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Towards elucidation of the lignin degradation pathway in actinomycetes

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Six biodegradative actinomycete strains were grown on a dimeric model lignin compound of the β-aryl ether type. Although only two strains, *Thermomonospora mesophila* and *Streptomyces badius*, utilized the compound as a carbon and energy source and produced substantial amounts of monomeric products, all of the strains could demethylate the substrate and oxidize Cα on the phenylpropane side-chain. *Streptomyces* sp. EC1 produced small amounts of aromatic acids and unidentified lignin-derived products when grown on straw. This organism also produced cell-bound demethylase requiring H_2O_2 and Mn^{2+}, protocatechuate 3,4-dioxygenase and β-carboxymuconate decarboxylase activity in response to growth on low-molecular-mass aromatic compounds but not lignocellulose or its polysaccharide components. Extracellular peroxidase and catalase activity were detected in all of the strains. These data are used to propose a scheme by which actinomycete attack of the lignin component of plant biomass can be envisaged.

Introduction

The detailed biochemistry of lignin degradation is beginning to be resolved in the wood-rotting fungus *Phanerochaete chrysosporium*. This organism can extensively depolymerize lignin by secreting oxidative enzymes, of which the peroxidases have been identified as the most important. Multiple forms of lignin peroxidase (Farrell *et al.*, 1989) and manganese peroxidase (Gold *et al.*, 1989) are known to be involved, together with glyoxal oxidase, which is reponsible for the generation of H_2O_2 (Kersten & Kirk, 1987) essential to the degradation process. Lignin degradation occurs only during secondary metabolism in *P. chrysosporium*, triggered by nutrient (particularly nitrogen) starvation (Keyser *et al.*, 1978), and this has helped in the implication of particular enzyme species in lignin attack. Lignin degradation by bacteria is by comparison poorly understood; it appears to be a primary metabolic process which results in modification and solubilization rather than depolymerization on any significant scale (for a review see Zimmermann, 1990).

The structural complexity of native and extracted lignins often requires that alternative substrates be used as indicators of degradative ability. These substrates have included simple monomeric aromatic compounds (Eriksson *et al.*, 1984; Sutherland *et al.*, 1983), but synthesized dimeric and trimeric aromatic ring compounds containing linkages similar to those found in native lignin (Crawford *et al.*, 1975; Gold *et al.*, 1984) are more relevant. Enzymes directly involved in attacking these compounds include not only phenol oxidases such as peroxidases and laccases, but also dioxygenases such as protocatechuate dioxygenase. In native lignin, the arylglycerol-β-aryl ether (β-O-4) bond accounts for almost 50% of the phenylpropane linkages (Adler, 1982) and its cleavage is thought to be crucial to depolymerization. The extracellular lignin peroxidases of *P. chrysosporium* have been shown, using model compounds, to cleave not only this bond (Kirk *et al.*, 1986; Miki *et al.*, 1986), but also several others, including diaryl propane, α-aryl and biphenyl linkages (Tien, 1987). A number of prokaryotes can cleave β-O-4 linkages in model compounds (see Zimmermann, 1990) and in *Streptomyces*

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Abbreviation: APPL, acid-precipitable polymeric lignin.

viridosporus, this activity was exhibited by a partially purified isoform of extracellular peroxidase (Ramachandra *et al.*, 1988).

Actinomycetes have received the most attention of the biodegradative prokaryotes. Their hyphal growth form is well-suited to the colonization of plant biomass and they secrete a range of enzymes active against lignocellulose. While they can generate some $^{14}CO_2$ from [^{14}C]lignin-labelled lignocellulose preparations, their ability to solubilize a lignin-rich fraction of the substrate is more impressive (for a review see McCarthy, 1987). Whether this solubilization of lignocarbohydrate involves any direct attack on polyphenolic lignin is equivocal (Ball *et al.*, 1989) but the product, which can be recovered from culture supernatants by simple acid precipitation (Crawford *et al.*, 1983), has a number of interesting and potentially exploitable properties (Crawford *et al.*, 1984; Ball *et al.*, 1990). Subsequent to the discovery of lignin peroxidase in *P. chrysosporium* (Tien & Kirk, 1983), Crawford and coworkers have described and partially characterized extracellular peroxidases in *Streptomyces* strains classified as ligninolytic (Ramachandra *et al.*, 1988; Adhi *et al.*, 1989; Lodha *et al.*, 1991). However, extracellular peroxidase activity is not restricted to those actinomycetes for which there is good evidence of lignin-degrading ability (Ball *et al.*, 1990; Wang *et al.*, 1990), and indeed preliminary screening shows that peroxidases are common in this group of organisms (D. Mercer & A. J. McCarthy, unpublished data). That lignocellulose degradation involves the activity of a range of hydrolytic and oxidative enzymes is established; in the work described here, biodegradative actinomycete strains were examined using different methods and substrates with a view to clarifying the enzymology of attack on the lignin component.

