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Short contribution

Substrate-dependent degradation patterns in the decay of wheat straw and beech wood by ligninolytic fungi

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Summary. The ability of 45 fungal strains to degrade wheat straw and beech wood was studied. Degradation patterns were defined in terms of chemical evolution of substrates and changes in lignin and polysaccharides. *Trametes versicolor* produced an important degradation of lignin and increased substrate digestibility, but it caused high weight losses and gave rise to similar decay patterns on both substrates. A preferential degradation of lignin was produced during straw transformation by *Pleurotus eryngii*. The increase of soluble lignin and decreases of lignin content and H/C ratio defined the degradation tendency after principal component analysis. The cation exchange capacity and water and alkali solubility presented the highest loading factors for the characterization of fungal transformation of beech wood.

Introduction

The white-rot of wood and other lignocellulosic substrates is characterized by the degradation of lignin and polysaccharides by certain basidiomycetes, including species responsible for very selective removal of lignin (Liese 1970; Blanchette 1984). Such selective delignification has been investigated for its potential use in wood biopulping (Eriksson and Kirk 1985), but also for the production of improved feed from agricultural wastes (Zadrazil 1980, 1985; Agosín et al. 1985a; Kamra and Zadrazil 1988). However, important increases in wood digestibility can be achieved without very selective lignin degradation (Zadrazil et al. 1982; González et al. 1987). The physical disruption of plant tissues, chemical alteration of the lignin polymer, breakdown of cellulose-lignin linkages and protein enrichment all contribute to improve the characteristics of these partially delignified materials.

This paper presents a comparative study on wood and straw degradation by a selection of strains representative of ligninolytic basidiomycetes.

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Materials and methods

Fungal strains. The 45 strains studied (Fig. 1) correspond to different species of basidiomycetes, and were isolated from decayed wood of *Fagus sylvatica* (A 140, A 141, A 144, A 159), *Eucryphia cordifolia* (A 130), *Drymis winteri* (A 148, A 149), and *Abies pinsapo* (A 196, A 198, A 200, A 201); from fungal fruit-bodies growing on the wood of *F. sylvatica* (A 135, A 136, A 157, A 160, A 166, A 180, A 181), *Picea abies* (A 175, A 176, A 178), *Quercus* sp. (A 184) and *A. pinsapo*, (A 185, A 187, A 188, A 189, A 190, A 191, A 197), on leaf litter of *F. sylvatica* (A 146, A 150, A 155) and *A. pinsapo* (A 186), on wheat straw (A 163, A 169) and on rabbit dung (A 224, A 225, A 226); or were obtained from the culture collections of the Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (IJFM), Madrid, Spain (A 104, A 110, A 111, A 112, A 137), and the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (CBS 316.75 = A 261). The cultures obtained from decayed lignocellulosic substrates were identified following the monographs of Käärik (1965), Nobles (1965) and Stalpers (1978) and by comparison with authentic cultures obtained from fruit-bodies.

Substrates and transformation conditions. Wheat straw and beech wood were ground to 5 mm fragments, and 100-ml erlenmeyer flasks containing 2 g wheat straw or 3 g beech wood with 4 ml water and 2 or 3 ml, respectively, of 0.75% NH_4NO_3, to obtain a similar *N* concentration, substrate volume and saturation of the water-holding capacity, were autoclaved at 120° C for 15 min. Two 3 × 3 mm portions of 10-day-old cultures on 2% malt-extract agar were used as inoculum, and the 45 strains were grown in triplicate at 28° C for 2 months.

Analytical methods. The water-soluble fraction, the alkali-soluble material and the Klason lignin content were determined by TAPPI methods (1974, 1976), and the 'acid-soluble lignin' was estimated absorptiometrically (Schöning and Johansson 1965). The element composition was determined with a Heraeus CHN-O Rapid analyser (Hanau, FRG), and the cation exchange capacity by following Harada and Inoko (1980). The crystallinity index was estimated according to Segal et al. (1959), and digestibility was determined by the method of Roughan and Holland (1977). The degree of colonization was evaluated from the chitin content in the transformed samples (Chen and Johnson 1983), and the sugars, after polysaccharide hydrolysis, were analysed as alditol acetates following Laine et al. (1972). For the infrared spectroscopy studies, a Perkin-Elmer 980B (Norwalk, Conn., USA) was employed, using 300 mg KBr and 4 mg lignin.

