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Lignin Degradation by *Streptomyces viridosporus*: Isolation and Characterization of a New Polymeric Lignin Degradation Intermediate†

DON L. CRAWFORD,[1]* ANTHONY L. POMETTO III, [1] AND RONALD L. CRAWFORD[2]

Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843,[1] and Gray Freshwater Biological Institute and Department of Microbiology, University of Minnesota, Navarre, Minnesota 55392[2]

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A new, quantitatively significant intermediate formed during lignin degradation by *Streptomyces viridosporus* T7A was isolated and characterized. In *Streptomyces*-inoculated cultures, the intermediate, an acid-precipitable, polyphenolic, polymeric lignin (APPL), accumulated in the growth medium. The APPL was a water-soluble polymer probably consisting of a heterogeneous mixture of molecular weight components of ≥20,000. APPLs were precipitable from culture filtrates after they had been acidified to pH <3 to 5. Noninoculated controls yielded little APPL, but supernatant solutions from inoculated cultures produced quantities of APPL that correlated with the biodegradability of the lignocellulose type. Maximal recovery of APPL was obtained from corn lignocellulose, reaching 30% of the initial lignin present in the substrate. APPLs contained small amounts of carbohydrate, organic nitrogen, and inorganic materials. The lignin origin of APPLs was confirmed by chemical analyses, which included acidolysis, permanganate oxidation, elemental analyses, functional group analyses, nuclear magnetic resonance spectroscopy, and ^{14}C isotopic techniques. Analyses of APPLs from corn lignocelluloses showed that *S. viridosporus*-degraded APPLs were lignin derived but significantly different in structure from APPLs derived from uninoculated controls or from a standard corn milled-wood lignin. Degraded APPLs were enriched in phenolic hydroxyl groups and, to a small extent, in carboxyl groups. Degradative changes appeared to be largely oxidative and were thought to involve substantial cleavage of *p*-hydroxy ether linkages and methoxyl groups in the lignin.

When *Streptomyces viridosporus* T7A is grown on lignocellulose, both lignin and carbohydrate are degraded (7, 10). Lignin is degraded by oxidative mechanisms, as shown by the chemical characterization of a streptomycete-degraded softwood lignin (7). A number of single-ring aromatic intermediates released during the degradation of hardwood, softwood, and grass lignins by *S. viridosporus* also have been identified (6). Recently we observed an additional lignin degradation intermediate present as a water-soluble polymer in culture media. This intermediate is a structurally modified lignin and precipitates from solution after acidification of the culture medium. We call this new intermediate acid-precipitable polymeric lignin (APPL), and it appears to be a very important degradation intermediate. In the present paper we discuss the production of APPL during lignin degradation by *S. viridosporus* and report some of the chemical properties of an APPL harvested from cultures of *S. viridosporus* after growth on corn stover lignocellulose. We also discuss the importance and relationship of APPLs to the lignin degradation pathway of *Streptomyces*.

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MATERIALS AND METHODS

Growth on lignocellulose and APPL harvest. Cultures were grown on corn stover lignocellulose prepared from *Zea mays* as previously described (6, 7). To summarize, a dampened lignocellulose culture system was used. Five-gram samples of air-dried lignocellulose in 1-liter Corning no. 1460 reagent bottles were autoclaved (121°C, 1 h), and then the bottles were plugged with cotton stoppers and autoclaved for an additional 1 h. The sterilized lignocellulose was inoculated with *S. viridosporus* T7A (10) grown for 48 h in 49.5 ml of mineral salts medium containing 3 g of yeast extract per liter (6). Each bottle was placed on its side and rolled on the bench top to distribute the lignocellulose over its inside surface. Inoculated cultures and uninoculated controls were incubated at 37°C for 8 weeks in a humid incubator.

Products were harvested and characterized by the following procedure. Distilled water (500 ml) was added to each bottle, which was then steamed at 100°C for 1 h. The mixture was then filtered through preweighed filter paper discs. Residues on the filters were air-dried and weighed to determine lignocellulose weight loss (5). The lignin contents of the residues and products were determined by a modified Klason procedure (15), and carbohydrate contents were determined by measurement of reducing sugars after acid hydrolysis of residues, using a Somogyi-Nelson procedure (1). Protein was estimated by the Kjeldahl procedure (15), and ash contents were determined by combusting 10- to 20-mg samples at 900°C and weighing the residues. Elemental and methoxyl analyses were performed by Galbraith Laboratories, Inc. (Knoxville, Tenn.). Carbon, hydrogen, and oxygen contents were corrected for ash content.

