

# POLYSACCHARIDE-DEGRADING COMPLEX PRODUCED IN WOOD AND IN LIQUID MEDIA BY THE BROWN-ROT FUNGUS *PORIA PLACENTA*

*Terry L. Highley, Karl E. Wolter, and Faye J. Evans*

Plant Pathologist, Plant Physiologist, and Chemist  
Forest Products Laboratory,<sup>1</sup> Forest Service  
U.S. Department of Agriculture  
Madison, WI 53705

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## ABSTRACT

The polysaccharide-degrading enzymes produced by *Poria placenta* in decayed wood and liquid media were compared qualitatively and quantitatively. A single carbohydrate-degrading complex was isolated and purified from wood and liquid cultures that was active on both polysaccharides and glycosides. Quantitative differences in enzyme activities from decayed wood versus liquid media were observed. However, the purified extracellular carbohydrate-degrading complex isolated from decayed wood and from liquid cultures must be structurally similar because of similar isoelectric points, electrophoretic properties, and molecular sieving properties.

*Keywords:* Hemicellulase, glycosidase, decay, degradation, enzyme purification.

## INTRODUCTION

The cellulose and hemicellulose-degrading enzymes of wood-degrading fungi have received a great deal of attention in recent years. Most of these studies, however, have employed preparations from liquid cultures rather than from wood. It is well established that cultural conditions can affect quantitatively and qualitatively the spectrum of polysaccharide-degrading enzymes secreted by fungi (Albersheim and Anderson-Pronty 1972). As the substrates and cultural conditions in liquid media differ greatly from those in solid wood, the polysaccharide-degrading enzymes produced by the fungus in liquid culture may differ from those it produces in decaying wood.

The ultimate aim of our work is to identify properties of extracellular wall-degrading enzymes produced by decay fungi that can be utilized to stop their decaying activities in wood. Thus, it is important that we use enzymes in the form in which they occur naturally in wood. In a previous communication (Wolter et al. 1980), we described an extracellular carbohydrase purified from a liquid culture preparation of the brown-rot fungus *Poria placenta* that appears to be a complex composed of different enzyme activities. In this paper we compare qualitatively and quantitatively the polysaccharide-degrading enzymes produced by *P. placenta* in wood with those produced in liquid culture.

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TABLE 1. *Effect of simple sugars on polysaccharidase production by Poria placenta in liquid cultures.*

Sugar (%)	Polysaccharidase activities <sup>a</sup> production on different sugars <sup>b</sup>								
	Xylanase (day)			Mannanase (day)			CMCase (day)		
	7	14	21	7	14	21	7	14	21
Glucose (0.1)	1.1	2.0	1.8	0.3	1.4	1.0	0.2	1.0	0.6
Glucose (1.0)	1.0	2.7	2.9	0.3	1.5	1.2	0.3	1.1	0.6
Galactose (0.1)	1.2	1.8	2.7	0.3	0.7	0.4	0.3	0.8	1.0
Galactose (1.0)	0.9	1.8	1.3	0.4	1.3	0.6	0.2	1.0	0.6
Mannose (0.1)	2.1	2.6	2.6	0.3	1.0	1.2	0.8	0.3	1.9
Mannose (1.0)	2.9	2.8	2.1	0.3	3.2	1.4	1.4	3.2	2.3
Xylose (0.1)	1.2	2.0	1.9	0.3	1.3	0.8	0.4	0.6	1.6
Xylose (1.0)	1.3	2.2	2.9	0.5	1.2	0.8	0.8	1.1	1.6
Arabinose (0.1)	1.4	2.0	1.6	0.2	0.8	0.5	0.1	0.6	0.9
Arabinose (1.0)	1.2	2.2	3.1	0.2	0.8	0.8	0.5	0.8	1.6

<sup>a</sup> A unit of enzyme activity is the amount which will liberate reducing power equivalent to 1  $\mu$ M of glucose/h at 40 C.

<sup>b</sup> Substrate for mannanase, galactomannan; xylanase, xylan (NBC); CMCase, sodium carboxymethylcellulose.

## METHOD

### *Culture and preparation of extracellular crude enzymes*

*Liquid.*—To estimate the effect of different monomer sugars on the production of the various enzyme activities, *P. placenta* was grown in a previously described basal salts solution (Highley 1973) containing 0.1% and 1.0% sugars (Tables 1 and 2). Enzymatic activities were determined in dialyzed culture filtrates after 7, 14, and 21 days.