Methods

Strains and growth conditions. Actinomycete strains (Table 1) were maintained as spore suspensions and hyphal fragments in 20% (v/v) glycerol at −70 °C, and routinely cultured on L-agar (Hopwood *et al.*, 1985). Distilled-water suspensions of sporulating growth were used to inoculate shake flasks containing basal salts medium supplemented with ball-milled wheat straw (Ball & McCarthy, 1988). Conical flasks (250 ml) containing 50 ml growth medium were used throughout. In cultures examined for the utilization of lignin-related compounds, ball-milled straw was replaced by 0·01% of the appropriate model compound. Cultures were incubated with shaking at 200 r.p.m. for up to 12 d at 30 °C, 37 °C or 50 °C, as appropriate (Ball *et al.*, 1989). Cultures were harvested by centrifugation, washed twice in distilled water and dried at 80 °C overnight for the determination of growth yields by dry weight measurement. For experiments on the induction of enzyme production in *Streptomyces* strain EC1, the basal medium and methods of Godden *et al.* (1989) were used.

Lignin model compounds. 3,4-dimethoxyphenyl-ω-(2-methoxyphenoxy)acetophenone (compound 1), 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)ethanol (compound 2), and 4-hydroxy-3-methoxyphenyl-ω-(2-methoxyphenoxy)acetophenone (compound 3) were synthesized as described elsewhere (Betts & Dart, 1988), and kindly supplied by Dr W. B. Betts, Department of Biology, University of York, UK. Their structures are illustrated in Fig. 1.

Recovery of degradation products. Cultures grown on model dimers were harvested by centrifugation at 10000 *g* for 10 min at 4 °C and the supernatants acidified to pH 2 with 0·5 M-HCl before extraction twice with ethyl acetate. The extracts were evaporated to dryness using a rotary evaporator and the residue dissolved in a small volume of acetone for application to thin-layer chromatography (TLC) plates.

Cultures grown on ball-milled wheat straw (0·1%) were centrifuged at 27000 *g* for 10 min, acidified to pH 1·5 with 0·5 M-H_2SO_4 and then recentrifuged to remove precipitate from the supernatant. The supernatant was extracted twice with diethyl ether or ethyl acetate and evaporated to dryness before redissolving in the appropriate solvent for further analysis.

Thin-layer chromatography (TLC). Samples were spotted on silica plates (Silica Gel 60F-254, Merck) and the products separated using toluene/methanol/ethyl acetate (90:16:8, by vol.), toluene/ethyl acetate (90:10, v/v), or chloroform/methanol (97:3, v/v). Compounds containing aromatic rings were visualized under short-wave UV light.

For analysis of straw degradation products, benzene/acetic acid/dioxane (90:5:25, by vol.) or acetone/propylene glycol (90:10, v/v) were used to resolve components. Non-fluorescent compounds were detected by spraying the plates with an indicator comprising $K_3Fe(CN)_6$ (1·0% w/v) and $FeCl_3$ (1·0%, w/v) mixed in equal volumes immediately prior to use (Eggen, 1969).

High-performance liquid chromatography (HPLC). Straw and model compound degradation products were also detected and identified using HPLC on a μBondapak CN column eluted with methanol/water/acetic acid (20:79:1, by vol.) at a flow rate of 0·5 ml min^{-1}. The column was loaded with ethyl acetate extracts (1 ml) of culture supernatants and elution monitored at 254 nm.

Enzyme preparations. Cultures were centrifuged at 10000 *g* for 10 min at 4 °C and the supernatant fluids assayed for the presence of enzyme activities or concentrated by ultrafiltration. Culture supernatants were filtered under pressure using a 10 kDa molecular mass cut-off filter (Amicon PM10) until a 20-fold reduction in supernatant volume had been achieved. Intracellular enzyme activities were assayed in culture extracts prepared by sonication in an ice bath (3 × 30 s at 18 μm peak to peak) of washed culture pellets. Protein concentration was determined by the Lowry method.