A dioxane lignin preparation was obtained by following Pepper et al. (1959), dialysed after precipitation, and used for spec-

troscopic studies with a Shimadzu UV-240 OPI-11 (Kyoto, Japan) spectrophotometer using solutions of 200 μg ml^{-1} and 20 μg ml^{-1} in 0.1 *N* NaOH for visible and UV spectroscopy respectively. The molecular size distribution of this lignin preparation was examined by gel permeation using Sephadex G-100 (Swift and Posner 1971), and the densitometric curves were recorded at 450 nm.

Data analyses. The programmes of Orlóci and Kenkel (1985) were used for the multivariate data treatment. The original values (except those for weight loss and fungal biomass) were transformed into percentages of their respective controls (undecayed wheat straw and beech wood) to examine the extent of the changes induced by the fungi.

Results and discussion

Screening of ligninolytic strains

Figure 1 shows the weight losses caused by the fungal strains studied in the lignocellulosic substrates, and the portion corresponding to lignin degradation. The greatest weight losses were produced by strains of *Phanerochaete chrysosporium* (52.5%), *Ganoderma* sp. (51.0%) and *Fomes fomentarius* (50.0%) in wheat straw, and by *Armillaria* sp. (33.3%), *Trametes versicolor* (27.6%) and *Ganoderma* sp. (25.4%) in beech wood. The most important degradation of lignin was produced by *T. versicolor* on wheat straw (79% of the lignin before decay) and beech wood (57%). A similar degradation of wheat straw lignin was produced by *F. fomentarius*.

The lowest lignin contents in the transformed wheat straw were found after solid-state fermentation with *Pleurotus eryngii, F. fomentarius, T. versicolor* and *Laccaria amethystina,* whereas those in beech wood were produced by *Ganoderma* sp. and *T. versicolor.* The present results with *P. eryngii* on wheat straw agreed with those reported by Agosín et al. (1985b) and Kamra and Zadrazil (1986). The intense degradation obtained with *T. versicolor* coincided with the results reported by Müller and Trösch (1986) and Otjen et al. (1988).

From the above results, five fungal strains (*G. applanatum* A 130, *T. versicolor* A 137, *Ganoderma* sp. A 181, *G. lucidum* A 189 and *P. chrysosporium* A 261) were selected to compare the changes in chemical composition of both lignocellulosic substrates after fungal decay.

Analyses of the decayed substrates

The extent of fungal colonization of wheat straw (3.3%–6.7%) was rather similar to that obtained by Agosín et

