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The Qualitative Analysis of the Cell Walls of Selected Species of Fungi

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Extensive studies have already been made on the chemistry, morphology and biosynthesis of bacterial cell walls. In view of their importance as pathogens both of plants and animals, it seems equally pertinent to make a study of the composition of the cell walls of fungi.

Much of the earlier work on fungal cell walls was concerned with attempts to detect chitin and cellulose by cytochemical techniques [see Foster & Webber (1960), and Fuller & Barshad (1960) for references]. In some investigations extraction procedures have been used, either to attempt selective removal of specific components (Garzuly-Janke, 1940*a, b*), or to prepare a basic cell-wall residue free from cytoplasmic contamination, which was then submitted to chemical analysis (Daoud & Ling, 1931; Norman & Peterson, 1932), X-ray analysis (Frey, 1950; Blank, 1953, 1954; Blank & Burke, 1954; Aronson & Preston, 1960; Fuller & Barshad, 1960; Winkler, Douglas & Pramer, 1960) or to electron microscopy (Aronson & Preston, 1960). X-ray diagrams and electron micrographs have been prepared of the residues left after sequential solubilization of various components from mechanically isolated cell walls of yeasts (Houwink, Kreger & Roelofsen, 1951; Houwink & Kreger, 1953; Kreger, 1954) and filamentous fungi (Kreger, 1954).

To gain more extensive information, hydrolysates of mechanically isolated cell walls have been prepared and examined by several authors from yeasts (Northcote & Horne, 1952; Roelofsen, 1953; Falcone & Nickerson, 1956; Eddy, 1958; Miller & Phaff, 1958; Shifrine & Phaff, 1958) and from filamentous fungi (Cummins & Harris, 1958; Aronson & Machlis, 1959). Hydrolysates of cell walls isolated by mild chemical and enzymic

cleaning procedures have also been examined (Rzucidlo, Stachow, Nowakowska & Kubica, 1958).

The following investigation of the hydrolysates of the mechanically isolated cell walls of selected species was undertaken to gain some idea of the types of components to be expected in a wide range of species.

MATERIALS AND METHODS

Total nitrogen. This was estimated by the micro-Kjeldahl procedure with $\text{Na}_2\text{SeO}_4 \cdot 10\text{H}_2\text{O}$ - $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ - HgSO_4 - K_2SO_4 (2:3:10:85, by wt.) as catalyst.

Fungi. These are listed in Table 1.

Media. For yeasts the medium of Kilkenny & Hinshelwood (1951) was used, with yeast extract (Difco; 3 g./l. of medium) as a source of vitamins and CaCO_3 (1.5 g./l.) as a buffer where required. For *Ustilago maydis* a glucose- $(\text{NH}_4)_2\text{SO}_4$ medium (Haskins, 1950; designed to give no crystal production) was used. For *Phytophthora cactorum* the complete medium of Hall (1959) was used. *Saprolegnia ferax* was grown in the following medium: glucose, 25 g., NH_4NO_3 , 1.6 g., K_2HPO_4 , 0.2 g., $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.16 g., DL-methionine, 25 mg., NaCl, 2.5 mg., together with the constituents of Reischer's (1951) inorganic basal medium (excluding K_2HPO_4 , KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), made up to 1 l. with water and the pH adjusted to 7.0. For *Nematospora gossypii* the following medium was used: glucose, 20 g., KH_2PO_4 , 1.0 g., $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g., asparagine, 5.0 g., trace-element soln. (Kilkenny & Hinshelwood, 1951), 1 ml., biotin, 5 μg ., thiamine, 100 μg ., inositol, 0.2 g., water to 1 l. For *Polystictus sanguineus* 2.5% (w/v) malt extract (Difco) was used. For all other fungi, the basal medium of Lilly & Barnett (1953) was employed, with 1 ml. of the solution of Kilkenny & Hinshelwood (1951) to supply trace elements, and for the growth of the strain of *Neurospora sitophila*, 0.25% of yeast extract (Difco) was included.

