

Evaluating Isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* for Use in Biological Pulping Processes

Robert A. Blanchette¹, Todd A. Burnes¹, Marjorie M. Eerdmans¹ and Masood Akhtar²

¹Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, U.S.A.

²University of Wisconsin Biotechnology Center, Madison, WI 53705, U.S.A.

Keywords

Biodegradation
White rot
Delignification
Biotechnology
Wood decay
Lignin
Cellulose
Biopulping
Extracellular enzymes
Polyacrylamide gel electrophoresis (PAGE)
Phanerochaete chrysosporium
Ceriporiopsis subvermispora

Summary

Decay of birch (*Betula papyrifera*), aspen (*Populus tremuloides*) and loblolly pine (*Pinus taeda*) wood by 19 isolates of *Phanerochaete chrysosporium* showed considerable variation in percent weight loss and loss of lignin and wood sugars. Birch and aspen woods are degraded to a greater extent than loblolly pine wood. Among the isolates tested, a great deal of variation was observed in their ability to preferentially degrade lignin. Isolate BKM-F-1767 resulted in the greatest loss of lignin on the deciduous woods tested. Many isolates of *P. chrysosporium* removed all cell wall components causing a nonselective type of white rot. In contrast, nine isolates of *Ceriporiopsis subvermispora* caused moderate weight losses and preferential degradation of lignin in aspen, birch and loblolly pine wood. Less variation among isolates was observed in the cell wall components removed from all woods tested. Lignin losses ranged from 19 to 38% in loblolly pine and 50-80% in aspen and birch wood. Polyacrylamide gel electrophoresis (SDS-PAGE) showed a wide variety of band patterns for extracellular enzymes among isolates of *P. chrysosporium*, but relatively uniform protein bands among isolates of *C. subvermispora*. Only a few isolates of *P. chrysosporium* appear to be strains that preferentially removed large amounts of lignin from wood, whereas all isolates of *C. subvermispora* tested are selective lignin degraders on deciduous as well as coniferous wood.

Introduction

The capacity of white-rot fungi to remove lignin from wood is a characteristic that makes them ideally suited for industrial applications where lignin or various phenolic compounds must be altered or removed (Blanchette 1991; Eriksson and Kirk 1985; Eriksson *et al.* 1990; Kirk and Farrell 1987). Since there are several thousand species of white-rot fungi worldwide, procedures to select the best species for industrial applications have been developed (Blanchette 1984; Kimura *et al.* 1990; Nashida 1989; Otjen *et al.* 1987). Although there are many different methods of screening white-rot fungi (Ander and Eriksson 1977; Blanchette 1984; Otjen *et al.* 1987; Setliff and Eudy 1980), one of the most appropriate procedures appears to be an assessment of decay (chemical analyses of lignin and wood sugar content) from inoculated wood blocks placed in accelerated decay chambers (Blanchette *et al.* 1988; Otjen *et al.* 1987). The species selected with this method also have been shown to be successful candidates for biological pretreatment of wood for mechanical pulping processes (Leatham *et al.* 1990a) or as an alternative to chemical pretreatment in high-yield wood pulping (Wegner *et al.* 1991). These fungi were able to reduce electricity requirements and improve various paper strength properties (Leatham *et al.* 1990 a,b; Myers *et*

al. 1988; Setliff *et al.* 1990). Although the actual removal of substantial amounts of lignin from the wood does not appear necessary to achieve these benefits, the fungi that have been selected for preferential lignin degradation appear to be the best species for use in biomechanical pulping.

Previous screening investigations have identified several species of white rot fungi that have been used successfully in pretreating wood chips for biopulping. Two species, *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* were among the best isolates tested (Blanchette *et al.* 1988; Leatham *et al.* 1990a,b; Otjen *et al.* 1987). The use of five different isolates of *P. chrysosporium* in a recent study showed a great deal of variation in decay capacity among different strains of the species (Blanchette *et al.* 1988). To further evaluate the variation present within species, 19 isolates of *P. chrysosporium* and 9 isolates of *C. subvermispora* were evaluated on three different wood substrates. A comparison of the extracellular proteins produced by each isolate in liquid culture media also was made using polyacrylamide gel electrophoresis (SDS-PAGE gels).

