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Hyphal Wall Chemistry in *Apodachlya*

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Abstract. Hyphal walls were isolated from the Oomycete, *Apodachlya* sp. Microscope examination of wall preparations showed that they were clean and relatively free of cellulin granules. The principal wall constituents, accounting for more than half of wall weight, were β-glucans with 1,3- and 1,6-glucosidic linkages. Apparently chitin was the second most abundant wall constituent (18%) and cellulose accounted for less than 10% of wall weight. Protein was a significant wall constituent (6.4%), and protein hydrolysis demonstrated nearly all common amino acids plus hydroxyproline; additionally, the unusual amino acid, hydroxylysine was tentatively identified. The lipid and ash constituents were small (1.7% and 0.4%, respectively) and no particular significance was assigned to them. The possible occurrence of wall glycoproteins and the relationship between wall chemistry and systematics in *Apodachlya* and related genera were discussed.

Key words: Hyphal walls – Chemical composition – *Apodachlya* – β-Glucan – Chitin – Cellulose – Fungal systematics.

Cell walls function in the determination of form in both vegetative and reproductive stages of fungi, and wall chemistry has assumed an important role in assessing taxonomic and phylogentic relationships (Aronson, 1965; Bartnicki-Garcia, 1968). In various genera among the aquatic fungi (Chytridiomycetes, Hyphochytridiomycetes and Oomycetes) cell wall composition as well as other biochemical features have shown noteworthy correlations with taxonomic assignments based upon morphologic criteria (Aronson, 1965; Bartnicki-Garcia, 1968, 1970). In Oomycetes, one of the more extensively investigated fungal classes, hyphal walls are characterized by a preponderance of β-1,3- and β-1,6-linked glucans and comparatively small amounts of cellulose, a β-1,4-linked glucan (Bartnicki-Garcia, 1966; Aronson et al., 1967; Novaes-Ledieu et al., 1967; Sietsma et al., 1969). Although most fungi have chitin in their walls and lack cellulose (Aronson, 1965), earlier investigations purporting to have shown the presence of chitin in walls of some oomycete species, were not confirmed in later studies (Aronson, 1965). However, uniformity of wall composition among oomycete species was questioned when Lin and Aronson (1970) demonstrated with X-ray diffraction the presence of both cellulose and chitin in hyphal walls of *Apodachlya* sp. This unexpected finding (Lin and Aronson, 1970) followed the work of Sietsma et al. (1969) demonstrating typical glucan components in walls of *Apodachlya brachynema* (Hildeb.) Pringsh. Although *A. brachynema* walls contained 3.2% hexosamines, Sietsma et al. (1969) were of the opinion that chitin was not present. The inability of mycelia to induce chitinase in *Streptomyces* species sensitive to low levels of chitin in their culture medium was the basis for their view.

Because of the apparent difference between *A. brachynema* and the species we investigated, a comparison of the quantitative composition of their walls is of interest. The work reported here makes such a comparison possible and also reports the unusual composition of cell wall protein in *Apodachlya* sp. Furthermore, it indicates a possible use for cell wall chemistry in assessing systematic relationships among leptomitalean fungi.

MATERIALS AND METHODS

Culture Methods. Apodachlya sp. 47-17 was obtained from Dr. Ralph Emerson, University of California, Berkeley. Methods for culturing the fungus were described previously (Lee and Aronson, 1975). Mycelia were harvested after 8 days of cultivation so that cellulin (chitin-glucan) granules would not be numerous.

Isolation of Walls. All steps in the following procedure (except sonication) were carried out at 3–5° C. Mycelia were harvested and washed free of culture medium with 0.1 M Tris-HCl (pH 7.5). Fungal material was suspended in Tris buffer and homogenized with three 1-min treatments in a Sorvall Omni-Mixer operated at 16000 rpm. After centrifugation at 1500 *g*, the pellets were resuspended in buffer and centrifuged again. This resuspension and centrifugation was repeated six times. The final pellets, containing partially cleaned walls, were suspended in Tris buffer and sonicated for 15 min in a Raytheon DF-101 sonicator operated at maximum output current. Liquid coolant, circulating around the sonicator treatment chamber, maintained a sample temperature of approximately 10° C. After sonication, the material was centrifuged and resuspended in buffer six times as before. Pellets were resuspended in distilled water, dispersed in the Omni-Mixer for 1 min and centrifuged again; this procedure was repeated twelve times. Hyphal walls in the final pellet were clean judging by the apparent absence of any cytoplasmic material in KI_3 stained wall segments examined in a microscope. We estimated that cellulin granule contamination did not exceed 1% of dry weight. Wall preparations were freeze-dried and stored at −18° C in a desiccator.