Culture conditions. The yeasts, *Ustilago maydis* (which grew in the yeast form) and *Nematospora gossypii* were grown in their respective media to prepare inocula. For *Chaetomium globosum*, a suspension of ascospores, and for

Aspergillus niger, *Penicillium notatum*, *Neurospora sitophila*, *Botrytis cinerea* and *Zygorhynchus vuilleminii*, a suspension of asexual spores, were used to prepare 24–30-hr. pregerminated cultures as inocula. For *Saprolegnia ferax* and *Phytophthora cactorum* pieces of mycelium were used to inoculate eight 700 ml. flasks (250 ml. of medium/flask, standing culture) and these were harvested after 17 and 20 days respectively. *Polystictus sanguineus* was grown in a Roux bottle for 16 days. All other fungi were grown in flasks on a reciprocating shaker (Townson and Mercer, Croydon, Surrey). Thus yeasts were grown in six 1 l. conical flasks (200 ml. of medium/flask) and shaken at 120 strokes/min. for about 45 hr. *Schizosaccharomyces octosporus* was grown for 70 hr. and *Nematospora gossypii* (150 ml. of medium/flask, shaking rate 140/min.) for 60 hr. Remaining fungi were grown in long-necked flasks (Laboratory Glassblowing Co. Ltd., High Wycombe, Bucks.) containing 150 ml. of medium, at 200 strokes/min. for 60 hr. Growth was at the following temperatures: *Saprolegnia ferax* and *Phytophthora cactorum*, 21°; *Botrytis cinerea* and *Zygorhynchus vuilleminii*, 23°; *Polystictus sanguineus*, 25°; other species were grown at 30°.

Preparation of cell-wall material. Washed yeast cells (8 g. wet wt.), or the same amount of mycelium dispersed in an overhead-drive MSE blender, were kept on ice and mixed with 12 ml. or 20 ml. respectively of ice-cold 0.1 M-NaCl solution. A portion (10 ml.) of the resulting suspension was then pipetted into each of two cooled Mickle cups containing 13.5 g. of Ballotini beads. For yeast cells no. 12 beads, and for mycelia no. 9 beads, were used (Kreger, 1954). Subsequent operations were carried out at 2°. Shaking in the Mickle disintegrator for 40–60 min. was necessary. The supernatant was separated, the beads were washed several times with M-sucrose solution, the washings added to the supernatant and the cell-wall fraction was recovered by centrifuging for 5–10 min. at 1400g. Whole cells or unbroken mycelial clumps formed a pellet beneath the cell-wall fraction. The wall fraction was easily loosened and separated by gentle shaking with small quantities of fresh wash fluid. The pellet was discarded. The suspension was washed three or four times in M-sucrose (0.5 M-sucrose for *Ustilago maydis*). Resuspension and centrifuging were repeated three or four times in 0.1 M-NaCl (adjusted to pH 7.9–8.0 with saturated NaHCO₃ soln.), and finally three or four times in water. At this stage the residual solid was resuspended in 5 ml. of 0.1 M-NaCl and shaken with 5 g. of beads in one cup of the Mickle disintegrator for 20–30 min., and the whole washing procedure was repeated. The cleaning process was followed under the light microscope. The final water washing of the *Botrytis cinerea* preparation was carried out at 30° to remove mucilage particles adhering to cell-wall fragments. The isolation procedure was judged complete when unbroken cells and other material staining intensely with methylene blue (1:10 000 soln.) had been removed. Preparations showed no free fat globules (tested with Sudan III). Baker's-yeast preparations had less than 0.1% of unbroken cells (Bürker haemocytometer cell, Hawksley and Sons Ltd., London, W. 1), and some had none at all (light or phase-contrast microscope). A preparation from the pressed-yeast cake contained 1.15% of N (on a dry-wt. basis), and from a young growing culture of the same strain a preparation containing 0.82% of N was obtained (cf. Eddy, 1958). *Sporobolomyces roseus* and *Penicillium notatum* cell walls

under the electron microscope showed no evidence of cytoplasmic particles.

A yield of cell walls (freeze-dried), equivalent to 1–2% of the wet wt. of material used, was usually obtained. This material, or in a few cases the wet pellet from 6 g. of blended mycelium, was used as described below.

