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Degradation of Cellulose by Culture Filtrates of *Poria placenta*

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1. Introduction

In wood, brown-rot fungi rapidly depolymerize cellulose; this effect is similar to acid hydrolysis of cellulose (E. B. COWLING, 1961). Fungal degradation of cellulose is usually attributed to synergistic effects of C_1 and C_x enzymes (K.-E. ERIKSSON and B. PETTERSSON, 1972; G. HALLIWELL and M. GRIFFIN, 1973; E. T. REESE, R. G. H. SIN, and H. S. LEVINSON, 1950). The C_x enzyme probably affects the substrate by creating free chains ends that are attacked by an exo-B-1-4-glucan cellobiohydrolase (C_1). White-rot fungi apparently depolymerize cellulose in this manner, although recently it has been suggested that an oxidative enzyme is also involved in the degradation of cellulose by these fungi (K.-E. ERIKSSON, B. PETTERSSON, and U. WESTERMARK, 1974).

Cellulase preparations from brown-rot fungi generally show no C_1 activity, but C_x is produced on media with simple sugars (T. L. HIGHLEY, 1973). Many brown-rot fungi exhibit sparse growth and low cellulolytic activity on isolated cellulose (T. L. HIGHLEY, 1973, 1975; T. NILSSON, 1974). The catalyst produced by brown-rot fungi to depolymerize cellulose in wood must be minute because it evidently penetrates the entire

secondary cell wall structure (E. B. COWLING, 1961). J. W. KOENIGS (1972 a, 1972 b, 1974 a, 1974 b) offers evidence that a small nonprotein system (hydrogen peroxide/iron) may be employed by brown-rot fungi to depolymerize cellulose in wood or to render it more susceptible to conventional cellulases.

The objective of this work was to determine if enzyme preparations can be obtained from cultures of a brown-rot fungus that can depolymerize native or insoluble cellulose and, if not, to determine if a nonprotein system can promote degradation of cellulose by culture filtrates that have C_x activity.

2. Materials and Methods

2.1. Substrates

Degradation of the following types of substrates[1] was investigated:

Cellulose - Avicel (FMC), cotton (purified, Hercules), Solka-floc (SW-40, Brown), filter paper (Schleicher and Schuell), and sodium carboxymethylcellulose (Fisher, purified with a degree of substitution, 0.65 - 0.85).

Holocellulose - Aspen (*Populus grandidentata* Michx. and hemlock (*Tsuga heterophylla* (Raf.) Sarg.).

Wood - Aspen, spruce (*Picea engelmannii* (Parry)) and hemlock sawdust, and ballmilled aspen and spruce sawdust (donated by John Obst, For. Prod. Lab.).

2.2. Chemical treatments of substrates

To determine if chemical treatment of cellulose would increase susceptibility to attack by enzyme solutions of a brown-rot fungus, the cellulose substrates were treated with the following chemical reagents: 0.44 mmol $FeSO_4 \cdot 7H_2O$ + 0.3 % H_2O_2 (J. W. KOENIGS, 1972 a); saturated oxalic acid for 3 days on a shaker; mercerization with 25 % NaOH for 3 h followed by dilution with 2% NaOH, and neutralization with acetic acid; and swelling with H_3PO_4 for 2 h (C. S. WALLSETH, 1952). Hemlock holocellulose was treated with both the H_2O_2/Fe and NaOH; the hemlock sawdust, with the H_2O_2/Fe.

2.3. Culture procedures

The brown-rot fungus *Poria placenta* (Fr.) Cke. (Madison 698) was used in this work because of the information available on its activities in degrading wood.

Liquid and nonliquid cultures were used for cellulase induction.

1. Liquid. - The fungus was grown in stationary liquid culture with a carbon source and a basal salts solution (T. L. HIGHLEY, 1973). Carbon sources (0.5%) were the following: Ballmilled aspen and spruce sawdust;

[1] Trade or proprietary names are included for informational purposes only and do not imply any endorsement by the Forest Service of the U.S. Department of Agriculture.

hemlock holocellulose; Avicel; Solka-floc; cotton; filter paper; carboxymethylcellulose; galactomannan (Sigma); xylan (Difco); cellobiose (Difco); glucose (Fisher); and various combinations of them. The chemically treated celluloses also were used as carbon sources. Unless indicated otherwise, flasks containing 25 ml of media were inoculated with 1 ml of mycelial suspension. Flasks were incubated without light for 21 days. Mycelial mats were separated by filtration with suction through a glass fiber filter; the filtrate passed through a Millipore filter (0.45 μ), and toluene (1 ml/l) was added to prevent contamination.

