INFLUENCE OF CARBON SOURCE ON CELLULASE ACTIVITY OF WHITE-ROT AND BROWN-ROT FUNGI

Terry L. Highley

Forest Products Laboratory,[1] Forest Service, U.S. Department of Agriculture

(Received 18 January 1973)

ABSTRACT

Three white-rot fungi, *Polyporus versicolor, Ganoderma applanatum,* and *Peniophora* "G," produce an adaptive cellulase complex that can degrade both soluble cellulose (C_x) and microcrystalline cellulose (C_1), a highly ordered form of cellulose. Production of C_x and C_1 by the white-rot fungi was repressed by simple sugars. Cellulase preparations from three brown-rot fungi, *Poria monticola, Lentinus lepideus,* and *Lenzites trabea,* exhibited only C_x activity; microcrystalline cellulose was not significantly degraded. Contrary to the cellulase (C_x) of the white-rot fungi, that of the brown-rot fungi apparently is constitutive, since activity was abundant in cultures with simple sugars or with non-cellulosic polysaccharides as the sole source of carbon. This work disclosed no differences between the cellulase-inducing effects of hardwoods versus those of softwoods that might help explain the preference of white rotters for hardwoods and brown rotters for softwoods.

Additional keywords: Polyporus versicolor, Ganoderma applanatum, Peniophora "G," Poria monticola, Lentinus lepideus, Lenzites trabea, enzyme, decay.

INTRODUCTION

The mechanism by which fungal cellulases break down celluloses in wood cell walls is not understood. However, it is generally accepted that two types of enzymes are involved: A "C_1" that acts on highly ordered cellulose to produce linear chains and a "C_x" that breaks down the linear chains [β(1→4)D-glucan 4-glucanohydrolase, E.C. no. 3.2.1.4].

Brown-rot fungi and white-rot fungi, the two major types of wood-rotting fungi, produce very different rates of change in average degree of polymerization of holocellulose during wood decay (Cowling 1961). Brown-rot fungi, characterized by *Poria monticola*, liberate cellulolytic enzymes (or nonproteinaceous catalysts) that are apparently capable of penetrating the entire secondary wall structure and of degrading cellulose without prior removal of lignin from the cell wall. The cellulose is depolymerized rapidly in the initial stages of attack, and the products of hydrolysis accumulate faster than they are used. In contrast, the cellulytic catalysts of the white rotter *Polyporus versicolor* do not penetrate the cell-wall capillaries, but act only on the lumen surface degrading both crystalline and amorphous regions of microfibrils. Cellulose is not depolymerized rapidly, and the products of hydrolysis are metabolized at about the same rate as they are being formed. Lignin is removed rapidly at almost constant rates during all stages of the infection; the removal is prior to or simultaneous with cellulose breakdown.

Studies of white-rot fungi indicate that cellulases are induced only in the presence of substrates containing cellulose (Jensen 1971 and Johansson 1966). With easily metabolized sources of carbon such as glucose, cellulase production was suppressed and was not induced until after the sugars had been consumed. Culture filtrates from white-rot fungi show both C_1 and C_x activity (Johansson 1966; Reese and Levinson 1952; Walch and Kühlwein 1968). In contrast, brown-rot fungi generally exhibit sparse growth and low cellulolytic activity on cellulose media, but grow well and produce cellulases on media with a simple carbohydrate such as glucose (Bailey et al. 1969; Johansson 1966; Keilich et al. 1969;

[1] The Laboratory is maintained at Madison, Wis., in cooperation with the University of Wisconsin.