Hydrolysis of cell walls. This was done in sealed tubes. For carbohydrates, 12–13 mg. of cell wall (one-quarter of the wet pellet) was heated in 3 ml. of formic acid (90%, w/w) for 15 hr. at 100°. Formic acid was removed *in vacuo*, formyl esters were removed by heating in 2 ml. of N-H₂SO₄ for 1–2 hr. at 100° and the mixture was neutralized with Ba(OH)₂. For amino acids, 30–33 mg. of cell wall (or three-quarters of the wet pellet) were heated in 6 ml. of 6N-HCl for 18 hr. at 110°; HCl was removed and the residue taken up in 3 ml. of water. A portion (1 ml.) of the resulting solution was treated to remove chromogens (Boas, 1953) and then used for amino sugar chromatography, and the remaining portion (2 ml.), after oxidation with ammonium molybdate and H₂O₂, was used for amino acid chromatography.

Paper chromatography and electrophoresis

Whatman papers nos. 1, 3 and 4 were used. Solvent compositions are in proportions by vol.

Carbohydrates. Two-way chromatograms were run in phenol-water (80:20) followed by butan-1-ol-acetic acid-water (120:30:50). One-way chromatography was in propan-1-ol-ethyl acetate-water (7:1:2), and for uronic acids only, pyridine-ethyl acetate-acetic acid-water (5:5:1:3). Electrophoresis (up to 60v/cm.) was in 0.05 M-sodium tetraborate, or, for loads up to 1 mg., saturated sodium tetraborate soln. was used. Detection methods: for reducing sugars, alkaline AgNO₃ (Trevelyan, Procter & Harrison, 1950), for aldoses, aniline hydrogen phthalate (Cummins & Harris, 1956); for terminal deoxy sugars, Edwards & Waldron (1952); for polyols, Baddiley, Buchanan, Handschumacher & Prescott (1956); for uronic acids, Abdel-Akher & Smith (1951). Loads up to 1.2 mg. of cell wall were used per chromatogram.

Amino acids. Two-way chromatograms were run in butan-1-ol-acetic acid-water (100:22:50), followed by phenol-water (80:20) in the presence of KCN and NH₃. Electrophoresis, when carried out, was by the method of Harris & Warren (1954). Detecting reagents were 0.2% (w/v) of ninhydrin in acetone and 0.2% (w/v) of isatin in 4% (v/v) acetic acid in acetone, the latter being followed by Ehrlich's reagent if hydroxyproline was suspected. For electrophoresis, ninhydrin reagent containing acetic acid and the Pauly reagents were used (see Smith, 1960, for details of reagents). Loads of 5–7 mg. of cell wall were applied.

Amino sugars. Pyridine-butan-1-ol-water (4:6:3) was used as solvent, and alkaline AgNO₃ and the Elson & Morgan reagents (Partridge, 1948) were the detecting reagents. Loads of 2.5–5.0 mg. of cell wall were used.

RESULTS

Neutral and amino sugars

The results are compiled in Table 1 and the approximate relative amounts of substances seen on chromatograms are scored. Estimating from

Table 1. Neutral and amino sugars of some fungal cell walls

The size of spots on chromatograms, judged by eye, varied from + + + + + (maximum size) to a trace (tr., just visible). -, Absent. NCYC, National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, Surrey; CMI, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey; CBS, Centraalbureau voor Schimmelcultures, Baarn, Holland; FPRL, Forest Products Research Laboratory, Princes Risborough, Bucks.; LSHTM, London School of Hygiene and Tropical Medicine.