APPLs were recovered from culture media by dialyzing the solutions for 3 days at 4°C against distilled water, followed by lyophilization. Alternately, APPLs were precipitated from culture filtrates by acidifying them to pH 1 to 2 with 12 M HCl. Precipitates were collected by centrifugation, washed twice with water, and then either air-dried and weighed or dissolved in water (pH 7), polyethylene glycol, or dimethyl sulfoxide. In the latter case, APPLs were first washed thoroughly in sequence with ethanol and ether.

APPL production in liquid cultures. Growth of *S. viridosporus* also was carried out in shake flask cultures. Duplicate 0.5-g lignocellulose samples were sterilized in 250-ml flasks as described above. The lignocelluloses used were from corn (*Zea mays*), quack grass (*Agropyron repens*), alfalfa (*Medicago sativa*), maple (*Acer platanoides*), and spruce (*Picea pungens*). Each flask was inoculated with spores of *S. viridosporus* suspended in 5.0 ml of sterile 0.3% (wt/vol) yeast extract solution and incubated without shaking at 37°C for 96 h. Then, 120 ml of sterile medium was added, and the flasks were incubated with shaking at 37°C. The medium consisted of our mineral salts solution supplemented with various amounts of yeast extract (0.0 to 0.8%, wt/vol).

The time course of APPL production was followed by periodic sampling of the shaking cultures. The relative concentration of APPL in a sample was determined by filtering the sample, acidifying a 2-ml portion to pH 1 to 2 with 0.1 ml of 12 M HCl, and recording the optical density of the acidified sample at 600 nm.

Milled-wood lignin preparation. A standard milled-wood lignin (MWL) was prepared from corn lignocellulose by the procedure of Björkman (3). This MWL was subjected to most of the characterizations described below.

Gel permeation chromatography. APPL or milled wood lignin (MWL) (1 mg) was dissolved in 1.0 ml of 0.1 M NaOH (11) containing 0.1 M LiCl as recommended by Connors (4), and its molecular weight distribution was determined on a Sephadex G-50 column under the following chromatographic conditions: column, 670 by 15 cm; solvent, 0.1 M NaOH containing 0.1 M LiCl; flow rate, 0.64 ml/min; void volume, 38 ml as shown by exclusion of dextran blue 2000 (Sigma Chemical Co., St. Louis, Mo.). Elution of APPLs was monitored by measuring the absorbance at 280 nm of 2-ml fractions.

Chemical characterizations. (i) Permanganate oxidation. Air-dried control and degraded APPLs and MWL (75 to 90 mg) were ethylated and oxidized with permanganate as described by Kirk and Adler (12). Trimethysilyl derivatives of oxidation products were gas chromatographed by the procedure of Lundquist and Kirk (14) as modified by Pometto et al. (18). Confirmation of product identities was accomplished by comparing the retention times of unknown compounds with those of ethylated standards as described by Crawford et al. (7). Mass spectrographic analyses of the effluent from the gas chromatograph were also utilized (7).

(ii) Acidolysis. APPLs and MWL were degraded by the acidolysis procedure of Lundquist and Kirk (14) as modified by Pometto et al. (17).

(iii) Ester hydrolysis. Aromatic acids esterified to APPLs or the MWL were cleaved from the lignin by mild alkaline hydrolysis. APPL or MWL (30 mg) was dissolved in 1.0 ml of 1 M NaOH and incubated with shaking for 48 h at room temperature (8, 16). The compounds released were quantified by gas chromatography of trimethylsilyl derivatives (7).

(iv) NMR spectroscopy. Nuclear magnetic resonance (NMR) spectra were obtained for APPLs and MWL dissolved in deuterated dimethyl sulfoxide or deuterium oxide (D_2O) on a Brüker 270-MHz NMR instrument run in the Fourier transform mode. The APPLs used for NMR studies were dissolved in 0.1 M NaOH, dialyzed against distilled water, lyophilized, redissolved in D_2O, lyophilized again, and dissolved in deuterated solvents.

(v) Ionization difference spectra. The content of *p*-hydroxyphenyl-carbonyl structures in the APPLs and MWL was determined by the ionization difference spectral procedure of Aulin-Erdtman (2).

