*Aust. J. Biol. Sci.*, 1975, **28**, 227–31

# Extracellular Polysaccharides of some Basidiomycetes

*Veronika J. Bender*

School of Biological Technology, University of New South Wales, Kensington, N.S.W. 2033; present address: Division of Animal Genetics, CSIRO, P.O. Box 90, Epping, N.S.W. 2121.

*Abstract*

Culture filtrates of four basidiomycete fungi, *Stereum strigoso-zonatum*, *Fomes australis*, *Trametes lilacinogilva* 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 $[\alpha]_D$ +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 lilacinogilva* (Berk.) Lloyd and *Stereum strigoso-zanatum* (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 $P_2O_5$ 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 hydrolyses 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 (1M $NaHCO_3$), dried ($Na_2SO_4$), 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. $N_2$ was the carrier gas. Glass columns (0·3 by 170 cm) were packed with 1·5% diethyleneglycol 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 1. Sugar composition of lyophilized culture media**

| Species | Glucose | Galactose | Mannose | Xylose | Fucose | Uronic acid |
|---|---|---|---|---|---|---|
| *S. strigoso-zonatum* | + | ++ | +++ | + | + | – |
| *F. australis* | – | ++ | +++ | + | + | – |
| *T. lilacinogilva* | + | ++ | ++ | + | + | – |
| *P. tumulosus* | + | +++ | + | + | + | – |

## 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

| Species | Total yield (g) | Yield (g) of: | | | Recovery (%) |
|---|---|---|---|---|---|
| | | Fraction A | Fraction B | Fraction C | |
| *S. strigoso-zonatum* | 4·03 | 1·32 (32·8) | 1·75 (43·6) | 0·42 (10·5) | 87 |
| *F. australis* | 2·61 | 0·67 (25·8) | 0·56 (21·8) | 1·27 (49·0) | 97 |
| *T. lilacinogilva* | 2·69 | 0·49 (18·5) | 1·77 (66·6) | 0·14 (5·4) | 90 |
| *P. tumulosus* | 2·50 | 0·51 (20·5) | 1·49 (59·6) | 0·27 (11·1) | 91 |

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**

| Species | Fraction | Ratio of alditol acetates[A] | | | | |
|---|---|---|---|---|---|---|
| | | Xylitol | Fucitol | Mannitol | Dulcitol | Sorbitol |
| *S. strigoso-zonatum* | A | 1·0 | 0·4 | 5·0 | 0·18 | 0·13 |
| | B | 1·0 | 1·4 | 6·2 | 4·0 | — |
| | C | 1·0 | 0·5 | 5·0 | 3·0 | — |
| *F. australis* | A | 1·0 | 0·8 | 8·0 | 4·8 | — |
| | B | 1·0 | 1·2 | 8·0 | 4·8 | — |
| | C | 1·0 | 0·2 | 14·0 | 8·2 | — |
| *T. lilacinogilva* | A | tr. | tr. | 1·7 | 1·0 | 0·6 |
| | B | 1·0 | 1·0 | 2·0 | 2·0 | — |
| | C | 1·0 | 0·5 | 6·0 | 5·0 | — |
| *P. tumulosus* | A | 1·0 | 0·3 | 0·8 | 1·7 | 0·8 |
| | B | 0·2 | 1·0 | 1·0 | 2·0 | — |
| | C | 1·0 | 0·8 | 3·0 | 5·4 | — |

[A] 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* (Bouveng *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 $[\alpha]_D$ of $-7°$.