Materials and Methods

Nineteen isolates of *P. chrysosporium* and 9 isolates of *C. subvermispora* from different geographic areas and diverse substrates

Table 1. Isolates of *Phanerochaete chrysosporium* and their sources

Isolates	Host	Locality	State/Country
HHB-6251-sp	<i>Populus fremontii</i>	Guadalupe Canyon	Arizona
5157-A-1	<i>Fagus sylvatica</i>	Stockholm	Sweden
ME-BIC-6	<i>Betula</i> sp.	Winslow	Maine
ML-26	<i>Sequoia sempervirens</i>	Marley County	Kansas
Gold-9-419-4	Beech wood chips	Beaverton	Oregon
ME-BC-10	<i>Fagus grandifolia</i>	Winslow	Maine
Gold-9-420-1	Beech wood chips	Beaverton	Oregon
BKM-F-1767	Grape vine	The All-Union Collection of Microorganisms	Kazakhstan, USSR
ME-461	Southern pine chips	Fargo	Georgia
ML-21	<i>Sequoia sempervirens</i>	Marley County	Kansas
MJL-98-sp	Hardwood slash	Rochester Junction	New York
ME-PC-8	<i>Pinus</i> sp.	Fargo	Georgia
FP-102169	<i>Populus deltoides</i>	Hawesville	Kentucky
FP-104297-sp	<i>Liriodendron tulipifera</i>	Etchison	Maryland
HHB-11741-sp	<i>Acer</i>	Jackson	Illinois
ME-OC-11	<i>Quercus falcata</i>	Brunswick	Georgia
ML-20	<i>Sequoia sempervirens</i>	Marley County	Kansas
5161 ME-8	<i>Liquidambar styraciflua</i>	Brunswick	Georgia
P-127-1	<i>Pinus</i> sp.	Stockholm	Sweden

Table 2. Isolates of *Ceriporiopsis subvermispora* and their sources

Isolate	Host	Locality	State/Country
ME-485	Douglas fir	Wauna	Oregon
L-14807-sp	Aspen	Candle lake	Saskatchewan, Canada
L-15225-sp	Dogwood	Ashokan	New York
FP-104027-T	Oak	Jacksonville	Florida
L-39292-sp	Conifer log	Newcomb	New York
FP-105752-sp	Hardwood board	Beltsville	Maryland
CZ-3	Western Hemlock	Port Townsend	Washington
L-6133-sp	Conifer wood	Trappers Lake	Colorado
FP-90031-sp	Oak house log	Beltsville	Maryland

(Tables 1 and 2) were used in this study. These isolates were obtained from the Center for Forest Mycology Research, Forest Products Laboratory, Madison, WI.

Wood blocks (1.6 × 1.6 × 0.7 cm) were cut from the sapwood of freshly harvested birch (*Betula papyrifera*), loblolly pine (*Pinus taeda*) and aspen (*Populus tremuloides*), dried for 72 hr at 60°C and weighed to determine dry weight. Wood blocks were placed in 30 ml glass bottles containing 10 ml vermiculite and 6.5 ml distilled water. Bottles were loosely capped, autoclaved and inoculated as previously described (Otjen *et al.* 1987). Ten blocks of each wood species were used for each fungal isolate. Noninoculated wood blocks served as controls. Bottles with inoculated wood blocks were loosely capped and incubated in the dark at 27°C for *C. subvermispora* and 39°C for *P. chrysosporium*, and approximately 90% relative humidity for 12 weeks. Blocks were then dried for 72 hr at 60°C and weight loss determined. Blocks with weight losses approximately equal to the mean of ten replications were chosen for chemical analyses. Lignin and wood sugar analyses were done using previously described techniques (Effland 1977; Petterson *et al.* 1985).

For polyacrylamide gel electrophoresis, Cultures of *P. chrysosporium* and *C. subvermispora* were grown in flasks containing 25 ml of glucose asparagine media (Reid 1979) supplemented with 2 mM veratryl alcohol at 25°C for 10 days. The culture fluid containing extracellular proteins was recovered by filtration through a glass fiber filter and a Super-800 membrane (Gelman Sciences, Ann Arbor,

Michigan). The culture fluid was concentrated by ultrafiltration using an Amicon YM-10 membrane (Amicon Corp., Danvers, Massachusetts). The retentate was desalted by diafiltration with distilled water, and the concentrated solution stored at -20° until used.

The molecular weight patterns of extracellular proteins were determined by discontinuous dissociating sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Hames (1981). A 4.5% T stacking gel and a 10% T (for *C. subvermispora*) or 12.5% T resolving gel (for *P. chrysosporium*) were used. For each isolate, 25 g of protein was applied and electrophoresis carried out for 4 hrs at 200 V. The gels were silver stained by the method of Morrissey (1981).

