the conidial arm and the cytoplasm had also degraded (Fig. 8). In the

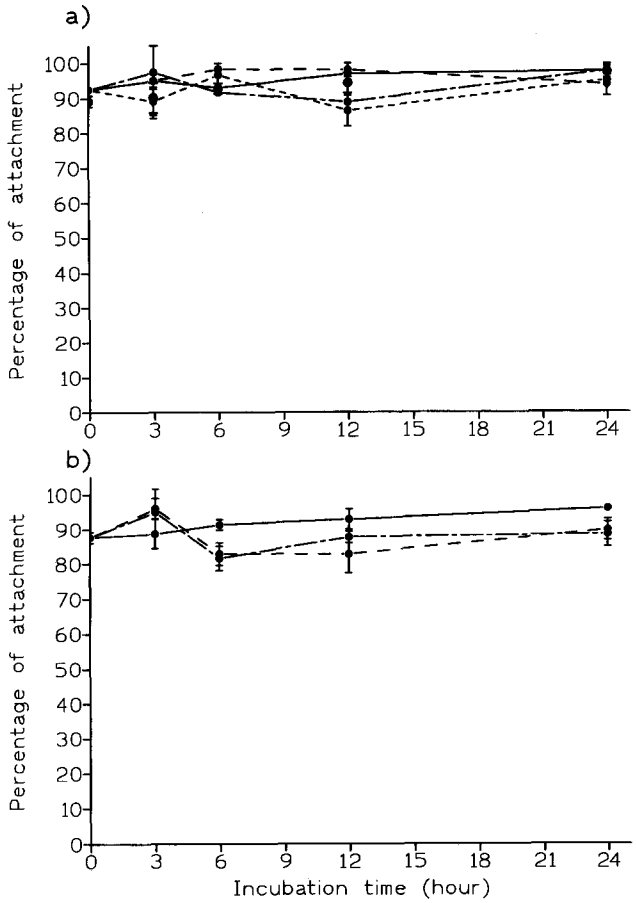


Fig. 2. The effect of up to 24 h incubation with proteolytic enzymes on percentage attachment of conidia at 3.2 Nm^{-2} shear force.
2a. *Lemonniera aquatica*; 2b. *Mycocentrospora filiformis*.
(—— Control; ---- α-chymotrypsin; – – – papain; —·— pronase E)

presence of β-glucuronidase, further degradation was observed on the germ hyphal sheath after 24 h of incubation (Fig. 9). Germ hyphae incubated in α-mannosidase showed no cytoplasmic damage, although the mucilaginous sheath was more dispersed (Fig. 10) than that of the control (Fig. 5).

The subtending hypha of *L. aquatica* was enrobed in an extensive mucilaginous sheath (Fig. 11). Following incubation in lyticase (1 h), the sheath was degraded and the hypha had partly collapsed (Fig. 12); after 6 h of incubation the hyphal cell wall was disrupted (Fig. 13).

Appressoria were attached to the substratum by an extensive electron-dense mucilaginous sheath (Figs. 14, 16). Numerous electron-transparent regions of cytoplasm were associated with the plasma membrane of the appressorium (Fig. 17). Although the α-mannosidase-treated appressoria showed no ultrastructural changes compared to the controls, both the β-glucuronidase- and lyticase-treated appressoria showed loss of the mucilaginous sheath (Figs. 15, 18); and in the case of lyticase, complete lysis of plasma membrane and leakage of cytoplasm were observed after 6 h of incubation in the enzyme (Fig. 19).

Germinated *Mycocentrospora filiformis* (Fig. 4) The germ hyphal sheath which attaches the hypha to the substratum in control material was smooth at the apex but warty subapically (Fig. 20). Transverse sections of a germ hypha showed that the sheath comprised electron-dense material with less electron-dense inclusions and was bounded externally by a continuous electron-dense layer (Fig. 21). After 6 h in lyticase, the hyphal apex had collapsed and the extracellular mucilage was partly degraded (Fig. 22), dispersed and separated from the cell wall (Fig. 23). In the presence of β-glucuronidase, the germ hyphal sheath showed advanced degradation and loss of sheath integrity after 24 h (Fig. 24). The extent of digestion was less severe in α-mannosidase than in β-glucuronidase (Fig. 25).

The subtending hyphae of appressoria were covered in a warty, compact mucilage (Fig. 26). In the presence of lyticase, the mucilaginous layer became dispersed and fibrillar (Fig. 27).