TABLE 1. *Cellulase activity (C_x and C_1) and growth of white-rot fungi on various sources of carbon*

Substrate	Cellulase activity[a,b] and growth[c] produced by:								
	Polyporus versicolor			Ganoderma applanatum			Peniophora "G"		
	C_x	C_1	Growth	C_x	C_1	Growth	C_x	C_1	Growth
Polysaccharide									
Arabinogalactan	2	--	4	--	--	6	--	--	10
Cellulose	48	0.58	13	33	0.39	10	56	0.80	12
CMC	33	--	6	8	--	8	2	--	8
Pectin	7	--	19	4	--	38	2	--	25
Starch	--	--	26	--	--	33	--	--	39
Xylan	3	--	10	4	--	25	--	--	20
Wood									
Aspen	27	0.30	+	17	0.25	+	27	0.25	+
Sweetgum	18	0.25	+	14	0.29	+	28	0.36	+
Southern pine	31	0.24	+	17	0.27	+	32	0.20	+
Engelmann spruce	21	0.36	+	13	0.35	+	24	0.25	+
Modified Wood									
Pine holocellulose	32	0.17	4	48	0.53	10	39	0.21	7
Sweetgum holocellulose	31	0.25	8	45	0.46	6	38	0.54	8
Ballmilled spruce	21	0.37	32	37	0.47	22	40	0.33	22
Ballmilled aspen	23	0.28	46	18	0.33	29	42	0.22	21
Simple sugars									
Glucose	--	--	38	--	--	49	--	--	42
Xylose	--	--	18	--	--	56	--	--	39
Lactose	--	--	7	--	--	24	--	--	20
Maltose	--	--	8	--	--	30	--	--	21
Cellobiose	--	--	18	--	--	22	--	--	20

a C_x activity expressed as $\frac{10,000}{t}$, where t equals sec required for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate;--, negligible activity.

b C_1 activity expressed as micromoles of glucose released from microcrystalline cellulose/24 hr/ml of culture filtrate; --, negligible activity.

c Growth expressed as milligrams of mycelium per culture; +, trace amounts of growth that could be detected by visual observation, but could not be quantitatively measured.

Reese and Levinson 1952). Culture filtrates from the brown-rot fungi showed no C_1 activity on highly ordered forms of cellulose such as cotton and filter-paper.

Comparison of cellulases produced by the different types of fungi is of interest because of these differences in the breakdown of cellulose by brown- and white-rot fungi. The purpose of this work was to investigate how the source of carbon influences the synthesis of cellulases by typical brown- and white-rot fungi. It was of particular importance to determine if differences exist between the cellulase-inducing effects of hardwoods versus softwoods that might explain, at least in part, the predominant occurrence of brown-rot fungi on softwoods and white-rot fungi on hardwoods.

METHOD

The following fungi were used: three white-rot—*Polyporus versicolor* (L. ex Fr.) (Madison 697), *Ganoderma applanatum* [Pers. ex Wallr. (Pat.)] (Madison 708), and *Peniophora* "G" (ME 461, unidentified to species)—and three brown-rot—*Poria monticola* (Murr.) (Madison 698), *Lenzites trabea* (Pers.) Fr. (Madison 617), and *Lentinus lepideus* (Fr.) (Madison 534). They were grown in stationary liquid cultures containing the following basal salts per liter: 2 g NH_4NO_3, 2 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.57 mg H_3BO_4, 0.036 mg $MnCl_2 \cdot 4H_2O$, 0.31 mg $ZnSO_4 \cdot 7H_2O$, 0.039 mg $CuSO_4 \cdot 5H_2O$, 0.018 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.015 mg $FeSO_4 \cdot 7H_2O$, and 0.001 g thiamine hydrochloride.

The carbon sources (0.5% wt/v ratio

TABLE 2. *Cellulase activity (C_x) and growth of brown-rot fungi on various sources of carbon*