Results

The percent weight loss caused by *P. chrysosporium* varied among the different isolates tested and the wood substrate used (Tables 3, 4 and 5). In aspen and birch woods, weight losses ranged from 7 to 73%, whereas losses were considerably lower on loblolly pine, ranging from 1 to 22%. Variation in the percent lignin and wood sugars lost during degradation was also seen among isolates. On birch and aspen woods, the greatest loss of lignin was caused by isolate BKM

Table 3. Percent weight, lignin and wood sugars lost in birch wood decayed by different isolates of *Phanerochaete chrysosporium* after 12 weeks at 39°C^{a)}

Isolate	Weight	% loss			
		Lignin	Glucose	Xylose	Mannose
BKM-F-1767	49.9	71.2	37.1	62.8	49.9
5161 ME-8	40.9	42.5	39.2	43.8	50.3
HHB-11741-sp	11.4	19.6	4.0	13.5	7.3
FP-104297-sp	43.8	49.5	46.9	49.9	40.9
FP-102169	54.7	59.7	55.0	65.9	54.7
ME-PC-8	45.8	50.8	51.6	53.0	42.9
MJL-98-sp	39.6	42.7	47.2	49.1	26.8
P-127-1	48.1	62.4	44.2	60.4	48.1
HHB-6251-sp	47.7	55.8	49.7	58.4	42.2
5157-A-1	27.4	47.4	19.2	35.3	8.3
ME-BIC-6	13.5	18.7	8.8	19.4	36.3
ML-26	30.6	30.6	29.6	39.0	30.6
Gold-9-419-4	48.7	61.3	41.2	58.6	56.8
ME-BC-10	32.8	41.2	24.0	34.1	78.8
Gold-9-420-1	55.1	61.4	49.8	70.1	66.9
ME-461	34.7	39.5	37.3	39.9	20.9
ML-21	27.7	34.2	30.3	32.9	31.5

^{a)} Isolate ML-20 and ME-OC-11 were not evaluated in this experiment.

Table 4. Percent weight, lignin and wood sugars lost in aspen wood decayed by different isolates of *Phanerochaete chrysosporium* after 12 weeks at 39°C

Isolate	Weight	% loss			
		Lignin	Glucose	Xylose	Mannose
BKM-F-1767	61.0	80.7	49.9	77.9	61.0
5161 ME-8	53.6	54.4	53.0	61.0	77.6
HHB-11741-sp	25.6	20.7	28.2	26.0	33.3
FP-104297-sp	55.3	47.5	51.3	63.0	81.5
FP-102169	50.2	56.7	51.4	52.0	55.3
ME-PC-8	64.2	60.8	69.2	67.9	77.8
MJL-98-sp	49.8	48.7	51.8	51.6	60.2
P-127-1	69.3	72.3	66.8	77.5	85.1
HHB-6251-sp	56.2	58.9	55.0	59.4	59.2
5157-A-1	23.0	30.6	5.2	27.4	60.2
ME-BIC-6	9.5	1.2	5.0	3.4	12.6
ML-26	21.6	15.5	22.1	19.6	32.4
Gold-9-419-4	61.2	73.4	56.1	71.7	67.9
ME-BC-10	59.9	72.9	54.6	77.4	80.6
Gold-9-420-1	73.7	69.7	76.4	78.9	79.1
ME-461	63.5	61.2	66.2	66.0	73.6
ML-21	30.1	31.7	28.8	26.5	34.9
ML-20	7.8	3.0	1.0	7.3	39.6
ME-OC-11	8.2	1.0	10.9	5.8	7.6

F-1767 with 71 and 80% loss of lignin, respectively. Several isolates resulted in a preferential removal of lignin but other isolates removed approximately the same percentages of lignin and cellulose (the cellulose content is represented by % glucose) (Tables 3 and 4). In all treatments, considerable losses of xylan and mannan, reflecting the amount of hemicellulose removed from the wood, were observed (Tables 3 and 4). On loblolly pine wood, lignin was not preferen-

tially degraded by any of the isolates tested (Table 5). Isolates that caused the greatest weight losses also removed considerable amounts of glucan. Several isolates failed to grow on the loblolly pine wood and resulted in less than a 4% weight loss. Among the isolates that successfully colonized the wood, considerable variation was evident in percent loss of lignin and wood polysaccharides (Table 5).