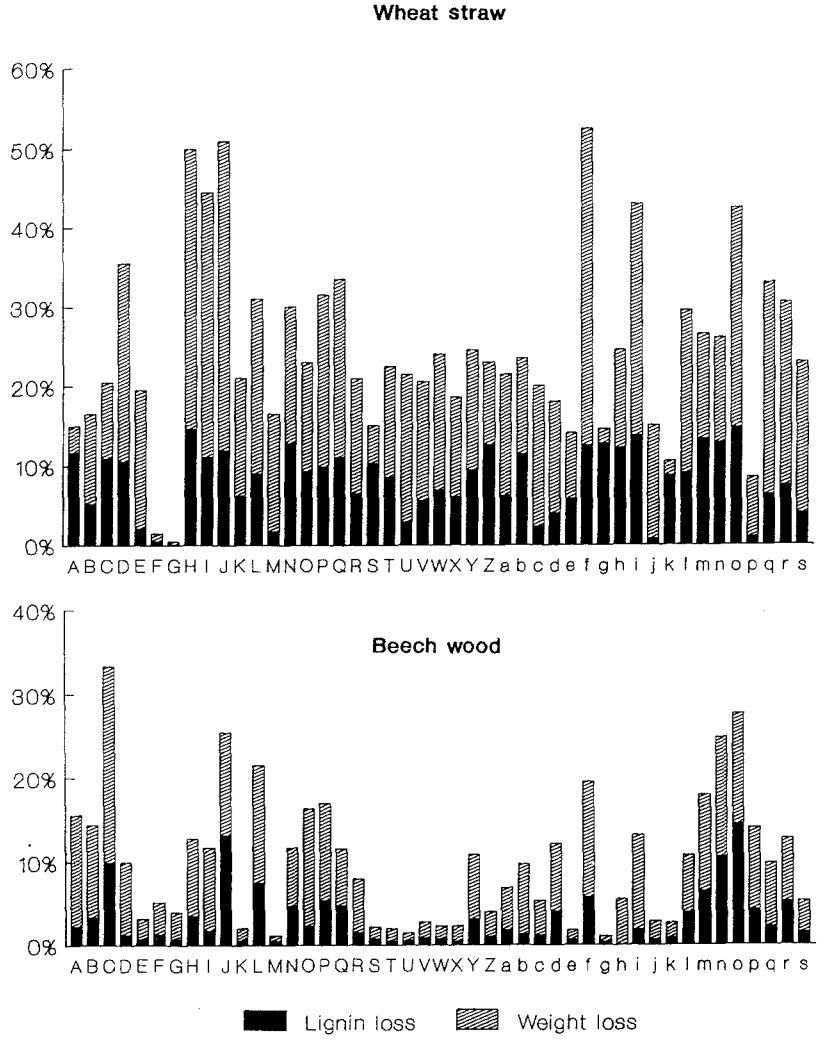

Fig. 1. Losses of weight and lignin in wheat straw and beech wood decayed by ligninolytic fungi: *A, Armillaria bulbosa* A 180; *B, A. mellea* A 186, *C, Armillaria* sp. A 196; *D, Cerrena unicolor* A 184; *E–G, Coprinus* sp. A 224, A 225, A 226; *H–I, Fomes fomentarius* A 159, A 166; *J–K, Ganoderma* sp. A 181, A 190; *L–N, G. applanatum* A 130, A 148, A 149; *O–P, G. lucidum* A 188, A 189; *Q, Gymnopilus junonius* A 146; *R, G. penetrans* A 175; *S, Laccaria amethystina* A 150; *T–Y, Heterobasidion annosum* A 176, A 187, A 197, A 198, A 200, A 201; *Z, Hypholoma sublateritium* A 157; *a, Marasmius alliaceus* A 155; *b, Merulius tremellosus* A 191; *c, Oudemansiella radicata* A 160; *d, Panellus stipticus* A 178; *e, Peniophora gigantea* A 104; *f, Phanerochaete chrysosporium* A 261; *g, Pleurotus eryngii* A 169; *h, P. ostreatus* A 163; *i, Pycnoporus cinnabarinus* A 168; *j–k, Schizophyllum commune* A 111, A 112; *l, Sphaerobolus stellatus* A 110; *m–o, Trametes versicolor* A 135, A 136, A 137; *p, Tyromyces caesius* A 185; *q–s,* unidentified basidiomycetes A 140, A 141, A 144. *Total heights* correspond to weight losses and *solid bars* indicate the portion corresponding to lignin degradation

al. (1985b), and lower values (1.3%–4.2%) were obtained on beech wood. An increase in acid-soluble lignin-derived products ('soluble lignin') was observed after fungal degradation of both substrates. The water solubility (21.3% in wheat straw and 5.3% in beech wood) increased after degradation, mainly in wheat straw degraded by *G. applanatum* (36.8%) and beech wood inoculated with *T. versicolor* (13.4%). The solubility in alkali, an index of the degree of wood rotting, showed the accumulation of altered polymers and tended to increase in decayed beech wood, but important decreases were observed in wheat straw (49.3%) degraded by *Ganoderma* sp. (26.7%) and *G. lucidum* (29.6%).

The digestibility of wheat straw (38%) and beech wood (15%) showed important increases, as reported by Agosin and Odier (1985) and Zadrazil (1980). *Trametes versicolor* produced a 40% increase in both substrates and significant increases in digestibility were also obtained with *G. lucidum* (45% increase in wheat straw) and *P. chrysosporium* (36% increase in beech wood). Important increases in wheat straw digestibility by *T. versicolor* were also found by Calzada et al. (1987). The three strains of *G. applanatum* studied were isolated from naturally decayed wood with high digestibility (Zadrazil et al. 1982) and raised the digestibility of both substrates, although a very limited effect of this species on wheat straw was reported previously by Zadrazil and Brunnert (1981).

Several changes in some physical and chemical characteristics of the holocellulose and lignin fractions were observed. The yields obtained after acid hydrolysis of both substrates showed no preferential degradation of pentosans, whereas the mannose content tended to decrease after fungal degradation. *Trametes versicolor* increased the glucose yields both in wheat straw and beech wood, but *G. applanatum* produced a greater increase in beech wood. The crystallinity index tended to decrease after fungal degradation, and the lowest values on both substrates were produced by *Ganoderma* sp. (reduction of 19.9% in wheat straw and 20.7% in beech wood).

An increase in the specific extinction at 465 nm of dioxane lignin was observed in most cases. The decrease (around 68%) in the ratio between the specific extinctions (E) at 465 and 665 nm (E_4/E_6) in the straw lignin suggests an increase in the molecular sizes (Chen et al. 1977), corresponding to a preferential degradation of the low molecular size lignin fractions by fungi. This coincides with the results from gel permeation of the wheat straw dioxane lignin, which showed an increase in the amount of the excluded fraction mol. wt. $>10^4$) (70.5% reduction by *P. chrysosporium*). In the case of beech wood, only *T. versicolor* produced an increase (24.8%) in this fraction.