Analytical Methods. Acid hydrolysis of isolated walls was accomplished employing four different methods. (1) Partial hydrolysis was carried out according to Aronson et al. (1967). (2) For quantitative determination of glucan constituents of wall polymers, 3 mg of walls were stirred in 0.3 ml of 27 N H_2SO_4 at 1° C until only a trace of insoluble material remained. The acid was diluted to 3 N and the mixtures were heated at 98° C for 5 h. The solutions were adjusted to pH 5–6 with 2 N NaOH and diluted to a known total volume. (3) For quantitative determination of amino acid constituents of wall protein, 20-mg samples were hydrolyzed in vacuo in 6 N HCl at 110° C for 20 h. (4) For quantitative determination of amino sugars and qualitative identification of monosaccharides, 15-mg wall samples were hydrolyzed in vacuo in 6 ml of 4 N HCl at 98° C for 8, 16 and 24 h. HCl was removed in a stream of dry N_2 at 40–45° C.

Hydrolysis with β-glucanases from *Penicillium brefeldianum* QM 1873 was done according to Pao and Aronson (1970).

Descending paper chromatography of sugars was carried out on Whatman No. 1 paper using the following solvent mixtures (all given as v/v): (a) n-butanol : pyridine : H_2O (6 : 4 : 3); (b) n-butanol : ethanol : H_2O (13 : 8 : 4); (c) n-propanol : ethyl acetate : H_2O (7 : 1 : 2); (d) n-butanol : pyridine : 0.1 N HCl (5 : 3 : 2). Irrigation times ranged from 24–48 h at 23° C. Alkaline $AgNO_3$ and ninhydrin were used to locate the sugars.

Preparative paper chromatography (solvent mixture c) of hydrolysis products prepared by method 4 (20 h hydrolysis) was used to collect a quantity of hexosamine for paper electrophoresis analysis. Hydrolyzates were applied as a band and, after a 30-h irrigation, longitudinal strips were sprayed with both $AgNO_3$ and ninhydrin to locate amino sugars which were then eluted with H_2O. Using a modification of the procedure of Maley and Maley (1959), paper electrophoresis of hexosamines was done at 20° C on Whatman No. 1 paper in 0.03 M $Na_2B_4O_7$ (pH 9.0) at a potential of 38 V · cm^{-1} for 2 h. Papers were rapidly passed through acidified methanol (95 ml absolute methanol + 5 ml of concentrated HCl) and dried to remove excess borate; amino sugars were detected with alkaline $AgNO_3$.

The following quantitative analyses of unhydrolyzed walls were made: total glucan with anthrone reagent (Ashwell, 1957); protein with Folin reagent (Lowry et al., 1951); free and bound lipids by extraction and gravimetric measurement (Bartnicki-Garcia and Nickerson, 1962); and total phosphorus with semidine (Dryer et al., 1957). Total nitrogen analyses were performed by Midwest Microlab, Inc., Indianapolis. Ash was analyzed gravimetrically following combustion of wall samples for 3 h at 850° C in an electric furnace.

Glucostat (Worthington Biochemical Corp.) was used for glucose analyses on hydrolyzates prepared by method 2. Total hexosamine in hydrolyzates prepared by method 4 was measured by the Elson-Morgan procedure as modified by Boas (1953). Amino acids in hydrolyzates prepared by method 3 were identified using a Beckman Model 120C automatic amino acid analyzer. To check the identities of certain amino acids, hydrolyzates (prepared by method 3) were applied to a column containing a 1 × 15 cm (approximate) height of Dowex-50 (H-form) resin. After passing 30 ml of H_2O through the column, amino acids eluted with 60 ml of 2 N HCl, followed by 100 ml of 4 N HCl were collected in 5-ml fractions. A crude diamino acid preparation, obtained by pooling fractions 18–24, was concentrated and HCl was removed as noted before. This material was examined by thin-layer chromatography (TLC) on cellulose MN-300 using three successive irrigations with isopropanol : formic acid : H_2O (20 : 1 : 5, v/v). Amino acids were detected with ninhydrin and isatin.