In contrast to results obtained with *P. chrysosporium*,

Table 5. Percent weight, lignin and wood sugars lost in loblolly pine wood decayed by different isolates of *Phanerochaete chrysosporium* after 12 weeks at 39°C^{a)}

Strain	Weight	Lignin	% loss		
			Glucose	Xylose	Mannose
BKM-F-1767	24.5	20.9	26.1	19.1	31.4
5161 ME-8	8.4	3.6	11.2	9.7	14.5
HHB-1174-sp	1.3	— ^{b)}	—	—	—
FP-104297-sp	22.7	12.9	30.5	23.8	39.3
FP-102169	19.7	16.9	23.0	17.4	24.3
ME-PC-8	1.6	— ^{b)}	—	—	—
MJL-98-sp	3.7	— ^{b)}	—	—	—
HHB-6251-sp	11.9	8.3	8.7	6.9	5.3
5157-A-1	13.6	12.8	10.3	14.8	11.5
MC-BIC-6	1.4	— ^{b)}	—	—	—
ML-26	1.4	— ^{b)}	—	—	—
Gold-9-419-4	19.2	12.4	19.2	16.9	23.9
ME-BC-10	7.9	1.8	8.7	4.0	10.2
Gold-9-420-1	18.7	18.3	17.5	25.7	20.7
ME-461	21.6	21.7	20.3	21.3	19.3
ML-21	12.9	9.0	9.9	12.9	13.6
ME-OC-11	1.2	— ^{b)}	—	—	—
ML-20	1.1	— ^{b)}	—	—	—

^{a)} Isolate P-127-1 was not evaluated in this study.

^{b)} Lignin and wood sugar analyses were not done due to the small weight loss (less than 4%).

Table 6. Percent weight, lignin and wood sugars lost in birch, loblolly and aspen wood decayed by different isolates of *Ceriporiopsis subvermispora* for 12 weeks at 27°C

Wood	Strain	Weight	Lignin	% loss		
				Glucose	Xylose	Mannose
<i>Betula papyrifera</i>	ME-485	31.5	79.8	6.9	35.8	52.1
	L-14807-sp	32.8	76.1	9.1	40.3	51.5
	L-15225-sp	31.5	78.3	13.7	37.5	8.7
	FP-104027-T	35.8	79.4	16.2	44.0	35.8
	L-39292-sp	25.9	57.2	11.3	30.5	1.2
	FP-105752-sp	27.8	70.8	4.5	32.0	11.8
	CZ-3	27.3	75.2	2.7	35.1	15.2
	L-6133-sp	33.6	78.7	9.4	41.3	44.7
	FP-90031-sp	28.2	60.7	7.9	48.1	52.1
	ME-485	22.8	31.0	20.3	0	24.2
<i>Pinus taeda</i>	L-14807-sp	22.5	37.0	14.7	33.2	29.9
	L-15225-sp	23.7	38.2	12.4	27.2	28.1
	FP-104027-T	28.3	40.6	18.8	33.8	26.9
	L-39292-sp	29.0	42.2	22.4	31.1	26.2
	FP-105752-sp	19.6	33.9	7.1	27.0	10.1
	CZ-3	21.3	31.8	14.0	31.0	20.3
	L-6133-sp	30.1	34.1	26.2	34.0	18.9
	FP-90031-sp	22.7	38.2	14.1	30.0	15.9
	ME-485	28.4	61.5	2.5	45.4	72.3
	L-14807-sp	24.4	57.2	6.8	36.9	39.1
<i>Populus tremuloides</i>	L-15225-sp	25.4	58.8	2.9	40.4	66.3
	FP-104027-T	26.4	65.9	2.2	44.6	66.8
	L-39292-sp	25.6	63.7	2.1	47.9	66.4
	FP-105752-sp	22.7	55.7	0.6	31.9	30.2
	CZ-3	23.8	71.2	6.3	43.8	28.7
	L-6133-sp	24.4	70.7	3.4	38.4	29.3
	FP-90031-sp	26.5	50.1	7.3	31.5	31.3

the amount of variation observed in losses of weight, lignin and wood sugars among isolates of *C. subvermispora* was not excessively large (Table 6). Weight

losses ranged from 26 to 36%, 19 to 30% and 23 to 28%, on birch, loblolly and aspen wood, respectively. Preferential degradation of lignin occurred in all

woods tested and by all isolates. In birch wood, as much as 80% of the lignin was degraded with relatively low losses of glucan and moderate losses of xylan or mannan. Although some variation was evident in the results obtained using *C. subvermispora*, the isolates all appear to selectively degrade lignin from birch, loblolly or aspen wood.

Polyacrylamide gel electrophoresis showed a wide variety of extracellular protein band patterns among the isolates of *P. chrysosporium* (Fig. 1). Differences in the number of protein bands and molecular weights were observed. In contrast, relatively uniform band patterns were observed for isolates of *C. subvermispora* (Fig. 2).