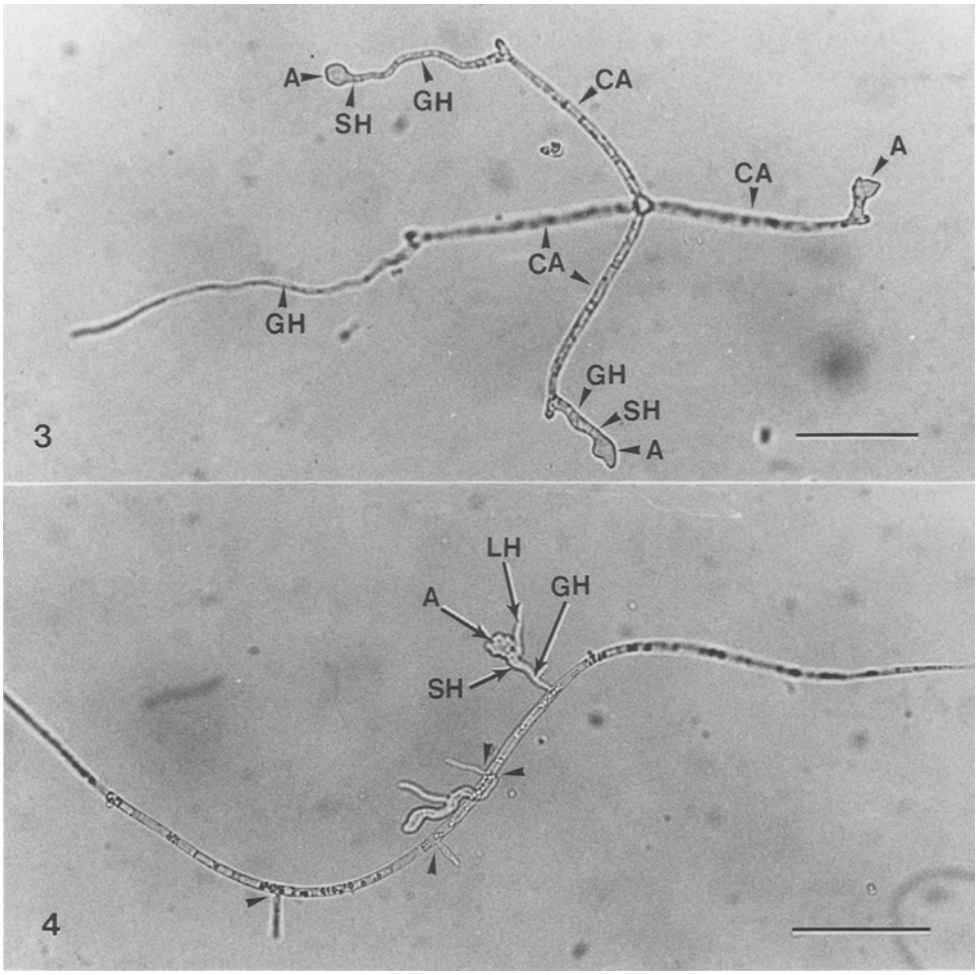
The appressorial sheath was continuous in *M. filiformis* and extended onto the substratum (Fig. 28). At the TEM level, the appressorial sheath comprised an electron-dense, undulating outer region and a thicker inner region which contained electron-transparent material within an electron-dense matrix. Material of similar morphology occurred adjacent to the plasma membrane (Fig. 30). After 6 h in lyticase, the appressorial sheath became highly dispersed with a fibrillar margin (Fig. 31). After 24 h in β-glucuronidase (Fig. 29) and α-mannosidase, the surface mucilage was lost.

Discussion

Results presented in this paper indicate that the carbohydrate-digestive enzymes lyticase, β-glucuronidase and α-mannosidase are effective in weakening the adhesion of *L. aquatica* and *M. filiformis* hyphae and appressoria to surfaces. The extent of conidial detachment and mucilage digestion increased with enzyme incubation time. However, the proteolytic enzymes α-chymotrypsin, papain and pronase E exhibited little or no effect on conidial detachment.