Cell wall components containing hexosamines were separated into alkali soluble, acid soluble, and insoluble fractions. A wall sample (20 mg) was treated with 2 ml of 1 N NH_4OH for 8 h at 23° C. The insoluble residue was washed with 1 ml of NH_4OH and then with 1 ml of H_2O. The extract and washes were combined and dried in a stream of N_2 at 40–45° C. The washed residue was treated twice for 15 min each with 2 ml of 1 N acetic acid at 98° C. After washing the insoluble residue with 1 ml of 1 N acetic acid (23° C), the extracts and wash were combined and dried. The insoluble residue was washed twice with 95% ethanol and dried. Each of these three fractions was hydrolyzed using method 4 for 16 h. Glucosamine, in each hydrolyzate was measured with a Beckman Model 120C automatic amino acid analyzer.

An estimation of the quantitity of cellulose in walls was made by treating samples (20–30 mg) with 2.5 ml of Schweitzer's reagent for 9 h at 23° C. After a second treatment with 1 ml of reagent for 3 h, the insoluble residue was washed with 1 ml of reagent. The extracts and wash were combined, cooled in ice water and acidified by dropwise addition of glacial acetic acid. The insoluble residue and the precipitated cellulose (regenerated) were washed once with 1 N acetic and twice with H_2O. Alkali soluble (noncellulosic) material, removed from the cellulose fraction by two extractions with 15 N NH_4OH, was added to the insoluble residue. The regenerated cellulose and residue were dried in vacuo at 55° C and then weighed.

Isolated walls were also fractionated with anhydrous ethylene diamine according to the procedure of Korn and Northcote (1960). Walls (1 g) were treated with 250 ml of reagent for 3 days at 37° C.

RESULTS

Hyphal Wall Isolation

Because of the similar composition of cell walls and cellulin granule inclusions (Lee and Aronson, 1975) produced by *Apodachlya* sp., it was necessary to prepare walls relatively free of cellulin as well as cytoplasmic contaminants. Since the number of cellulin granules increases with age of cultures, this problem was partially solved by using a short cultivation time (see "Materials and Methods"). Analytical data (Table 1) showing only small amounts of lipid and phosphorus in isolated walls precluded any significant contamination from intracellular membranes and nucleic acids. These observations, combined with microscope examination of wall preparations, demon-

Table 1. Chemical composition of *Apodachlya* cell walls

Component	% of dry weight
1. Glucan[a]	65.4
2. Glucan[b]	68.9
3. Alkali soluble hexosamine[c]	3.1
4. Acid soluble hexosamine[c]	1.5
5. Insoluble hexosamine (chitin)[c]	18.2
6. Protein	6.4
7. Readily extracted lipids	0.9
8. Bound lipids	0.8
9. Ash	0.4
10. Phosphate, as $(H_2PO_3)_n$	0.2
11. Total nitrogen	2.5
Sum of 2–9	100.2

[a] Determined (as glucose) with anthrone on unhydrolyzed walls; calculated as anhydroglucose.
[b] Determined in hydrolyzates with Glucostat and calculated as anhydroglucose.
[c] Based on the total (22.8%) anhydro-*N*-acetylglucosamine content (Table 3); soluble and insoluble hexosamine values were computed in relation to their respective proportions (Table 4).

strated that *Apodachlya* walls were sufficiently clean to permit satisfactory analyses of their composition.

Chemical Composition of Hyphal Walls

Carbohydrates. Examination of products derived from acid hydrolysis of *Apodachlya* walls consistently showed only two monosaccharides. One reacted with $AgNO_3$ and had chromatographic mobilities in all solvent mixtures tested that were the same as those of D-glucose (Table 6). The other sugar reacted with both $AgNO_3$ and ninhydrin and had a chromatographic mobility corresponding to that of D-glucosamine (Table 6). The apparent predominance of glucose on chromatograms was substantiated as a result of a direct anthrone analysis of walls indicating a glucose content of 65.4% (Table 1). With a complete hydrolysis procedure (method 2) designed to give maximum yields, we found a slightly higher glucose content. Moreover, the analysis of hydrolyzates employed a glucose oxidase (Glucostat) method and 68.9% of wall weight was accounted for specifically as D-glucose (Table 1). Thus, glucose polymers are the principal wall components, but they occur in significantly lower quantities in *Apodachlya* sp. as compared with other investigated Oomycetes (e.g. Bartnicki-Garcia, 1966; Cooper and Aronson, 1967; Novaes-Ledieu et al., 1967; Sietsma et al., 1969; Pao and Aronson, 1970).

