Extracellular Polysaccharides of some Basidiomycetes

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Abstract

Culture filtrates of four basidiomycete fungi, Stereum strigoso-zonatum, Fomes australis, Trametes lilacinogilba and Polyporus tumulosus were fractionated and examined for polysaccharide content. Acid hydrolysis showed the presence of galactose, mannose, xylose, fucose and glucose. Their relative amounts were estimated by gas chromatography of the corresponding alditol acetates. Galactose and mannose were the major constituent sugars, amounting to more than 50% of the total. One of the polysaccharides, a fucogalactomannan elaborated by P. tumulosus, was isolated in a purified form. It was shown to have [α]D 41 +41° and contained galactose, mannose, fucose and xylose in the relative proportions 2 : 1 : 1 : 0.2.

Introduction

A close correlation has been recognized between the chemical composition of cell wall polysaccharides of yeasts and fungi and the main taxonomic groupings established on the basis of morphology. A study dealing with this subject was carried out on 23 species of various Basidiomycetes and Ascomycetes by O'Brien and Ralph (1966). Attempts to relate the structure of extracellular polysaccharides to species differences and immunological properties of yeasts has also been the subject of a number of investigations. The studies of Gorin and Spencer (1968) on the extracellular polysaccharides of yeasts of the Rhodotorula, Candida and Cryptococcus species have demonstrated that such a correlation does exist. Similar comparative studies on fungal extracellular polysaccharides are limited. The present work investigates the types of polysaccharides elaborated by some basidiomycete fungi. The culture filtrates of four Basidiomycetes were examined, three of which are members of the family Polyporaceae and one belonging to the Stereaceae.

Materials and Methods

Organisms and Growth Conditions

Cultures of Polyporus tumulosus (Cooke) Massee, Fomes australis (Fr.) Cooke, Trametes lilacinogilba (Berk.) Lloyd and Stereum strigoso-zonatum (Sw.) G. H. Cunn. were obtained from the culture collection of the Botany Department, University of New South Wales, Sydney, and were maintained on agar slopes. Mycelial slips, obtained from 14-day-old agar slopes, were inoculated into Roux culture bottles containing 1 litre of modified William-Saunders medium (Ralph and Robertson 1950). The cultures were maintained at 27°C for 8 weeks after which the mycelial mats were removed by filtration through cheesecloth and washed with distilled water (4×50 ml each). The combined culture filtrates were clarified by filtration through sintered glass and lyophilized in 1-litre lots.
Fractionation of the Polysaccharides

The lyophilized culture filtrates were blended with a few drops of ethanol to give a thick paste. Water was then added dropwise to make a 2% aqueous solution of the total solids. The insoluble residue was collected by centrifugation at 2000 g for 20 min, washed first with 75%, then with 95% ethanol, and dried in a vacuum pistol to constant weight to give fraction A. Fraction B was obtained by precipitating the polysaccharides with ethanol from the supernatant. The ethanol was added dropwise with mechanical stirring until permanent turbidity appeared and then faster, to a final 75% ethanol concentration. The mixture was stirred for another 20 min, allowed to stand overnight, and the precipitate removed by centrifugation at 2000 g for 20 min. It was washed successively with 75% and 95% ethanol and dried. The washings combined with the supernatant from fraction B were concentrated to a small volume (10–15 ml) on a rotary film evaporator under reduced pressure at 35°C and subsequently freeze-dried, giving fraction C.

Analytical Techniques

Dry weights were determined in triplicates as weight loss at 105°C in vacuo over P₂O₅ to constant weight. Optical rotations were measured at room temperature (20°C) on a Bellingham and Stanley model A polarimeter in concentrations of 0·5–1% (w/v). For sedimentation analysis, aqueous solutions of polysaccharides (0·5–1%) were examined at 20°C in a Spinco model E analytical ultracentrifuge at 60,000 g for 60 min.

Hydrolysis. Acid hydrolysates and paper chromatography were carried out as previously described (Angyal et al. 1974). For quantitative analyses the sugars were converted to their corresponding alditol acetates and estimated by gas chromatography. Acid hydrolysates, containing about 25 mg/ml of sugars, were reduced with sodium borohydride at 5°C and the alditols acetylated without the removal of inorganic ions. This was carried out by heating the residue in a mixture of dry pyridine and acetic anhydride (1:1) at 100°C for 1 h. The solution so obtained was injected directly onto a column for gas chromatography, or, alternatively it was extracted with chloroform and the chloroform solution washed (1 ml NaHCO₃), dried (Na₂SO₄), and concentrated prior to injection onto the column. No differences were shown by the two methods for alditol acetates on gas chromatography.

The final concentration of alditol acetates in these solutions was 50 mg/ml. Gas chromatography was carried out on a Beckman GC4 gas chromatograph equipped with a temperature programmer and hydrogen flame ionization detector. N₂ was the carrier gas. Glass columns (0·3 by 170 cm) were packed with 1·5% diethylene glycol adipate on Chromosorb W. The temperature program was 150°C for 10 min, then to 200°C over 3 min and then kept at 200°C until completion of each run. The proportion of each individual alditol acetate in the mixtures was determined relative to internal inositol and xylitol acetates. Measurement of the areas under the peaks compared to the areas given by standards gave the amounts of components in the hydrolysates. A correction, obtained by analysis of synthetic mixtures under the same conditions, was applied for the relative response of alditol acetates. However, corrections for the losses occurring during acid hydrolysis were not applied and alditol acetates were expressed in proportion to xylitol acetate.