For purification studies, *P. placenta* was grown in stationary culture on the basal salts solution (Highley 1973) containing 0.1% galactomannan (Sigma), 0.5% Solka-Floc (Brown), 0.5% glucose (MCB), and 0.5% hemlock (*Tsuga heterophylla* [Raf.] Sarg.) or sweetgum (*Liquidambar styraciflua* L.) sawdust. Culture vessels were 2-liter Erlenmeyer flasks containing 500 ml of media. After sterilization at 121 C for 15 min, cultures were inoculated with 10 ml of washed mycelial suspension precultured on the basal medium containing 1% glucose. After incubation at 27 C in the dark for 30 days, mycelial mats were separated by filtration with suction through glass filter paper. Enzyme adhering to the residue was eluted in 0.1 M, pH 5.0 acetate buffer for 12 h and then squeezed through cheesecloth. The residue was eluted twice with this procedure. The liquid from the two squeezings was filtered through glass filter paper and combined with culture filtrate. This solution was concentrated by hollow-fibre P-10 (Amicon) ultrafiltration. Further concentration and dialysis were accomplished with an S and S collodion membrane [10,000, 25,000, 75,000 molecular weight (MW) cutoff].

*Solid.*—Sweetgum blocks (1 by 1 by 1/8 in., the long axis parallel to the grain) were decayed in chambers patterned after the standard ASTM soil block method (ASTM 1971). Thirty blocks were placed on sterilized soil in quart jars laid horizontally. Blocks were inoculated with a mycelial suspension of *P. placenta* and incubated in the dark at 27 C for 60 days. After removal from chambers, blocks were homogenized in 0.1 M, pH 5.0 acetate buffer, in a Waring blender for 1 min, and the homogenate was squeezed through cheesecloth and filtered through glass-fibered filter paper. The residue from the squeezings was eluted twice in 0.1 M, pH 5.0 acetate buffer, filtered, and the liquid combined with the previous filtrate.

TABLE 2. Effect of simple sugars on glycosidase production by *Poria placenta* in liquid cultures.

Sugar (%)	Glycosidase activities <sup>a</sup> × 10 <sup>2</sup> produced on different sugars														
	β-D-glucosidase (day)			α-D-glucosidase (day)			β-D-galactosidase (day)			α-D-galactosidase (day)			α-β-xylosidase (day)		
	7	14	21	7	14	21	7	14	21	7	14	21	7	14	21
Glucose (0.1)	1.2	1.8	0.5	0.5	0.8	0.8	8.3	19.0	8.3	3.6	6.3	22.2	0.5	1.0	0
Glucose (1.0)	1.2	2.7	4.0	0.5	2.2	2.0	10.6	24.0	13.1	5.6	7.2	22.2	1.0	1.0	1.8
Galactose (0.1)	1.0	1.2	0.5	0.5	0.8	0.8	5.0	13.4	5.9	3.4	3.6	19.0	0.5	0	0
Galactose (1.0)	1.0	2.0	2.0	0.5	1.0	1.0	5.6	6.0	9.8	6.0	1.8	16.5	0.5	0	0
Mannose (0.1)	3.4	3.1	2.2	1.8	2.0	5.0	22.9	22.2	16.5	30.6	11.8	21.8	3.4	2.7	2.7
Mannose (1.0)	1.7	3.1	7.0	1.8	3.4	3.1	12.7	22.4	22.2	42.4	22.4	33.0	6.5	9.9	9.5
Xylose (0.1)	1.0	1.0	2.0	0.5	0.8	1.8	5.2	19.0	7.6	4.7	11.5	19.0	1.2	0.8	1.0
Xylose (1.0)	2.0	1.4	3.4	1.2	1.8	1.0	13.4	22.4	14.4	15.3	10.6	20.3	2.0	0.8	1.0
Arabinose (0.1)	1.0	2.4	2.0	0.5	1.8	1.8	42.5	10.3	8.7	9.0	6.5	22.2	1.0	0.8	1.0
Arabinose (1.0)	1.4	2.4	2.0	0.5	3.4	3.4	9.8	22.4	22.2	4.2	6.0	19.0	0.5	0.8	1.8

<sup>a</sup> A unit of enzyme activity is the amount which will release 1 μM of p-nitrophenol/h at 40 C.

