APPLIED AND ENVIRONMENTAL MICROBIOLOGY. Dec. 1980, p. 1145–1147 Vol. 40. No. 6
0099-2240/80/12-1145/03$02.00/0

# Cellulose Degradation by Cellulose-Clearing and Non-Cellulose-Clearing Brown-Rot Fungi

**TERRY L. HIGHLEY**

***Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison. Wisconsin 53705***

Cellulose degradation by four cellulose-clearing brown-rot fungi in the *Coniophoraceae*—*Coniophora prasinoides. C. puteana, Leucogyrophana arizonica,* and *L. olivascens*—iscompared with that of a non-cellulose-clearing brown-rot fungus, Poria placenta. The cellulose- and the non-cellulose-clearing brown-rot fungi apparently employ similar mechanisms to depolymerize cellulose; most likely a nonenzymatic mechanism is involved.

There is a definite need for new approaches to protect wood in use from fungal degradation, because increasing environmental concerns are limiting use of hazardous chemicals. External enzymes are the agents of fungal degradation. Thus, it is critical to understand how the enzymes involved in decay operate to develop nontoxic, environmentally acceptable control measures. The cellulolytic system of brown-rot fungi is of particular interest because of the unique manner in which these fungi degrade cellulose in wood—rapid depolymerization at low weight loss. Since no known enzyme system reproduces the effects of these fungi in lignified wood, they possibly employ a nonenzymatic system ($H_2O_2$/Fe?) to degrade cellulose (8).

Most brown-rot fungi exhibit sparse growth on pure cellulose despite their rapid degradation of cellulose in wood (6, 7, 12). Enzyme preparations from these fungi are also inactive on insoluble cellulose (C, activity according to Reese et al. [15]), but are active on soluble celluloses such as carboxymethylcellulose (C. according to Reese et al. [15]). White-rot fungi can degrade pure cellulose, and enzyme preparations from them have the $C_1$-$C_x$ complex (8, 5, 12). Nilsson and Ginns (13) recently reported several brown rotters in the family *Coniophoraceae* to be exceptions because they can degrade (clear) pure swollen cellulose (17) in an agar medium. They propose that this unusual combination of capability to cause a brown rot and to degrade pure cellulose is characteristic of brown-rot species of the family *Coniophoraceae.*

The question arises, do these brown-rot fungi, capable of clearing swollen cellulose in agar, degrade cellulose by a mechanism similar to most other brown-rot fungi that are unable to cause clearing of cellulose media? To gain insight into this question, I compared cellulose degradation by four cellulose-clearing brown-rot fungi in the *Coniophoraceae* with that by *Poria placenta,* a non-cellulose-clearing brown-rot fungus.

In this work, the following areas were investigated: (i) weight loss and change in chain length, or degree of polymerization (DP), of pure cellulose caused by the fungi and (ü) activity of culture filtrates against pure cellulose.

Fungi. The cellulose-clearing brown-rot fungi used were *Coniophora prasinoides* (Bourd. and Galz.) Bourd. and Galz., *C. puteana* (Schum. ex Fr.) Karst., *Leucogyrophana arizonica* (Ginns), *L. olivascens* (Berk. and Curt.) Ginns and Mem.; the non-cellulose-clearing fungus used was *Poria placenta* (Fr.) Cke.

Degradation of cellulose and DP measurements. Degradation of cellulose (purified cotton, Hercules) by the fungi was determined by the American Society for Testing and Materials soil block procedure (1). The percentage of weight loss of cellulose (50 mg) was determined after 4, 8, and 12 weeks by comparing air-dry weights (27°C and 70% relative humidity) before and after exposure of the fungi. Average DP was measured viscometrically (2) with cupriethylenediamine hydroxide solvent (Ecusta Paper Corp.).

Liquid culture and extracellular enzyme preparation. The fungi were grown in stationary liquid culture, pH 5.5, with 25 ml of a basal salts solution (5) and with the following carbon sources: 1% glucose, 1% microcrystalline cellulose (Avicel, FMC), and 0.18 glucose plus Avicel. Flasks were inoculated with 1 ml of washed mycelial suspension and incubated at 27°C without light for 21 days. Mycelium was separated by filtration with suction, and the filtrate was dialyzed for 24 h against distilled water. The dialyzed filtrate was used for enzyme assays.