Species	Glucose	Galactose	Mannose	Ribose	Xylose	Fucose	Glucos- amine	Galactos- amine	Source
Phycomycetes									
<i>Phytophthora cactorum</i>	+ + + + +	-	+	+	-	-	+	-	CMI 62471
<i>Saprolegnia ferax</i>	+ + + + +	-	tr.	+	-	-	+	-	Dr B. C. Parker, Botany Dept., University College London
<i>Zygorhynchus vuilleminii</i>	-	+	+	-	-	+	+	-	CMI 21994
Ascomycetes									
(a) Yeasts									
<i>Saccharomyces cerevisiae</i>	+ + + + +	-	+	-	-	-	tr.	-	Baker's yeast (The Distillers Co. Ltd.)
<i>Candida utilis</i>	+ + + + +	-	+	-	-	-	tr.	-	NCYC 359
<i>Sporobolomyces roseus</i>	+ +	+	+	-	-	+	+	-	NCYC 424
<i>Rhodotorula aurantiaca</i>	+	+	+	-	-	+	+	-	NCYC 323
<i>Schizosaccharomyces octosporus</i>	+ + + + +	-	tr.	-	-	-	-	-	NCYC 131
<i>Nematospora gossypii</i> *	+ + + + +	-	+	-	-	-	-	-	CMI 31268
(b) Filamentous forms (inc. Fungi Imperfecti)									
<i>Chaetomium globosum</i>	+ + + + +	+	+	-	-	-	+	-	Botany Dept., Birkbeck College, University of London
<i>Neurospora sitophila</i>	+ + + + +	+	+	-	-	-	+	+	CMI 21944
<i>Aspergillus niger</i>	+ + + + +	+	+	-	-	-	+	+	Botany Dept., Birkbeck College, University of London
<i>Penicillium notatum</i>	+ + + + +	+	+	-	-	-	+	-	LSHTM/BB 102
<i>Botrytis cinerea</i>	+ + + + +	+	+	-	-	-	+	+	CMI 52058
Basidiomycetes									
<i>Ustilago maydis</i>	+ + +	+	tr.	-	-	-	+	-	CBS/NRRL 2321
<i>Polystictus sanguineus</i>	+ + +	-	+	-	+	+	+	-	FPRL/150A

* This species, which is no longer regarded as yeast, is included here for convenience in presenting the data.

spot sizes, these constituents would account for most of the cell wall in all species examined. Six neutral sugars, glucose, galactose, mannose, ribose, xylose and fucose, and two amino sugars, glucosamine and galactosamine, have been detected. Polyols and uronic acids were not found. The occurrence of ribose in two species is probably not due to contamination by RNA, since Dr B. C. Parker has found ribose present in the cell-wall polysaccharides of *Saprolegnia ferax*, remaining after extraction of the mycelium with 24% (w/v) KOH at room temperature for two periods of 4 hr. (personal communication, Dr B. C. Parker). Absence of any trace of ribose on sugar chromatograms of other species indicated freedom of cell-wall preparations from cytoplasmic contamination.

Traces of material in the oligosaccharide region could have been due to oligosaccharides, higher sugars or uronic acids. However, uronic acids could not be detected after special search, in the six species *Phytophthora cactorum*, *Saprolegnia ferax*, *Polystictus sanguineus*, *Aspergillus niger*, *Neurospora sitophila* and *Ustilago maydis*. Four spots in the oligosaccharide region of *Penicillium notatum* were shown by elution and hydrolysis to be oligosaccharides.

Hexosamines were detected in all species except *Schizosaccharomyces octosporus* and *Nematospora*

gossypii. With regard to the results for amino sugars, it is important to emphasize that these approximate proportions are those seen after drastic hydrolysis (6N-HCl/110°/18 hr.), which would probably cause 30–40% destruction of any amino sugar not combined in chitin (cf. Boas, 1953).

Amino acids

From the intensity of colour and size of spot produced by known amounts of amino acids, it appeared that most chromatograms carried about 150–300 µg. of amino acids (2–5% of the cell wall on a dry-wt. basis). The results are summarized in Table 2.

In most species, the amino acids commonly found in protein hydrolysates were observed. In *Schizosaccharomyces octosporus* only traces of amino acids were evident. *Saprolegnia ferax* and *Phytophthora cactorum* contained hydroxyproline in addition to the other amino acids. *Phytophthora cactorum* also contained two unidentified amino acids giving purple colours with ninhydrin and having R_{alanoine} values in phenol-water of 1.40 and 1.03 and in butan-1-ol-acetic acid-water of 1.30 and 0.76. In the yeasts *Sporobolomyces roseus* and *Rhodotorula aurantiaca*, evidence was obtained for the occurrence of γ -aminobutyric acid. Thus on

Table 2. *Amino acids of some fungal cell walls*

Slight variations of certain amino acids from the average relative amounts shown are not included. Maximum, intermediate and minimum amounts of amino acids are given by + + +, + + and + respectively. –, Absent; + ?, possibly present.