Copper number (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1968 a), carboxyl Content (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1968 b), and degree of polymerization (DP) (E. B. COWLING, 1960) measurements were made on residual cellulose in cultures to determine if *P. placenta* had depolymerized or oxidized the cellulose.

2. *Nonliquid.* - Cotton and sweetguin (*Liquidambar styraciflua* L.) blocks (0.3 cm × 2.9 cm × 3.5 cm) were degraded by P. placenta by the soil-block method of testing decay resistance (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 1971). For enzyme preparations, a Waring Blendor was used to homogenize the material with an extraction medium. After holding 1 h at 4° C, the homogenate was squeezed through four layers of cheesecloth. The residue was separated by filtration with suction through glass fiber filter, the filtrate passed through a Millipore filter (0.45 μ), and toluene (1 ml/l) was added. Extraction media were the following: 0.1 M EDTA (ethylenediamine-tetra-acetate (Harleco)); 0.2 M 2-mercaptoethanol (Eastman); 10 mmol ascorbic acid; 10 mmol glutathione (NBC); 10 % polyvinylpyrolidone (K-90, NBC); 2 mmol sodium metabisulfite (NBC); 0.1 % lysozyme (NBC): 0.1% bovine albumin (NBC); 1 % Triton X-100 (NBC); 1 % Tween-80 (NBC); 0.5 M sucrose (Fisher); 3 - 10 % NaCl; phosphate; McIlvaine and acetate buffers (pH 2.0 - 7.6); distilled H_2O; and various combinations of the extracting solutions.

2.4. Release of bound enzymes

To release enzymes that might be bound to mycelium or substrate, the residues from both the liquid and the nonliquid cultures were fragmented in a Waring Blendor with various combinations of the following: 0.1 M EDTA; 0.2 M 2-mercaptoethanol; 0.1 % lysozyme; 0.1 % bovine albumin; 1% Triton X-100; 1% Tween-80; 0.5 M sucrose; 3 - 10% NaCl; phosphate and acetate buffers (pH 5.0 - 7.6); and distilled H_2O. The suspensions were held for 24 h at 4° C, then squeezed through cheesecloth, and filtered through glass fiber filters. Enzyme activity remaining in the residue was determined by placing the residue directly into the assay medium after treating with 1% thimerosal to kill the mycelium.

2.5. Measurement of substrate degradation by filtrates

For enzyme assays 2 ml of filtrate were incubated with 1 ml of 0.1 M acetate buffer, pH 5.0, and 10 mg of substrate for 24 h. The following two methods were used to measure degradation of substrate:

1. Reducing groups. - Increase in reducing groups was measured by Nelson's modification of the Somogyi method (N. NELSON, 1944).

2. Change in degree of depolymerization. - Change in DP of cellulosic substrates after incubation with enzyme preparations was determined with cupriethylendiamine hydroxide (E. B. COWLING, 1960).

3. Results

3.1. Liquid culture filtrates

Various carbon sources had little effect as inducers of enzyme activities in culture filtrates of P. placenta. Activities of filtrates from typical liquid cultures, indicated by an increase in reducing groups, are shown in Table 1. Filtrates released insignificant reducing groups from cotton, Avicel, filter paper, and sawdust. A slight increase of reducing groups was detected from Solka-floc, but this was attributed to a xylan impurity in the Solka-floc. Only the filtrates from cultures containing ballmilled aspen and holocellulose released measurable reducing groups from holocellulose. Degree of polymerization measurements also indicated no degradation of Avicel, cotton, filter paper, Solka-floc, and holocellulose by filtrates.

Fitlrates from cultures inoculated with a sweetgum plug (10 mm) decayed by *P. placenta* also failed to significantly degrade the various cellulosic substrates.

Of the chemically treated insoluble celluloses, filtrates of P. placenta were most active on cellulose swollen in H_3PO_4, indicated by release of reducing groups (Table 1). However, activity on the cellulose swollen with H_3PO_4 was considerably less than that on carboxymethylcellulose. Some filtrates released reducing groups from mercerized cellulose that had not been dried. None of the filtrates attacked dried mercerized cellulose, but all of them attacked dried mercerized holocellulose. Ball-milling substantially increased susceptibility of wood to enzymatic attack by culture filtrates.

