Ultrastructure and Chemistry of Cell walls of *Geotrichum candidum*: With Special Reference to Conidiogenesis

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The conidiogenesis of *Geotrichum candidum* grown on Czapek agar containing 1% yeast extract was examined by electron microscopy. The results showed that the entire wall of the fertile hypha is incorporated into the conidial wall and also that increase in thickness of the inner wall layer and differentiation of crosswalls are likely to be due to new wall synthesis which occur during conidium formation. Furthermore, the chemical composition was analysed for the purified conidial walls, and was compared with that of haloarthric conidial walls produced by *Trichophyton mentagrophytes*.

Geotrichum candidum Link ex Pers. is an imperfect fungus which is commonly isolated from soil¹⁾ and frequently incriminated in food contamination and spoilage²⁾. In addition, *G. candidum* has been reported as part of the indigenous microflora of the gastrointestinal tract of animals, including man³⁾. Under conditions which compromise the host's immunological defenses, this apparently innocuous resident of the gut may become pathogenic and cause oral, gastrointestinal or bronchopulmonary infections in man^{3,4)} and cutaneous or renal infections in other animals^{5~7)}. The fungus typically produces conidia by simple fragmentation and disarticulation of fertile hyphae (i.e. haloarthric conidium formation⁸⁾). The fertile hyphae are produced from either aerial mycelia on solid substrates, or immersed mycelia in liquid growth media. Rapid growth and sporulation in simple liquid media have permitted extensive developmental and biochemical studies of *G. candidum* in chemostat culture and allowed investigators to examine endogenous and exogenous factors controlling conidiogenesis^{9~17)}. In this paper, we have reviewed significant developmental and ultrastructural aspects of conidium formation

in *G. candidum* and examined the fine structure and chemical composition of conidial walls. A comparison of chemical data derived from other investigations of hyphal and haloarthric conidial wall composition is also presented. The potential of utilizing *G. candidum* as an experimental model for studies of various morphogenetic aspects of septate fungi is discussed.

MATERIALS AND METHODS

Culturing Procedures. The strain of *Geotrichum candidum* used in this study was obtained from J. W. Carmichael, University of Alberta, Canada (UAMH 178) and maintained in the University of Texas fungal culture collection (UT 22). The fungus was grown on Czapek agar (Difco) plus 1% yeast extract in Petri dishes for 7–10 days at 28°C in continuous darkness. Blocks of agar (5 mm square) supporting growth of conidia and mycelium were excised from the edge of the sporulating colony and a single agar block was used to inoculate each 400 ml Erlenmeyer flask containing 150 ml of liquid media (composition of media: sucrose, 30 g; NaNO₃, 2 g; K₂HPO₄, 1 g; KCl, 0.5 g; MgSO₄ • 7H₂O, 0.5 g; FeSO₄, 0.01 g; yeast extract 10 g; 1 liter of distilled water). The inoculated flasks were placed on an enclosed, horizontal shaker (Takasaki Seisakusho Co., Japan)

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and agitated (about 90 cycles/min.) at 28°C for 48 hours in continuous darkness.

Electron Microscopy. Coverslip cultures of *G. candidum* (Fig. 1) were prepared for the scanning electron microscope (SEM) as described earlier¹⁸. Isolated wall samples examined in the SEM (Fig. 7) were initially fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.1) at 4°C for one hour. The wall material was then washed in buffer, dehydrated in an ethanol series, and finally transferred to absolute acetone. Droplets of the wall sample were air dried on the surface of clean coverslips which were then mounted on specimen stubs and coated in a Denton DV 502 evaporator equipped with a triode sputter coating device. Specimens were examined in an AMR model 1000 SEM using 30 kV and photographed with Polaroid Type 55 P/N film.

Conidia examined by freeze-etching were grown on agar in Petri dish cultures and prepared according to the procedure outlined by Cole (1973)¹⁹. Replicas were obtained using a Balzers BA 360 M Freeze-etch Device and examined in a Hitachi HU-11E transmission electron microscope.

Thin sections of *G. candidum* conidia were prepared by the procedure described in an earlier report⁸.