Substrate	Cellulase activity[a] and growth[b] produced by:					
	Poria monticola		Lentinus lepideus		Lenzites trabea	
	C_x	Growth	C_x	Growth	C_x	Growth
Polysaccharide						
Arabinogalactan	33	18	9	3	29	8
Cellulose	3	+	6	+	34	+
CMC	19	8	23	8	130	8
Pectin	62	22	22	46	32	30
Starch	91	45	16	31	81	42
Xylan	33	5	19	14	29	24
Wood						
Aspen	14	+	9	+	37	+
Sweetgum	18	+	21	+	50	+
Southern pine	17	+	28	+	35	+
Spruce	25	+	22	+	33	+
Modified Wood						
Pine holocellulose	37	+	29	+	47	10
Sweetgum holocellulose	28	+	24	+	37	11
Ballmilled spruce	77	16	77	29	58	14
Ballmilled aspen	79	23	79	21	79	15
Simple Sugar						
Glucose	83	52	39	59	75	46
Xylose	63	54	4	26	28	26
Lactose	45	16	21	28	40	37
Maltose	45	41	2	12	46	24
Cellobiose	129	29	48	31	127	24

a C_x activity expressed as $\frac{10,000}{t}$, where t equals seconds required for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate.

b Growth expressed as milligrams of mycelium per culture; +, trace amounts of growth that could be detected by visual observation, but could not be quantitatively measured.

unless otherwise indicated) included the following: microcrystalline cellulose (FMC); sodium carboxymethylcellulose (Fisher, purified with degree of substitution 0.65–0.85); pectin (Eastman); lactose, maltose, starch (Difco); cellobiose, glucose, xylose, xylan (NBC), arabinogalactan (Pfaltz and Bauer); holocellulose from southern pine (*Pinus* sp.) and sweetgum (*Liquidambar styraciflua* L.); ballmilled bigtooth aspen (*Populus grandidentata* Michx.) and Engelmann spruce (*Picea engelmannii* Parry); and sawdust (40 mesh) of southern pine, sweetgum, bigtooth aspen, and Engelmann spruce. In some experiments the microcrystalline cellulose was supplemented with glucose, cellobiose, asparagine (Fisher); peptone (Difco); and yeast extract (Difco) in a 0.5% wt/v ratio to give 1.0% wt/v total carbon source.

The effect of various glucose concentrations on cellulase production by *Polyporus versicolor* and *Poria monticola* in the presence of microcrystalline cellulose at 7, 14, and 21 days was studied. Fungi were grown on the basal medium containing microcrystalline cellulose and either 0.0, 0.1, 0.5, or 1.0% glucose. In a variation of this experiment, the concentrations of glucose were added to cellulose-containing cultures of *P. versicolor* after 7 days' growth, and cellulase activity determined immediately and 14 days later.

Culture vessels were erlenmeyer flasks (250 ml) with 25 ml of culture medium. After sterilizing at 121 C for 15 min, the pH was adjusted to approximately 5.5 with 1N HCl or 1N NaOH, and cultures were inoculated with 1 ml of washed mycelium suspension precultured on the basal salts solution with 1% malt extract and 0.5% yeast extract. Flasks were incubated in the dark for 21 days unless indicated otherwise. The cultures were harvested by filtering the

TABLE 3. *Cellulase activity (C_x and C_1) and growth of white-rot fungi in cellulose medium supplemented with various growth-promoting substances*

Cellulose supplement (0.5%)	Cellulase activity[a,b] and growth[c] produced by:								
	Polyporus versicolor			Ganoderma applanatum			Peniophora "G"		
	C_x	C_1	Growth	C_x	C_1	Growth	C_x	C_1	Growth
Glucose	4	--	18	--	--	40	--	--	25
Cellobiose	4	--	17	4	--	18	--	--	40
Peptone	77	1.14	40	36	0.42	34	110	2.13	58
Asparagine	75	1.06	24	56	0.80	30	110	1.84	34
Yeast extract	101	0.76	28	19	--	52	118	1.94	58
Cellulose alone	48	0.58	13	33	0.39	10	56	0.80	12

a C_x activity expressed as $\frac{10,000}{t}$, where t equals seconds required for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate; --, negligible activity.

b C_1 activity expressed as micromoles of glucose released from microcrystalline cellulose/24 hr/ml of culture filtrate; --, negligible activity.

c Growth expressed as milligrams of mycelium per culture.

culture medium through glass filter paper, and NaN_3 (0.31 g/l) was added to the filtrate to prevent contamination. The mycelial mats were held for growth measurement. The filtrate was stored at 4 C until used.