The culture filtrates had little effect on DP of any of the treated celluloses or holocelluloses. Chromatographic analysis of the breakdown product of cellulose treated with H_3PO_4 showed predominantly glucose with traces of cellobiose. Thus the cellulase in filtrates of P. placenta from liquid culture apparently is an exoglucanase.

Treating the residual mycelium from culture filtrates with various chemical reagents to release possible bound enzyme did not increase activity on the insoluble celluloses or did placing nonliving mycelium in contact with the substrates.

Degree of polymerization of residual cellulose from cultures of P. placenta was no different than that of unexposed cellulose. The copper number and ion exchange capacity of the residual cellulose in cultures

also was unchanged indicating that *P. placenta* had not oxidized the cellulose.

A high concentration of enzyme might have to be produced by the fungus on the surface of the substrate to depolymerize cellulose and our filtrates might have been too dilute. To examine this possibility, 500 ml of dialyzed and undialyzed filtrate from five liquid cultures (Table 1) were concentrated to 10 ml both *in vacuo* and by ultrafiltration through a PM-10 Amicon filter. Assays of the concentrate at pH's 2 through 7 for up to 5 days revealed no significant increase in the amount of degradation of insoluble cellulose substrates as analyzed by increases in reducing groups and change in DP.

The culture filtrate of *P. placenta* may contain inhibitors to C_1 activity. These could be materials in the synthetic medium, breakdown products, or materials such as polysaccharides and peptides that could complex with C_1 and inactivate it. Removal of some of these impurities by fractional precipitation with $(NH_4)_2SO_4$ and ethanol and by dialysis failed to increase activity toward the insoluble celluloses. To obtain further information on possible inhibitors in the culture filtrate, culture filtrate of *P. placenta* was incubated with filtrate of *Coriolus versicolor* (L. ex Fr.) Quél. with C_1 activity, after which C_1 activity was determined. The filtrate of *P. placenta* did not affect C_1 activity in *C. versicolor* filtrate.

3.2. Nonliquid culture filtrates

Filtrates prepared from wood with brown rot had very little effect on reducing groups released from insoluble celluloses. However, when the extract was precipitated in 4 volumes of ethanol and dialyzed, activity was substantially increased. This suggested that the crude extract might contain inhibitors to C_1 activity. To verify this, crude extract was prepared by homogenizing two blocks of decayed wood in 0.1 M acetate buffer and incubating the extract with the partially purified enzyme solution from *P. placenta.* Enzyme activity was inhibited by 38% when 0.5 ml of crude enzyme was incubated with 2 ml of enzyme solution; C_1 activity was completely inhibited by 1 ml of crude extract. Inhibitors in the extract, however, did not affect C_1 activity in *C. versicolor* filtrates.

The enzyme solutions prepared with the various extraction media did not significantly increase activity more than did extraction with 0.1 M acetate buffer, pH 5. Residue from the enzyme solutions was also incubated with the extraction media to elute any enzyme adhering to the residue. Generally about one-half of the enzyme activity remained with the residue. Elution of the residue with water was about as satis-

Table 1. Capacity of filtrates from *Poria placenta* to degrade cellulosic substrates

Substrate for enzyme assay		Enzyme activity[a] in filtrates from						
		Liquid cultures[b]					Nonliquid cultures	
		Ballmilled aspen sawdust (0.5 %) and glucose (0.1 %)	Hemlock holocellulose (0.5 %) and glucose (0.1 %)	Avicel (0.5 %) and glucose (0.1 %)	Avicel (0.5 %) and cellobiose (0.1 %)	Avicel (0.5 %) and mannan (0.1 %)	Wood[c]	Cellulose[d]
Cellulose	Avicel	0	0	0	0	0	0.33	0.27
	Cotton	0	0	0	0	0	.33	.14
	Filter paper	0	0	0	0	0	.44	.19
	Solka-floc	0.16	0.30	0.16	0.19	0.19	.61	.42
	Carboxymethylcellulose	2.36	3.02	2.6	3.05	3.22	2.14	2.27
	H_2O_2/Fe Avicel	.27	.36	.19	.27	.41	1.11	.38
	H_2O_2/Fe cotton	0	0	0	0	0	.44	.19
	Never-dried H_2O_2/Fe cotton	0	0	0	0	0	.44	.19
	H_2O_2/Fe filter paper	0	0	0	0	0	.75	.33
	H_2O_2/Fe Solka-floc	.25	.27	.30	.27	.19	.27	1.0