Although the hexosamine found in wall hydrolyzates corresponded to D-glucosamine on paper chromatograms, the chromatographic procedures employed did not give complete resolution of glucosamine, mannosamine and galactosamine. Consequently, we used preparative paper chromatography to isolate amino sugars obtained by hydrolysis of walls. When we examined this amino sugar fraction by paper electrophoresis (Table 2), the compound was definitively identified as glucosamine and we found no evidence for the presence of other hexosamines. Hyphal walls of *Apodachlya* sp. are unusual as compared with those of other investigated Oomycetes because Lin and Aronson (1970) demonstrated that they contained chitin. Therefore, we made a detailed quantitative analysis and determined glucosamine solubility in dilute alkali and acid. Table 3 demonstrates that we determined optimum conditions for glucosamine analyses taking into account the destruction of the compound during various hydrolysis periods. Although most of the glucosamine was insoluble under the conditions used (Table 4), significant proportions were found in alkali and acid soluble fractions.

Table 2. Resolution of hexosamines by paper electrophoresis

Compound	Migration (cm) from origin to	
	Cathode	Anode
Glucosamine[a]	7.3	–
Galactosamine[a]	2.3	–
Mannosamine[a]	–	9.5
Apodachlya hexosamine[b]	7.2	–

[a] Authentic aminosugars.
[b] Isolated by preparative paper chromatography.

Table 3
Liberation and destruction of glucosamine during hydrolysis

Hours	% Glucosamine destroyed	% Glucosamine detected[a]	% Glucosamine corrected[b]	% *N*-acetylglucosamine calculated[c]
8	–	14.5	–	–
16	9.7	15.9	17.6	22.2
24	13.1	15.7	18.1	22.8

[a] Determined in hydrolyzates and calculated as anhydroglucosamine.
[b] Corrected for % destruction of authentic glucosamine during corresponding hydrolysis time.
[c] Calculated as anhydro-*N*-acetylglucosamine; for purposes of calculation, it was assumed that all glucosamine residues were *N*-acetylated but *N*-acetyl determinations were not made.

Protein. The significant quantity of protein (Table 1) found in *Apodachlya* walls falls within the range of

Table 4. Solubility of glucosamine containing components of *Apodachlya* walls

Fraction	% of recovered glucosamine[a]
1 N NH_4OH, 23° C	13.6
1 N Acetic acid, 98° C	6.5
Insoluble residue	79.9

[a] Total recovery (calculated as anhydro-*N*-acetylglucosamine) was 75.1% of the maximum value obtained from Table 3.

Table 5. Amino acid composition of protein associated with *Apodachlya* walls

Amino acid[a]	nmoles/20 mg of walls[b]	Residues/ 1000
Glycine	21.0	128
Alanine	19.3	117
Aspartic acid	15.6	95
Leucine	13.1	80
Glutamic acid	13.0	79
Lysine	12.9	78
Valine	11.4	69
Serine	11.2	68
Threonine	9.7	59
Isoleucine	9.0	55
Proline	8.0	49
Arginine	5.1	31
Phenylalanine	4.4	26
Histidine	3.1	19
Tyrosine	3.0	18
Hydroxylysine[c]	3.0	18
Hydroxyproline	0.8	5
Half-cystine	0.7	4
Methionine	0.5	3

[a] An unusually large quantity of NH_3 was assumed to have been derived from glucosamine decomposition (Kent and Whitehouse, 1955).
[b] The sum of determined amino acids was 6.5% of wall weight.
[c] Tentative identification.

values generally reported for other investigated Oomycetes (e.g. Novaes-Ledieu et al., 1967; Sietsma et al., 1969). Percent protein nitrogen (calculated from Table 1 assuming a protein-N content of 16%) and percent hexosamine nitrogen (based on 6.89% N per anhydro-GlcNAc residue) provided the sum of 2.6%, which is in good agreement with the independently measured value of total nitrogen (Table 1). In addition, the total weight of amino acids recovered in analyses of protein hydrolyzates (Table 5) accounted for approximately the same percentage of walls as did the direct (Lowry procedure) analysis.