(vi) Titration-precipitation curves. Control and degraded APPLs and MWL were dissolved in 0.015 M NaOH, and the pH was recorded (pH 11.5 to 11.8). The solution was then titrated with 0.01 N HCl, and the pH was monitored along with absorbance of the solution at 600 nm.

RESULTS AND DISCUSSION

Lignocellulose degradation and APPL production. After 8 weeks of incubation, *S. viridosporus* had degraded 5 g of corn lignocellulose to a weight loss of 36.2% (Table 1). Lignin and carbohydrate components were depleted by 19.7 and 44.4%, respectively. The uninoculated control showed a weight loss of 0.0%, with lignin and carbohydrate losses of 0.0 and 2.5%, respectively. About 500 mg of APPL was recovered from the acidified aqueous extracts of the degraded lignocellulose compared with 3 mg from the control.

On a Klason lignin basis, the APPL recovered from the degraded lignocellulose represented approximately 36% of the starting lignin (27.1% initial lignin content in the corn lignocellulose). This value is considerably larger than the 19.7% lignin loss indicated by Klason assay of the lignocellulose residue. The discrepancy probably resulted in part from inaccuracies inherent in the Klason procedure (8, 9) and from the pres-

TABLE 1. Corn stover lignocellulose degradation by *S. viridosporus* T7A after 8 weeks of incubation

Prepn	Weight loss of lignocellulose (%)	% Carbohydrate loss[a]	% Lignin loss[a]	APPL recovered (mg)[b]
Incubated control	0.0	2.5 ± 0.8	0.0 ± 0.3	3.0
Streptomyces inoculated	36.2	44.4 ± 0.6	19.7 ± 0.4	490.0

[a] Values are averages of three determinations ± standard deviation.
[b] 5 g of initial lignocellulose containing 27.1% lignin; the Klason lignin content of different lots of the same lignocellulose may vary by a small percentage (see Table 2).

ence of nonlignin contaminants in the APPL. Actinomycete-mediated polymerization of low-molecular-weight aromatic compounds into the APPL might also account for the higher recovery. Analyses of the APPLs for nonlignin components showed that those from degraded and control lignocelluloses contained some carbohydrate (3.0 and 9.9%, respectively), organic nitrogen compounds (3.3 and 4.1%, respectively), and ash (3.3 and 0.7%, respectively). For comparison, the standard corn MWL contained more crude protein (10.9%), less carbohydrate (3.7%), and very little ash (≤0.1%). After correction of weight loss data for nonlignin materials, the APPL recovered from the degraded lignocellulose was 417 mg or 30.8% of initial Klason lignin.

Gel chromatography. Sephadex G-50 chromatography was used to determine the molecular weight distribution of the APPLs. Both control and degraded APPLs from acidified filtrates were eluted in a sharp peak with the void volume (Fig. 1). They contained none of the lower-molecular-weight components present in MWL. Their exclusion by Sephadex G-50 indicated a molecular weight of ≥20,000, as confirmed by passing polyethylene glycols (Matheson Coleman and Bell, Inc., Cincinnati, Ohio) of 15,000 to 20,000 daltons through the column. The degraded APPL probably consisted of a heterogeneous mixture of molecular weight components of ≥20,000. Chromatography of lyophilized APPLs (never acidified) resulted in different molecular weight distribution patterns than those for APPLs isolated by acid precipitation. The control APPL was eluted in a broad band centered around 10,000 daltons. The strain T7A APPL, however, still eluted with the void volume. This indicated that further polymerization of the control APPLs and possibly of the degraded APPLs occurred when culture filtrates were acidified. The data also showed that before acidification the control APPL was considerably lower in average molecular weight than the degraded APPL. When lyophilized control APPL was subjected to ultrafiltration (molecular cut-off, 10,000 daltons) and the filtrate was acidified to pH 1 to 2, a precipitate formed. When chromatographed on the Sephadex G-50 column, this precipitate eluted with the void volume. This confirmed that acid-mediated polymerization occurred in the control APPLs.