Growth was expressed on a dry weight basis (after 48 hr at 40 C). If the culture media contained a source of insoluble carbon, direct mycelial weights could not be determined. Therefore the protein content of washed mycelial mats was used as an indication of relative growth if the culture contained an insoluble carbon source (Lowry et al. 1951; Lumsden 1969). Protein content was converted to mycelial weight from a standard curve constructed for each fungus from mycelial weights and protein content of mycelium in culture media containing soluble carbon sources.

The mycelial mats from *Poria monticola* cultures on microcrystalline cellulose and 0.5% glucose and *Polyporus versicolor* cultured on microcrystalline cellulose were used to determine the cellulase activity in mycelial mats. They were rinsed with distilled H_2O, stripped from the filter paper, and fragmented in 25 ml of distilled H_2O in a semi-micro Waring blender cup. Portions of the mycelial suspension were assayed for enzyme activity. To determine the enzyme activity remaining on the mycelial mats after blending, the suspensions were then centrifuged, and the supernatant and the mycelial residue assayed for C_x and C_1 activity.

To determine the effect of various sugars on activity of C_x cellulase *in vitro*, glucose, galactose, mannose, xylose, and cellobiose (1.0% wt/v) were incubated with *Polyporus versicolor* culture filtrate for 4 hr.

C_x activity was determined by a viscometric assay in which 1 ml of filtrate was added to 9 ml of 0.6% carboxymethyl cellulose (CMC) buffered to pH 5.0 with 0.1 M acetate buffer. Viscometric data are expressed as $10,000/t_{50}$ per ml of enzyme solution, where t_{50} equals time (sec) for the relative viscosity of the solution to be reduced by 50% at 40 C.

To determine C_1 activity, the increase in reducing groups from microcrystalline cellulose was measured by Nelson's modification of the Somogi method (Nelson 1944). Filtrates were dialyzed against running tapwater for 16 hr at room temperature before assaying the reducing groups. Reaction mixtures consisted of 1 ml of fil-

TABLE 4. *Cellulase activity (C_x) and growth of brown-rot fungi in cellulose-containing medium supplemented with various growth-promoting substances*

Cellulose supplement (0.5%)	Cellulase activity[a] and growth[b] produced by:					
	Poria monticola		*Lentinus lepideus*		*Lenzites trabea*	
	C_x	Growth	C_x	Growth	C_x	Growth
Glucose	100	24	33	20	64	44
Cellobiose	130	40	33	50	113	36
Peptone	2	5	--	8	96	11
Asparagine	3	5	3	8	103	5
Yeast extract	3	12	3	10	99	17
Cellulose alone	3	+	6	+	34	+

a C_x activity expressed as $\frac{10,000}{t}$, where t equals seconds required for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate; --, negligible activity.

b Growth as expressed milligrams of mycelium per culture; +, trace amounts of growth that could be detected by visual observation, but could not be quantitatively measured.

trate, 1 ml of 0.1 M acetate buffer, and 0.01 g of microcrystalline cellulose. Reducing-group data were expressed as micromoles of glucose released in 24 hr per ml of culture filtrate at 40 C.

RESULTS

Extracellular C_x and C_1 activity in culture filtrates of the three white-rot fungi was abundant only with cellulosic substrates (Table 1); none or only trace amounts of C_x and C_1 activity were detected in culture filtrates with simple sugars or noncellulosic polysaccharides. C_x and C_1 activity of the white rotters was no greater on hardwood sawdust—intact and modified by ballmilling and removing lignin (holocellulose)—than on softwood sawdust. Growth of the white-rot fungi on bigtooth aspen and Engelmann spruce was increased by ballmilling, and except for *Polyporus versicolor*, C_x and C_1 production was slightly increased as well. Extracellular C_x and C_1 activity in culture filtrate from *Ganoderma applanatum* was higher in cultures containing pine or sweetgum holocellulose than with intact pine or gum sawdust. Enzyme activity by the other two test white-rot fungi in media with pine or gum holocellulose did not differ greatly from that with intact pine or gum sawdust. C_x and C_1 activity in culture filtrates of *P. versicolor* and *Peniophora* "G" was greatest with cellulose as the only source of carbon, and *G. applanatum* produced the most C_1 and C_x activity with pine holocellulose as the only carbon source.