	H_3PO_4 cotton	.36	.75	.77	.65	.58	1.47	.88
	Never-dried H_3PO_4 cotton	.47	.86	.77	.65	.58	1.50	1.61
	Mercerized cotton	0	0	0	0	0	.69	.14
	Never-dried mercerized cotton	.19	.42	.44	.44	.38	1.38	.66
	Oxalic acid cotton	0	0	0	0	0	.33	.14
Holo-cellu-lose	Aspen	0.58	0.52	0	0	0	0.47	0
	Hemlock	.38	.44	0	0	0	.44	0
	Mercerized hemlock	.58	.63	0.83	0.58	0.58	1.16	0.41
	H_2O_2/Fe hemlock	.22	.50	.36	.19	.19	1.27	.31
Wood	Aspen sawdust	0	0	0	0	0	0.38	0
	Spruce sawdust	0	0	0	0	0	.30	0
	H_2O_2/Fe hemlock sawdust	0	0	0	0	0	.65	0
	Ballmilled aspen	.58	1.33	0.58	0.50	1.0	.50	0.83
	Ballmilled spruce	.66	1.11	.83	1.58	1.58	.58	2.02

a) Expressed as micromoles of reducing groups (as glucose) released in 24 h from 10 mg of substrate. — b) Two ml of filtrate + 1 ml of 0.1 M acetate buffer, pH 5.0. — c) Approximately 16 g of decayed sweetgum (40 - 50 % weight loss) extracted in 100 ml of 0.1 M acetate buffer, pH 5,0. Precipitated in ethanol and dialyzed. Final volume 40 ml. Two ml of filtrate + 1 ml of 0.1 M acetate buffer, pH 5.0 used for assay. — d) Approximately 8 g of decayed cotton (40 - 50 % weight loss) extracted in 100 ml of 0.1 M acetate buffer, pH 5.0. Precipitated in ethanol and dialyzed. Final volume 40 ml: Two ml of filtrate + 1 ml of 0.1 M acetate buffer, pH 5.0 used for assay.

factory as any of the materials, indicating that the enzyme removed was loosely bound.

Table 1 shows release of reducing groups from the various untreated and treated substrates by the ethanol-precipitated and dialyzed enzyme preparations from decayed wood. Release of reducing groups from the untreated celluloses was still relatively weak compared to that obtained with filtrates from liquid cultures of white-rot fungi. For example, unconcentrated filtrates from the white-rot fungus *Coriolus versicolor* released from Avicel about 1 μm of glucose/24 h/ml of filtrate (T. L. HIGHLEY, 1973), whereas in this work filtrates of *P. placenta* from the decayed wood released only 0.33 μm of glucose/24 h/2 ml of filtrate.

Of the chemical treatments, phosphoric acid most effectively enhanced susceptibility of cellulose to attack by the enzyme preparation from wood. Contrary to the results with filtrates from liquid culture, H_2O_2/Fe treatment increased susceptibility of some celluloses to degradation. Treatment of the holocellulose with H_2O_2/Fe and with NaOH enhanced its degradation. Ballmilling enhanced wood degradation as did the H_2O_2/Fe.

The enzyme solution from wood was assayed at different pH's (2 through 7) with H_3PO_4 swollen cotton as the substrate; pH5 was found the optimum. Assays from 24 to 72 h showed most of the reducing groups were released within 24 h. Chromatography of reaction products with H_3PO_4 swollen cotton yielded only one spot, glucose, indicating endwise cleavage by the enzyme preparation.

Table 2 shows the effects of crude and dialyzed filtrates of decayed sweetgum on DP of cotton cellulose. The filtrates from decayed wood lowered the DP of cellulose; the nonheated crude filtrates were the most active. There was some lowering of DP with the heated filtrate, which suggests some nonenzymatic attack or heat-stable enzyme activity. The crude filtrate was more active than was the dialyzed suggesting an active component was removed during dialysis. pH had little effect on the degradation of cellulose by filtrates. Iron alone lowered DP, but did not enhance degradation with filtrate added.