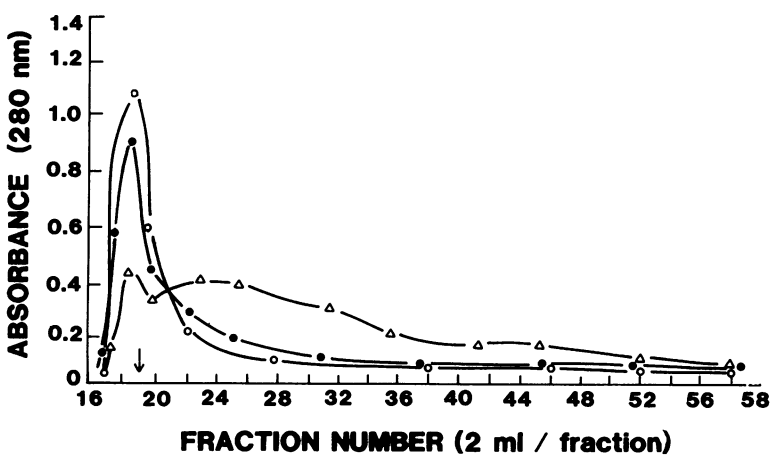

FIG. 1. Elution patterns of corn lignocellulose APPLs after gel permeation chromatography through Sephadex G-50. Symbols: ○, control APPL; ●, APPL from *S. viridosporus* T7A-degraded lignocellulose; △, MWL prepared from undecayed corn lignocellulose.

Lignins are known to undergo condensation reactions under acidic conditions (8).

APPL production over time. The time course of APPL production from corn lignocellulose was followed in liquid shake cultures (Fig. 2). APPL began to accumulate after 72 h of incubation, and the rate of APPL production from 0.5% (wt/vol) lignocellulose was markedly affected by the yeast extract concentration. With no added yeast extract, APPL accumulated at a slow, steady rate over the duration of the experiment (450 h). The yield and rate of production increased with increasing yeast extract concentration up to a 0.6% (wt/vol) level. At 0.8% yeast extract, APPL production was slightly lower than it was at 0.6%. At the 0.6% yeast extract level, the maximal rate of APPL production occurred between 80 and 160 h of incubation. APPL accumulated at a slowly decreasing rate between 160 and 450 h. The same pattern was observed for APPL production when another grass (*Agropyron repens*) was used as substrate (data not shown). Measurable APPL accumulation continued in these cultures for at least 800 h. No APPL was produced by cells grown on yeast extract in the absence of lignocellulose.

APPL production from softwood, hardwood, and grass lignocelluloses. Previous work has shown that *S. viridosporus* cells decompose grass-type lignins more efficiently than softwood or hardwood lignins (1). Table 2 shows the APPL yields from liquid cultures after growing *S. viridosporus* for 875 h on lignocelluloses prepared from a softwood (spruce), a hardwood (maple), two grasses (corn and quackgrass), and alfalfa. The data show that the grass-type lignocelluloses yielded significantly more APPL than did the other lignocelluloses. APPL yields appeared to correlate with the biodegradability of the lignocellulose used.

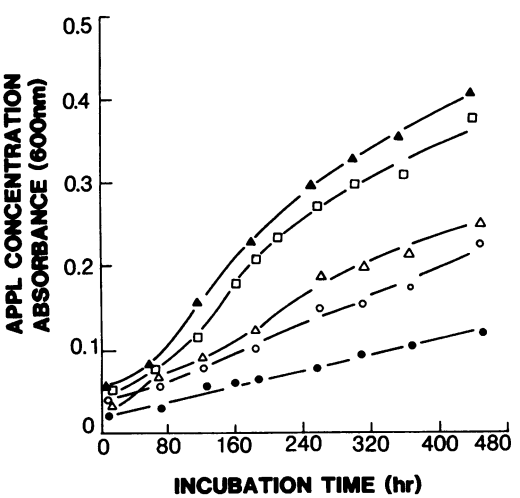

FIG. 2. Rate of APPL production from 0.5% (wt/vol) corn lignocellulose by *S. viridosporus* T7A as influenced by yeast extract concentration in the growth medium (●, 0.0; ○, 0.2; △, 0.3; ▲, 0.6; and □, 0.8% yeast extract). Each point is an average of two replicate flasks sampled at each time.

TABLE 2. APPL yields from softwood, hardwood, grass, and alfalfa lignocelluloses after an 875-h incubation in liquid culture with *S. viridosporus* T7A

Lignocellulose source	APPL produced (mg)	% of initial lignin[a]
Corn	16.3	13.4
Quackgrass	17.7	13.7
Spruce	5.8	3.7
Maple	8.6	5.9
Alfalfa	11.0	8.8

[a] Based on 0.5 g of lignocellulose and initial Klason lignin contents of 24.3 (corn), 25.8 (quackgrass), 34.5 (spruce), 29.3 (maple), and 25.0% (alfalfa).