The three brown-rot fungi produced very little or no extracellular C_1 on all the sources of carbon, but produced C_x on most of the sources of carbon. Contrary to the white-rot fungi, the brown-rot fungi produced abundant C_x with simple sugars or with noncellulosic polysaccharides as the sole source of carbon (Table 2). Trace amounts of growth and C_x activity occurred in cultures of *Poria monticola* and *Lentinus lepideus* with cellulose as the only source of carbon. *Lenzites trabea* also grew poorly in cultures containing cellulose, but C_x activity was significant. Just as with the white-rot fungi, C_x activity in the brown-rot cultures with modified or intact sawdust of softwoods did not differ greatly from that in cultures with modified or intact sawdust of hardwoods. C_x activity and growth of the three brown-rot fungi on the wood substrates was greatest with

TABLE 5. *Effect of concentrations of glucose on C_x and C_1 activity*[a, b] *by* Polyporus versicolor (*P.v.*) *and* Poria monticola (*P.m.*) *in cellulose medium (.5%)*

Incubation time (days)	C_x and C_1 activity per medium															
	No glucose				0.1% glucose				0.5% glucose				1.0% glucose			
	P.v.		P.m.		P.v.		P.m.		P.v.		P.m.		P.v.		P.m.	
	C_x	C_1	C_x	C_1	C_x	C_1	C_x	C_1	C_x	C_1	C_x	C_1	C_x	C_1	C_x	C_1
7	52	0.80	1	--	36	0.67	53	--	2	--	59	--	--	--	61	--
14	42	0.80	1	--	22	0.67	70	--	1	--	100	--	--	--	88	--
21	50	0.59	2	--	39	0.20	83	--	4	--	80	--	--	--	88	--

a C_x activity expressed as $\frac{10,000}{t}$, where t equals seconds regained for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate; --, negligible activity.

b C_1 activity expressed as micromoles of glucose released from microcrystalline cellulose/24 hr/ml of culture filtrate; --, negligible activity.

the ballmilled bigtooth aspen and Engelmann spruce as the carbon source. Growth and C_x activity of the brown-rot fungi in media with pine or gum holocellulose generally differed very little from that with intact pine or gum sawdust. C_x activity was greatest with cellobiose as the source of carbon for *Poria monticola*; with ballmilled aspen for *Lentinus lepideus*; and with CMC for *Lenzites trabea*.

Both glucose (0.5%) and cellobiose (0.5%) in cellulose medium greatly inhibited the formation of extracellular C_x and C_1 by the white-rot fungi (Table 3). With the exception of *Ganoderma applanatum* on cellulose medium with yeast extract, the synthesis of cellulase by the three white-rot fungi was increased by adding 0.5% yeast extract, peptone, or asparagine to the cellulose medium.

The three brown-rot fungi produced abundant extracellular C_x in the cellulose medium supplemented with glucose (0.5%) or cellobiose (0.5%) (Table 4), but as before, no C_1. Culture filtrates of *Lenzites trabea* had abundant C_x activity in the cellulose medium supplemented with 0.5% asparagine, peptone, and yeast extract. The addition of these substances to the cellulose medium, however, resulted in only trace amounts of C_x activity in culture filtrates of *Lentinus lepideus* and *Poria monticola*.

The various sugars incubated with culture filtrate of *P. versicolor* had no effect on C_x activity. The sugars, then, did not inactivate C_x of *Polyporus versicolor in vitro*.