Filtrates prepared from decayed cotton released some reducing groups from most substrates (Table 1). As with preparations from decayed wood, the extraction medium had little effect on the activity in the filtrate. Again cellulose swollen in H_3PO_4 was the most readily attacked of the chemically treated celluloses. None of the enzyme releasing agents was significantly more effective than water in releasing C_1 activity from the residue; about one-half of the activity remained in the residue.

Table 2. Effect of filtrates from Poria placenta decayed sweetgum[a] on degree of polymerization (DP) of cotton cellulose[b]

Assay conditions	Crude			Dialysed		
	Heated	Non-heated	No enzyme	Heated	Non-heated	No enzyme
pH 3	1848	1102	2284	—	—	—
pH 3 + 0.44 mmol Fe	1498	1230	1520	—	—	—
pH 4	1728	1204	1630	—	—	—
pH 4 + 1.44 mmol Fe	1728	1204	1630	—	—	—
pH 5	1812	1258	2284	2156	1590	2252
pH 5 + 0.44 mmol Fe	1752	1132	1740	1858	1512	1952
pH 5 + 1 % glucose oxidase	—	—	—	2316	2078	2400
pH 5 + 1 % glucose oxidase + 0.5 glucose	—	—	—	2012	1740	1930

a) Approximately 16 g of decayed sweetgum (40 - 50 % weight loss) extracted in 100 ml of 0.1 M acetate buffer pH 5.0. Concentrated to 10 ml. Two ml of filtrate + 1 ml of 0.1 M acetate buffer used for assay.
b) DP determined after 72-h incubation with filtrate.

4. Discussion

In this investigation, efforts were made to obtain enzyme preparations from liquid cultures of the brown-rot fungus *P. placenta* and from wood and cellulose decayed by this fungus that could significantly degrade highly ordered types of cellulose. The effect of different induction conditions, enzyme releasing agents, antioxidants, concentration of filtrates, assay conditions, and chemical pretreatments of cellulose were considered.

Enzyme preparations from liquid cultures of *P. placenta* ineffectively degraded insoluble celluloses such as Avicel and cotton. This is in accord with other workers who also failed to find significant degradation of insoluble cellulose by filtrates in different kinds of incubation experiments with concentrated culture filtrates and cell extracts (J. GOKSØYR et al., 1975).

Filtrates from wood and cellulose decayed by a brown-rot fungus caused weak attack on insoluble cellulose. The attack, however, did not occur in the same manner as that by brown-rot fungi on wood or isolated celluloses. The enzyme preparations had little effect on DP of cellulose; glucose was the main degradation product, whereas brown-rot fungi in wood rapidly decrease DP of cellulose (E. B. COWLING, 1961).

This effect of the enzyme preparations from decayed wood and cellulose on cellulose is similar to that produced by C_1 of white rot and other fungi (K.-E. ERIKSSON and B. PETTERSSON, 1972; G. HALLIWELL and M. GRIFFIN, 1973; T. L. HIGHLEY, 1973).

Swelling cellulose in NaOH or acid generally will render cellulose susceptible to attack by cellulase preparations that show weak activity toward insoluble celluloses. Susceptibility is increased because of increased accessibility of the cellulose. Likewise, oxidation reactions such as with H_2O_2 open the structure of cellulose crystallites. G. HALLIWELL (1961), for example, found the culture filtrate of *Myrothecium verrucaria* produced only about 3% weight loss in cotton, but when swollen in NaOH, 30% of the weight of cotton was rapidly lost. Therefore, K. SELBY (1963) suggested that an enzyme or enzyme system must be missing from the extracellular extracts as normally prepared. The enzyme or enzyme system is near a growing fungus and is necessary for the breakdown of native fibrous cellulose but not necessary for the breakdown of swollen cellulose.

In this investigation, chemical pretreatments of cellulose with NaOH, H_2O_2/Fe, and oxalic acid generally did not increase susceptibility of cellulose to attack by filtrates from liquid culture or from decayed wood and cellulose. Swelling in H_3PO_4 increased susceptibility as, in some cases, did never-dried alkali-swollen cellulose, but attack was weak compared with that of carboxymethylcellulose. J. W. KOENIGS (1972 a) reported increased susceptibility of cellulose treated with H_2O_2/Fe to degradation by *Trichoderma viride* cellulase, but this cellulase complex contains a very active C_1.