Species	Asp, Glu, Ser, Gly, Ala	Leu, Ileu, Pro, Val, Lys, Arg, Thr	Cys, Tyr, Phe, His	Hypro	Met	γ -Amino- butyric acid
Phycomycetes						
<i>Phytophthora cactorum</i> *	+ + +	+ +	+	+	–	–
<i>Saprolegnia ferax</i>	+ + +	+ +	+	+	–	–
<i>Zygorhynchus vuilleminii</i>	+ + +	+ +	+	–	+ ?	–
Ascomycetes						
(a) Yeasts						
<i>Saccharomyces cerevisiae</i>	+ + +	+ +	+	–	+	–
<i>Candida utilis</i>	+ + +	+ +	+	–	–	–
<i>Sporobolomyces roseus</i>	+ +	+	+ (Tyr –)	–	–	+
<i>Rhodotorula aurantiaca</i>	+ + +	+ +	+	–	–	+
<i>Schizosaccharomyces octosporus</i>	+	(Arg + ?)	–	–	–	–
<i>Nematospora gossypii</i>	+ + + (Gly + + +)	+ +	+	–	–	–
(b) Filamentous forms (inc. Fungi Imperfecti)						
<i>Chaetomium globosum</i>	+ + +	+ +	+	–	+ ?	–
<i>Neurospora sitophila</i>	+ + +	+ +	+	–	–	–
<i>Aspergillus niger</i>	+ + +	+ +	+	–	–	–
<i>Penicillium notatum</i>	+ + +	+ +	+	–	–	–
<i>Botrytis cinerea</i>	+ + + (Ala + + +)	+ +	+	–	–	–
Basidiomycetes						
<i>Ustilago maydis</i>	+ + +	+ +	+	–	–	–
<i>Polystictus sanguineus</i>	+ + +	+ +	+	–	–	–

* Two additional unidentified amino acids.

two-way chromatograms, whether or not the hydrolysate was oxidized with molybdate- H_2O_2 , a spot running in the correct position for γ -aminobutyric acid was observed. Cochromatography of the hydrolysate with authentic material intensified the spot, and electrophoresis gave a band between lysine and histidine, a position also occupied by authentic γ -aminobutyric acid. Chromatography of a 70% (v/v) ethanol extract of 20 mg. of *Sporobolomyces roseus* cell wall showed no ninhydrin-positive material to be initially present. Hence γ -aminobutyric acid is probably a structural component of the walls of these yeasts.

Formic acid-insoluble residue

After the formic acid hydrolyses, small insoluble residues were obtained from all the Basidiomycetes, Phycomycetes and filamentous Ascomycetes examined and from the yeast *Sporobolomyces roseus*. From this species and from *Polystictus sanguineus*, *Saprolegnia ferax* and *Phytophthora cactorum* this fraction was removed by centrifuging and washed with water. After heating for 22 hr. at 100° in 5-6N-HCl, residues still remained from *Saprolegnia ferax* and *Phytophthora cactorum*. Paper chromatography of the HCl-soluble portions of the residues from the four species showed the presence of glucosamine as the main component from *Sporobolomyces roseus* and *Polystictus sanguineus*, and glucose and oligosaccharides from the residues of *Saprolegnia ferax* and *Phytophthora cactorum*. Glucosamine was completely absent from the residues of the two last-named species. Further, the formic acid-insoluble residues of these last two species were soluble in freshly prepared cuprammonium hydroxide reagent and were reprecipitated on acidification. These results strongly suggest the presence of chitin in the cell walls of *Sporobolomyces roseus* and *Polystictus sanguineus*, and of cellulose in the walls of *Saprolegnia ferax* and *Phytophthora cactorum*.

DISCUSSION

It is clear at this stage of our knowledge of fungal cell walls that the term 'cell wall', as applied to the resultant of a given isolation procedure, cannot sensibly be equated with the functional cell wall of the organism from which it was isolated. For purposes of the present study, 'cell-wall material' was defined as all components remaining after mechanical rupture of the wall and vigorous washing with dilute aqueous solutions of a pH near 7.0. Much material here regarded as 'cell wall' would have been removed by exhaustive chemical cleaning such as that used by Norman & Peterson (1932), Frey (1950), Blank (1953, 1954), Blank & Burke (1954) and by Aronson & Preston (1960).