Conidial Wall Isolation and Purification. Liquid cultures were slowly strained through eight layers of cheesecloth to remove mycelium from the cell suspension. The conidial filtrate was examined in the light microscope to check that essentially all hyphae has been removed. The conidial suspension was then centrifuged at 120 g for ten minutes. The pellet was washed and centrifuged at 120 g three times to remove residual growth media from the cells. The cells were fractured using acid-cleaned, 0.45–0.50 mm glass beads in a Bronwill mechanical cell homogenizer (Braun Model MSK; Bronwill Sci. Inc., Rochester, New York). A ratio of 1 g wet weight of packed cells (after centrifugation at 120 for ten minutes) to 5 g glass beads was employed. The 75 ml glass homogenization flask was cooled with liquid CO₂ during the cell fraction procedure. The suspension of ruptured cells was subsequently centrifuged at 180 g for 15 minutes. The supernatant was discarded and the wall pellet was washed with distilled water and centrifuged at 180 g three times. The sample was then resuspended in distilled water and subjected to mild sonication (five minutes, setting 4, Branson Sonifier, Branson

Sonic Power Co., Danbury, Conn.) to remove cytoplasmic debris attached to the wall surface. The suspension was subsequently centrifuged at 180 g, the supernatant discarded and the pellet washed an additional three times. A sample of the wall material was then processed for SEM examination while the rest was lyophilized in preparation for chemical analyses.

Chemical Analyses. The procedures used in analysis of wall components of *C. candidum* were the same as those described by Cole *et al.*²⁰.

OBSERVATIONS AND DISCUSSION

When grown at 28°C on Czapek agar supplemented with 1% yeast extract, prolific conidial production occurs after four to five days. The same mode of conidiogenesis is demonstrated when the fungus is grown at 23°C on a thin layer of potato dextrose agar (Difco) which coats the surface of a glass coverslip¹⁸. The cylindrical hypha in Fig. 1 has undergone septation and has partially disarticulated. In a time-lapse analysis of this process, Cole and Kendrick (1969)²¹ have shown that a rapid succession of septation of the fertile hypha occurs shortly after the arrest of hyphal tip growth. It has been postulated that the latter event is associated with dispersion of a cluster of secretory vesicles at the hyphal apex which had been previously involved in maintaining the polarity of wall synthesis and intussusception at the apical dome of the vegetative filament^{22–27}. It seems that during hyphal elongation microvesicles which are components of the cluster of secretory vesicles fuse with the plasmalemma and thereby transport chitin synthetase to, or activate the zymogenic form of this enzyme necessary for wall fibrillogenesis at the hyphal tip²⁸. Just prior to the succession of septation which leads to conidium differentiation in *G. candidum*, however, vesicle fusion is apparently arrested at the hyphal tip. From the standpoint of current molecular models which explain membrane fusion as a Ca²⁺-induced phase change²⁹, the programmed inhibition of membrane fusion and related morphogenetic events in *G. candidum* may be useful for studying the association between calcium ions and calcium binding proteins (e.g. calmodulin), and the process of fusion of secretory vesicles with the plasmalemma in fungal hyphae.

Septation of the determinate, fertile hypha is at first acropetal but is later random. In spite of this apparent haphazard sequence of crosswall forma-

tion, each conidium shown in Fig. 1 contains a single nucleus³⁰). Just prior to septation, nuclei are more or less regularly spaced within the hypha. Crosswalls subsequently form so that large hyphal compartments are delimited, each containing two nuclei. These compartments then divide, resulting in differentiation of conidia each containing a single nucleus. Such observations suggest that both temporal and spatial associations may exist between initiation of septation on the one hand, and positioning of nuclei and nuclear division on the other. Recent ultrastructural studies of septation in hyphae utilizing improved techniques of cryo-fixation³¹) and chemical fixation have revealed a ring or belt of microfilaments attached to the plasmalemma prior to the first signs of crosswall formation³²). This latter author has pointed out that microfilaments can attach to membranes of other organelles in fungal hyphae, including microvesicles, which "may partially fulfill the demand for sequestration of factors needed for septum formation. It is possible..." that the microfilamentous belt works mainly as a mechanical device for entrapment of vesicles... bearing chitin synthetase and... other vesicles with proteases for activation of the zymogen". Although cytochalasin B has no apparent effect on these microfilaments³²), cytochalasin A has been shown to influence intracellular organization associated with wall deposition in hyphae (i.e. arrangement and fusion of microvesicles with the cell membrane)^{33,34}). Employing cytochalasin A for studies of septation in *G. candidum* during conidium formation may prove useful for determining the interrelationships of microfilaments, microvesicles, wall deposition and wall growth in fungal hyphae²⁶).