Inability to obtain a filtrate from *P. placenta* cultures and other brown-rot fungi more active on insoluble cellulose could be due to (1) instability or loss of essential enzyme components during isolation, (2) surface topology (configuration and arrangement of molecules on a surface), and (3) the traditional C_1-C_x concept not being applicable to brown-rot fungi. The first explanation is not probable because the enzymes depolymerizing native celluloses must operate outside the cell; therefore, they must be stable (J. GOKSØYR et al., 1975). In addition, in this work, nonliving mycelium added to filtrates was no more active than culture filtrates. Thus, inactivation by solubilization in the culture media is not likely a factor in the lack of C_1 activity in culture filtrates of *P. placenta*.

B. HOFSTEN and B. BERG (1972) have used surface topology to explain how bacterial enzymes in vivo can attack cellulose fibers, whereas isolated enzymes have little ability to attack. They presented evidence that fibers are degraded by cell-bound cellulase and not extracellular

enzymes. The reason suggested is that enzymes may be more effective when cell-bound because of their high concentration and favorable alinement on the cell surface. It is doubtful that the brown-rot fungi employ this kind of mechanism because the catalyst causing decomposition evidently diffuses from the mycelium and penetrates the entire secondary cell wall. It is possible, however, that mycelium might have to be near the cell wall to produce high concentrations of a material that loosens the structure making it susceptible to attack by the typical cellulases. Microscopic observation of wood degraded by the same brown-rot fungus used in this study indicated that at least one hyphal strand was in the lumen of each degraded wood cell (W. WILCOX, 1968).

The evidence suggests that brown-rot fungi employ a mechanism of cellulose degradation different from typical cellulose-degrading fungi because no known enzyme system reproduces the effects of these fungi in lignified wood - a rapid decrease of DP at a low weight loss. In addition, the catalyst must be small enough to penetrate the entire secondary cell wall. No cellulase has been isolated small enough to accomplish this.

J. W. KOENIGS offers convincing evidence that the mechanism of the brown-rot fungi is via an H_2O_2/Fe system. He found that brown-rot fungi produce sufficient H_2O_2 in wood to degrade cellulose. In addition, the effects of H_2O_2/Fe on cellulose mimic those of brown-rot fungi (J. W. KOENIGS, 1972 a, 1974 a, b). The hydrogen peroxide molecule would be small enough to penetrate the microstructure of the secondary cell wall, attack the cellulose, and perhaps predispose the cellulose to degradation by conventional cellulases. This kind of mechanism would be oxidative (J. W. KOENIGS, 1974 a). T. L. HIGHLEY (1977) and others (E. C. BARTON-WRIGHT and J. G. BOSWELL, 1931; J. G. BOSWELL, 1938; M. W. BRAY and T.M. ANDREWS, 1924) present evidence that brown-rot fungi do oxidize cellulose.

In the investigation reported here, the H_2O_2/Fe treated celluloses were generally not more susceptible to degradation by enzyme preparations from P. placenta than were the untreated celluloses. The ratio of H_2O_2 to iron and the pH determine the products of cellulose degradation. Thus other treatment combinations may give products more susceptible to degradation by enzyme preparations from *P. placenta.*

Poria placenta did not break down or oxidize cellulose in liquid culture with glucose present. Brown-rot fungi are reported to produce H_2O_2 from glucose and several other carbohydrates (J. W. KOENIGS, 1972 b, 1974 b). Why then was cellulose not affected by P. placenta in liquid culture? Possibly H_2O_2 concentration was too low in liquid culture to degrade cellulose or it was present for only a short time because of

destruction by enzymes such as catalase or peroxidase. In wood, H_2O_2 might be expected to diffuse more rapidly than enzymes that destroy the H_2O_2, and the chemical would be free to react chemically before it was destroyed (J. W. KOENIGS, 1972 b).

In conclusion, failure to obtain enzyme preparations from cultures of the brown-rot fungus *P. placenta* capable of reproducing the effects of brown-rot fungi on cellulose in wood is further evidence that at least some brown-rot fungi employ a mechanism of cellulose degradation different from most other types of fungi. The possibility that they employ a nonprotein system such as an H_2O_2/Fe system apparently is most likely. This aspect should be considered in future investigations on the mechanism of degradation by this very important type of fungal decay.