Analyses of amino acid composition (Table 5) in isolated walls demonstrated the presence of nearly all commonly encountered protein constituents; the less prevalent amino acids, hydroxyproline and hydroxylysine, were found also. Hydroxyproline has been found in walls of other Oomycetes (Bartnicki-Garcia, 1966; Novaes-Ledieu et al., 1967; Pao and Aronson, 1970) but, to the best of our knowledge, hydroxylysine has not been found previously in the walls of any fungus, although minute quantities were reported to be present in extracts from whole cultivated mushrooms (Altamura et al., 1967). Although identification of this unusual amino acid was based mainly on amino acid analyzer retention times and must be regarded as tentative, the identity was corroborated by a TLC examination that resolved two major components in a crude diaminoacid fraction of wall hydrolyzates: lysine, R_f, 0.55 and hydroxylysine, R_f, 0.46 (R_f values of authentic L-lysine and DL-hydroxylysine, respectively were 0.56 and 0.46).

Other Constituents. Because carbohydrate and protein constituents comprised 98% of wall weight, the significance of other components was not ascertainable. Nevertheless, small quantities of readily extracted and bound lipids were found along with a minute ash content (Table 1). The latter was probably derived mainly from some unidentified phosphorus compounds.

Structure and Proportions of Wall Components

Glucans. Examination of the disaccharides liberated during partial acid hydrolysis of walls showed the presence of β-1,3-, β-1,4- and β-1,6-glucosidic linkages (Table 6). The β-1,4-linkage probably arose primarily from the cellulose component of the walls, since Lin and Aronson (1970) demonstrated the presence of this polysaccharide with X-ray diffraction analysis. We observed extensive lysis of walls treated with a crude β-glucanase preparation that had high exo-β-1,3-glucanase activity but apparently lacked β-1,4-glucanase (cellulase) activity. The nature of the β-1,3- and β-1,6-linked glucose components is unknown but they may constitute branched polymeric structures of the type found in the walls of the Oomycetes, *Phytophthora cinnamomi* (Zevenhuizen and Bartnicki-Garcia, 1969) and *Pythium acanthicum* (Sietsma et al., 1975). The proportion of β-1,3- and β-1,6-glucan in relation to cellulose could not be determined with great accuracy, but was estimated by extraction of a cellulose fraction with Schweitzer's reagent. In one analysis of a 24-mg wall sample, 16.4 mg and 1.7 mg, respectively, were recovered as noncellulosic residue and regenerated cellulose. The latter accounted for 7.1% of the initial wall sample and 9.4% of recovered material. These data indicated that cellulose was present in a relatively small quantity.

Table 6. Chromatographic mobilities of acid hydrolysis products from *Apodachlya* walls

Sugar[a]	Linkage	R_G[b]			
		Solvent b		Solvent c	
		Authentic	Hydrolyzate	Authentic	Hydrolyzate
Glucose	–	1.00	1.00	1.00	1.00
Glucosamine	–	0.59	0.59	0.82	0.82
Laminaribiose	β-1,3	0.52	0.52	0.67	0.66
Cellobiose	β-1,4	0.35	0.35	0.49	0.49
Gentiobiose	β-1,6	0.28	0.29	0.42	0.43

[a] Monosaccharides were detected in both complete and partial hydrolyzates and the identities were confirmed in solvents a and d; disaccharides identified in partial hydrolyzates only (method 1).
[b] Chromatographic mobility relative to D-glucose.

Glucosamine Polymers. Previous work (Lin and Aronson, 1970), providing a qualitative demonstration of chitin in *Apodachlya* walls, suggested that the chitin content was appreciable. Although there is uncertainty in measurements of chitin content, the solubility of hyphal wall glucosamine provided a basis for a satisfactory approximation. We regarded the insoluble glucosamine (nearly 80% of total; Table 4) as an indicator of cell wall chitin and calculated from this the 18.2% value appearing in Table 1. This may be an under-estimate of insoluble amino sugar since an unknown (although small) quantity of highly dispersed wall particles might have been carried over into soluble fractions. In spite of any assumptions or analytical errors, the apparent chitin content of *Apodachlya* walls is substantially greater than is the cellulose content. The insolubility of a major portion of the hexosamines was corroborated with the observation that approximately 95% of the recovered amino sugar was in the insoluble residue remaining after a 3-day treatment with anhydrous ethylene diamine.