Extracellular C_x and C_1 activity of *Polyporus versicolor* was markedly reduced after 7, 14, and 21 days of growth if 0.5 or 1.0% glucose was added to the cellulose medium; even 0.1% glucose caused a decrease in enzyme activity values compared with those for cellulose alone (Table 5). When 0.5 or 1.0% glucose was added to the cellulose-containing medium after 7 days' growth, cellulase activity by *P. versicolor* after 21 days was markedly reduced (Table 6).

No extracellular C_1 activity by *Poria monticola* was detected in any of the media after 7, 14 and 21 days of growth (Table 5). In contrast to *P. versicolor, P. monticola* did not form C_x on the cellulose medium, but only in the cellulose medium containing glucose (0.1 to 1%).

Mycelial suspensions of *Poria monticola* and *Polyporus versicolor* prepared from mycelial mats after filtration had only about 0.5% of the culture filtrate C_x activity. Assay of the liquid and the mycelium

TABLE 6. *Effect of adding glucose after 7 days' growth in cellulose containing medium on cellulase activity (C_x and C_1) of* Polyporus versicolor

Percent glucose added after 7 days	C_x and C_1 activity[a,b] at: 7 days		21 days	
	C_x	C_1	C_x	C_1
0	50	0.61	50	0.59
0.1	-	-	40	0.72
0.5	-	-	1	0
1.0	-	-	1	0

a C_x activity expressed as $\frac{10,000}{t}$, where t equals seconds required for the relative viscosity of the carboxymethylcellulose reaction mixture to be reduced by 50%/ml of culture filtrate.

b C_1 activity expressed as micromoles of glucose released from microcrystalline cellulose/ 24 hr/ml of culture filtrate.

after being separated by centrifugation showed that essentially all of the C_x activity was in the liquid portion of the suspension. No significant C_1 activity was detected in the mycelial suspension of *P. monticola*. The mycelial suspension of *P. versicolor* contained about 20% of the culture filtrate C_1 activity, all in the liquid portion of the suspension.

DISCUSSION

Extracellular cellulase production by three white-rot fungi and by three brown-rot fungi was affected differently by carbohydrates present in liquid culture media. The three white-rot fungi produce a cellulase complex capable of degrading both soluble cellulose (C_x) and microcrystalline cellulose (C_1). These enzymes were produced by the white rotters only if the culture medium contained cellulose. Thus the cellulase system of the white rotters, as in most microorganisms, is an adaptive, or an inductive system. C_x and C_1 activity by the white rotters was repressed when the cellulose-salts medium was supplemented with glucose or cellobiose. Incubation of *Polyporus versicolor* filtrates with various sugars did not affect C_x activity. Thus the effect of the sugars is on production and not inactivation. Glucose added to cellulose-containing medium after 7 days'growth by *Polyporus versicolor* evidently stopped production of further cellulase and that already formed must have been degraded since cellulase activity in filtrates after 21 days was substantially reduced. This type of repression by simple sugars is a commonly observed phenomenon among microorganisms (Jensen 1971; Johansson 1966; Mandels and Weber 1969).

Increased activity in filtrates from cellulose-containing medium supplemented with peptone, asparagine, or yeast extract can probably be attributed to the increased fungal growth after addition of these compounds to the cellulose medium. The favorable effect of yeast extract on cellulase production by white-rot fungi was also observed by Johansson (1966). However, with the white-rot fungi, growth-supporting and inducing abilities of the carbon source generally were unrelated.