5. Summary

Enzyme preparations from liquid cultures of the brown-rot fungus *Poria* placenta and from wood and cellulose decayed by this fungus only slightly degraded highly ordered types of cellulose. The type of attack differed from that by this fungus on wood or on isolated cellulose. The effect of different induction conditions, enzyme releasing agents, antioxidants, concentration of filtrates, assay conditions, and chemical pretreatments was considered. Chemical treatments that swell cellulose had slight effect on increasing susceptibility to attack by the preparations. Thus this brown-rot fungus apparently employs an alternate mechanism to degrade cellulose, possibly a small nonprotein catalyst.

Zusammenfassung

Abbau von Cellulose durch Kulturfiltrate von *Poria placenta*

Enzympräparate aus Flüssigkulturen des Braunfäulepilzes Poria placenta sowie aus Holz und Cellulose, die von diesem Pilz befallen waren, bauten hochpolymerisierte Cellulose nur schwach ab. Der Cellulose-Angriff unterschied sich von dem durch Pilze im Holz. Der Einfluß verschiedener Induktionsbedinqungen, wie enzymabgebende Mittel, Antioxidationsmittel, Konzentration der Filtrate, Versuchsbedingungen und chemische Vorbehandlungen, wurde untersucht. Eine chemische Behandlung, die zu einem Aufquellen der Cellulose führte, erhöhte die Anfälligkeit durch die Präparate geringfügig. Daher verwendet dieser Braunfäulepilz offenbar einen alternierenden Mechanismus, um Cellulose anzugreifen, wahrscheinlich über einen nicht Protein-haltigen Katalysator kleinerer Molekülgröße.

Résumé

Degradation de la cellulose par des filtrats de culture de *Poria placenta*

Des preparations enzymatiques en provenance de cultures liquides du champignon de pourriture brune *Poria placenta* et à partir du bois et de la cellulose degrades par ce champignon n'attaquent que légèrement les types de cellulose hautement polymerisés. Le type d'attaque est different de celui

du bois. On a considere l'effet de diverses conditions d'induction, les agents de liberation de l'enzyme, les antioxydants, la concentration des filtrates, les conditions d'essai et les pretraitements chimiques. Les traitements chimiques de gonflement de la cellulose ont eu un effet négligeable sur la plus grande sensibilité à l'attaque par les preparations enzymatiques. Donc ce champignon de pourriture brune emploie apparemment un autre mécanisme pour degrader la cellulose, peut être un catalyseur non protéinique.

Resumen

Degradaeión de celulosa mediante filtrados de cultivos de *Poria placenta*

Preparados enzimáticos procedentes de cultivos liquidos de *Poria placenta,* hongo causante de la pudrición parda, asi como de madera y celulosa por éste infestados, sólo produjeron una leve degradación en celulosas altamente polimerizadas. El tipo del ataque a la celulosa resultó distinto del observado en la madera. Se procedió a investigar la influencia de diferentes efectos de inducción, tal como agentes generadores de enzimas, antioxidantes, la concantración de filtrados, las condiciones de ensayo y los tratamientos químicos previos. Tratamientos químicos causantes de un hinchamiento de la celulosa aumentaron ligeramente su susceptibilidad a los preparados. Por lo tanto, este hongo causante de la pudrición parda debe de emplear un mecanismo alterno para la degradación de la celulosa, posiblemente un catalizador no proteínico de reducida magnitud molecular.

References

American Society for Testing and Materials: Standard method of test for copper number of paper and paperboard. ASTM Desig. D 919 - 49. In: Ann. ASTM Standards, Philadelphia, Pa., 1968 a.

— Standard method of test for carboxyl content of cellulose. ASTM Desig. D 1926. In: Ann. ASTM Standards, Philadelphia, Pa., 1968 b.

— Standard method of test for accelerated laboratory test of natural decay resistance of woods. ASTM Desig. D 2017. In: Ann. ASTM Standards, Philadelphia, Pa., 1971.

Barton-Wright, E. C. and J. G. Boswell: LX. The biochemistry of dry-rot in wood. II. An investigation of the products of decay of spruce wood rotted by *Merulius lacrymans.* Biochem. J. *25* (1931) 494 - 506.

