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Factors Involved in the Regulation of a Ligninase Activity in *Phanerochaete chrysosporium*

B. D. FAISON AND T. K. KIRK*

Forest Products Laboratory, Forest Service, Department of Agriculture, Madison, Wisconsin 53705, and Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

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The regulation of an H_2O_2-dependent ligninolytic activity was examined in the wood decay fungus *Phanerochaete chrysosporium*. The ligninase appears in cultures upon limitation for nitrogen or carbohydrate and is suppressed by excess nutrients, by cycloheximide, or by culture agitation. Activity is increased by idiophasic exposure of cultures to 100% O_2. Elevated levels of ligninase and, in some cases, of extracellular H_2O_2 production are detected after brief incubation of cultures with lignins or lignin substructure models, with the secondary metabolite veratryl alcohol, or with other related compounds. It is concluded that lignin degradation (lignin → CO_2) by this organism is regulated in part at the level of the ligninase, which is apparently inducible by its substrates or their degradation products.

Phanerochaete chrysosporium, a white-rot wood-decaying basidiomycete, produces a potent lignin-degrading system that oxidizes lignin completely to CO_2. The biochemistry of this process is only partially understood.

A body of literature has accumulated on the gross regulation of the complete lignin-degrading system (lignin → CO_2) in this organism. The complete system is idiophasic (7, 14), appearing upon starvation for carbon, nitrogen, or sulfur (13). Activity is rapidly abolished by cycloheximide (14). The ligninolytic system is particularly active in cultures grown under high oxygen tension (3, 17, 20). Agitation of cultures during growth suppresses ligninolysis (17, 28). The system is produced in the absence of lignin (14), and its appearance is associated with the accumulation of a secondary metabolite, 3,4-dimethoxybenzyl (veratryl) alcohol (14, 23).

P. chrysosporium has recently been shown to produce an extracellular enzyme that catalyzes oxidative C_α-C_β cleavage in two important lignin substructures (18, 25) and thereby causes the partial depolymerization of natural lignins (25). This enzyme also oxidizes veratryl alcohol to veratraldehyde (18, 26). The ligninase requires extracellular H_2O_2 (18, 25), which is also generated by this organism (8, 11). Initial studies have concentrated on the enzyme's biochemistry—its mode of catalysis, physicochemical properties, and substrate range (26). The work described here identifies factors involved in the regulation of this ligninolytic activity in cultures. We show that the ligninase is regulated in tandem with the complete lignin-degrading system, and that it is apparently inducible by several of its substrates (or their transformation products), including veratryl alcohol and lignins.

MATERIALS AND METHODS

Organism and culture conditions. *P. chrysosporium* Burds ME-446 (ATCC 34541; maintained by the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis.) was grown in shallow liquid culture, i.e., 10 ml of fluid in a 125-ml Erlenmeyer flask (17). Conidial inocula were prepared as described previously (13, 17). Cultures were routinely grown in defined liquid Basal III medium (22) containing 2.2 mM nitrogen and 1.0% glucose as the carbon source. This medium contains nitrogen in growth-limiting amounts. Cultures to be assayed for H_2O_2 production were grown in defined Basal II medium (9, 17) containing 2.2 mM nitrogen and 1.0% glucose. Carbohydrate-limited cultures were grown in Basal II medium containing 6.6 mM nitrogen and 0.15% glucose. The same buffer, 10 mM potassium 2,2-dimethylsuccinate, pH 4.5 (8), was used in all media. Cultures were routinely incubated unshaken at 39°C. Agitated cultures were grown on a rotary shaker (200 rpm; 2.5-cm diameter cycle) at 39°C. Unless otherwise indicated, nitrogen-limited cultures were equilibrated with air at the time of inoculation (day 0) and were flushed with oxygen on day 3 (shifted cultures). For studies of the effect of atmosphere on ligninolytic activity, cultures were flushed with air on day 3 (air grown), or with oxygen on days 0 and 3 (oxygen grown). Cultures were assayed on day 6. Carbohydrate-limited cultures were routinely equilibrated with air on day 0 and flushed with oxygen on day 2. For studies to determine the time course for ligninolytic activities, nitrogen-limited cultures were equilibrated with oxygen on day 0 and were flushed with oxygen on days 2, 3, 4, 5, and 6; carbohydrate-limited cultures were sparged with 60% O_2–40% N_2 by the same schedule (13). Aeration regimens for the culture additive studies are described below.