Chemical characterizations. (i) Acidolysis. To establish the lignin origin of the APPLs, we subjected them to chemical degradation in acidic dioxane. The principal single-ring aromatic acidolysis products from lignins are phenylpropanoid ketols (PKs), such as 1-hydroxy-3-[4-hydroxy-3-methoxyphenyl]-2-propanone (derived from guaiacyl monomeric units) and its coumaryl and syringyl analogs (7, 14). The yield ratio of PK to vanillic acid (VA) can be used to determine the structural integrity of lignin after its partial degradation by microorganisms (13, 14). In the present work, PK and VA yields from the standard MWL were 5.4 and 0.7% of the original lignin, respectively. These values are reasonable for a native lignin (13). The respective yields from the degraded APPL were 2.9 and 2.2%, respectively. The increased yield of VA concomitant with a decreased yield of PK is indicative of significant actinomycete-mediated oxidative degradation of the APPL, as has been reported for the residual insoluble lignin of spruce after decay by *S. viridosporus* (7). As an additional confirmation of the lignin origin of APPLs, we isolated APPLs from *S. viridosporus* cultures grown on [^{14}C]lignin-labeled lignocellulose (10) and found them to be labeled with ^{14}C (unpublished data).

Acidolytic analyses of control APPLs gave very low yields of both VA (0.2%) and PK (0.3%). These data indicate that control APPLs are not representative fractions of native corn lignin. This was confirmed by studies of the elemental and functional group contents of the APPLs compared with those of MWL (see below). The control APPLs were also distinctly different chemically from the microbiologically formed APPLs.

(ii) Permanganate oxidation and ester hydrolysis. Permanganate oxidation products of lignin are derived from phenolic units containing hydroxyl substituents *para* to propyl side chains. From ethylated lignins, these products are recovered as ethoxylated benzoic acids (13). Yields of the principal acids after oxidation of the MWL and APPLs are shown in Table 3. As expected from a grass lignin, the MWL yielded significant amounts of *p*-hydroxybenzoic acid (PHB), VA (4-hydroxy-3-methoxybenzoic acid), and syringic acid (SA; 4-hydroxy-3,5-dimethoxybenzoic acid), which would be derived, respectively, from coumaryl, guaiacyl, and syringyl units within the lignin (8, 13). A small amount of protocatechuic acid (PCA; 3,4-dihydroxybenzoic acid) was also recovered, indicating the presence of some free *o*-diphenolic structures in the MWL (12, 13). The control APPL yielded similar products; however, recoveries of PHB, VA, and SA were lower than from the MWL, and the recovery of PCA was greater. The degraded APPL gave much higher yields of PHB and VA and somewhat higher recoveries of SA than did the MWL and control APPL. Degraded APPLs yielded no PCA.

These substituted benzoic acids could have been derived in part from phenolic acids esterified to the lignin. Bamboo grass lignins, for example, contain significant amounts of *p*-coumaric acid (CA) esterified with γ-hydroxyl groups of lignin side chains (16). CA would be recovered as PHB after permanganate oxidation of such a lignin. Any other esterified phenylpropanoid acids would be recovered as their corresponding benzoic acids. To selectively cleave these esterified acids, we subjected the MWL and APPLs to mild alkaline hydrolysis (8, 16), and free acids were quantified by gas chromatography (17) (Table 4).

As a percentage of the total lignin, CA (3-[4-hydroxyphenyl]-2-propenoic acid) and ferulic acid (3-[4-hydroxy-3-methoxyphenyl]-2-propenoic acid) were the most abundant esterified acids. VA, PHB, and SA were found in lesser amounts, and small amounts of a few unidentified compounds were detected. The degraded APPL contained a substantial amount (5.1%) of esterified CA. Data (Table 5) were obtained for permanganate oxidation yields of lignin-derived VA, PHB, and SA after correction for esterified acids. Even after correction, there was an increase in recovery of all three acids from the degraded APPL compared with recoveries from the MWL and control APPL, which is indicative of a greater number of free (ethylatable) phenolic hydroxyl groups in the streptomycete-produced APPL, resulting from streptomycete-mediated cleavage of ether linkages of *para*-oxygen atoms of lignin. The data suggest that *S. viridosporus* freed significant numbers of hydroxyls as it decomposed and solubilized the polymer.