The values of cation exchange capacity in decayed samples revealed the formation of carboxyl groups, and agreed with the increased intensities of the 1720 cm^{-1} peak in the lignin infrared spectra. The greatest values on wheat straw were obtained with *G. applanatum* (95.3% increase), whereas *T. versicolor* was responsible for a 237.1% increase on beech wood. The intensity of the 280-nm band of the dioxane lignin was quantified in the first derivative of the spectral curves. The data obtained showed important decreases in wheat straw, as found by Zadrazil (1974). *Ganoderma applanatum* was responsible for the most intense decrease both in wheat straw and beech wood. The infrared spectra of the dioxane lignins showed an increase in the relative intensity of the alkyl vibrations around 2920 cm^{-1}, and of the 1720 cm^{-1} C=O vibrations after fungal decay.

An important reduction of the syringyl/guaiacyl ratio (S/G), as estimated from the ratio between the absorbances at 1330 and 1270 cm^{-1} (Fengel and Wegener 1984) was also observed. Such an effect coincides with the results obtained by Kirk et al. (1975) after acidolysis of lignin obtained from decayed wood. The most important decrease in the S/G ratio were obtained with *T. versicolor* and *P. chrysosporium* in wheat straw, and with *G. applanatum* in beech wood.

Transformation patterns of wheat straw and beech wood

The results of principal components analysis revealed that the influence of the substrate tended to be more important than that of the fungal species inoculated. Figure 2 represents data of the first two components. The loading factors of the original variables on the second component (Y axis) show that fungal transforma-

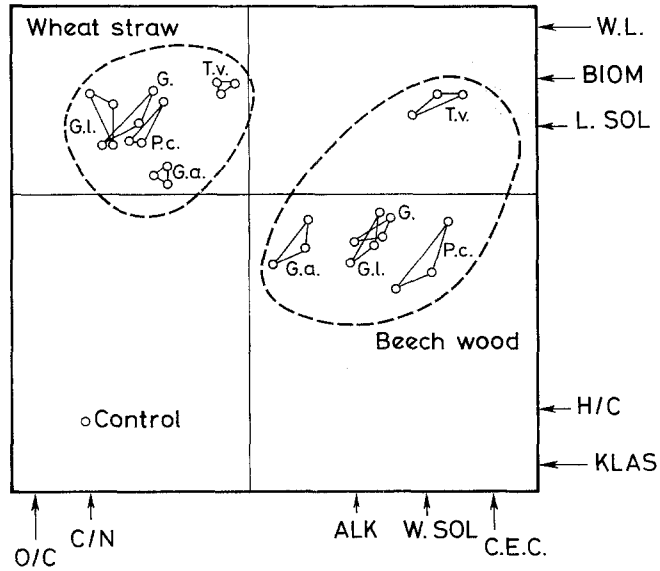

Fig. 2. Sample arrangement after principal component analysis from the relative values of the analytical characteristics of wheat straw and beech wood transformed by ligninolytic fungi (variables were divided by the values of the respective undecayed substrate); G.a. = *Ganoderma applanatum;* G.l. = *G. lucidum;* G. = *Ganoderma* sp.; P.c. = *Phanerochate chrysosporium;* T.v. = *Trametes versicolor;* Control = undecayed control for both substrates. The total variance explained was 60%, and the variables with the highest loading factors on every axis are indicated; ALK = solubility in alkali; BIOM = fungal biomass; C.E.C. = cation exchange capacity; C/N ratio; H/C ratio; KLAS = Klason lignin; L.SOL = acid-soluble lignin; O/C ratio; W.L. = weight loss; and W.SOL = water soluble

tion is defined mainly by an increase in soluble lignin, and by a decrease in the Klason lignin content and the H/C atomic ratio. On the other hand, the first component (X axis) reveals the different pattern of changes in the substrates. When compared with wheat straw, fungal transformation of beech wood is characterized by a higher increase in the cation exchange capacity and in the water- and alkali-soluble fractions. The great influence of the substrates on the degradative activity of the fungi studied was recognized by a tendency to independent decay patterns in wheat straw and beech wood As a whole, the most remarkable changes in both substrates were obtained with *T. versicolor,* producing increases in acid-soluble lignin and in vitro digestibility, and decreases in the H/C ratio and Klason lignin content, as well as in the E_4/E_6, S/G and carboxyl content of the dioxane lignin.

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Note added in proof. The *Ganoderma* species (strains A 130, A 148 and A 149) producing extensive delignification of Chilean wood (Zadrazil et al. 1982) and formerly identified as *G. applanatum*, is actually considered as *G. australe*