Lignins and related compounds. Synthetic [ring-*U*-^{14}C]lignin (specific activity, 1.5×10^4 Bq/mg) and unlabeled synthetic lignin were prepared as described previously (14, 15). Milled wood lignins (4) of spruce (*Picea glauca*) and birch (*Betula verrucosa*) were provided by H.-m. Chang and K. Lundquist, respectively. [*U*-^{14}C]glucose (specific activity, 4.0×10^5 kBq/mmol) was from ICN Pharmaceuticals (Irvine, Calif.). 1-(3-Methoxy-4-[^{14}C]methoxyphenyl)-2-(2-methoxyphenoxy)-propane-1,3-diol (compound I; specific activity 2.2×10^5 kBq/mmol) and unlabeled compound I (Fig. 1) were prepared from 4-hydroxy-3-methoxy-α-(2-methoxyphenoxy)-β-hydroxypropiophenone (19) by methylation with $^{14}CH_3I$ (or CH_3I), followed by reduction with $NaBH_4$. Methylation with CH_3I, without subsequent reduction, provided compound II. 1,2-Bis-(4-methoxyphenyl)-propane-1,3-diol (compound III) was prepared as described previously (16). Syringyl; vanillyl; anisyl; 3,4,5-trimeth-

* Corresponding author.

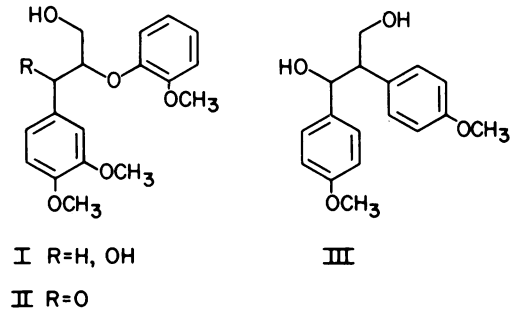

FIG. 1. Molecular formulae.

oxybenzyl; veratryl alcohol; and veratraldehyde were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). Veratrylglycerol was prepared from 1-(4-hydroxy-3-methoxyphenyl) glycerol (obtained from Knut Lundquist) by 4-*O*-methylation with CH_2N_2. 4-Hydroxybenzyl alcohol was prepared by $NaBH_4$ reduction of 4-hydroxybenzaldehyde (Aldrich).

Other chemicals and reagents. Cycloheximide, *o*-dianisidine, and peroxidase (type II; specific activity, 152 U/mg) were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of reagent grade.

Assays. Lignin degradation was detected in whole cultures by a radiorespirometric assay (15). Cultures (six replicates) were labeled with approximately 833 Bq of a synthetic [ring-^{14}C]lignin, carried in an aqueous suspension (0.5 ml containing <2 μl of *N*,*N*-dimethylformamide); $^{14}CO_2$ was trapped in a basic scintillation cocktail (14, 15, 17). Glucose oxidation was measured in an analogous fashion. Unless otherwise indicated, the labeled substrates were added to cultures on day 6.