The crude enzyme preparation was then centrifuged and the supernatant dialyzed and concentrated with an S and S collodion membrane (10,000, 25,000, or 75,000 MW).

#### Enzyme assays

Enzymes that catalyze the depolymerization of cell-wall polymers were assayed by increasing reducing groups at 40 C using Nelson's modification of the Somogyi method (Nelson 1944) as described previously by Highley (1976). Substrates used were xylan (Nutritional Biochemical Company), carboxymethylcellulose or CMC, galactomannan (Sigma), and glucomannan (prepared from loblolly pine, *Pinus taeda* L.). A unit of enzyme activity was defined as the amount needed to liberate reducing power equivalent to 1 μM of glucose per unit of time.

α-D-galactosidase, β-D-galactosidase, β-D-glucosidase, α-D-glucosidase, and β-D-xylosidase activities were assayed by determining the liberation of p-nitrophenol from the respective p-nitrophenol substrate as described previously by Highley (1976). A unit of enzyme activity was defined as the amount that will liberate 1 μM of p-nitrophenol per hour.

#### Protein concentration

Protein was monitored during column isoelectric focusing, or column chromatography, by absorbance at 280 nm. Protein concentration was also determined by the method of Lowry et al. (1951) using bovine albumin as a standard.

#### Isoelectric focusing

*Column.*—The separation was performed essentially as described by Nelson et al. (1974) in an ISCO Model 212 electrophoresis column. It was cooled by water at 4 C from a low-temperature circulator (Landa K-2/R). The density gradient was made up of water solutions of ethylene glycol with the denser solution containing 70% (v/v) glycol. Commercial carrier ampholytes (pH 3.5 to 10, pH 3.5 to 5.0, and pH 2.0 to 4.0) were used as marketed by LKB and BioRad. The material was used in a 2% concentration. The anode compartment contained

ethylene glycol (10%, v/v) in 1% phosphoric acid while the cathode compartment was filled with ethylene glycol (75%, v/v) in 1% NaOH. The run was performed at a constant power of 0.42 W (initially 200 V and 2.1 mA), and carrier ampholytes were focused for 48 h prior to application of the sample. We followed progress of sample separation by raising and lowering the gradient through an absorbance monitor cell and obtaining a scan. When focusing was complete, the gradient was removed from the top and 0.5-ml fractions collected in a fraction collector. The pH's of the fractions were measured with a Corning Model 12 pH meter.

*Thin layer isoelectric focusing (TLIEF).*—This process was performed with a Desaga/Brinkmann Double Chamber. Carrier ampholytes were incorporated into a Sephadex G-75 or G-200 (Pharmacia) gel slurry and poured onto glass plates. Samples were streaked approximately in the middle of the plate. The anode electrolyte solution was 0.2 N H<sub>2</sub>SO<sub>4</sub> and the cathode solution 0.4 N ethylenediamine. A low-temperature circulator (Lauda K-2/R) at 4 C was used to cool the chamber during focusing. Samples were focused for approximately 20 h at a constant voltage of 250 V, with additional focusing at 500 V for 2 h. After focusing, pH measurements were made directly on the gel with a pH electrode. Protein bands were located with a strip of print paper placed directly on the wet gel for 1 to 2 min. The paper was dried for 15 min at 115 C, rinsed three times with trichloroacetic acid (10%), and bands detected with 2% Coomassie Brilliant Blue R 250. Gel was removed from plates where bands occurred and protein eluted from the gel in 0.1 M, pH 5.0 acetate buffer.

#### *Molecular weight determinations*

Molecular weights of the TLIEF purified proteins were determined with Ultragel AcA 34 according to a procedure suggested by Pharmacia Fine Chemicals Company. The protein solution was applied to a 43- by 1.5-cm column (Pharmacia) of Ultragel AcA 34, equilibrated and eluted with degassed phosphate buffer, pH 6.0. Fractions were collected and assayed for enzyme activity, and molecular weights were determined by comparisons of elution volumes on a standard curve of known proteins.