Thin sections have revealed that the mature septa of vegetative and fertile hyphae of *G. candidum* are structurally distinct²⁵). The crosswalls of vegetative hyphae are uniperforate and demonstrate morphological features which are typical of the anamorphs of ascomycetous fungi³⁵). As long as the septal pore is open, adjacent cells are cytoplasmically connected and the pore is usually large enough to permit passage of nuclei, mitochondria and other organelles between cells. On the other hand, the septa which delimit conidia are multiperforate, and each micropore^{36,37}) or plasmadesmal canal³⁸) is approximately $0.02\mu\text{m}$ in diameter. In Fig. 2, a tangential section of a conidial

septum reveals several micropores (M). When the conidium is mature, the pores of the multiperforate septum are occluded by electron dense material so that adjacent cells are cytoplasmically separated. The cytological events and details of wall differentiation associated with multiperforate septum formation are underscribed.

Ultrastructural examinations of conidiogenesis in *G. candidum* have shown that the entire wall of the fertile hypha is incorporated into that of the conidia. Thickening of the inner wall layer and differentiation of crosswalls appear to be the only events involving new wall synthesis which occur during conidium formation. This is reflected in certain similarities in chemical composition between the hyphal and conidial walls which will be discussed later. During secession of adjacent conidia, the crosswalls split centripetally, a process which is referred to as schizolysis in septate fungi^{25,27,39,40}). As a result of disarticulation of the fertile hypha, the outer wall layer ruptures at the septa but persists as a frill at the ends of the conidia (Fig. 3). The significance of these data is that all wall layers of the fertile hypha become integrated into the conidial wall. This mode of conidiogenesis is called holoarthritis development²⁵). However, another method of conidium formation has been reported for *G. candidum* and *G. loubieri* in which chains of conidia are formed endogeneously in the fertile hypha, disarticulate and are then released by breakdown of the outer hyphal wall^{41,42}). This kind of conidiogenesis is referred to as enteroarthritis development and has been considered to characterize such septate fungi as *Sporendonema*, *Coremiella* and *Coccidioides*^{8,25}). A diagrammatic interpretation of wall differentiation during holoarthritis and enteroarthritis conidium formation is presented in Fig. 4²⁵). Most strains of *G. candidum* demonstrate holoarthritis conidium formation. However, when Saez⁴¹) grew his isolate on Sabourand's glucose (2%) agar at 25–30°C for two to three weeks, abundant enteroarthritis conidia were formed especially near the outer perimeter of the colony. Using both holoarthritis and enteroarthritis strains of *G. candidum*, a comparison of wall chemistry and the ultrastructural events associated with wall differentiation during these two developmental processes may be performed. Of special interest is the suggested presence of digestive enzymes associated with wall lysis during both enteroarthritis and

Fig. 1. Coverslip culture of *Geotrichum candidum* showing conidia which have formed by disarticulation of a fertile hypha. $\times 2,750$. (from Cole and Samson, 1979).

Fig. 2. Tangential section of conidial septum showing multiperforate nature of cross wall. L, lipid droplets; M, micropores; Mt, mitochondria. $\times 36,000$.

Fig. 3. Adjacent conidia showing ruptured outermost wall layer which remains as a frill of wall material (arrows) at the ends of the cells. M, micropores; V, vacuole. $\times 12,000$.

Fig. 4. Diagrammatic interpretation of holoarthritis (A, B, F) and enteroarthritis (A–E) conidium formation. (after Cole and Samson, 1979).

holoarthric conidium formation^{10,25}) but their apparent differences in sites of action and/or substrates are unknown.

