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IDENTIFICATION OF TERMINAL STRUCTURES IN CELLULOSE DEGRADED BY THE BROWN-ROT FUNGUS POSTIA PLACENTA

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SUMMARY

To gain insight into the biochemical mechanism employed by brown-rot fungi to depolymerize cellulose, we identified the end-groups of chemically pure cellulose that had been depolymerized by the brown-rot fungus, *Postia placenta*. The depolymerized cellulose was acid hydrolyzed and the anion fractions isolated by ion chromatography. Sugar acids were identified by gas chromatographic and mass spectrocopic analysis. Cellulose degraded by Fenton's reagent (H_2O_2/Fe^{2+}) was also analyzed. The two systems generated the same sugar acids but not in the same quantities. The acids identified include glyceric and erythronic, indicating oxidative cleavage of the vicinal diol carbon-carbon bonds within glucosyl residues in the cellulose polymer. Gluconic and arabonic acids were also identified as major products. No uronic acids were produced in either systems.

Key words: Brown-rot, wood decay, cellulose degradation, *Postia placenta*, Fenton's reagant.

INTRODUCTION

In previous work we (Highley, *et. al.*, 1988) established the cultural parameters for cellulose decomposition by various brown-rot fungi and some of the physical and chemical properties of the degraded cellulose. Various culture conditions, such as low nitrogen and elevated O_2 levels, did not induce degradation of cellulose by the brown-rot fungi. Extensive degradation of cellulose occurred only in cellulose exposed over a soil or agar medium. Analysis of the molecular weight distribution of brown-rotted cellulose indicated a fairly tight distribution centered around DP 200. From x-ray diffraction analysis it appeared that there was preferential attack on the smaller crystallites and amorphous regions of the cellulose, yielding

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crystallites similar to these produced on acid hydrolysis. Infrared spectroscopy and carboxyl determinations with methylene blue showed that carboxyl groups were present in the degraded cellulose. Uronic acids were not detected in acid hydrolysates of the brown-rotted cellulose, indicating that oxidation was not at C-6 had not occurred.

To gain further insight into the biochemical mechanism employed by brown-rot fungi to depolymerize cellulose, we identified the end-groups of chemically pure cellulose depolymerized by the brown-rot fungus, Postia placenta.

MATERIAL AND METHODS

Culture Procedures. The brown-rot fungus, Postia placenta (Fr.) M. Lars. et. Lomb. (Mad-698) [syn:Poria placenta (Fr.) Cke.] was used in this study.

Cotton cellulose (Herculus, type A-600) was exposed to P. placenta in chambers (1 liter jars) patterned after the ASTM soil-block procedure (American Society for Testing and Materials, 1971). The cotton was placed in chambers on previously inoculated pine feeder strips (0.3 x 2.9 x 3.5 cm, with the long axis in the grain direction). After incubation, mycelium was separated from residual cellulose, and the cellulose was washed in 0.1N NaOH.

Treatment of Cellulose with Fenton's reagent. Cellulose was treated with ferrous salts in the presence of H_2O_2 (Fenton's reagent) according to Halliwell (1965).

Acid Hydrolysis and Ion Exchange Chromatography. The brown-rotted cellulose was chemically degraded by acid hydrolysis (Effland, 1977) and the anionic fraction isolated by ion exchange chromatography.

Gas Chromatographic and Mass Spectroscopic Analysis of Trimethysilyl Ethers. Sugar acids in the anionic fraction were identified by conversion to their silyl ethers by the method of Hyppanen, et. al., 1983, and comparison with authentic compounds by gas chromatographic and mass spectroscopic analysis.

RESULTS AND DISCUSSION

Four major acids were identified by GC mass spectroscopic analysis of the acid hydrolsates of brown-rotted cellulose (figure 1): glyceric, erythronic, gluconic and arabonic acids. No uronic acid was produced. The presence of glyceric and erythronic acids indicates oxidative cleavage of the vicinal diol carbon-carbon bond within glucosyl residues. Reaction of cellulose with Fenton's reagent (H_2O_2/Fe^{2+}) generated the same sugar acids but not in the same amounts.

Quantitation of the acids was difficult because of the low yields (0.5% of the degraded cellulose). We may be able to circumvent this problem by degrading the depolymerized cellulose with commercial preparations of cellulase, followed by quantitation with gas chromatography.