The nature of the 1 N NH_4OH soluble hexosamine (3.1%; Table 1) is not known, but it may be indicative of the presence of cell wall glycoproteins (discussed below). Similarly, the 1 N acetic acid soluble fraction is of unknown structure. Although chitosan, a glucosamine polymer with little or no *N*-acetyl substitution, is soluble under these conditions, there are no data indicating the presence of this polysaccharide and, in any event, the quantity is small.

Proteins. We have not made detailed analyses on the hyphal wall protein of *Apodachlya* sp. but we can report some initial results that should be interpreted cautiously. A 3-day treatment of walls (1 g) with anhydrous ethylene diamine yielded three fractions: (1) 30 mg soluble in ethylene diamine and H_2O; (2) 46 mg ethylene diamine soluble, but H_2O insoluble; and (3) 743 mg ethylene diamine insoluble (residue).

Table 7. Composition of *Apodachlya* cell wall fractions produced by ethylene diamine treatment

Fraction	% of fraction		
	Protein[a]	Glucan[b]	Glucosamine[c]
1	55.1	16.4	20.9
2	76.2	2.8	4.4
3	2.2	67.2	24.7

[a] Measured by the Lowry method.
[b] Measured as glucose with anthrone.
[c] Determined in hydrolyzates using amino acid analyzer.

Although all fractions contained protein, glucan and glucosamine (Table 7), it is probable that they were heterogenous and the data permit few conclusions. Nevertheless, fraction 3 is of interest because it contained most of the cell wall chitin and glucan but only one-fourth to one-third of the wall protein. The hydroxylysine content of the protein in this fraction was three times higher than that of protein in unfractionated walls and indicated that hydroxylysine residues or peptide chains containing hydroxylysine are tightly bound to a polysaccharide component.

DISCUSSION

Sietsma et al. (1969) demonstrated that *Apodachlya brachynema* walls were composed of between 80% and 90% glucans containing β-1,3-, β-1,4- and β-1,6-linkages. Less than 10% of wall weight was accounted for as cellulose. Our work with a different *Apodachlya* species also demonstrates that glucans are the most abundant wall components, and that cellulose is a comparatively minor constituent. Although the two investigated species are similar, a major quantitative difference exists. Walls of *Apodachly* sp. contain an appreciable amount of hexosamine, most of which

we believe to be in the form of chitin, and there is a corresponding reduction in total glucan; *A. brachynema* walls had a hexosamine content of only 3.2% (Sietsma et al., 1969). While the methods used for acid hydrolysis of *A. brachynema* walls were different from ours, quantitative differences in hexosamine composition in the two investigations can not be attributed solely to different methodologies. Therefore, there is an unquestionable difference between investigated *Apodachlya* species as far as quantitative wall composition is concerned. This is an unexpected conclusion, since previous Oomycete investigations permitting comparison of wall composition in different species within a genus, have generally demonstrated striking similarities (Bartnicki-Garcia, 1966; Cooper and Aronson, 1967; Novaes-Ledieu et al., 1967). Although we found chitin to be a significant wall component in *Apodachlya* sp., Sietsma et al. (1969) were of the opinion that this polysaccharide was not present in *A. brachynema*. Their view was based upon the inability of mycelia to induce chitinase in Streptomycetes known to produce the enzyme when grown on culture media containing as little as 0.01% chitin. The sensitivity of these chitinase producing organisms was certainly adequate, but, as Lin and Aronson (1970) pointed out, if chitin was present in minute quantities in *A. brachynema*, it might have been ineffective as a chitinase inducer because of masking effects by other cell components, particularly the preponderant glucans. Admittedly, this speculation can end only when hexosamines in *A. brachynema* walls are reexamined.