In a pioneering study of exogenous and endogenous factors controlling conidiogenesis in *G. candidum*, Park and Robinson⁹) demonstrated that vegetative growth is maintained under conditions of high nutrient levels of carbon and nitrogen sources but may be induced as these nutrients are depleted. The authors suggested that a certain endogenous condition is necessary for maintenance of "apical integrity" and polarized growth of vegetative hyphae. This endogenous condition is sustained by the availability of exogenous carbon and nitrogen sources. After several days of starvation, it was proposed that the endogenous condition may break down in some, but not all, of the hyphae, which then revert to the resting phase with production of arthrospores (holoarthric conidia). Another suggested possibility was that depletion of exogenous nutrients essential for vegetative growth leads to accumulation of a diffusible metabolite which induces sporulation to occur. The effects of differing nutrient levels of carbon and nitrogen sources on growth and differentiation of *G. candidum* have since been investigated using chemostat and batch culture techniques^{11,12,15~17}. Robinson and Smith (1979)¹⁵) compared hyphal growth of *G. candidum* on solid agar with that in chemostat culture. At high initial glucose concentrations in solid media, the marginal hyphae of the colony establish a steep concentration gradient in glucose from the "apices of the leader hyphae to the more proximal non-apical cells". The gradient steepens as apical branching occurs. Metabolites released by non-apical cells may participate in the inhibition of extension growth and stimulation of septation and conidium development. When grown either in steady-state glucose-limited chemostat culture or on solid media with low initial glucose concentrations, the cell dimensions and hyphal extension rates of *G. candidum* are similar¹⁶). It was suggested that similar nutrient concentration gradients exist under these two growth conditions. In both cases, an increase in temperature results in a decrease in hyphal diameter, cell length and cell volume. Aeration also plays a role in hyphal differentiation. Cultures grown on solid media reveal differentiated surface and submerged, marginal hyphae. While the former are broad, septate and branched, the latter

are unbranched, have longer cells and are narrow. Such differences are probably the result of competition for oxygen⁴³).

Park and Robinson⁹) proposed that the supply of carbon and nitrogen have separate effects on conidium formation: Time to sporulation is reduced when glucose only is present, whereas a nitrogen source alone increases the time to sporulation. When grown in both culture using simple, synthetic media with an initial glucose concentration of 0.5%, two different exponential growth phases are demonstrated^{11,12}). The first phase involves an increase in mycelial growth while the second, after only 15% of the original glucose and 30% of the ammonium have been consumed, involves conidium formation. At low initial glucose concentration in the growth medium (e.g. 0.1%), one exponential growth phase occurs. The fungus uses up all the exogenous glucose and conidia are then formed in stationary phase. The holoarthric conidia produced under these different growth conditions are morphologically and biochemically distinct (Table 4)¹⁷). At the higher initial glucose concentration (0.5%), short ellipsoidal conidia were predominant, while in media with a lower initial glucose concentration (0.1%), long, cylindrical conidia were formed^{11,17}).

Thin sections of *G. candidum* conidia reveal a fine, outer electron translucent wall layer which seems to be discontinuous (OW, Fig. 5). Wall material has also apparently sloughed off the surface of the cell (arrows in Fig. 5). Freeze-fractures of conidial walls show fascicles of short fibrils called 'rodlets' (Fig. 6) which have been observed on the surface of conidia produced by many imperfect fungi^{19,20,25}). The apparent discontinuity of the rodlet layer in Fig. 6 corresponds with the discontinuous outer wall (OW) shown in Fig. 5. The chemistry of rodlets of conidial fungi has been examined by several authors and identified as a crystallized protein^{44~46}) or a lipoprotein complex⁴⁷). When conidia of *Aspergillus niger* were treated with non-ionic detergents (e.g. Nonidet P-40), wall components were extracted without any significant structural changes to the rodlet layer⁴⁷). This suggests that the latter may function as a sieve through which macromolecular components of the inner wall layer may be leaked. Isolated rodlets are hydrophobic which suggests that they facilitate dissemination of propagules in moist or aquatic environments⁴⁸). In fact rodlets have been observed

Fig. 5. Thin section of the conidial wall of *G. candidum*. The outer wall layer (OW) is discontinuous and some wall material (arrows) has apparently sloughed off the cell. Mt, mitochondria; Pm, plasmalemma. $\times 61,200$.

Fig. 6. Freeze-fracture of the conidial wall of *G. candidum* showing fascicles of 'rodlets'. Arrows indicate regions of apparent discontinuity of the rodlet layer. $\times 111,000$. (after Cole, 1973).

Fig. 7. Isolated conidial walls of *G. candidum* examined with the SEM. $\times 3,240$.