The molecular weight of the TLIEF purified protein was also determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis by comparing the relative electrophoretic mobility (Rf) to standards of known molecular weights (Weber and Osborn 1969; Wolter and Gordon 1975). Low molecular weight [14,300–94,000 daltons (D)] and high molecular weight (43,000–200,000 D) natural proteins were obtained from Bio-Rad. Purified enzyme and standards were denatured in a 0.25 M Tris buffer (7.4) with 1% SDS and 1% mercaptoethanol and heated for 5 min at 100 C. Standard and sample aliquots (100  $\mu$ l) were applied to a vertical discontinuous polyacrylamide slab of Cyanagum 41 (95% acrylamide and 5% bisacrylamide), in a chamber, which was polymerized with N,N,N,N-tetra-methylethylenediamine and ammonium persulfate (Gordon 1971; Wolter and Gordon 1975). The gel consisted of 4% spacer gel (0.1 M, pH 6.7) and a 10% running gel (0.5 M, pH 8.9). Buffer compartment contained tris/glycine buffer (0.04 M, pH 8.3). Gels were run at 200 mA until samples were stacked and then 3 h at 300 mA, which effected adequate separation. Gels were stained for 75 min with a 2% Coomassie Brilliant Blue R 250 stain in a methanol-water-acetic acid

solution (4.5:4.5:1.0, v:v:v) and destained until protein bands were clear with water, methanol, and acetic acid (6.5:2.5:0.75, v:v:v).

## RESULTS AND DISCUSSION

### *Induction-repression studies*

For studies of polysaccharide breakdown, it is important to know how enzyme activity is affected by its own reaction products. Tables 1 and 2 show the polysaccharidase and glycosidase activities produced on monomer sugars that predominate from breakdown of carbohydrate polymers in wood cell walls. The polysaccharidases and glycosidases of *Poria placenta* were not specific to any type of sugar. For example,  $\beta$ -galactosidase activity with galactose as the substrate was not greater than with glucose, xylose, or arabinose as substrates. For most other fungi, synthesis of enzymes is normally specifically induced by the monomer or dimer predominant in the polymer degraded by the enzyme, provided the concentration of the inducer does not exceed certain low values (Albersheim and Anderson-Pronty 1972; Cooper et al. 1978). In general, the hemicellulases maximized at day 14. However, the glycosidases were much more variable.

Synthesis of polysaccharidases is generally controlled by catabolic repression, a term for the inhibition of synthesis of catabolic enzymes by readily metabolized carbon sources (Cooper et al. 1978). Eriksson (1977) has shown this to be very important in cellulose degradation by several white-rotters. Synthesis of the carbohydrases from *P. placenta* apparently is not controlled by catabolic repression as the enzyme activities were not repressed by simple sugars.

Carbohydrases can be substrate-adsorbed or mycelium-bound. Thus, release of enzyme from substrate and mycelium was studied in the presence of 0.1 M EDTA, 0.2 M 2-mercaptoethanol (Eastman), 10 mM ascorbic acid, 0.1% bovine albumin (NBC), 0.1% Triton-X-100 (NBC), 1% Tween-80 (NBC), 1 to 10% NaCl, phosphate, McIlvaine and acetate buffers (pH 2.0 to 7.6), 10% polyvinylpyrrolidone (K-90, NBC), distilled H<sub>2</sub>O, and various combinations of the above. Additional enzyme was released after treating the residue from decayed wood and liquid cultures with these materials, but none of them proved significantly better than distilled H<sub>2</sub>O for releasing enzyme from mycelium and substrate. Apparently the carbohydrases of *P. placenta* are loosely bound to the mycelium.

### *Purification studies*

Initial purification work concerned separation of glycoside enzymes from polysaccharide-degrading enzymes. Fractional precipitation of crude preparations with 20 to 90% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, alcohol, and acetone did not separate any of the enzyme activities.

In most instances enzymes capable of hydrolyzing high molecular weight substances are small molecules compared with the glycosidases that hydrolyze low molecular weight substrates. Thus, molecular sieving on gel columns has been a very effective method to separate polysaccharidases from their corresponding glycosidases. However, gel chromatography with Sepharose 6B, Sephadex-100, Sephadex-200, and Ultragel AcA 34 did not separate any of the enzyme activities in the crude preparation from *Poria placenta* liquid cultures or from decayed