TABLE 3. Yields of principal permanganate oxidation products from an ethylated corn MWL and ethylated APPLs from control and *S. viridosporus* T7A-degraded corn lignocelluloses

Lignin source	Yield (%)[a] of:			
	VA	PHB	SA	PCA
Control	2.9	3.9	3.5	2.5
T7A APPL	10.4	11.8	4.6	ND[b]
Corn MWL	4.5	7.6	2.9	2.3

[a] Yield was calculated as a percentage of the ethylated lignin subjected to oxidation. Yields were not corrected for esterified compounds in the original preparation.
[b] ND, Not detected.

TABLE 4. Recovery of esterified phenolic compounds after mild alkaline hydrolysis of corn MWL and APPLs from control and *S. viridosporus* T7A-degraded corn lignocelluloses

Lignin source	Yield (%)[a] of:					
	CA	Ferulic acid	SA	VA	PHB	Total
Control APPL	0.31	ND[b]	0.08	ND	ND	0.39
T7A APPL	5.10	0.78	0.05	ND	0.03	5.96
Corn MWL	1.20	1.06	0.13	ND	0.05	2.44

[a] Yields were calculated as a percentage of the lignin subjected to alkaline hydrolysis. Yields are not corrected for nonlignin substances.
[b] ND, Not detected.

(iii) Titration-precipitation curves. All three lignins showed two regions of significant buffering capacity. pKs for these regions were pH 10.0 and 6.3 for the MWL, pH 9.6 and 6.4 for the control APPL, and pH 9.8 and 6.3 for the degraded APPL. The pKa at pH 9.8 to 10.0 corresponded to phenolic hydroxyl groups. The pKa at pH 6.3 to 6.4 was not readily assignable, but may also represent hydroxyl moieties.

By following the absorbance at 600 nm of each lignin solution during titration, it was possible to determine the pH at which lignin precipitation began. For the MWL, control APPL, and degraded APPL, the pH values were 4.0, 3.8, and 5.0, respectively. The degraded APPL began precipitating from solution a full pH unit higher than the other two lignins. APPL precipitation appeared to begin at pH's at which most phenolates have been converted to their free phenols.

(iv) Functional group analyses. Ionization difference spectra (ΔE_i), obtained spectrophotometrically by the procedure of Aulin-Erdtman (2), were used to estimate the conjugated phenolic hydroxyl content of the APPLs. Our interpre-

TABLE 5. Yields of lignin-derived products after permanganate oxidation of corn MWL and APPLs from control and *S. viridosporus* T7A-decayed corn lignocelluloses

Lignin source	Yield (%)[a] of:		
	VA	PHB	SA
Control APPL	2.0	3.6	3.4
T7A APPL	9.6	6.7	4.5
Corn MWL	3.4	6.4	2.8

[a] Yields were calculated as percentage of the lignin subjected to oxidation after subtracting the amount of each compound which would be derived from esterified phenolic compounds.

tation of these spectra is based on the observation that ΔE_i spectra of phenolic residues conjugated to benzyl carbonyls show absorption maxima near 364 nm. Based on a molar extinction coefficient of 2.2×10^7 cm^2/mol for conjugated phenols (2), the conjugated phenolic hydroxyl content of the degraded APPL was 0.774×10^{-2} g/g of APPL, or about 0.8% (wt/wt). The corresponding value for the control APPL was 0.17×10^{-2} g/g of APPL, or approximately 0.2% (wt/wt). The MWL contained about 1.0% conjugated phenolic hydroxy content, about fourfold higher than the control APPL and about the same as the MWL. Nonconjugated phenols would not be discernible by our ΔE_i procedure due to the much higher extinction coefficient of conjugated phenols.