Although lyticase is a combination of β-1,3 glucanase and a protease, our data clearly showed that proteolytic enzyme had little effect on the digestion of mucilage. We, therefore, concluded that the primary effect of lyticase on mucilage digestion is due to the enzyme β-1,3-glucanase. In summary, the results indicate that the mucilage of *L. aquatica* and *M. filiformis* is largely polysaccharide (e.g., highly glycosylated glycoprotein), comprising β-1,3-glucan, β-glucuronide and α-mannosyl residues.

Of the six enzymes studied, lyticase was the most effective in the detachment of settled conidia under shearing forces. Lyticase not only digested the mucilaginous sheath but also the cell wall of *L. aquatica* and *M. filiformis*. The conidial arm of *L. aquatica*, which lacked extracellular mucilage, was also degraded by lyti-



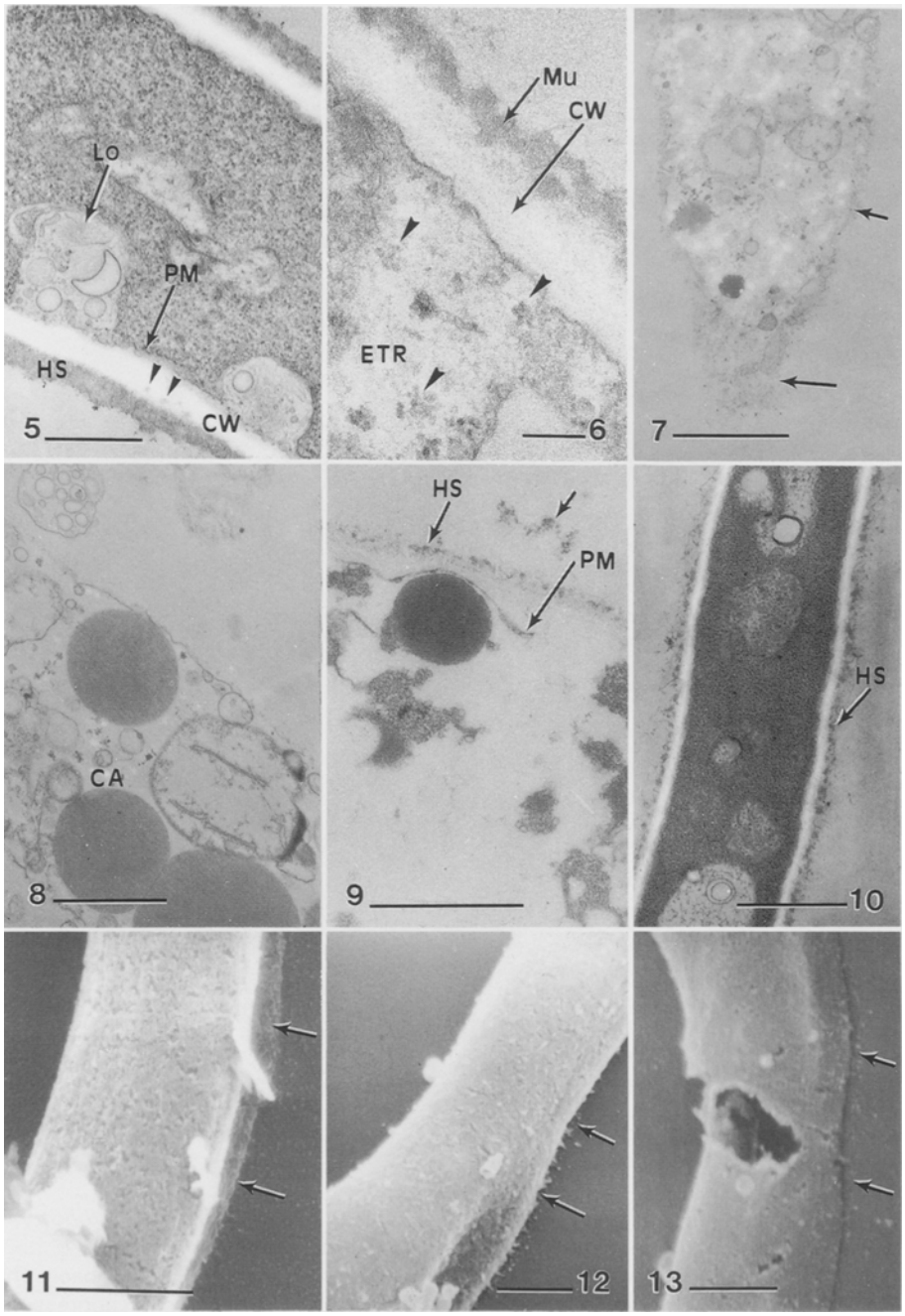
Figs. 3, 4. Germinated conidia after settlement on Thermanox coverslip for 12 h. Light micrographs.
3. Tetraradiate conidium of *Lemonniera aquatica*. Germ hypha (GH) produced from the apex of each divergent arm, the conidial arm (CA). The transition region between the germ hypha and the appressorium (A) is the subtending hypha (SH) of the appressorium. Scale bar=40 μm. 4. Sigmoid conidium of *Mycocentrospora filiformis*. Germination (darts) occurs along the conidium. An appressorium (A) has formed at the apex of germ hypha (GH). SH=subtending hypha; LH=lateral hypha. Scale bar=40 μm.