*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 (galactose : mannose : fucose : 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 D-galactopyranosides to which 3-*O*-$\alpha$-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 D-galactopyranosyl residues, substituted with either mannosylfucose, fucopyranosyl or 3-*O*-methylgalactosyl side-chains were isolated from fruiting bodies of the Basidiomycetes, *Armillaria mellea* (Bouveng *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 : fucose 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 glucuronoglucans 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**

Angyal, S. J., Bender, V. J., and Ralph, B. J. (1974). *Biochim. Biophys. Acta* **362**, 175.
Bhavanandan, V. P., Bouveng, H. O., and Lindberg, B. (1964). *Acta Chem. Scand.* **18**, 504.
Bjorndal, H., and Lindberg, B. (1969). *Carbohydr. Res.* **10**, 79.
Bouveng, H. O., Fraser, R. N., and Lindberg, B. (1967). *Carbohydr. Res.* **4**, 20.
Cunningham, G. H. (1954). *Trans. Br. Mycol. Soc.* **37**, 44.
Fraser, R. N., Karacsonyi, S., and Lindberg, B. (1967). *Acta Chem. Scand.* **21**, 1783.
Fraser, R. N., and Lindberg, B. (1967). *Carbohydr. Res.* **4**, 12.
Gorin, P. A. J., and Spencer, J. F. T. (1968). *Adv. Carbohydr. Chem.* **23**, 367.
O'Brien, R. W., and Ralph, B. J. (1966). *Ann. Bot. (Lond.)* **30**, 831.
Ralph, B. J., and Robertson, A. (1950). *J. Chem. Soc.* 3380.
Rosik, J., Zitko, J., Bauer, S., and Kubala, J. (1966). *Collect. Czech. Chem. Commun.* **31**, 375.

Manuscript received 26 November 1974

# Notice to Authors

## AUSTRALIAN JOURNAL OF BIOLOGICAL SCIENCES

Papers will be considered for publication if they make an original contribution, either experimental or theoretical, in any of the following fields: biochemistry, cell physiology and ultrastructure, animal physiology, endocrinology, reproductive biology, genetics or microbiology. Submission of a paper implies that the results reported have not been published or submitted for publication elsewhere. All papers are refereed.

**General Presentation.** The work should be presented concisely and clearly in English. Introductory material, including a review of the literature, should not exceed what is necessary to indicate the reason for the work and the essential background.

***Title.*** This should be concise and appropriately informative and should contain all key words to facilitate retrieval by modern searching techniques.

If the paper is one of a numbered series, a reference to the previous part should be given as a footnote on the first page. If a part not yet published needs to be consulted for a proper understanding of the paper, a copy of that manuscript should be supplied to assist the referees.

***Abstract.*** This should state concisely the scope of the work and the principal findings, and should be suitable for direct use by abstracting journals; this will seldom require more than 200 words.

***Accessory Publication.*** Supplementary material of a detailed nature which is not essential in the printed paper but may be useful to other workers may be lodged with the Editor-in-Chief if submitted with the manuscript for inspection by the referees. Such material will be made available on request and a note to this effect should be included in the paper.

**Manuscripts.** The original and two copies of the manuscript should be typed on good quality paper with 1 cm spacing between lines *throughout*. The marginal space on the left-hand side should not be less than 4 cm. All pages of the manuscript must be numbered consecutively, including those containing references, tables and captions to illustrations, which are to be placed after the text.

Authors are referred to the 'Style Manual for Authors, Editors and Printers of Australian Government Publications' (2nd edition, 1972; Australian Government Publishing Service, Canberra) for conventions to be generally adopted in the preparation of their paper. A more specific 'Style Guide for CSIRO Publications' is available on application to the Editor-in-Chief.

Authors are also advised to note the layout of headings, tables and illustrations exemplified in the latest issues of the Journal. Strict observance of these and the following requirements will shorten the interval between submission and publication.

***Footnotes*** within the text should be used only when essential. They should be placed within horizontal rules immediately under the lines to which they refer.

***References*** are cited in the text by the author and date and are not numbered. All references in the text must be listed at the end of the paper, with the names of authors arranged alphabetically; all entries in this list must correspond to references in the text. No editorial responsibility can be taken for the accuracy of the references; authors are requested to check these with special care. Titles of published papers should be included; a reference to any paper accepted for publication but not yet published must include the title. Papers which have not been accepted for publication may not be included in the list of references and must be cited either as 'unpublished data' or as 'personal communication'; the use of such citations is discouraged. Authors are referred to the latest issues of the Journal for the style used in citing references to books, periodicals and other literature. Abbreviations of titles of periodicals should conform to those given in the latest edition of *BIOSIS List of Serials*, but capital letters are to be used only for the first letter of each word, e.g. *Aust. J. Biol. Sci.* **27**, 123–9.