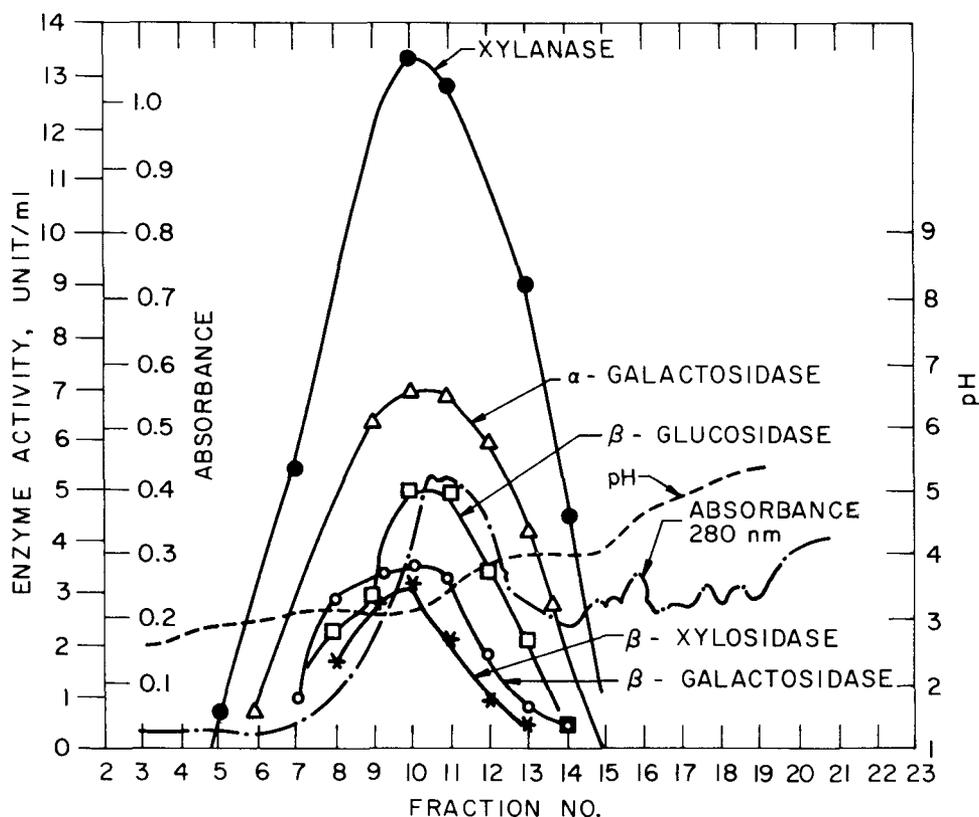


FIG. 1 pH diagram with enzyme activities from isoelectric focusing of decayed wood extract from *Poria placenta*. Mannanase and CMCase activities occurred with xylanase activities but were much less than xylanase. 0.5-ml fractions were collected. Ampholyte range 3.5–5.0 pH.

wood. Additional column chromatography separation attempts with hydroxylapatite and DEAE Bio-Gel A were unsuccessful. The above results did indicate, however, that the carbohydrate-degrading complex from both wood and liquid culture filtrates was of high molecular weight. Ultrafiltration with filters of 100,000 MW cutoff confirmed this; the complex did not pass through the filter.

Isoelectric focusing is probably the most powerful protein separation technique available today. Proteins can be resolved that differ in isoelectric points (pI) by only 0.0025 pH units. It also has the advantage that only small amounts of protein are required. Thus, this separation technique was used almost exclusively in the remaining work. Column isoelectric focusing employing an ISCO 212 column and absorbance monitor was used initially. With this method, protein and enzyme activities were readily recovered after focusing. However, all the enzyme activities still occurred essentially together in both liquid and decayed wood filtrates. Figure 1 shows typical data obtained from a decayed sweetgum extract. A problem that could not be overcome with this system was the breakage of membranes in the central column resulting in leakage. Thus, for the remaining isoelectric focusing experiments, we used thin layer isoelectric focusing (TLIEF) on glass plates with Sephadex G-75 or G-200 gels. Sample preparation for TLIEF required

only dialyzation and concentration of the crude enzyme preparation with collodion membranes (75,000 MW cutoff).

Samples were run on prep plates first. Wide-range LKB ampholytes (pH 3.5–10) were used initially, but the protein band containing the enzyme activities was always at a pI below 3.0. Therefore, pH 2.0–4.0 and 3.5–5.0 ampholytes (Brinkman, LKB, Serva) were used in subsequent work. In all the preparations, no more than four bands ever appeared. This result differs considerably from white-rot fungi, where, for example, Eriksson (1977) reported as many as 20 bands for the white-rotter *Sporotrichum pulverulentum*. Bands were scraped from the gel and eluted in 0.1 M acetate buffer, pH 5.0. The suspension was filtered and the filtrate dialyzed and concentrated with a collodion 75,000 MW membrane. All the various enzyme activities were found to be in one or two bands in the same location for both wood and liquid culture filtrates at a pI of about 1.8 to 2.0. The active eluant from prep plates was run on analytical TLIEF plates, and all enzyme activities tested were localized in one protein band in the same location for both wood and liquid filtrates. The pI was the same as on the prep plates.