case. Cell wall digestion leads to leakage of cytoplasmic contents and collapse of the germ hyphae (cell wall damage was not observed on conidia treated with heat-denatured lyticase). This may indicate that both the mucilage and the cell wall of *L. aquatica* and *M. filiformis* are at least partially composed of β-1,3-glucan. Glucans have been reported to be part of the cell wall components in the majority of fungal taxa (Bartnicki-Garcia, 1968, 1970; Gomez-Miranda and Leal, 1981).

Mucilage on germ hyphae Results presented here indicate that the germ hyphal sheaths of *L. aquatica* and *M. filiformis* comprise more β-1,3-glucans than β-glucuronide and α-mannosyl residues. However, the mucilage in *M. filiformis* contains more β-glucuronide and α-mannosyl residues but less β-1,3-glucans compared to that in *L. aquatica* (Table 2). Glucan microfibrils have been reported on the hyphal sheath of other fungi, e.g., *Schizophyllum commune* Fries (Wessels et al., 1972), *Phanerochaete chrysosporium* Burdsall (Ruel and Joseleau, 1991) and *Arthrobotrys oligospora* Fres. (Tunlid et al., 1991).

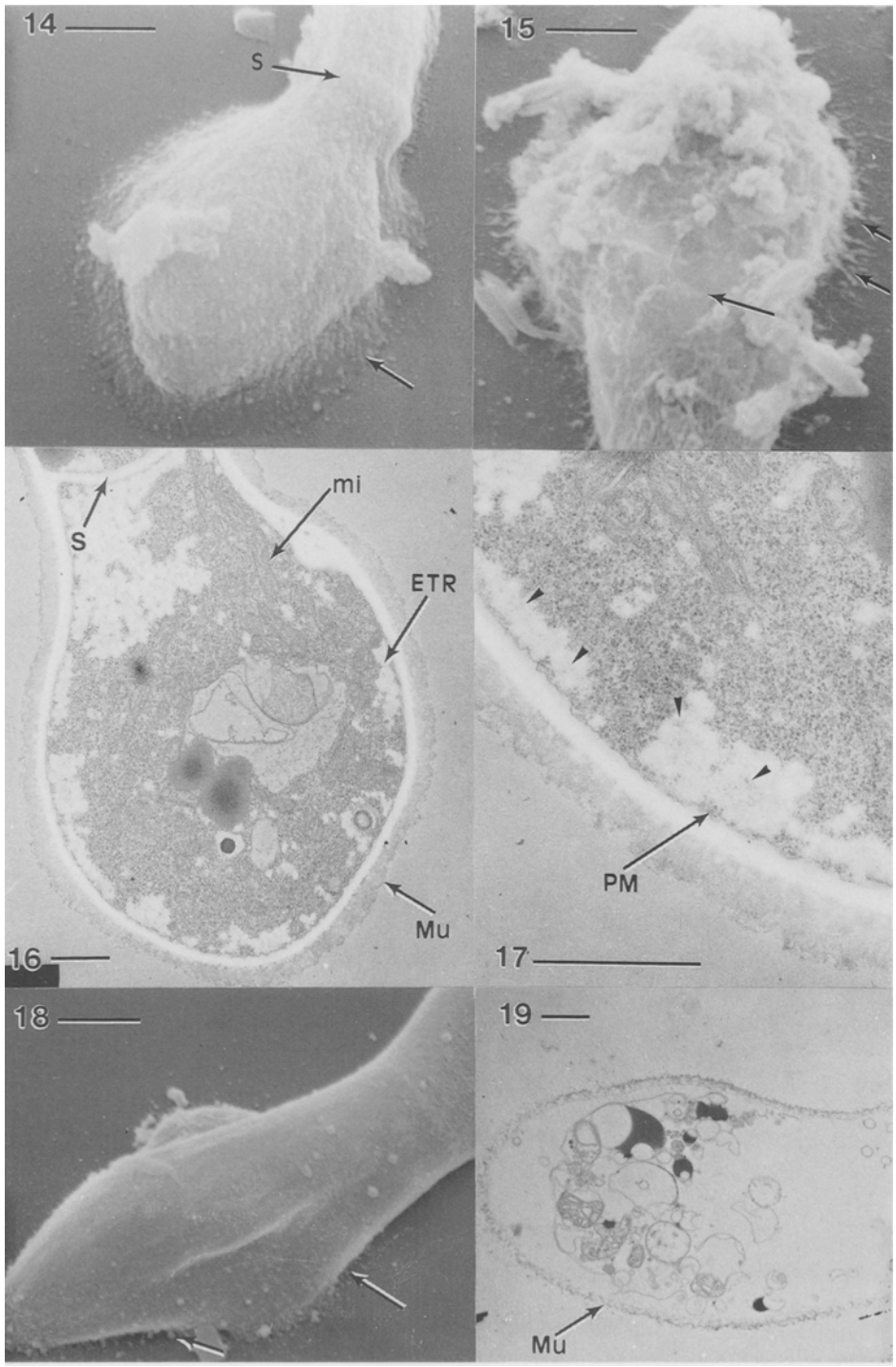
Mucilage on subtending hyphae The apex of a germ hypha may swell to form an appressorium, and the transition region between the germ hypha and the appressorium is the subtending hypha of the appressorium (Read et al., 1992a). All these structures were enrobed in mucilage (Read, 1990). The amount of mucilage becomes more extensive from a germ hypha to a subtending hypha. The extent of mucilage digestion by lyticase, β-glucuronidase and α-mannosidase on *L. aquatica* and *M. filiformis* appeared less severe on the subtending hyphal sheath than on the germ hyphal sheath. This may be attributed to changes in mucilage composition from germ hypha to subtending hypha (Table 2).