Fig. 8. Isolated conidial walls of *G. candidum* obtained by cell homogenization showing high degree of purity. $\times 800$.

only on the wall surface of aerially grown cells and in the Fungi Imperfecti, only on conidia. Wessels *et al.*⁴⁹⁾, however, has reported rodlet fascicles on the hyphal wall of the *Basidiomycete*, *Schizophyl-*

lum commune and identified rodlet composition as primarily S-glucan. In *G. candidum*, rodlets have not been found on the hyphal wall surface. Only aerial chains of holoarthric conidia produced by

mycelia grown on solid agar media have been shown to have a rodlet layer which is exposed by freeze-fracturing. Direct replication of these cells does not expose the rodlet fascicles and not all surface fractures of aerial conidia reveal the rodlet layer. We suggest that the outermost wall material which is bound to and may have been released through the rodlet layer from inner wall layers (Fig. 5). This outermost layer affects the plane of fracture during freeze-fracturing so that only rare surface fractures occur over or between the rodlet fascicles. An outermost amorphous layer has been demonstrated on the conidial walls of *Trichophyton mentagrophytes*⁴⁵⁾, *Cladosporium macrocarpum* and *Aspergillus niger*²⁰⁾. Our knowledge of rodlet ultrastructure, chemical composition and function is still rather scanty. The apparent morphological similarity of rodlet fascicles on the surface of aerial propagules produced by members of all groups of septate fungi as well as filamentous bacteria⁵⁰⁾, suggests a common function. The degree of similarity in chemical composition of rodlets requires additional comparative investigations.

Mechanisms of conidiogenesis and the chemical composition of conidial and fertile hyphal walls are considered to be interrelated. On the basis of this premise, a comparison of the wall chemistry of various stages of fungal cell development should reveal certain trends which reflect relationships between alterations in wall composition and morphogenesis⁵¹⁾. Since *G. candidum* demonstrates a simple process of conidium ontogeny, this fungus is a good model for correlating such data. In this study, conidia were harvested from stationary phase cultures using media with an initially high carbon source nutrient level (30 g sucrose/liter). Extreme care was taken to isolate conidia only from the culture media. Our procedure of homogenization resulted in 95% cell rupture (Fig. 7, 8). Care was also taken to thoroughly wash the conidia before and after homogenization to remove media and cytoplasmic debris, respectively, from the wall surface. A summary of the chemical composition of the conidial wall is presented in Table 1. The values represent averages of data from four different wall preparations. No significant variations in data for the individual analyses or total recovery were found. The total carbohydrate content was high while total peptide and lipid contents were low. The percentage of

hexosamine in the conidial wall was also relatively high. Mannose was the predominant monosaccharide while galactose and glucose occurred in approximately equal proportions. Threonine and aspartic acid were the major amino acids (Table 2). Comparable percentages were determined for serine, valine and alanine on the one hand, and glutamic acid, proline and glycine on the other. The fatty acid composition of the readily extractable and bound lipids obtained from the conidial walls were examined by gas-liquid chromatography (Table 3). Oleic, linoleic and iso-palmitic acid occurred in the largest amounts. The concentration of palmitoleic acid was about two times greater in the bound than the readily extractable lipid form.

In Table 4, a comparison is presented between the composition of hyphal and holoarthric conidial walls produced by *T. mentagrophytes* and *G. candidum*. In each case, the ratio of total neutral carbohydrate/protein is high. A distinct similarity is also evident between the total hexosamine content of hyphal and conidial walls. Cole and Samson²⁶⁾ have suggested that such similarities in the composition of hyphal and holoarthric conidial walls is a reflection of the mode of conidiogenesis: holoarthric conidia are formed by simple fragmentation and involve little new wall synthesis. Their chemical composition, therefore, would be expected comparable to that of the hyphae from

Table 1. Summary of chemical composition of the conidial wall of *Geotrichum candidum*

Component	%
Carbohydrate	69.7
Mannose	(54.7) ^a
Galactose	(21.6)
Glucose	(23.7)
Hexosamine	12.2
Peptide	8.3
Lipid	
Readily extractable	
Lipid	0.5
Bound Lipid	0.8
RNA	1.7
DNA	—
	93.2

^a Monosaccharide content expressed as percentage of total carbohydrate.