The results reported above with pH 2.0–4.0 ampholytes from Brinkmann produced a distinct band resolution at pH 1.8–2.0. These ampholytes are no longer available. Further work using pH 2.0–4.0 ampholytes from Serva and LKB produced a linear pH gradient, but the sample protein was diffused throughout the gel rather than in a distinct band. As yet, we have not discovered the cause of this discrepancy.

The low pI for the carbohydrase complex from *P. placenta* is very unusual. To the authors' knowledge, such a low pI has never been reported prior to this study. However, in most studies ampholytes with pH's below 3.0 have not been used. Ishihara et al. (1978) found the purified xylanase from the brown-rotter *Tyromyces palustris* to have a pI of 3.6 when using ampholytes with a pH range 3 to 10. Perhaps a lower pH range would have produced different results. Ampholytes below pH 2.0 were not available for this study. It is possible that an extended pH range would resolve the carbohydrase complex into additional bands. Methods are available to accomplish this (Stenmann and Grasbeck 1972), and further work along these lines is planned.

The specific enzyme activities decreased after focusing (Table 3). Richards and Shambe (1976) found similar results with the hemicellulase from *Cephalosporium sacchari*. They propose that enzyme protein is deactivated during focusing because of conformational changes that may occur at the isoelectric point.

#### *Molecular size*

In preliminary separation for molecular size, ultrafiltration of the crude protein indicated that all the various enzyme activities were in a fraction greater than 100,000 D. Further determinations of the purified TLIEF protein via comparisons with standard proteins, on Ultragel AcA 34, indicated one active peak at approximately 185,000 ( $\pm 5,000$ ) D. This peak contained all the active proteins found in the initial preparations. However, SDS-polyacrylamide runs indicated at least five protein bands of lower molecular weight to be present in the TLIEF eluant.

Apparently the SDS is breaking down the enzyme complex from *P. placenta* to small subunits. Similar results were reported by Kemp et al. (1979). He found that purified nopaline synthase eluted from a column of Ultragel AcA 34 with a mo-

TABLE 3. Comparison of polysaccharidase and glycosidase activities in crude, collodion, and TLIEF eluate from decayed wood and liquid media.

Substrate	Enzyme activity (units/mg protein) <sup>a</sup>								
	Sweetgum decayed wood			Sweetgum liquid			Hemlock liquid		
	Crude dialyzed	Collo-dion concen-trate	TLIEF eluate	Crude dialyzed	Collo-dion concen-trate	TLIEF eluate	Crude dialyzed	Collo-dion concen-trate	TLIEF eluate
Xylan	2.1	5.0	1.6	14.5	15.3	7.5	4.4	7.3	3.5
Glucomannan	2.4	6.9	0.6	8.0	7.3	2.0	2.5	6.0	1.8
Carboxymethylcellulose	1.4	3.5	0.7	2.5	3.7	1.0	1.4	2.1	0.8
$\beta$ -D-glucoside <sup>b</sup>	9.7	18.5	3.8	6.0	6.0	0.10	7.2	13.1	0.26
$\alpha$ -D-glucoside <sup>b</sup>	0.4	0.9	0.2	1.0	0.6	0.09	1.3	5.8	0.95
$\beta$ -D-galactoside <sup>b</sup>	12.9	20.0	3.1	40.6	40.7	23.8	18.7	61.9	29.6
$\alpha$ -D-galactoside <sup>b</sup>	15.2	26.2	4.4	62.0	51.8	28.7	25.7	61.9	35.1
$\beta$ -D-xyloside <sup>b</sup>	3.6	11.1	2.8	1.4	1.6	0.7	1.9	7.6	0.17

<sup>a</sup> A unit of enzyme activity is the amount which will release 1  $\mu$ M of p-nitrophenol or reducing power equivalent to 1  $\mu$ M of glucose/h at 40 C.