Cytochemical and X-ray techniques, which have been used to search for polymers such as chitin, cellulose or (X-rays only) yeast glucan in fungal cell walls, suffer from the limitation that they are able to detect only those components which have already been isolated and studied in a pure form. Further, selective extraction procedures that have been used to study specific components such as mannans (Garzuly-Janke, 1940b; Kreger, 1954) may not be rigorously applicable to all polysaccharides of this class. We decided to begin with a detailed qualitative analysis of the monomer units involved in the wall complex, to obtain guidance in any subsequent attempt to fractionate the complex and study its components.

The range of components found (neutral sugars, amino sugars and amino acids) suggests the possibility of the existence of protein-carbohydrate complexes in a wide range of fungal cell walls. Other studies (Kessler & Nickerson, 1959; Korn & Northcote, 1960) have demonstrated these in yeast cell walls. Bacterial and actinomycete cell walls are known to contain a similar range of components. Glucose, galactose, mannose, rhamnose and arabinose (Cummins, 1956), heptoses (Salton, 1960), ribitol (Baddiley, Buchanan & Carss, 1958) and glucuronic acid (Janczura, Perkins & Rogers, 1961) are known to occur in bacterial cell walls. Actinomycetes have cell walls resembling those of bacteria (Cummins & Harris, 1958), but, although polyols, heptoses and uronic acids are not yet known to occur in them, *Actinomyces bovis* cell wall has recently been shown to contain three 6-deoxy sugars, namely fucose, rhamnose and 6-deoxy-L-talose (MacLennan, 1961). Fucose occurred in large amounts in the cell wall of *Zygorhynchus vuilleminii*. It is also present in the cell walls of brown algae (Mori, 1953).

The amino acid patterns found were those of protein hydrolysates, and the limited amino acid content of bacterial cell-wall mucoprotein and of actinomycete cell walls was not observed in any species. However, the probable occurrence of γ -aminobutyric acid in the cell walls of the two yeasts *Sporobolomyces roseus* and *Rhodotorula aurantiaca* parallels the finding of ornithine as a structural component of the mucus of the marine snail *Busyon canaliculatum* (Shashova & Kwart, 1959). Further, the presence of a non- α -amino acid must lead to interesting structural features in these cell walls.

The absence of glucosamine in the cell walls of *Schizosaccharomyces octosporus* and *Nematospora gossypii* is consistent with earlier conclusions based on cytochemical studies (Roelofsen & Hoette, 1951; Nabel, 1939), in which chitin could not be detected in these two species. However, glucosamine has been detected in the cell walls of *Rhodotorula aurantiaca*, *Ustilago maydis* and *Sac-*

charomyces cerevisiae; but Frey (1950), using X-ray methods on chemically cleaned walls, reported that chitin was absent in *Rhodotorula rubra*, *Ustilago violacea* and *Saccharomyces cerevisiae*, although Nabel (1939), using cytochemical methods, had already reported *Ustilago zeae* (*U. maydis*) to contain chitin. Both Frey (1950) and Kreger (1954) have produced X-ray evidence for chitin in the cell wall of *Sporobolomyces roseus*, in which we have found glucosamine to be abundant. Glucosamine has also been found in cell walls of *Saprolegnia ferax* and *Phytophthora cactorum*, although, according to the surveys by Wettstein (1921), Nabel (1939) and Frey (1950), chitin is absent from these genera. Thus the glucosamine in the cell wall is not necessarily involved in the structure as chitin. Some evidence of this has been obtained (Korn & Northcote, 1960). The finding of galactosamine in the cell walls of *Aspergillus niger*, *Neurospora sitophila* and *Botrytis cinerea* is interesting in view of the discovery of this amino sugar in the acid-washed residue of mycelia of several fungi including *Aspergillus niger*, *Neurospora crassa* and *Neurospora tetrasperma* (Distler & Roseman, 1960). Muramic acid was not detected.