Enzyme assays. A 1-ml amount of filtrate was incubated for 16 h with 1 ml of 0.1 M citrate buffer (pH 5.0) and 10 mg of substrate at 40°C. Substrates used were Avicel, xylan (Aldrich), Walseth cellulose (phosphoric acid-swollen cellulose [17]), and carboxymethyl cellulose (Hercules). Increase in reducing groups was measured by Nelson's modification of the Somogyi method (10).

Degradation of pure cellulose by the cellulose-clearing brown-rot fungi and the non-cellulose-clearing brown-rot fungus *P. placenta* was similar because both types produced substantial decrease in chain length (DP) while causing only minor weight losses in the cellulose (Table 1). Thus, both types of brown-rot fungi differ from white-rot fungi that cause only a very slow change in DP of cellulose as it is degraded. White-rot fungi produce the conventional $C_1$-$C_x$ complex (4, 5, 12). In addition oxidative enzymes may be involved in cellulose breakdown by white-rot fungi (4).

The cellulose-clearing brown-rot fungi differed from *P. placenta* in their capacity to degrade cellulose over filter paper in soil block testa without an alternate carbon source (Table 1). In earlier work (6, 7) it was found that *P. placenta* could not degrade isolated cellulose in soil block tests over filter paper feeder strips without a "starter sugar," such as glucose. Evidently, the cellulose-clearing fungi do not require a starter sugar to become established on cellulose. However, when established on cellulose, both types of brown-rot fungi apparently employ similar cellulolytic activity on cellulose as indicated by the DP results described.

Extracellular enzyme preparations from the cellulose-clearing fungi were similar to those from the non-cellulose-clearing fungus, *P. placenta* (Table 2). None of the preparations was active on crystalline cellulose (Avicel); thus, there was a lack of the so-called $C_1$ activity. All of the preparations degraded soluble cellulose (carboxymethyl cellulose) and phosphoric acid-swollen cellulose (Walseth). Therefore, the filtrates from both cellulose- and non-cellulose-clearing brown-rot fungi do not contain the necessary component ($C_1$?) that enables them to degrade cellulose in wood. Cellulose must be predisposed by swelling or solubilization before filtrates from the brown-rot fungi will attack it.

Rautela and Cowling (14) propose that dissolution of Walseth cellulose mainly involves $C_x$ enzymes. However, Nilsson (11) discusses several factors that support the necessity of $C_1$ enzymes for degrading Walseth cellulose. Nilsson reasons that $C_x$ enzymes are produced in some brown-rot fungi and in other fungi that cannot clear Walseth cellulose. He thus proposes that if only $C_x$ enzymes are necessary for dissolution of Walseth cellulose, these fungi will have produced clearing of Walseth cellulose. My results support Rautela and Cowling's (14) proposal that degradation of Walseth cellulose involves $C_x$-type enzymes, because both the cellulose- and the non-cellulose-clearing brown-rot fungi produce filtrates active on Walseth and carboxymethyl cellulose but inactive on crystalline cellulose.

Furthermore, degradative studies with isolated cellulose indicate that the cellulose- and the non-cellulose-clearing brown-rot fungi depolymerize cellulose by a similar mechanism that most likely does not involve the conventional $C_1$-$C_x$ type of cellulose breakdown. Thus, both the cellulose- and the non-cellulose-clearing brown-rot fungi probably employ a nonenzymatic mechanism to depolymerize cellulose as originally proposed by Cowling and Brown (3) and later supported by Koenigs (8) and Highley (7).

The inability of some brown-rot fungi to clear swollen cellulose could be caused by the following.