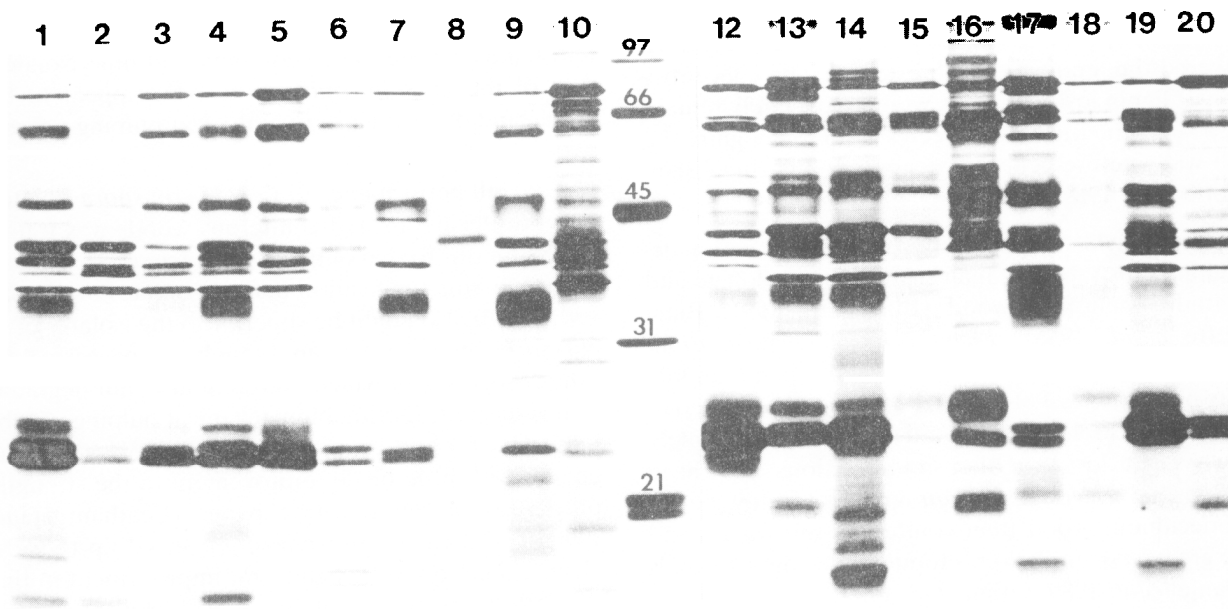


Fig. 1. Polyacrylamide SDS-PAGE gel showing extracellular proteins of different isolates of *Phanerochaete chrysosporium*. Silver stained. Lane #1 ML-21, 2 ME-461, 3 Gold 9-420-1, 4 ME-BC-10, 5 Gold 9-419-4, 6 ML-26, 7 ME-BIC-6, 8 5157-A-1, 9 HHB-6251-sp, 10 P-127-1, 11 Molecular weight markers, 12 MJL-98-sp, 13 ME'PC-8, 14 FP-102169, 15 FP-104297, 16 HHB-11741-sp, 17 ME-OC-11, 18 ML-20, 19 5161-ME-8, 20 BKM-F-1767.

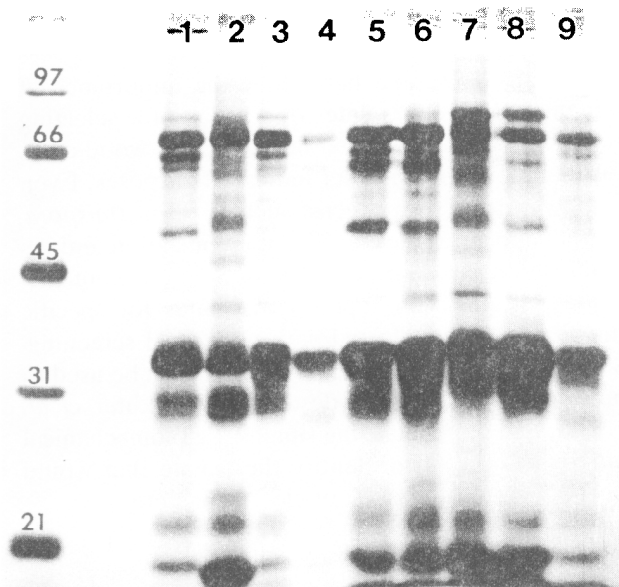


Fig. 2. Polyacrylamide SDS-PAGE gel showing extracellular proteins of different isolates of *Ceriporiopsis subvermispora*. Silver stained. Lane #1 L-6133-sp, 2 CZ-3, 3 FP-105752-sp, 4 L-39292-sp, 5 FP-104027-T, 6 L-15225-sp, 7 L-14807-sp, 8 ME-485, 9 FP-90031-sp. Molecular weight markers are shown at left of gel.