Ligninase activity was determined in the unconcentrated supernatant solutions of cultures that had been centrifuged at 12,000 × *g* for 10 min at 4°C in a Sorvall model RC-5 centrifuge. Cultures were assayed as pooled duplicates; little variation was found among replicate cultures (standard deviation, ≤10%). Unless otherwise indicated, the ligninase activity was assayed spectrophotometrically (26) as the H_2O_2-dependent formation of veratraldehyde from veratryl alcohol. The standard reaction mixture (0.5-ml volume) contained 0.4 mM veratryl alcohol and 0.15 mM H_2O_2 in 100 mM sodium tartrate (pH 3.0). Each assay tube contained 335 μl of culture supernatant. The reaction was started by H_2O_2 addition, and the linear increase in absorbance at 310 nm was monitored over 3 to 5 min at room temperature. Optical measurements were performed with a Gilford model 250 spectrophotometer. Where noted, ligninase activity was assayed by the radiometric quantitation (25) of veratraldehyde produced by the cleavage of ^{14}C-labeled β-*O*-4 compound I. The reaction mixture (0.5-ml volume; 335 μl of culture supernatant) contained 8 × 10^3 Bq of the labeled compound, adjusted to 2.5 mM with the unlabeled substrate (added in 5 μl of dimethylformamide), 0.15 mM H_2O_2, and 0.01% Tween 80, and was buffered with 100 mM sodium tartrate (pH 3.0). The reaction was initiated by H_2O_2 addition; incubation was at room temperature for 3 min. Radioactive veratraldehyde was isolated by thin-layer chromatography and quantified (25).

Hydrogen peroxide release from whole cultures was assayed as described previously (8). Cultures were assayed in duplicate; variation among cultures has been established at ±20%.

Additive studies. Idiophasic cultures were spiked with various compounds in aqueous solution (0.5-ml volume) and then flushed with oxygen and incubated for an additional 12 h at 39°C. Cultures were then monitored for ligninase activity, H_2O_2 production, lignin oxidation to CO_2, and glucose respiration.

In nutritional studies, nitrogen-starved (5.5-day-old) cultures were incubated with 2.8 mM (final concentration) sodium glutamate, or with 5.7 mM NH_4Cl (9). Carbohydrate-limited (3.5-day-old) cultures were supplemented with 0.1% glucose, or with 2.8 mM glutamate or α-ketoglutarate (13), supplied as their sodium salts. Cultures treated with cycloheximide received 70 μg of that inhibitor. Control cultures received distilled water only. Cultures to be used for induction studies were supplied with various lignin-related compounds, carried in an aqueous suspension or solution also containing 10% dimethylformamide. All monomeric lignin substrates were added at 0.2 mM final concentration; each culture received 0.38 mg of dimeric and polymeric substrates. Control cultures received 10% dimethylformamide only. Cultures that had been spiked with lignin-related compounds were assayed for ligninase activity in reaction mixtures containing an excess of substrate (20 mM veratryl alcohol or 25 mM unlabeled compound I).

RESULTS

Nitrogen-limited cultures. Under our experimental conditions, mycelial growth ceases within 1.5 to 2.0 days (14). In the work described here, ligninase activity was detected in the extracellular culture fluid 24 to 36 h after the end of the active growth phase. The appearance of the ligninase coincided with the appearance of the complete lignin-degrading system (lignin → CO_2). Although ligninolysis (CO_2 evolution) proceeded at an essentially constant rate from days 4 to 6, ligninase activity peaked on day 5 and declined sharply thereafter (Fig. 2A). The addition of either glutamate or NH_4^+ to these nitrogen-starved cultures strongly suppressed ligninase activity (Table 1), as did the addition of the protein synthesis inhibitor cycloheximide.

Incubation of cultures under high oxygen tension affected enzyme activity in a complex fashion (Table 2). Cultures grown under air produced substantially less ligninase activity than did those grown under oxygen. Activity was highest in cultures held under air during primary growth and then shifted to an oxygen atmosphere. A similar effect was observed for the complete ligninolytic system (lignin → CO_2). Oxygen was stimulatory toward glucose oxidation as well; however, this effect was restricted to the primary growth phase.

Ligninase activity was abolished by agitating the growing cultures, which resulted in formation of mycelial pellets. Activity was also absent in shaken cultures in which a mat had been allowed to develop before agitation.