Mucilage on appressorium About 80% of *L. aquatica* and *M. filiformis* conidia produced appressoria after settlement for 12 h on Thermanox coverslips (Au, 1993; Au et al., 1996) and were covered with extensive

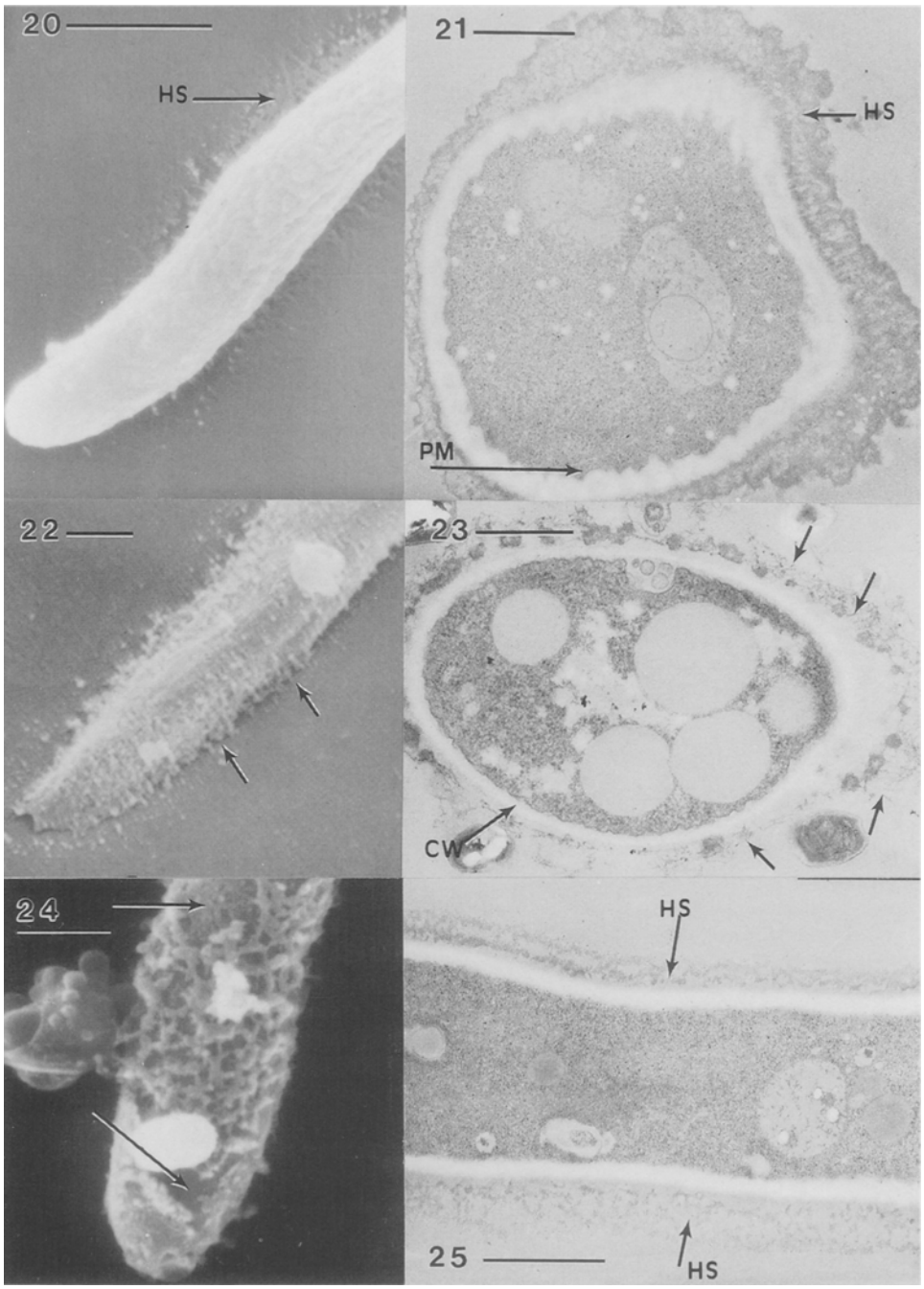


Figs. 5–13. *Lemonniera aquatica*.

5. TEM of germ hypha in control buffer for 3 h. The germ hyphal sheath (HS) is electron-dense and homogeneous. Inclusions of similar electron-density (darts) to the mucilage are present in the cell wall (CW). Lomosome-like bodies (Lo) are associated with the convoluted plasma membrane (PM). Scale bar = 0.5 μm. 6. TEM of germ hypha in control buffer for 6 h. High magnification of the mucilaginous sheath (Mu) and cell wall (CW) interface showing the electron-dense inclusions (darts) within the electron-transparent region (ETR). Scale bar = 0.2 μm. 7. TEM of germ hypha after 1 h of incubation in lyticase. The plasma membrane and cell wall are absent, cytoplasmic contents are degenerate and the mucilaginous sheath (arrowed) is incomplete. Scale bar = 0.2 μm. 8. TEM of conidial arm after 6 h of incubation in lyticase. The cell wall of the conidial arm (CA) and the cytoplasm are degraded. Scale bar = 1 μm. 9. TEM of germ hypha after 24 h of incubation in β-glucuronidase. The hyphal sheath (HS) is degraded with amorphous, electron-dense materials (arrowed) detached from the sheath. The plasma membrane (PM) is retracted, discontinuous and the cytoplasmic organization is disrupted. Scale bar = 1 μm. 10. TEM of germ hypha after 24 h of incubation in α-mannosidase. The hyphal sheath (HS) is diffuse. Scale bar = 1 μm. 11. SEM of subtending hypha of appressorium in control buffer for 1 h. The mucilaginous sheath (arrowed) is extensive and extends onto the substratum. Scale bar = 2 μm. 12. SEM of subtending hypha of appressorium after 1 h of incubation in lyticase. The extracellular mucilage is degraded (arrowed) and the hypha has partly collapsed. Scale bar = 2 μm. 13. SEM of subtending hypha of appressorium after 6 h in lyticase. The extracellular mucilage is degraded (arrowed) and the hyphal cell wall is disrupted. Scale bar = 2 μm.