Discussion

Exceedingly large amounts of variation exists among different isolates of *P. chrysosporium*. The results presented here show differences in weight loss and the type of degradation among the 19 isolates tested. Preferential degradation of lignin is evident in wood decayed by some isolates, such as BKM-F-1767, P-127-1 and Gold 9-420-1 in birch and BKM-F-1767, Gold-9-419-4, and ME-BC-10 in aspen, but a non-selective attack is evident for several other isolates including ME-PC-8, MJL-98-sp, ML-26 and others (Tables 3 and 4). The large amount of variation that exists within this species is clearly evident in extracellular proteins produced when the isolates are grown under identical conditions (Fig. 1). These results help explain the differences observed by researchers using different isolates of *P. chrysosporium*. Selective degradation occurred using isolates of BKM-F-1767 (Blanchette *et al.* 1988, 1989a, b, 1990; Otjen *et al.* 1987) and nonselective type of white rot occurred using other isolates (Daniel *et al.* 1989; Eriksson *et al.* 1980; Ruel *et al.* 1981; Rue] and Joseleau 1991). In nature, there appears to be a wide range of decay patterns

produced by *P. chrysosporium* depending upon the isolates ability to produce wood destroying enzymes. The results presented here indicate that a wide range of selective, intermediate and nonselective types of degradation are possible among different isolates of *P. chrysosporium* when they are used to decay wood for biological pulping processes. Although the extent of cellulose degradation in wood may be low for some isolates, after 12 weeks of laboratory decay considerable amounts of cellulose may be removed by other isolates. Analyses of wood blocks degraded for a reduced amount of time (4–8 weeks) would undoubtedly have lowered the amount of cellulose test lost. The concentration of lignin lost, however, would most likely remain high since the fungus has been shown to delignify the cell walls before cellulose degradation occurs (Blanchette *et al.* 1989a, b).

In general, white rot fungi are commonly found on deciduous substrates but many species can colonize and degrade coniferous wood (Blanchette 1991; Blanchette *et al.* 1988). *Phanerochaete chrysosporium* grows substantially better on birch and aspen than loblolly pine (Tables 3, 4 and 5). Several isolates failed to grow on the loblolly pine wood and showed extremely low weight losses. Other investigations also have shown that *P. chrysosporium* appears to grow better on deciduous wood than coniferous substrates, and the greatest degradation is found in deciduous woods (Daniel *et al.* 1989; Otjen *et al.* 1987).

A recent study that evaluated lignin peroxidase activity among 53 homokaryotic strains of *P. chrysosporium* showed large differences among the strains (Raeder *et al.* 1989). In a similar study using another white-rot fungus, *Dichomitus squalens*, cellulose as well as lignin-degrading abilities varied among the 20 monokaryotic strains used (Pham *et al.* 1990). These investigations demonstrate that large amounts of genetic diversity exists, in relation to wood-destroying enzymes, among some species of white rot fungi. The polyacrylamide gel electrophoresis results presented in this study show the variation of all extracellular proteins produced in liquid culture among different isolates. Although information on the concentration of each enzyme is not available, there are distinctly different extracellular proteins excreted by many of the *P. chrysosporium* isolates tested. A comparison of protein bands produced by 19 different isolates of *P. chrysosporium* by gel electrophoresis showed that five (HHB-11741, MJL-98, 5157-A-1, ME-BIC-6 and ML-26) were missing or had decreased concentrations of proteins at approximately 40 Kd. Of these strains, most had poor growth on loblolly pine wood and were among the isolates causing the lowest weight losses on aspen and birch wood. Eleven of the 12 isolates that exhibited moderate banding at approximately 40 Kd had lignin losses, when grown on hardwood, of 39.5%

or greater, and included isolates with the highest percent lignin lost. The bands at approximately 40 Kd most likely represent lignin peroxidases (41 Kd) or manganese-dependent peroxidases (45 to 47 Kd) (Farrell *et al.* 1989), which may be essential for significant wood degradation to occur.

Our results indicate that the isolates of *C. subvermispora* used in this study have relatively little variation in their capacity to degrade wood. This also is evident in the extracellular protein banding patterns observed for the isolates (Fig.2). The same basic pattern of protein bands can be observed among all nine isolates. Any of the *C. subvermispora* isolates appear to be good candidates for use in biological pulping processes.