On all sources of carbon, the three brown-rot fungi produced either no C_1 or barely detectable quantities. Contrary to the white-rot fungi, C_x of the brown rotters apparently is constitutive since activity was generally abundant in cultures with simple sugars or with noncellulosic polysaccharides as the sole source of carbon. The isolate of *Lenzites trabea* was the most adaptable of the fungi studied; it could secrete C_x regardless of the carbon source. *Lentinus lepideus* and *Poria monticola* had trace amounts of growth, and no C_x activities were detected in cultures with cellulose as the only source of carbon. Significant C_x activity was detected in filtrates of these fungi from cellulose medium only when the medium contained glucose or cellobiose; low activity was detected in filtrates from cellulose medium supplemented with asparagine, yeast extract, and peptone. C_x activity may have been low because growth was still relatively low in the cellulose medium containing these substances. Abundant C_x activity by *Poria monticola* and *Lentinus lepideus* was usually coupled with substantial growth.

There was no relationship between the amount of extracellular cellulase activity in culture filtrates of white-rot and brown-

rot fungi and the nature (hardwood and softwood) of the woody substrate used as sole source of carbon. Thus preferential formation of cellulase apparently does not contribute to the predominant attack of white rotters and brown rotters on hardwoods and softwoods, respectively.

Growth of both white- and brown-rot fungi was greater with ballmilled wood than with intact wood or holocellulose as the source of carbon. However, C_x and C_1 activity of the white rotters was increased only slightly or not at all in filtrates from ballmilled wood, whereas C_x activity by the brown rotters was greatest on the ballmilled wood substrates. Growth and C_x production by the brown-rot fungi were generally related; thus increased growth may account for the increased C_x activity in filtrates of brown-rot fungi from ballmilled wood.

The results of this study suggest that treating wood with nonmetabolizable compounds related to glucose might effectively reduce white rot, but since glucose did not repress cellulase of the brown rotters, the compounds would not be effective against brown rot.

During decay the cellulases of brown-rot fungi, exemplified by *Poria monticola*, are exposed to a relatively large amount of decomposition products of soluble cellulose, whereas cellulases of white-rot fungi, exemplified by *Polyporus versicolor*, are not (Cowling 1961). Data from this study suggest that simple carbohydrates such as glucose or cellobiose formed during degradation of wood by brown-rot fungi will cause production of greater amounts of cellulase. In white rot the cellulose breakdown products are utilized as they are formed; therefore, the fungi are not exposed to simple carbohydrates that may, as indicated by this work, repress cellulase production.

Inability of culture filtrates of brown-rot fungi to degrade highly ordered cellulose is still unexplained. Some factors that may be responsible for a lack of C_1 in culture filtrates are: (1) Culture conditions did not permit induction, (2) C_1 is bound to the cell surface, (3) inactivation by fungal secretions, (4) C_1 in filtrates is too dilute to produce measurable breakdown products, and (5) C_1 is not produced by brown-rot fungi.

Although a large number of different carbon sources were used, liquid culture conditions cannot duplicate those in nature. Thus the first factor cannot be eliminated. The second factor was investigated by fragmenting mycelia and introducing them directly into the assay medium; no activity was detected. Treating mycelia with acids, by homogenization or by freezing, also did not produce detectable activity (Johansson 1966). C_1 may not have been released by any of these methods, particularly if bound to the fungus mycelium by covalent bonds such as disulfide bonds. Barash and Klein (1969) found that release of polygalacturonase from cells of *Geotrichum candidum* was greatly enhanced by treating with mercaptoethanol, which apparently reduced disulfide bonds and facilitated liberation of the enzyme into the medium. In the case of the third factor, fungi may secrete materials such as polysaccharides and peptides into synthetic media that could complex with the C_1 enzyme and inactivate it. These complexing materials could vary with the medium. For the fourth factor, the C_1 in culture filtrates would probably be much more dilute than during decay when the concentrated enzyme may be secreted directly into the site of hydrolysis. With a concentrated filtrate and a longer incubation period with the cellulose substrate, C_1 activity may have been detected in filtrates of brown-rot fungi.