<table>
<thead>
<tr>
<th>Table 1. Sugar composition of lyophilized culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>S. strigoso-zonatum</td>
</tr>
<tr>
<td>F. australis</td>
</tr>
<tr>
<td>T. lilacinigilba</td>
</tr>
<tr>
<td>P. tumulosus</td>
</tr>
</tbody>
</table>

Results and Discussion

The culture media of four Basidiomycetes were filtered and lyophilized and the sugar composition of the acid hydrolysates examined by paper chromatography. In all four species mannose and galactose were the main constituents and were
present in varying ratios whilst fucose and xylose were present in smaller amounts. No glucose was found in the hydrolysates of the polysaccharides from the culture medium of *F. australis* although most *Fomes* spp. examined by O'Brien and Ralph (1966) contain approximately 50% glucose in their cell wall. It was also interesting to find galactose in abundance in hydrolysates of polysaccharides from the culture media of these fungi, when none was found in their cell wall polysaccharides (O'Brien and Ralph 1966; Angyal et al. 1974). Table 1 shows the sugar composition of the hydrolysed polysaccharides as obtained from the relative intensity of spots on paper chromatograms.

**Table 2. Fractionation of lyophilized culture media**

Yields expressed as grams per litre of lyophilized media. Values in parentheses are percentages of total yield.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total yield (g)</th>
<th>Yield (g) of:</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fraction A</td>
<td>Fraction B</td>
</tr>
<tr>
<td><em>S. strigoso-zonatum</em></td>
<td>4.03</td>
<td>1.32 (32.8)</td>
<td>1.75 (43.6)</td>
</tr>
<tr>
<td><em>F. australis</em></td>
<td>2.61</td>
<td>0.67 (25.8)</td>
<td>0.56 (21.8)</td>
</tr>
<tr>
<td><em>T. lilacinogilva</em></td>
<td>2.69</td>
<td>0.49 (18.5)</td>
<td>1.77 (66.6)</td>
</tr>
<tr>
<td><em>P. tumulosus</em></td>
<td>2.50</td>
<td>0.51 (20.5)</td>
<td>1.49 (59.6)</td>
</tr>
</tbody>
</table>

The medium from each fungal culture yielded three fractions on precipitation with alcohol. Table 2 shows the weight distribution and recoveries of each fraction. The variation found in the proportion of fractions could be due to the great differences in physiological age of the organisms observed after the incubation period of 8 weeks. Homogeneity of polysaccharides in the fraction was assessed by ultracentrifugation. The monosaccharide contents of these fractions are given in Table 3.

**Table 3. Alditol acetate composition of fractions from the culture media of four Basidiomycetes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Fraction</th>
<th>Xylitol</th>
<th>Fucitol</th>
<th>Mannitol</th>
<th>Dulcitol</th>
<th>Sorbitol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. strigoso-zonatum</em></td>
<td>A</td>
<td>1.0</td>
<td>0.4</td>
<td>5.0</td>
<td>0.18</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.0</td>
<td>1.4</td>
<td>6.2</td>
<td>4.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.0</td>
<td>0.5</td>
<td>5.0</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td><em>F. australis</em></td>
<td>A</td>
<td>1.0</td>
<td>0.8</td>
<td>8.0</td>
<td>4.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.0</td>
<td>1.2</td>
<td>8.0</td>
<td>4.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.0</td>
<td>0.2</td>
<td>14.0</td>
<td>8.2</td>
<td>—</td>
</tr>
<tr>
<td><em>T. lilacinogilva</em></td>
<td>A</td>
<td>tr.</td>
<td>tr.</td>
<td>1.7</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.0</td>
<td>0.5</td>
<td>6.0</td>
<td>5.0</td>
<td>—</td>
</tr>
<tr>
<td><em>P. tumulosus</em></td>
<td>A</td>
<td>1.0</td>
<td>0.3</td>
<td>0.8</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.2</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.0</td>
<td>0.8</td>
<td>3.0</td>
<td>5.4</td>
<td>—</td>
</tr>
</tbody>
</table>

*Related to standard xylitol acetate. Each value represents the numerical average of four determinations.*

Mannose was the major component in all three fractions of *S. strigoso-zonatum*. Fraction A consisted mainly of mannose and xylose, suggesting the presence of a high molecular weight xylomannan. This fraction comprised 33% of the lyophilized
medium, higher than that obtained from the other three species. Xylomannans have been isolated from cell walls of *P. tumulosus* (Angyal et al. 1974), the mycelium of *Armillaria mellea* (Bouven et al. 1967), and a laevorotatory fucoxylomannan from the fruiting bodies of *P. pinicola* (Fraser et al. 1967), but none of these resembled the extracellular polysaccharide elaborated by *S. strigoso-zonatum*.