The amount of ligninase detected in cultures was affected by lignin and by compounds structurally related to lignin subunits (Table 3). A two- to fivefold increase in ligninase activity was seen in cultures incubated during the idiophase with various lignins or with either of two dimeric arylglycerol-β-aryl (β-*O*-4)-type model compounds (compounds I and II); in fact, β-*O*-4 model I was the most stimulatory compound tested. Incubation with the 1,2-diarylpropane-1,3-diol (β-1-type) model (III), however, suppressed ligninase activity. A slight increase in activity was seen in cultures after incubation with either syringyl or vanillyl alcohol. In contrast, 4-methoxybenzyl (anisyl) and 3,4,5-trimethoxybenzyl alcohols were slightly suppressive, whereas 4-hydroxyben-

zyl alcohol decreased activity by 80%. A marked increase in ligninase activity was observed in cultures incubated with the fungal idiolite veratryl alcohol, which is closely related to the above-mentioned compounds and which accumulates in ligninolytic cultures (21, 23). Veratraldehyde and veratrylglycerol, the immediate biosynthetic precursors of veratryl alcohol (23), were somewhat less stimulatory. With the exception of anisyl alcohol, which had no effect, all compounds tested inhibited (competed with) veratryl alcohol oxidation by the crude enzyme in vitro at the concentrations added to cultures. In addition to their effects on ligninase activity, birch lignin and two of the three dimeric compounds, as well as veratryl alcohol, also slightly enhanced idiophasic H_2O_2 production (Table 3). Moreover, those compounds most stimulatory toward ligninase activity, i.e., the β-*O*-4 model I and veratryl alcohol, caused a small, but statistically significant, increase in the rate of lignin oxidation to CO_2. This increase could not be accounted for by an overall stimulation of general oxidative metabolism (glucose respiration) (Table 3).

Growth of cultures in the presence of veratryl alcohol from the time of inoculation increased ligninase titer by 20%, but did not alter the time of appearance of the ligninase activity (data not shown). Under these conditions, veratryl alcohol had no apparent effect on the complete lignin-degrading system; cultures became ligninolytic at the same age and oxidized lignin to CO_2 at the same rate as in the absence of added veratryl alcohol.

To test the validity of the veratryl alcohol oxidation assay, with which the results described above were obtained, cultures were further assayed for their ability to catalyze the oxidative C_α-C_β cleavage of dimeric (β-*O*-4) lignin model I. This reaction is catalyzed in vitro by the ligninase (26). We found that the two assays give essentially the same results. Activity against the dimer appeared on day 3 and peaked on day 5 (Fig. 2A). This cleavage activity was quenched by incubation of cultures with either glutamate or NH_4^+, but was enhanced in cultures incubated with veratryl alcohol or lignin.

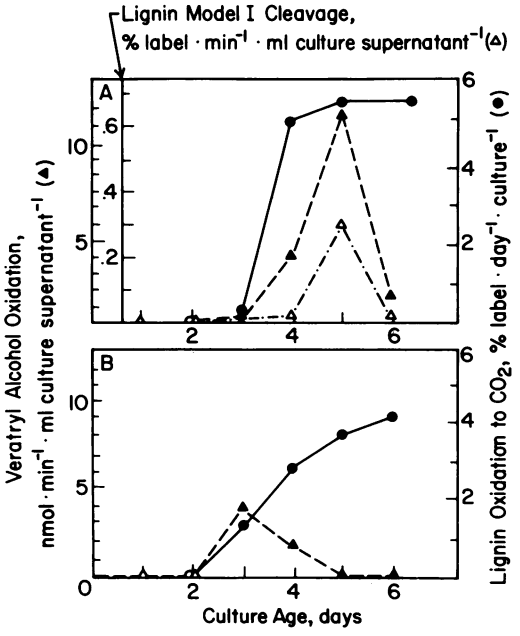

FIG. 2. Time course for ligninolytic activities in *P. chrysosporium*. Nitrogen-limited (A) or carbohydrate-limited (B) cultures were prepared and incubated as described in the text. Lignin oxidation (●) was assayed radiorespirometrically; cultures received [^{14}C]lignin at the time of inoculation. Each point represents the mean of six replicates (±15% standard deviation). Ligninase activity was assayed spectrophotometrically as veratryl alcohol oxidation (▲) or radiometrically as lignin model compound I cleavage (△). Each point represents pooled duplicates.