For the fifth factor there is some evidence that a C_1 stage is not necessary if cellulose has never been dried (remains swollen). Cotton fibers that had never dried were readily hydrolyzed by culture filtrates of various organisms (Marsh and Reese 1963). King (1968) found that never-dried holocellulose was degraded by culture filtrates of the brown-rotter *Coniophora cerebella*; α-cellulose prepared from the holocellulose was much more slowly attacked because the open structure of the never-dried holo-

cellulose evidently did not survive the treatments necessary in preparing α-cellulose. Cellulases that have been isolated thus far are too large to penetrate the fine structure of wood fibers (Cowling and Brown 1969). Thus the brown-rot fungi may not produce a C_1 enzyme *in vitro*, but produce a small nonenzymatic catalyst that swells and opens cellulose so that conventional cellulases can attack it (Cowling and Brown 1969; Koenigs 1972). Koenigs (1972) presents evidence that the swelling factor in the cellulose complex of brown-rot fungi may be a H_2O_2-Fe system. He proposes that the acidic conditions that brown-rot fungi create could solubilize Fe and furnish a favorable pH for the system to operate optimally. Exposure of H_2O_2-Fe-treated dewaxed cotton samples to *Trichoderma viride* cellulase predisposed a portion of the substrate to attack. The effects of H_2O_2-Fe on the predisposition of cellulose and holocellulose to degradation by brown-rot cellulases should be considered in future studies.

REFERENCES

Bailey, P. J., W. Liese, R. Roesch, G. Keilich, and E. G. Afting. 1969. Cellulase (B-1, 4-Glucan, 4-Glucanohydrolase) from wood-degrading fungus *Polyporus schweinitzii* Fr. I. Purification. Biochim. Biophys. Acta 185: 381–391.

Barash, I., and L. Klein. 1969. The surface localization of polygalacturonase in spores of *Geotrichum candidum*. Phytopathology 59: 319–324.

Cowling, E. B. 1961. Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown-rot fungi. USDA For. Serv. Tech. Bull. 1258. 79 pp.

Cowling, E. B., and W. Brown. 1969. Structural features of cellulosic materials in relation to enzymatic hydrolysis. *In* G. J. Hajny and E. T. Reese, eds., Cellulases and their applications. Adv. Chem. Ser. 95:152–187.

Jensen, K. F. 1971. Cellulolytic enzymes of *Stereum gausapatum*. Phytopathology 61: 134–138.

Johansson, M. 1966. A comparison between the cellulolytic activity of white and brown rot fungi. I. The activity on insoluble cellulose. Physiol. Plant. 19:709–722.

Keilich, G., P. J. Bailey, E. G. Afting, and W. Liese. 1969. Cellulase (B-1, 4-Glucan, 4-Glucanohydrolase) from the wood-degrading fungus *Polyporus schweinitzii* Fr. II. Characterization. Biochim. Biophys. Acta 185: 392–401.

King, N. J. 1968. Degradation of holocellulose by an enzyme preparation from a wood-degrading fungus. Nature 218(5147):1173–1174.

Koenigs, J. W. 1972. Effects of hydrogen peroxide on cellulose and on its susceptibility to cellulose. Mater. Org. 7(2):133–147.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193:265–275.

Lumsden, R. D. 1969. *Sclerotinia sclerotinum* infection of bean and the production of cellulase. Phytopathology 59:653–657.

Mandels, M., and J. Weber. 1969. The production of cellulases. *In* G. J. Hajny and E. T. Reese, eds., Cellulases and their applications. Adv. Chem. Ser. 95:391–414.

Marsh, P. B., and E. T. Reese. 1963. Measurement of cellulase. Page 91 *in* E. T. Reese, ed., Advances in enzymic hydrolysis of cellulose and related materials. Pergamon, Oxford.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375–380.

Reese, E. T., and H. S. Levinson. 1952. A comparative study of the breakdown of cellulose by microorganisms. Physiol. Plant. 5: 345–366.

Walch, H., and H. Kühlwein. 1968. Zur Kenntnis der cellulolytischen Aktivatät in der Gattung *Ganoderma* (Lackporlinge). Arch. Mikrobiol. 61:373–380.