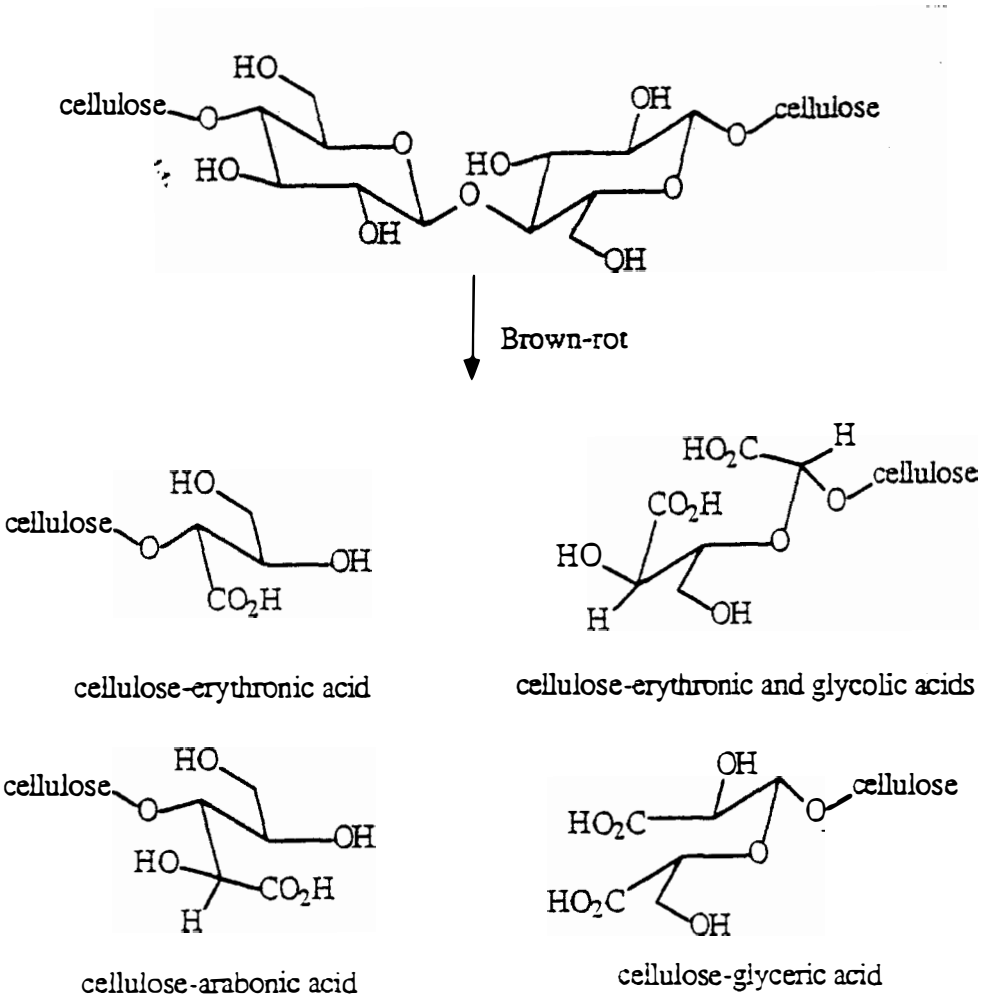

Figure 1. Proposed structure of oxidized cellulose.

Although in this work we did not identify the degrading agent, knowing the cellulose breakdown products produced by brown-rot fungi should permit us to mimic the brown-rot system in vitro which should facilitate identification of the agent. Our working hypothesis is that the initial depolymerization of cellulose is caused by a small diffusible agent. This hypothesis is based on measurements of pore size in uninfected wood and pulp (Stone and Scallan, 1965, 1968; Kerr and Goring, 1975) and size estimates of cellulolytic enzymes (Cowling, 1961; Cowling and Brown, 1969) and diffusion properties of cellulase. Cellulases isolated thus far are too large to penetrate the microstructive of cellulose, and therefore cellulolytic action is restricted to larger capillaries. Furthermore, a strong affirmity exists between enzyme and substrate which would retard diffusion of cellulolytic enzymes. Thus, based on our findings and the model studies of Halliwell (1965) and Koenigs (1972), it is clear that the mechanism employed by brown-rot fungi to breakdown cellulose is (1) oxidative in nature, (2) the type of oxidation that occurs suggests involvement of a transition metal, and (3) because of the slight modification of lignin and the absence of uronic acid residues in degraded cellulose, the agent apparently is fairly selective in its attack.

When the nature of the degrading system is established the information could lead to the identification of potential inhibitors of the system that would thwart brown-rot decomposition of wood.

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