<sup>b</sup> p-nitrophenyl-glycoside.

molecular weight of 158,000 D and migrated on SDS-polyacrylamide gels with a molecular weight of 40,000 D. He therefore proposed that the "native" enzyme is composed of four equal-weight subunits. Umezurike (1971) reports a  $\beta$ -glucosidase from *Botryodiplodia theobromae* with a molecular weight of 320,000 to 332,000, which under the right conditions dissociates into lower molecular weight polypeptides. He estimated the  $\beta$ -glucosidase to be an aggregate of about 32 polypeptide units. Recently Ait et al. (1979) reported that the cellulolytic system from culture filtrates of *Clostridium thermocellum*, when partially purified by preparative electrophoresis, showed a single band equal to 125,000 D on analytical polyacrylamide gel electrophoresis. However, SDS-polyacrylamide gel electrophoresis indicated at least five proteins of lower molecular weight, results similar to the one obtained in our filtrates from *P. placenta*.

Thus, the carbohydrate-degrading complex isolated from decayed wood and liquid cultures of *P. placenta* may also be composed of an aggregate of polypeptides that under the right conditions, as indicated by SDS-polyacrylamide electrophoresis, breaks down into small polypeptide subunits. Such small units would be able to penetrate the microstructure of the wood cell walls. The big complex cannot degrade crystalline cellulose; it would be interesting to determine if the small polypeptides can.

#### Discussion of complex

The purified enzyme complex from decayed wood and liquid culture filtrates of *Poria placenta* is active on macromolecules as well as glycosides. To the authors' knowledge, such multiple action by one carbohydrase has not been reported. However, recent work has shown multiple enzyme activities associated with a single enzyme fraction even after extensive purification (Cooper et al. 1978; Urbanik et al. 1978). Cooper et al. found two isozymes of polygalacturonase pectin lyase of *Verticillium albo-atrum* which retained almost identical activity profiles after wide- and narrow-range isoelectric focusing. When working with *Phoma hibernica*, Urbanik et al. found one band after isoelectric focusing with

activities toward CMC-insoluble cellulose, xylan, galactomannan, glucomannan, and galactoglucomannan. Nitrophenyl derivatives of carbohydrates were hydrolyzed slowly. In both of these cases, it was suggested that the purified enzyme preparation is a complex most probably composed of different enzyme activities. The large size of the purified carbohydrase complex from decayed wood and liquid cultures of *P. placenta* also suggests that the multiple activities are derived from a complex.

Only a few carbohydrate-degrading enzymes from brown-rot fungi have been purified, but those that have are not substrate specific. A purified  $\beta$ -glucosidase from the brown-rot fungus *Lenzites trabea* also hydrolyzed  $\beta$ -xyloside (Herr et al. 1978); the purified xylanase from the brown-rotter *Tyromyces palustris* also had CMCase activity but no glycosidase activity. The purified endocellulase from the brown-rotter *Polyporus schweinitzii* was accompanied by mannanase and xylanase activities (Keilich et al. 1969). The purified carbohydrase from *P. placenta* appears to be more nonspecific than the above cases in that the enzyme can degrade both polysaccharides and glycosides.

With most cell-wall-degrading fungi, the nutritional environment of the fungus influences the nature of the cell-wall-degrading enzymes that it produces. For example, Cooper et al. (1978) found that polysaccharidases produced by some fungi *in vivo* can differ qualitatively from those produced *in vitro*. The differences indicated variation in enzyme type and isoenzymes of a single enzyme. *Poria placenta* produced the same type of enzyme activities in both liquid cultures with simple sugars or gum and hemlock sawdust and in extracts from decayed wood. However, there were differences in amounts of enzyme activity produced per milligram of protein from decayed wood and liquid media (Table 3).  $\beta$ - and  $\alpha$ -galactosidase and xylanase activities were higher in liquid culture than in extracts from decayed wood, while  $\beta$ -xylosidase activities were slightly higher in decayed wood extracts. Exposure of the enzyme complex to phenolic oxidative breakdown products in preparation of the decayed wood extracts may account for some of these quantitative differences.

Despite these quantitative differences, the extracellular carbohydrase complex from liquid culture must be structurally similar to that of decayed wood because of similar isoelectric points, electrophoretic properties, and molecular sieving properties. Thus, it appears from these results that enzyme preparations from either decayed wood or liquid cultures are suitable for identifying enzyme properties that can be utilized to inhibit carbohydrate cell-wall-degrading enzymes produced by the brown-rot fungus *P. placenta*.

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