Reports claiming the absence of mannan in the cell walls of *Sporobolomyces roseus* (Kreger, 1954) and *Rhodotorula* sp. (Garzuly-Janke, 1940a; Kreger, 1954) are suspect, since mannose was apparently the major building unit of the cell walls in the two species studied in this work. The finding that the typical insoluble copper complex is not given by a mannan-protein (Cifonelli & Smith, 1955) or by a glucomannan-protein (Kessler & Nickerson, 1959) may explain the results of Kreger and of Garzuly-Janke. In addition, there is no guarantee that all mannans will give insoluble copper complexes.

SUMMARY

1. The mechanically isolated cell walls from 16 species of fungi have been hydrolysed and examined for their qualitative composition.

2. A range of six neutral sugars (glucose, galactose, mannose, fucose, xylose and ribose) and two amino sugars (glucosamine and galactosamine) have been found. Polyols and uronic acids were not detected.

3. The amino acids found were those typical of protein hydrolysates. In addition to these, however, two species of Phycomycetes, *Saprolegnia ferax* and *Phytophthora cactorum*, contained hydroxyproline, and two yeasts, *Sporobolomyces roseus* and *Rhodotorula aurantiaca*, contained γ -aminobutyric acid.

4. The occurrence of neutral sugars, hexosamines and amino acids suggests that the walls are of a protein-carbohydrate nature.

5. The definition of 'cell-wall' material used in this study is discussed together with the usefulness of cytochemical, X-ray and selective-extraction techniques used in work on fungal cell walls. The results are discussed in relation to the type of constituents found in the cell walls of some other organisms.

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Biochem. J. (1962) **83**, 331

Almond-Emulsin β -D-Glucosidase and β -D-Galactosidase

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The existence of different types of β -glucosidase and β -galactosidase with variations in C-4 specificity has clearly emerged from investigations of many sources by various authors.

Horikoshi (1942) investigated some preparations in which only one of these activities could be detected. A β -glucosidase from the sorghum group of grasses and the β -galactosidase from pig spleen were each inhibited by derivatives of the corresponding hexose only, but the β -glucosidase of *Proteus vulgaris* and the β -galactosidase of *Escherichia coli* were each inhibited by derivatives of both hexoses. Cohn & Monod (1951) reported an induced bacterial β -galactosidase with no activity on β -glucoside and no inhibition by glucose. Duerksen & Halvorson (1958) found an induced β -glucosidase in yeast with no β -galactosidase activity or inhibition by galactose.

Several preparations containing both activities have been studied. Miwa (1939) changed the ratio of β -glucosidase activity to β -galactosidase activity of Taka-diastase by adsorption, heat inactivation and solvent precipitation; Ezaki (1940) and Horikoshi (1942) showed that each was inhibited only by derivatives of the corresponding hexose. Dahlqvist (1961) failed to separate the activities of pig intestinal mucosa by chromatography or heat inactivation and concluded that they were associ-

ated with a single active protein. Conchie & Levvy (1957) showed that the activities in a preparation from rumen micro-organisms were each inhibited only by the corresponding hexonolactone, whereas those in limpet visceral hump were inhibited by both glucono- and galactono-1 $\rightarrow$ 4-lactone.

Previous studies of fractionation and inhibition of β -glucosidase and β -galactosidase in sweet-almond emulsin have led to conflicting results (see Discussion section). This paper describes a study of the inhibition of emulsin β -glucosidase and β -galactosidase by glucose, galactose, glucono- and galactono-1 $\rightarrow$ 4-lactone and of the competition between substrates of each kind. A preliminary account of part of this work has been published (Heyworth & Walker, 1961).

MATERIALS AND METHODS

Substrates. *p*-Nitrophenyl β -D-glucoside was supplied by California Corp. for Biochemical Research.

p-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl- β -D-galactoside was prepared by condensing 2.7 g. of 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl bromide (Ohle, Marecek & Bourjau, 1929) with 1.1 g. of *p*-nitrophenol in 38 ml. of acetone and 7.9 ml. of *N*-sodium hydroxide. After 24 hr. the mixture was extracted with 75 ml. of chloroform, washed with sodium hydroxide and water, and dried over anhydrous magnesium sulphate. The syrup obtained on evaporation