***Units.*** Authors are requested to use the International System of Units (Système International d'Unités) for exact measurements of physical quantities and as far as practical elsewhere.

***Mathematical Formulae*** should be carefully typed with symbols in correct alignment and adequately spaced. At least two clear lines should be left above and below all displayed equations. If special symbols must be hand-written, they should be inserted with care and identified by pencilled notes in the margins. Judicious use should be made of the solidus to avoid two-line mathematical expressions wherever possible and especially in the running text. All long formulae should be displayed.

***Enzyme Nomenclature.*** The names of enzymes should conform to the Recommendations (1972) of the Commission on Biochemical Nomenclature on the Nomenclature and Classification of Enzymes, as published in either 'Enzyme Nomenclature' (1972, Elsevier, Amsterdam) or Volume 13 of 'Comprehensive Biochemistry' (Eds. M. Florkin and E. Stotz, 3rd Ed., 1973, Elsevier, Amsterdam). Where enzymes are referred to only in the course of discussion, or are obtained from commercial sources and are used solely as a reagent, it will be adequate to use the recommended name without the identifying EC number. For enzymes which are not the main subject of the paper, the recommended names should be used in the title, abstract and throughout the paper, but should be identified by their EC number at the first mention in the body of the paper. Where an enzyme is the main subject of a paper, the recommended name should be used in the title, abstract and throughout the paper but it should be identified by its code number, source, and either its systematic name or *reaction equation* at its first mention in the text. If there is good reason to use a name other than the recommended name, at the first mention of the alternative name in the text it should be identified by the recommended name and code number. The editor should be advised of the reasons for using the alternative name.

***Chemical Nomenclature.*** The nomenclature of compounds such as amino acids, carbohydrates, lipids, steroids, vitamins, etc. should follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature. Other biologically active compounds, such as metabolic inhibitors, plant growth regulators, buffers, pesticides, etc. should be referred to once by their correct chemical name (which is in accordance with IUPAC Rules of Chemical Nomenclature) and then by their most widely accepted common name. Where there is no common name, trade names or letter abbreviations of the chemical may be used.

***Tables*** should be numbered with arabic numerals and be accompanied by a title. They should be arranged having regard to the dimensions of the printed page and the number of columns should be kept to a minimum. Long headings to columns should be avoided by the use of explanatory footnotes. Each table must be referred to in the text. Only in exceptional circumstances will presentation of essentially the same data in tabular and graphical form be permitted; where adequate the latter form should be used. Short tables can frequently be incorporated into the text as a tabulation or sentence.

**Illustrations,** both line drawings and half-tones, are to be numbered in a common sequence, with half-tones grouped together as much as possible. Each figure must be referred to in the text. A typed list of captions is required.

***Line Drawings.*** The originals and two copies are required. Originals must be drawn with black ink on white board or on drawing or tracing paper, and must *not* be lettered or mounted; copies should be lettered clearly. Authors should note the size of comparable drawings in recent issues of the Journal and submit originals that are two-and-a-half times as large. In this case the axes and curves should be 0·6–0·8 mm thick and of uniform density, and symbols should be 3–4 mm in diameter. Allowance should be made for the effect of reduction on dots and stipples. The dimensions of drawings must not exceed 30 by 50 cm. If the originals are larger than this they should be photographically reduced and good quality prints submitted.

***Half-tone Illustrations.*** Photographic prints for half-tone reproduction must be of the highest quality with a full range of tones and good contrast. They should neither be lettered nor mounted. They should be trimmed to exclude features not relevant to the paper, or the maximum permissible trim may be indicated on copies. Two sets of mounted, clearly lettered copies, suitable for sending to referees, must be submitted with the originals. A scale must be supplied for each photomicrograph or electron micrograph, but it should be inserted **only** on the copies. Important features to which special attention should be given by the blockmaker may be indicated on an overlay of the copies.