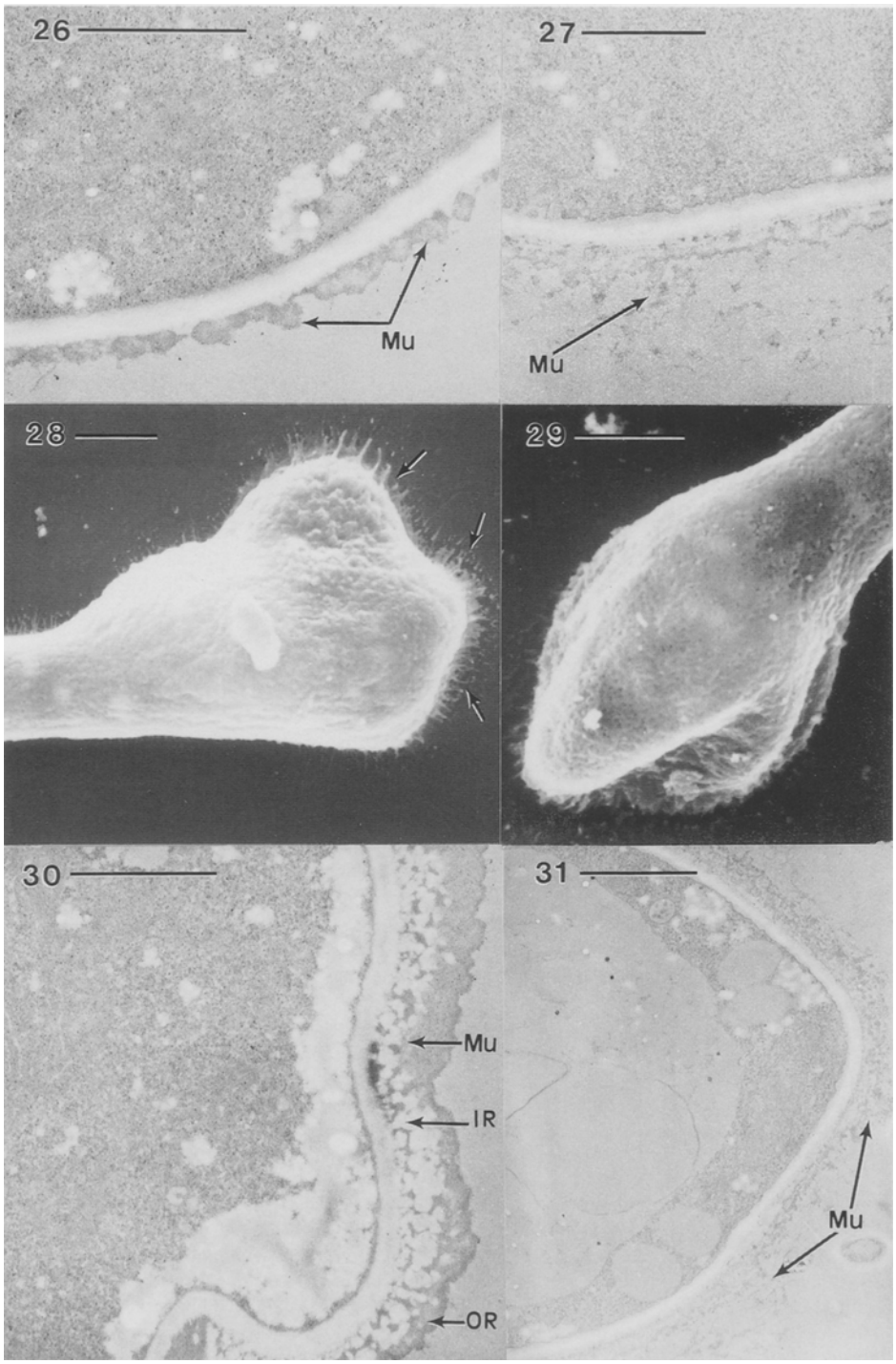


Figs. 14–19. *Lemonniera aquatica*.
14. SEM of appressorium in control buffer for 24 h. The appressorium is attached to the substratum by an extensive mucilaginous sheath (arrowed). S=septum. Scale bar=2 μm. 15. SEM of appressorium after 24 h in β-glucuronidase. The appressorium has degenerated, both the mucilaginous sheath and cell wall are degraded (arrowed). Scale bar=2 μm. 16. TEM of appressorium in control buffer for 24 h. The appressorial sheath (Mu) is electron-dense. Numerous electron-transparent regions (ETR) are associated with the plasma membrane; mitochondrial profiles (mi) are abundant. S=septum. Scale bar=1 μm. 17. Higher magnification of the appressorial wall showing the ETR-containing electron-dense inclusions (darts) associated with the plasma membrane (PM). Scale bar=1 μm. 18. SEM of appressorium after 6 h of incubation in lyticase. The appressorium has collapsed and the mucilaginous sheath has degraded (arrowed). Scale bar=1 μm. 19. TEM of appressorium after 6 h of incubation in lyticase. The lysed appressorium has disrupted organelles and only a poorly defined mucilaginous sheath (Mu). Scale bar=1 μm.