The overall performance of *C. subvermispora* isolates on both deciduous and coniferous woods was found to be superior to that of the *P. chrysosporium* isolates. This gives some indication that the isolates of *C. subvermispora* also might be superior to the isolates of *P. chrysosporium* for use in biopulping processes or other processes in which preferential lignin degradation is desired. During biomechanical pulping of loblolly pine, *P. chrysosporium* saved only 23% electrical energy with little or no improvement in the strength properties of the resulting paper (Leatham *et al.* 1990a), whereas *C. subvermispora* saved up to 68% electrical energy with significant improvement in the strength properties (Leatham *et al.* 1990a, b; Akhtar *et al.* 1992). Performance of *C. subvermispora* also was evaluated during biomechanical pulping of aspen wood chips. Substantial amounts of electrical energy were saved with significant improvement in the strength properties of the resulting paper (Akhtar *et al.* unpublished).

The results presented here show the importance of screening different isolates of a species for selecting superior strains to be used in pretreating wood chips for biopulping or for other industrial purposes. Even among isolates of a species such *C. subvermispora*, that all appear to have excellent colonization and delignification capabilities on a variety of different substrates, strains with superior attributes for specific functions can be selected with additional screening. Selected isolates of *C. subvermispora*, can be used to pretreat wood chips as described by Akhtar *et al.* (1992), and screened using small scale biomechanical pulping processes to identify the isolate that would save the greatest amount of electrical energy.

Acknowledgements

The authors thank Dr. Kent Kirk, Forest Products Laboratory, Madison, WI for his review of the manuscript. Published as paper No. 19,095 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 22-69H. This work was supported in part by the Biopulping Consortium. The consortium consists of the University of Wisconsin

Biotechnology Center, USDA Forest Products Laboratory, Madison, WI and 20 member companies involved in pulp and paper production and associated fields.