TABLE 1. Effect of culture additives on ligninase activity

Culture addition	Ligninase activity (relative rates)	
	Nitrogen limited	Carbohydrate limited
Control	(1.0)[a]	(1.0)[b]
NH_4^+	0.1	NA[c]
Glucose	NA	0.7
Glutamate	0.1	ND[d]
α-Ketoglutarate	NA	0.4
Cycloheximide	ND	ND

[a] Control cultures oxidized 7.2 nmol of veratryl alcohol per min per ml of culture supernatant (pooled duplicate cultures).
[b] Control cultures oxidized 5.2 nmol of veratryl alcohol per min per ml of culture supernatant (pooled duplicate cultures).
[c] NA, Not assayed.
[d] ND, None detected.

Carbohydrate-limited cultures. Cultures grown in medium containing excess nitrogen, but limited for glucose, cease mycelial growth within 1.5 to 2.0 days (13). Ligninase activity appeared simultaneously with the activity of the complete ligninolytic system (lignin → CO_2) on day 3 and peaked immediately (Fig. 2B); at no time did the level of ligninase activity approach that maximum observed under nitrogen limitation. Similarly, the titer of the complete system was lower than that obtained in nitrogen-starved cultures. Ligninase activity declined slowly with increasing culture age; the rate of ligninolysis (CO_2 evolution) decreased more gradually through day 6. Incubation of cultures with 0.1% glucose partially suppressed ligninase activity (Table 1). The addition of 2.8 mM glutamate completely quenched ligninase activity; a much lesser effect was observed after incubation with a corresponding amount of α-ketoglutarate, which is the deamination product of glutamate. Activity was abolished by cycloheximide. Incubation of cultures with 0.2 mM veratryl alcohol caused a twofold increase in ligninase activity; activity was partially suppressed, however, by the addition of lignin itself.

DISCUSSION

Our results demonstrate several parallels between the regulation of the complete lignin-degrading system (9, 13, 14) and that of the recently described ligninase in *P. chrysosporium*. Both activities are idiophasic; they appear simultaneously in cultures starved for either nitrogen or carbohydrate and are suppressed by supplementation with the limiting macronutrient. Both activities are suppressed by glutamate, under either nitrogen or carbohydrate limitation. Substantially higher levels of either activity are obtained under nitrogen limitation. Both activities are abolished by cycloheximide within 12 h, demonstrating rapid protein turnover and a requirement for continuing protein synthesis. Both activities are affected by oxygen in the same complex fashion. Both activities are absent in agitated cultures. Thus, the gross regulation of the ligninase activity correlates well with that of the complete ligninolytic system. We found, however, that ligninase activity peaks sharply during idi-

TABLE 2. Effect of growth atmosphere on ligninolytic activities (nitrogen-limited cultures)

Aeration mode[a]	Relative rates		
	Ligninase activity	Lignin oxidation to CO_2	Glucose oxidation to CO_2
Oxygen grown	(1.0)[b]	(1.0)[c]	(1.0)[d]
Air grown	0.06	0.06	0.7
Shifted	1.4	1.6	0.7

[a] Aeration protocols are described in text. Cultures were assayed on day 6.
[b] Control cultures oxidized 5.6 nmol of veratryl alcohol per min per ml of culture supernatant (pooled duplicates).
[c] Control cultures respired 0.20% of the added label per h (six replicates; standard deviation, ≤15%).
[d] Control cultures respired 0.041% of added label per h (six replicates; standard deviation ≤15%).

ophase, whereas the rate of CO_2 evolution from lignin declines much more slowly (carbohydrate limitation) or remains essentially constant (nitrogen limitation). The biochemical basis for this decrease in enzyme activity and its significance are not known. Degradation of the lignin polymer (1, 9, 11, 14, 27) and of dimeric model compounds (29) by *P. chrysosporium* occurs only in the idiophase. At least certain lignin-related monomers, however, are degraded during the tropophase (1). These results suggest that the ligninase, which is idiophasic as shown here, is involved in the initial attack on lignin.