Figs. 20–25. *Mycocentrospora filiformis*.

20. SEM of germ hypha in buffer control for 3 h. The hyphal sheath (HS) is smooth at the apex but warty at the subapical region. The mucilaginous sheath attaches the hypha to the substratum. Scale bar = 2 μm. 21. TEM transverse section of germ hypha in buffer control for 6 h. The sheath (HS) comprises electron-dense material with less electron-dense inclusions and is bounded externally by a continuous electron-dense layer; the plasma membrane (PM) is convoluted. Scale bar = 1 μm. 22. SEM of germ hypha after 6 h of incubation in lyticase. The hyphal apex has collapsed and the extracellular mucilage has degraded. Note the residues of a mucilaginous sheath on the substratum (arrowed). Scale bar = 1 μm. 23. TEM transverse section of germ hypha after 6 h of incubation in lyticase. The mucilaginous material (arrowed) is dispersed and separated from the cell wall (CW). Scale bar = 0.5 μm. 24. SEM of germ hypha after 24 h of incubation in β-glucuronidase. The hyphal sheath has degraded, lost integrity and is absent in some regions (arrowed). Scale bar = 1 μm. 25. TEM of germ hypha after 24 h of incubation in α-mannosidase. The hyphal sheath (HS) is diffuse. Scale bar = 1 μm.



Figs. 26–31. *Mycocentrospora filiformis*.

26. TEM of subtending hypha of appressorium in control buffer for 1 h. The mucilaginous sheath (Mu) is warty and compact. Scale bar = 1 μm. 27. TEM of subtending hypha of appressorium after 1 h of incubation in lyticase. The mucilaginous sheath (Mu) is dispersed and fibrillar. Scale bar = 0.5 μm. 28. SEM of appressorium in control buffer for 3 h. Extensive appressorial mucilage (arrowed) extends onto the substratum. Scale bar = 1 μm. 29. SEM of appressorium after 24 h of incubation in β-glucuronidase. The warty appearance of surface mucilage has been lost. Scale bar = 2 μm. 30. TEM of appressorium in control buffer for 1 h. The appressorial sheath (Mu) comprises an electron-dense, undulating outer region (OR) and a thick inner region (IR) which contains electron-transparent material within an electron-dense matrix. Material of similar morphology occurs adjacent to the plasma membrane. Scale bar = 1 μm. 31. TEM of appressorium after 6 h of incubation in lyticase. The appressorial sheath (Mu) is highly dispersed with a fibrillar margin. Scale bar = 1 μm.

Table 2. Comparison of mucilage composition between settled conidia of *Lemonniera aquatica* (La) and *Mycocentrospora filiformis* (Mf) on germ hypha, subtending hypha and appressorium.

	Germ hypha		Subtending hypha		Appressorium	
	La	Mf	La	Mf	La	Mf
β-1,3-glucan residues	+++(+)[a]	+++	+++	++(+)	++	+(+)
β-glucuronide residues	+(+)	++(+)	+	++	+(+)	+
α-mannosyl residues	+	++	(+)	+(+)	(+)	+

a) +: relative abundance; (+): partially abundant.

mucilaginous sheaths. The action of β-glucuronidase and α-mannosidase on appressorial mucilage, especially in *M. filiformis*, was slower than that on the hyphal sheaths. This implies that β-glucuronide and α-mannosyl residues are not the major components of appressorial mucilage in these two species.

In conclusion, the mucilage on the settled conidia of *L. aquatica* and *M. filiformis* is mainly polysaccharide, comprising β-1,3-glucans, β-glucuronide and α-mannosyl residues. The protein component is either very low or absent. Variability of mucilage composition exists between fungi (e.g., β-1,3-glucan, *L. aquatica* > *M. filiformis*) and also among different structures on the same fungus (e.g., β-glucuronide in *M. filiformis*, germ hyphae > appressoria).

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