References

- Akhtar, M., M.C. Attridge, G.C. Myers, T.K. Kirk, and R.A. Blanchette. 1992. Biomechanical pulping of loblolly pine with different strains of the white-rot fungus *Ceriporiopsis subvermispora*. Tappi J. (In press.)
- Ander, P., and K.-E. Eriksson. 1977. Selective degradation of wood components by white rot fungi. *Physiol. Plant.* 41: 239–248.
- Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. *Appl. Environ. Microbiol.* 48: 647–653.
- Blanchette, R.A. 1991. Delignification by wood decay fungi. *Annu. Rev. Phytopathol.* 29: 381–398.
- Blanchette, R.A., A.R. Abad, R.L. Farrell, and T.D. Leathers. 1989a. Detection of lignin peroxidase and xylanase by immunocytochemical labeling in wood decayed by basidiomycetes. *Appl. Environ. Microbiol.* 55: 1457–1465.
- Blanchette, R.A., A.R. Abad, K.R. Cease, R.E. Lovrien, and T.D. Leathers. 1989b. Colloidal gold cytochemistry of endo-1, 4- β -glucanase, 1, 4- β -D-glucan cellobiohydrolase, and endo-1, 4- β -xylanase: Ultrastructure of sound and decayed wood. *Appl. Environ. Microbiol.* 55: 2293–2301.
- Blanchette, R.A., A.R. Abad, K.R. Cease, R.L. Farrell, R.E. Lovrien, and T.D. Leathers. 1990. Enzyme immunocytochemistry and ultrastructural localization of cell wall components by enzyme-gold complexes. In: Kirk, T.K. and H.-M. Chang (Eds.) *Biotechnology in Pulp and Paper Manufacture, Applications and Fundamental Investigations*, pp. 69–81. Butterworth-Heinemann, Boston.
- Blanchette, R.A., T.A. Burnes, G.F. Leatham, and M.J. Effland. 1988. Selection of white-rot fungi for biopulping. *Biomass* 15: 93–101.
- Daniel, G., T. Nilsson, and B. Pettersson. 1989. Intra- and Extracellular localization of lignin peroxidase during the degradation of solid wood and wood fragments by *Phanerochaete chrysosporium* by using transmission electron microscopy and immunogold labeling. *Appl. Environ. Microbiol.* 55: 871–881.
- Effland, M. 1977. Modified procedure to determine acid-insoluble lignin in wood and pulp. *Tappi* 60: 143–144.
- Eriksson, K.-E., R.A. Blanchette, and P. Ander. 1990. *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer-Verlag, Berlin. 407 p.
- Eriksson, K.-E., A. Grunewald, T. Nilsson, and L. Vallander. 1980. A scanning electron microscopy study of the growth and attack on wood by three white-rot fungi and their cellulase-less mutants. *Holzforschung* 34: 207–213.
- Eriksson, K.-E., and T.K. Kirk. 1985. Biopulping, biobleaching and treatment of kraft bleaching effluents with white-rot fungi. In: Moo-Young, M. (Ed.) *Comprehensive Biotechnology: The Principals, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine*, pp. 271–294. Pergamon Press, New York.
- Farrell, R.L., K.E. Murtagh, M. Tien, M.D. Mozuch, and T.K. Kirk. 1989. Physical and enzymatic properties of lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. *Enzyme Microb. Technol.* 11: 322–328.
- Hames, B.D. 1981. An introduction to polyacrylamide gel electrophoresis. In: Hames, B.D., and D. Rickwood (Eds.) *Gel Electrophoresis of Proteins, a Practical Approach*, pp. 1–19. IRC Press, Oxford.
- Kimura, Y., Y. Asada, and M. Kuwahara. 1990. Screening of basidiomycetes for lignin peroxidase genes using a DNA probe. *Appl. Microbiol. Biotechnol.* 32: 436–442.
- Kirk, T.K., and R.L. Farrell. 1987. Enzymatic “combustion”: The microbial degradation of lignin. *Annu. Rev. Microbiol.* 41: 465–505.
- Leatham, G.F., G.C. Myers, T.H. Wegner, and R.A. Blanchette. 1990a. Energy savings in biomechanical pulping. In: Kirk, T.K., and H.M. Chang (Eds.) *Biotechnology in Pulp and Paper Manufacture, Applications and Fundamental Investigations*, pp. 17–25. Butterworth-Heinemann, Boston.
- Leatham, G.F., G.C. Myers, T.H. Wegner, and R.A. Blanchette. 1990b. Biomechanical pulping of aspen chips: Paper strength and optical properties resulting from different fungal treatments. *Tappi J.* 73: 249–254.
- Myers, G.C., G.F. Leatham, T.H. Wegner, and R.A. Blanchette. 1988. Fungal pretreatment of aspen chips improves strength of refiner mechanical pulp. *Tappi J.* 71: 105–108.
- Morrissey, J.H. 1981. Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced sensitivity. *Anal. Biochem.* 177: 307–310.
- Nashida, T. 1989. Lignin biodegradation by wood-rotting fungi V. A new method for evaluation of the ligninolytic activity of lignin-degrading fungi. *Mokuzai Gakkaishi* 35: 675–677.
- Otjen, L., R.A. Blanchette, M. Effland, and G. Leatham. 1987. Assessment of 30 white rot basidiomycetes for selective lignin degradation. *Holzforschung* 41: 343–349.
- Peterson, R.C., V.H. Schwandt, and M.J. Effland. 1985. An analysis of the wood sugar assay using HPLC: A comparison with paper chromatography. *J. Chromatogr. Sci.* 22: 478–484.
- Pham, T.T.T., A. Maaroufi, and E. Odier. 1990. Inheritance of cellulose- and lignin-degrading ability as well as endoglucanase isozyme pattern in *Dichomitus squulens*. *Appl. Microbiol. Biotechnol.* 33: 99–104.
- Raeder, U., W. Thompson, and P. Broda. 1989. Genetic factors influencing lignin peroxidase activity in *Phanerochaete chrysosporium* ME 446. *Mol. Microbiol.* 3: 919–924.
- Reid, I.D. 1979. The influence of nutrient balance on lignin degradation by the white-rot fungus *Phanerochaete chrysosporium*. *Can. J. Bot.* 57: 2050–2058.
- Ruel, K., F. Barnoud, and K.-E. Eriksson. 1981. Micromorphological and ultrastructural aspects of spruce wood degradation by wild-type *Sporotrichum pulverulentum* and its cellulaseless mutant Cel 44. *Holzforschung* 35: 157–171.
- Ruel, K., and J.-P. Joseleau. 1991. Involvement of an extracellular glucan sheath during degradation of *Populus* wood by *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 57: 374–384.
- Setliff, E.D., and W.W. Eudy. 1980. Screening white-rot fungi for their capacity to delignify wood. In: Kirk, T.K., H.M. Chang, and T. Higuchi (Eds.) *Lignin Biodegradation: Microbiology, chemistry and potential applications*, Vol. 1, pp. 135–149. CRC Press, Boca Raton, Fla.
- Setliff, E.C., R. Marton, S.G. Granzow, and K.L. Eriksson. 1990. Biomechanical pulping with white-rot fungi. *Tappi J.* 74: 141–47.
- Wegner, T.H., G.F. Leatham, G.C. Myers, and T.K. Kirk. 1991. Biological treatments as an alternative to chemical pretreatments in high-yield wood pulping. *Tappi J.* 74: 189–193.