The ligninolytic system in this organism has been considered noninducible by lignin: growth in the presence of low (50 mg·liter^{-1}) concentrations of a synthetic lignin does not significantly increase the rate of CO_2 evolution from lignin (14). The results presented here, however, show that the activity of the ligninase is markedly increased by a 12-h preincubation of idiophasic cultures with either synthetic or natural lignins (38 mg·liter^{-1}). Birch lignin also stimulated the production of extracellular H_2O_2, which is a cofactor for the ligninase (18, 25) and which is required for lignin degradation (8). Indeed, a slight increase in the rate of CO_2 evolution from lignin was noted here after preincubation of cultures with birch lignin. The synthetic lignin used here and in previous work was, however, only marginally effective in increasing ligninase titer, which may help to explain its failure to increase the activity of the complete ligninolytic system (14).

Ulmer et al. (27) recently reported an apparent induction of the total ligninolytic system after incubation of cultures with high concentrations (2 g·liter^{-1}) of a dioxane-HCl lignin from wheat straw. Similarly, Greene and Gould (12) have noted an acceleration of H_2O_2 production in this organism by incubation with lignocellulosic materials in vitro. Our new findings also suggest that the lignin-degrading system is inducible by lignin, at least in part, at the level of the ligninolytic enzyme. Our data suggest that lignin affects the production of extracellular H_2O_2 as well. The loose correlation between the amount of ligninase activity or extracellular H_2O_2 generated (or both) by induced cultures and the rate at which lignin is oxidized to CO_2, however, indicates that this effect does not encompass the entire ligninolytic system. Moreover, as discussed elsewhere (12, 27), it is unlikely that lignin itself could act as an inducer; its size and insolubility preclude its crossing the cytoplasmic membrane to interact with DNA. Thus, if true induction is involved, the actual effector would most plausibly be a soluble, low-molecular-weight compound derived from or related to lignin.

Lignin degradation by whole cultures proceeds through the depolymerization of the substrate (7), generating a variety of low-molecular-weight products (5, 6, 24). These initial reactions must occur extracellularly. Some of these reactions can be catalyzed by the extracellular ligninase, which attacks lignin yielding, presumably, the single-ring products vanillin and syringaldehyde (25). In the presence of excess glucose, as is the case here, such compounds are rapidly reduced to the corresponding alcohols (2). We found the ligninase activity to be increased only slightly by incubating cultures with syringyl or vanillyl alcohol. This modest stimulation may reflect the rapid degradation of these phenols via the action of phenol-oxidizing enzymes. In fact, the ligninase itself has phenol oxidase (peroxidase) activity (26).

Intriguingly, the nonphenolic 4-*O*-methylated analog of vanillyl alcohol, veratryl alcohol (which, as noted, is an idiolite of this fungus), increased the ligninase titer much more than vanillyl alcohol when added in physiological (23) concentrations and indeed was the most stimulatory monomeric compound tested. The greatest enhancement of activity was seen with the dimeric lignin model I; model II was somewhat less stimulatory, whereas model III markedly quenched ligninase activity. The β-*O*-4 dimeric models (I and II) are both degraded by cultures to yield veratryl alcohol (10), which probably is the actual effector in their cases; model II is degraded more slowly than I (10), presumably explaining its lesser effect. The action of the cultures on the β-1 model (III) generates anisaldehyde, which is reduced

TABLE 3. Effect of lignin-related compounds on ligninolytic activities (nitrogen-limited cultures)