Although hyphal walls of *Apodachlya* sp. do not contain an unusual amount of protein, they have what appears to be a novel amino acid composition. Hydroxyproline, an amino acid found in walls of other investigated Oomycetes, but not in chitinous walls of other investigated taxa (e.g., Bartnicki-Garcia, 1968), occurs in *Apodachlya* walls. This amino acid and chitin occur together in *Apodachlya*, demonstrating that they are not mutually exclusive wall constituents. A more surprising observation was the detection of a wall component tentatively identified as hydroxylysine. This hydroxylated amino acid has not been found previously in fungal walls, but was detected in walls of certain green algae (Thompson and Preston, 1967). Interestingly, the frequency of hydroxylysine residues in the walls of investigated algae and in *Apodachlya* walls is similar. The function of hydroxylated amino acids in fungal walls is unknown, but work with cell walls of green plants has demonstrated the occurrence of hydroxyproline arabinosides and provided evidence for the presence of serine galactosides also (Lamport, 1973). Lamport (1970, 1973) speculated on the role of these glycosides in cross-linking of cell wall polysaccharides and considered the possible function of such cross-links in the regulation of cell wall extensibility. It is premature to consider a function for hydroxylated amino acids in *Apodachlya* walls, but the strong binding observed between hydroxylysine and the polysaccharide components is not inconsistent with a concept of hydroxylysine glycosides within the wall fabric. However, the only characterized hydroxylysine glycosides occur in animal constituents such as glomerular basement membrane glycoproteins and collagens both of which contain significant amounts of hydroxylysine galactosides (Spiro, 1973). Thus, further investigations of wall protein in *Apodachlya* should prove interesting.

The results of our investigation have some significant implications in relation to fungal systematics also. Correlations between wall composition and fungal taxonomy have been made by a number of workers, the earliest of whom focused attention on the lower (aquatic) fungal taxa (see review by Aronson, 1965). More recently, Bartnicki-Garcia (1968) designed a comprehensive fungal classification system based upon dual combinations of the principal wall constituents. Accordingly, the Oomycetes were designated as a cellulose-glucan (β-1,3- and β-1,6-linked) group (Bartnicki-Garcia, 1968). With our work, establishing the presence of appreciable amounts of chitin in *Apodachlya* walls, it might be argued that the cellulose-glucan designation of Oomycetes is no longer tenable. On the other hand, one might conclude that the Oomycetes (certainly most of the investigated genera) can only be regarded as a cellulose-glucan group and that *Apodachlya* sp. represents an anomaly. In our view, the latter alternative is the only acceptable one, but it requires modification. *Apodachlya* sp. can not be regarded as anomalous since X-ray diffraction analyses of the related species, *Leptomitus lacteus* (Roth) Agardh., have shown that cell walls in this species contain chitin also (Aronson and Lin, unpublished observations). Since *Apodachlya*, *Leptomitus* and a third genus, *Apodachlyella*, are combined in the Family Leptomitaceae, it is conceivable that this Oomycete family is characterized by chitinous cell walls. The scope of these taxonomic considerations enlarges considerably upon taking into consideration the alliance of the Leptomitaceae and the Family Rhipidiaceae in the Order Leptomitales. In *Sapromyces*, the only studied rhipidiaceous genus, hyphal walls contained cellulose and other β-glucans but not chitin (Pao and Aronson, 1970). Should other rhipidiaceous genera be found lacking in chitin also, cell wall composition would provide a biochemical basis for distinguishing members of the two families. This could be a significant innovation because conventional approaches to systematics with this group of fungi has not been without problems, even for the most expert

aquatic fungus investigators (Emerson and Weston, 1967; references cited therein).

Except for this work, more recent investigations of Oomycete cell walls, particularly those employing X-ray methods, repeatedly failed to demonstrate any chitin. Consequently, claims by earlier workers that both cellulose and chitin occurred in some Oomycetes were discounted (see reviews of Aronson, 1965; Bartnicki-Garcia, 1968). However, the issue of the possible occurrence of chitin in the walls of other Oomycete genera has been revived by the recent work of Dietrich (1973). Working with seven oomycete species, she treated methanolic-NaOH insoluble frations with snail-gut enzymes and found that *N*-acetylglucosamine was released from all but one preparation. The digestion products were believed to have been produced by the action of a chitinase and Dietrich (1973) suggested that small quantities of chitin were present in the walls of *Achlya*, *Dictyuchus*, *Saprolegnia* and *Pythium* species. However, this work did not rule out the possible action of other enzymes in the snail digestive juice and their effect on some *N*-acetylglucosamine containing polymer other than chitin. Consequently, the presence of chitin in these other Oomycetes is still questionable.

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