(i) Movement of the cellulose-degrading enzyme through the agar medium is retarded. This could result from the large size of the enzyme or the complexing of the enzyme with the agar. Recent work by me at the Forest Products Laboratory showed the cellulose (carboxymethyl cellulose)-degrading enzyme from *P. placenta* was very large (180,000 daltons). Montenecourt and Eveleigh (9) found that *Trichoderma viride* did not produce clearing zones with acid-swollen cellulose as the substrate, unless Phosfon D was added to the media. The biochemical effect of Phosfon D on the production of cellulose was

TABLE 1. *Weight loss and DP of cellulose exposed to brown-rot fungi in soil block lest*

| Fungus | Cellulose[a] in wood contact for (wk): | | | | | | Cellulose[a] in filter paper contact for (wk): | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | | 8 | | 12 | | 4 | | 8 | | 12 | |
| | Wt loss (%) | DP | Wt loss (%) | DP | Wt loss (%) | DP | Wt loss (%) | DP | Wt loss (%) | DP | Wt loss (%) | DP |
| *C. prasinoides* | 0 | 286 | 24 | 242 | 92 | <196[b] | 10 | 304 | 40 | 242 | 95 | <196[b] |
| *C. puteana* | 10 | 716 | 20 | 322 | 60 | 573 | 10 | 340 | 40 | 180 | 76 | <196[b] |
| *L. arizonica* | 0 | 500 | 16 | 436 | 24 | 399 | 0 | 636 | 16 | 322 | 36 | 216 |
| *L. olivascens* | 0 | 250 | 10 | 212 | 24 | 180 | 0 | 304 | 16 | 304 | 21 | 180 |
| *P. placenta* | 5 | 242 | 20 | 196 | 76 | 170 | 0 | 2,130 | 0 | 2,111 | 0 | 2,111 |

[a] DP of unexposed cellulose, 2,340.

[b] Flow in viscometer too fast to determine depolymerization with concentration of substrate used.

TABLE 2. *Effect of carbon source on cellulase and xylanase activity in filtrates of brown-rot fungi*

| Source of filtrate | | Reducing groups[a] produced by filtrates on cellulose and xylan substrates | | | |
|---|---|---|---|---|---|
| | | Cellulose | | | Xylan |
| Fungus | Carbon source | Avicel | CMC[b] | Walseth | |
| *C. prasinoides* | 1% Avicel | 0 | 2.7 | 0.77 | 1.6 |
| | 1% Avicel + 0.1% glucose | 0 | 2.7 | 0.97 | 2.9 |
| | 1% Glucose | 0 | 1.4 | 0.53 | 2.3 |
| *C. puteana* | 1% Avicel | 0 | 1.9 | 0.58 | 2.1 |
| | 1% Avicel + 0.1% glucose | 0 | 1.6 | 1.1 | 2.3 |
| | 1% Glucose | 0 | 1.6 | 0.80 | 1.8 |
| *L. arizonica* | 1% Avicel | 0 | 1.4 | 1.0 | 3.5 |
| | 1% Avicel + 0.1% glucose | 0 | 1.6 | 0.91 | 2.8 |
| | 1% Glucose | 0 | 1.4 | 0.72 | 3.1 |
| *L. olivascens* | 1% Avicel | 0 | 3.1 | 0.83 | 2.0 |
| | 1% Avicel + 0.1% glucose | 0 | 2.6 | 1.1 | 3.4 |
| | 1% Glucose | 0 | 1.4 | 0.64 | 2.0 |
| *P. placenta* | 1% Avicel | 0 | 1.1 | 0.36 | 2.3 |
| | 1% Avicel + 0.1% glucose | 0 | 1.0 | 0.39 | 3.2 |
| | 1% Glucose | 0 | 1.1 | 0.55 | 2.7 |

[a] Expressed as micromoles of reducing groups (as glucose) released in 16 h from 10 mg of substrate.
[b] CMC, Carboxymethyl cellulose.

not established, but they suggest that Phosfon D either promotes release of cell-bound enzymes or affects enzyme diffusion in agar to permit increased digestion of cellulose.

(ii) Some brown-rot fungi require a starter-sugar to degrade cellulose. A readily metabolizable sugar, such as glucose, was not incorporated into the cellulose-agar medium by Nilsson (12).

(iii) Zones produced by some brown-rot fungi are indistinct. Smith (16) reports that zones produced by cellulolytic fungi in cellulose-agar medium are often indistinct and difficult to identify. He found that using dyed cellulose powder as the substrate greatly facilitated detection of cellulolytic activity. It would be interesting to test some of the non-cellulose-clearing brown-rot fungi with dyed cellulose in the presence of a simple sugar.

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