Culture addition	Relative rates			
	Ligninase activity	H_2O_2 production	Lignin oxidation to CO_2	Glucose oxidation to CO_2
Control	(1.0)[a]	(1.0)[b]	(1.0)[c]	(1.0)[d]
Lignins				
Birch	4.0	1.4	1.2	1.0
Spruce	3.1	NA[e]	1.0	1.2
Synthetic (coniferyl)	2.2	NA	1.2	1.2
Lignin dimers				
I (β-*O*-4; C_αHOH)	5.2	0.8	1.6	0.9
II (β-*O*-4; C_α=O)	2.6	1.4	1.2	0.9
III (β-1)	0.4	1.4	1.2	0.9
Lignin monomers				
Syringyl alcohol	1.5	NA	1.0	NA
Vanillyl alcohol	1.1	NA	0.8	NA
4-Hydroxybenzyl alcohol	0.2	NA	0.9	NA
Anisyl alcohol	0.9	NA	0.9	NA
3,4,5-Trimethoxybenzyl alcohol	0.9	NA	1.0	NA
Fungal metabolites				
Veratryl alcohol	4.5	1.4	1.6	0.8
Veratraldehyde	3.7	1.0	1.3	1.0
Veratrylglycerol	3.0	1.1	NA	NA

[a] Control cultures oxidized 4.5 nmol of veratryl alcohol per min per ml of culture supernatant (pooled duplicate cultures).
[b] Control cultures generated 0.38 nmol of H_2O_2 per min (duplicate cultures).
[c] Control cultures respired 0.20% of added label per h (six replicates; standard deviation, ≤15%).
[d] Control cultures respired 0.04% of added label per h (six replicates; standard deviation ≤15%).
[e] NA, Not assayed.

to anisyl alcohol in cultures (2, 16). This latter compound has little effect on the ligninase titer or on the crude enzyme in vitro; the basis for the β-1 model's suppression of ligninase activity, therefore, is not clear.

Our results add to the growing body of evidence that links lignin degradation to general secondary metabolism, specifically to veratryl alcohol production, in this organism. The ligninase has been shown to catalyze in vitro several of the reactions involved in veratryl alcohol synthesis (26), including the oxidation of cinnamyl alcohols to phenylglycerols and their subsequent oxidation to aromatic aldehydes as well as the oxidation of veratryl alcohol itelf. These reactions are also common to lignin model compound degradation. An induction of ligninase by veratryl alcohol would explain the previously recognized (9, 23) similarities in the gross regulation of ligninolytic activity and veratryl alcohol synthesis, and the production of ligninolytic activities in the absence of lignin, reported here and elsewhere (14). The apparent connection between veratryl alcohol synthesis and lignin oxidation has been discussed by Shimada et al. (23), who postulated a metabolic overlap between the two activities based on the similarities in their biochemistry and regulation. It is not known, however, whether the ligninase is involved in veratryl alcohol metabolism in vivo; our unpublished results have shown that the addition of catalase to idiophasic cultures does not halt the accumulation of veratryl alcohol, indicating that extracellular H_2O_2 plus ligninase are probably not involved in metabolite synthesis.

In addition to its origin from fungal metabolism, veratryl alcohol may be produced indirectly from lignin; vanillic acid, a major aromatic product of ligninolysis by *P. chrysosporium* (5, 24), is in part 4-*O*-methylated to veratric acid by this organism (2, 5, 24); the latter is rapidly reduced to veratryl alcohol (2). The observed effects of the lignins on ligninase titer in nitrogen-limited cultures may therefore reflect in part their conversion to veratryl alcohol. The low levels of activity produced under carbohydrate limitation, however, may preclude this sort of feedback, perhaps explaining the lack of increase in ligninolytic titer observed after the addition of lignin to these cultures. Veratryl alcohol itself stimulates ligninase activity under these conditions.

Whatever the relationship between veratryl alcohol and lignin degradation, it should be noted that the presence of the metabolite alone is not sufficient to trigger ligninolysis; the addition of veratryl alcohol to growing (tropophasic) cultures does not cause an early appearance of either the ligninase or the complete system. Thus, although the ligninolytic system may be inducible, a primary, overriding control exists at the level of macronutrient repression.

In conclusion, our results establish that the gross regulation of the H_2O_2-dependent ligninase in *P. chrysosporium* correlates well with that of the complete lignin-degrading system. We find also that the ligninase is apparently induced by lignin degradation products and, perhaps significantly, by the secondary metabolite veratryl alcohol. These observations are consistent with a role for this enzyme in the initial steps of ligninolysis, although it does not appear to be the rate-limiting component.

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