NMR spectra of APPLs and MWL (using deuterated dimethyl sulfoxide) showed an enrichment in the degraded APPL of carboxyl groups (− 11.2 ppm, D_2O exchangeable) compared with MWL and the control APPL (no carboxyl resonances detected). However, the number of carboxyl groups in the decayed APPL appeared to be quite small. Even more apparent in these spectra were greatly increased numbers of D_2O-exhangeable phenolic protons in the decayed APPLs compared with controls or MWL. Spectra obtained in D_2O indicated a decreased methoxyl and enriched benzylic proton content relative to aromatic ring protons in the degraded APPL as compared with the control. Broad line widths made integration of our spectra difficult; therefore, we have not quantified these differences. The data show, however, that *S. viridosporus* significantly altered APPL by largely oxidative mechanisms, increasing or enriching its content of phenolic hydroxyls, benzylic protons, and, to a small extent, carboxyl groups. Methoxyl groups were depleted in the decayed polymer (see below).

(v) Elemental and methoxyl contents. The carbon, hydrogen, oxygen, and methoxyl contents of the control and *Streptomyces*-degraded APPLs and the standard MWL are shown in Table 6. The *S. viridosporus*-degraded APPL was substantially lower in carbon content and higher in oxygen content than the standard MWL. The hydrogen content was slightly lower, and the methoxyl content was reduced by 35%. These values are indicative of oxidative degradation of APPLs by *S. viridosporus* (7, 13) and show that, in addition to the previously discussed *Streptomyces*-mediated cleavage of ether linkages in the lignin, there are also other extensive oxidative modifications of the solubilized polymer.

The control APPL was lower in carbon and hydrogen content, higher in oxygen content, and 77% lower in methoxyl content that the MWL. Because of its extremely low methoxyl content, the lower recoveries of substituted benzoic acids after permanganate oxidation and of acidolysis products after acidolysis, its lower average molecular weight before acidification, and the very low yields of control APPLs (≤2.2% on an initial lignin basis), the control APPL clearly represents a fraction not representative of corn lignin as a whole. In contrast, the high yields of degraded APPL (25 to 30% of the initial lignin), their higher average molecular weight, and their substantially greater methoxyl content as compared with the control APPL indicate that this fraction must be principally of lignin origin. Standard C_9 formulas, calculated by the procedure of Ramirez and Delgado (18), were $C_9H_{11.8}O_{4.1}(OCH_3)_{0.17}$, $C_9H_{11.6}O_{4.1}(OCH_3)_{0.6}$, and $C_9H_{10.0}O_{2.7}(OCH_3)_{0.7}$ for the control APPL, strain T7A-degraded APPL, and MWL, respectively. The degraded APPL was considerably more oxidized and contained fewer methoxyl groups per C_9 unit than the standard MWL.

S. viridosporus degrades lignin oxidatively and in the process releases water-soluble intermediates into the culture medium. These intermediates include single-ring aromatic compounds (6) and, as found in the present study, an oxidatively modified polymer (APPL). During degradation of corn lignocellulose by *S. viridosporus*, single-ring aromatic intermediates such as PHB, VA, and SA accumulate to total concentrations equivalent to 1 to 2% of the initial

TABLE 6. Elemental and methoxyl group contents of control and *S. viridosporus* T7A-degraded APPLs and the standard MWL

Lignin source	Contents (%)[a]			
	C	H	O	OCH_3
Control APPL	52.62	5.92	32.87	2.53
T7A APPL	50.31	5.86	32.94	7.79
Corn MWL	59.93	6.29	28.35	11.21

[a] Reported as percent of dry weight corrected for ash content.

lignin (6). In contrast, APPL accumulations can be >30%. The low-molecular-weight aromatic molecules may be metabolized further; therefore, it is not possible to determine their quantitative significance in lignin catabolism by *S. viridosporus*. Most are probably released enzymatically from terminal residues on the lignin polymer. The APPL fraction is clearly an important catabolic intermediate liberated from the intact lignin polymer, probably by streptomycete-catalyzed cleavage of *para* ether linkages in the lignin. However, streptomycete-mediated or spontaneous polymerization of lower-molecular-weight intermediates to form APPL cannot be ruled out. Further oxidative degradation of the APPL intermediate undoubtedly occurs. Our data suggest that degradation involves oxidation of side chains, demethylation of aromatic rings, and ring cleavage reactions such as those previously described for insoluble softwood lignin residues after partial degradation by *S. viridosporus* (7).

Since streptomycete-produced APPLs are polyphenolic and may be prepared in high yield from waste lignocelluloses such as corn stover, they have considerable potential as chemical feedstocks for a number of industries. Some potential industrial applications include the use of APPLs as surfactants or surfactant feedstocks and as polyurethane or adhesive precursors (6).

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