Since the ratio of mannose : galactose in fractions B and C was essentially constant (1-52 and 1-66 respectively) it can be assumed that the same polysaccharides occur in these fractions differing only in size. Fraction B had \([\alpha]_D = +19°\) but ultracentrifugation indicated that it was not homogeneous. A solution of fraction B failed to give an insoluble copper complex when tested with Fehling’s solution and at this stage was not further purified.

The ratio of mannose : galactose was constant (1-7) in all three fractions from *F. australis*. Fraction B had a low positive rotation \([\alpha]_D = +21°\), but it was heterogeneous and could not be purified via an insoluble copper complex. Table 3 shows that the extracellular polysaccharides of *F. australis* contain considerable fucose and no glucose. In contrast other workers have shown that cell wall or mycelial polysaccharides from different species of *Fomes* have a high glucose content but no fucose (O’Brien and Ralph 1966; Rosik et al. 1966). Thus the secreted polysaccharides differ from those found in cell walls and mycelia, and would point to different functional roles.

In the fractions obtained from *T. lilacinogilva* the mannose : galactose ratio in fractions B and C was 1-0 and 1-2 respectively. From this species fraction B was obtained in 66-5\% yield, which was the highest in the four species examined. It also gave a single peak on ultracentrifugation which, although not well defined, showed a higher degree of homogeneity than the fractions previously examined; it had an \([\alpha]_D = -7°\).

*P. tumulosus* also showed mannose and galactose as the main components in all three fractions. In contrast with the mixtures obtained from the other three species, fraction B showed a higher proportion of galactose and gave an insoluble copper complex with Fehling’s solution. It was purified in this manner and it was shown that the proportion of sugars (mannose:galactose:xylose 2:1:1:0.2) did not change on two further fractionations with Fehling’s solution, nor on dialysis of an aqueous solution of the polysaccharide. The fraction had a low positive rotation \([\alpha]_D = +41°\) and gave a well-defined single peak on ultracentrifugation. Certain similarities of this polysaccharide with the heterogalactan isolated from *P. giganteus* (Bhavanandan et al. 1964) is apparent. In the *P. giganteus* polysaccharide the ratio of galactose : mannose : fucose was 2:6:1:1 and a slightly higher optical rotation \([\alpha]_D = +65°\) was observed. These authors have shown the structure to consist of a \(\beta\)-1,6-linked backbone of \(\alpha\)-galactopyranosides to which 3-\(\alpha\)-D-mannopyranosyl-L-fucopyranose side-chains are linked (1,2) to each second or third galactose residue.

Polysaccharides consisting of a main chain of \(\alpha\)-1,6-linked \(\alpha\)-galactopyranosyl residues, substituted with either mannosylfucose, fucopyranosyl or 3-\(\alpha\)-methylgalactosyl side-chains were isolated from fruiting bodies of the Basidiomycetes, *Armillaria mellea* (Bouven et al. 1967; Fraser and Lindberg 1967), *P. pinicola* (Fraser et al. 1967), *P. fomentarius* and *P. igniarius* (Bjorndal and Lindberg 1969). All of these had higher positive optical rotations than the *P. giganteus* and *P. tumulosus
heterogalactans and the ratio of galactose:mannose was about 6:1:2. Thus the P. tumulosus heterogalactan is likely to consist of a $\beta$-1,6-linked galactan backbone and have other structural similarities with the P. giganteus heterogalactan rather than those isolated from the above species. Structural studies are needed to support this suggestion.

With the exception of the heterogalactan from P. tumulosus the extracellular polysaccharides require further fractionation prior to studies of their chemical structure. None of the polysaccharides from each fungal culture contained uronic acid, and only small amounts of glucose were found. Therefore, no glucuronoglycans such as found in fruiting bodies of P. fomentarius and P. igniarius (Bjorndal and Lindberg 1969) are present in the culture media of the four species investigated here.

The different patterns obtained for the sugar composition of polysaccharides elaborated by F. australis and P. tumulosus may be of some taxonomic significance. Cunningham (1954) has separated Fomes and Polyporus species on the basis of their hyphal system especially the colour of the hyphae. A comparative study of the cell wall polysaccharides of these species by O’Brien and Ralph (1966) tended to support Cunningham’s classification. In the present work, Table 3 shows that there is a high proportion of mannose in all three fractions isolated from the culture medium of F. australis and that the ratio of mannose to galactose is significantly different from that found in P. tumulosus. In Table 2 a significant difference in the proportion of fraction B to C is demonstrated between the two species. These results seem to provide further evidence in favour of Cunningham’s classification.

However, meaningful comparative studies of the various fungal polysaccharides can only be achieved when the fungi have been grown under standardized conditions. Thus, whilst comparison of the extracellular polysaccharides with polysaccharides isolated from cell walls or fruiting bodies of fungi might be of interest it cannot be used for taxonomic purposes.

Acknowledgments

I wish to express my thanks to Professors S. J. Angyal, B. J. Ralph and G. N. Richards for their helpful discussions during this study. Thanks are also due to Mr A. Netschey, University of Sydney, for carrying out the ultracentrifugation studies.

References


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1. vi. 75