Boswell, J. G.: XXX. The biochemistry of dry-rot in wood. III. An investigation of the products of the decay of pine wood rotted by *Merulius lacrymans.* Biochem. J. *32* (1938) 218 - 229.

Bray, M. W. and T. M. Andrews: Chemical changes of groundwood during decay. Ind. Eng. Chem. *16* (1924) 137 - 139.

Cowling, E. B.: Methods for chemical analysis of decayed wood. U. S. Dep. Agric., For. Serv. Rep. FPL 2177. 1960, 24 p.

— Comparative biochemistry of the decay of sweetgum by white-rot and brown-rot fungi. U. S. Dep. Agric., For. Serv. Techn. Bull. 1258. 1961, 79 p.

Eriksson, K.-E. and B. Pettersson: Extracellular enzyme system utilized by the fungus *Chrysosporium lignorum* for the breakdown of cellulose In: A. H. Walters and E. H. Hueck van der Plas (Eds.): Biodeterioration of materials, vol. 2. London (Appl. Sci. Publ. Ltd.) 1972, p. 116 - 120.

ERIKSSON, K.-E., B. PETTERSSON and U. WESTERMARK: Oxidation: An important enzyme reaction in fungal degradation of cellulose. Fed. Eur. Biochem. Soc. Letters *49* (1974) 282 - 288.

GOKSØYR, J., E. GUDMUNN, J. ERIKSEN and K. OSMUNDSVÅG: A comparison of cellulases from different microorganisms. In: M. BAILEY, T.-M. ENERI and M. LINKOY (Eds.), Symposium on enzymatic hydrolysis of cellulose. Helsinki, Techn. Res. Center of Finland. 1975.

HALLIWELL, G.: The action of cellulolytic enzymes from *Myrothecium verrucaria*. Biochem. J. *79* (1961) 185 - 192.

HALLIWELL, G. and M. GRIFFIN: The nature and mode of action of the cellulolytic component C_1 of *Trichoderma koningii* on native cellulose. Biochem. J. *135* (1973) 587 - 594.

HIGHLEY, T. L.: Influence of carbon source on cellulase activity of white-rot and brown-rot fungi. Wood and Fiber *5* (1973) 1, 50 - 58.

— Can wood-rot fungi degrade cellulose without other wood constituents? For. Prod. J. *25* (1975) 7, 38 - 39.

— Requirements for cellulose degradation by a brown-rot fungus. Material u. Organismen *12* (1977) 25 - 36.

HOFSTEN, B. and B. BERG: Cellulase formation in *Cellvibrio fulvus*. Proc. 4th Int. Ferment. Symp., Ferment Technol. Today. 1972, 731 p.

KOENIGS, J. W.: Effects of hydrogen peroxide on cellulose and its susceptibility to cellulase. Material u. Organismen *7* (1972 a) 133 - 147.

— Production of extracellular hydrogen peroxide and peroxidase by wood-rotting fungi. Phytopath. *62* (1972 b) 100 - 110.

— Hydrogen peroxide and iron: A proposed system for decomposition of wood by brown-rot basidiomycetes. Wood and Fiber *6* (1974 a) 1, 66 - 79.

— Production of hydrogen peroxide by wood-decaying fungi in wood and its correlation with weight loss, depolymerization and pH changes. Arch. Mikrobiol. *99* (1974 b) 129 - 145.

NELSON, N.: A photometric adaption of the Somogyi method for the determination of glucose. J. Biol. Chem. *153* (1944) 375 - 380.

NILSSON, T.: Comparative study in the cellulolytic activity of white-rot and brown-rot fungi. Material u. Organismen *9* (1974) 3, 173 - 198.

REESE, E. T., R. G. H. SIN, and H. S. LEVINSON: The biological degradation of soluble cellulose derivatives and the relationship to mechanism of cellulose hydrolysis. J. Bact. *59* (1950) 485 - 497.

SELBY, K.: The effect of cellulolytic enzymes on some properties of cotton fibers. In: E. T. REESE (Ed.): Advances in enzymic hydrolysis of cellulose and related materials. Oxford (Pergamon) 1963.

WALSETH, C. S.: Occurrence of cellulases in enzyme preparations from microorganisms. Tappi *35* (1952) 5, 228 - 233.

WILCOX, W.: Changes in wood microstructure through progressive stages of decay. U. S. Dep. Agric., For. Serv. Res. Pap. FPL 70. 1968, 46 p.

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