which they formed. In contrast, the authors have pointed out that the chemical composition of "blastic" conidia (Kendrick, 1971)⁵²⁾, which arise by *de novo* growth and involve a considerable

amount of new wall synthesis, shows marked differences to the composition of corresponding hyphal walls. However, some differences do exist in the peptide, lipid and monosaccharide content between hyphal and holoarthric conidial walls which may be significant in morphogenesis. For example, Hashimoto *et al.*⁵³⁾ have reported marked decreases in peptide and lipid content of conidial walls compared to hyphal walls of *T. mentagro-*

Table 2. Amino acid composition of the conidial wall of *Geotrichum candidum*

Amino Acid	Mol % ^a
Asparatic Acid	15.0
Threonine	17.6
Serine	10.2
Glutamic Acid	7.1
Proline	7.6
Glycine	7.4
Alanine	8.7
Cystine	0.5
Valine	9.0
Methionine	n.d. ^b
Isoleucine	5.2
Leucine	3.0
Tyrosine	1.3
Phenylalanine	1.4
Histidine	1.2
Lysine	3.9
Arginine	0.9

a Based on maximum yield values after acid hydrolysis except tryptophan.
b Not detected.

Table 3. Fatty acid composition of the readily extractable and bound lipid components of the conidial wall of *Geotrichum candidum*

Fatty acid		Readily extractable lipid ^a	Bound lipid ^a
Lauric	C12:0	0.6	0.8
Myristic	C14:0	3.5	5.0
Pentadecylic	C15:0	2.1	3.1
isopPalmitic	iso-C16:0	18.7	26.1
Palmitoleic	C16:1	6.1	13.9
Stearic	C18:0	5.0	6.4
Oleic	C18:1	27.7	20.4
Linoleic	C18:2	27.3	13.0

a Values expressed as percentages of the total lipid in each fraction.

Table 4. Comparison of cell wall composition of vegetative hyphae and holoarthric conidia

Component	<i>Trichophyton^a mentagrophytes</i> (hyphae) %	<i>Trichophyton^b mentagrophytes</i> (holoarthric conidia) %	<i>Geotrichum^c candidum</i> (hyphae) %	<i>Geotrichum^d candidum</i> (holoarthric conidia) %
Total neutral carbohydrates	47.9	53.0	60.3 [56.8]	57.3 [50.6/51.2]
Mannose	(11.7) ^e	+	(5.0)	(7.0)
Galactose	tr ^f	+	(4.0)	(4.0)
Glucose	(36.2)	+	(86.0)	(83.0)
Hexosamine	30.4	30.0 ^g	9.7	8.0
Peptide	7.8	3.0	8.8 [5.0]	11.9 [6.7/7.5]
Lipid	6.6	1.0	5.3 [1.0]	5.8 [1.0/1.0]

a Data from Noguchi *et al.*, 1971.
b Data from Hashimoto *et al.*, 1979.
c Two sets of data for hyphal wall composition of *G. candidum* from Ebina *et al.*, 1978 (left column) and Kier *et al.*, 1980 (data enclosed by square brackets).
d Two sets of data reported for holoarthric conidia from Ebina *et al.*, 1978 (left column) and Kier *et al.*, 1980 (data enclosed by square brackets indicate composition of two kinds of holoarthric conidia produced under different growth conditionals; cylindrical conidia/ellipsoidal conidia).
e Monosaccharide content expressed as percentage of total carbohydrate.
f Trace.
g Expressed as chitin in original source.

phytes (Table 4). Similarly, our determination of the total lipid content of conidial walls of *G. candidum* (1.3%, Table 1) contrasts with the lipid composition of hyphal walls of this same fungus (Table 4) reported by Ebina *et al.*¹³⁾. Our data on monosaccharide composition of *G. candidum* conidial walls differs sharply from that reported by other authors^{13,17)}. One possible explanation for these differences could be the variation in original carbohydrate content of the media. While our initial sucrose concentration was 3%, the original concentration of glucose in the media used by Ebina *et al.*¹³⁾ was 1%, and the initial glucose concentrations used by Kier *et al.*¹⁷⁾ for growth of cylindrical and ellipsoidal conidia were 0.1% and 0.5%, respectively. The importance of carbon nutrient levels of the growth media to morphogenesis in *G. candidum* has long been recognized⁹⁾. Selective incorporation of monosaccharides into existing and newly synthesized walls may give rise to differences in structure and physical properties of cell walls which, in turn, may lead to different cellular morphologies⁵⁴⁾.

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