Enzyme assays. Veratryl alcohol oxidation was measured by the formation of the product, veratraldehyde (Ball *et al.*, 1989). Peroxidase activity was recorded by measuring the formation of the dopachrome pigment (A_{470}) from L-DOPA (Sigma) (Deobald & Crawford, 1987). Catalase activity was measured by recording O_2 production by culture supernatants from selected strains grown on straw medium, using an oxygen electrode. Concentrated supernatant (200 μl) and potassium phosphate buffer (1·8 ml of a 10 mM solution, pH 7·0; final volume 2·0 ml), were maintained at 37 °C, and the reaction started by the addition of 10 μl of 10 mM-H_2O_2.

Protocatechuate 3,4-dioxygenase (EC 1.13.11.3) activity was assayed in crude cell extracts according to the spectrophotometric procedure of Stanier & Ingraham (1954) with slight modifications as follows. Extracts were prepared by sonication (10 min at 4 °C; Vibracell 500, Ultrasonic Processor) of *Streptomyces* sp. EC1 culture pellets obtained by membrane filtration (Millipore, 0·45 μm pore diameter) of 2-d-old cultures (200 ml) prepared as described above, and resuspended in 5 ml 50 mM-sodium phosphate buffer (pH 7·5). The assay was optimized at 30 °C under these conditions. Increase in

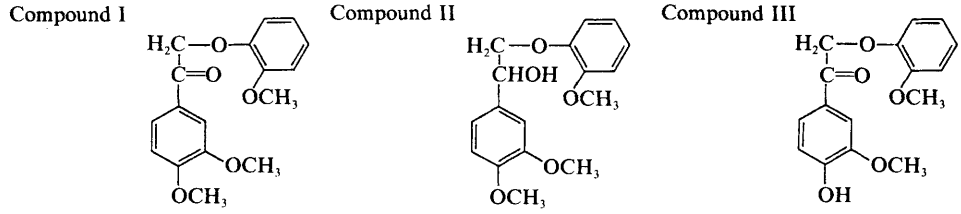

Fig. 1. Structures of dimeric lignin model compounds used.

Table 1. *Degradation of lignin model dimer (compound I) by actinomycetes*

Strain*	Growth [μg dry weight (ml culture)$^{-1}$]†		Products detected by TLC‡			
	Basal medium	Basal medium + compound I	Compound I (54)	Compound III (45)	Vanillic acid (49)	Protocatecheuic acid (67)
Streptomyces sp. EC1	20	25	+	+	−	−
Streptomyces sp. EC22	27	10	+	+	−	−
S. cyaneus MT813	11	21	+	+	−	−
S. badius 252	20	80	+	+	+	+
Thermomonospora mesophila DSM 43048	25	75	+	+	+	+
Amycolata autotrophica DSM 43099	41	30	+	+	−	−

* For strain designations and sources refer to Ball *et al.* (1989).
† Growth yield results are means of duplicate experiments. The variation was <5%.
‡ Identical product profiles were identified by the different solvent systems; R_F values for toluene/methanol/ethyl acetate (90:16:8) are given in parentheses. The structures of compounds I and III are given in Fig. 1.

absorbance was found to be linear with time up to 10 min as a function of the amount of protein in the enzyme preparation. A subsequent decrease in absorbance, and attributable to α-carboxymuconate decarboxylase (MacDonald *et al.*, 1954), was always shown when the dioxygenase was detected. Units of protocatechuate dioxygenase activity were expressed as increase in A_{270} min^{-1}.

Aromatic methylester demethylase, H_2O_2-dependent and activated by Mn^{2+}, was assayed in crude cell extracts by a slight modification of the procedure of Huynh & Crawford (1985). The sodium tartrate buffer (pH 5·0) used in the original procedure was replaced by sodium phosphate buffer (100 mM; pH 7·5), in the presence of 50 mM-H_2O_2 and 100 mM-$MnSO_4$. No activity was detected at pH < 6·5 and pH > 8·3. The assay was shown to be linear with time for up to 10 min, as a function of protein concentration. The model substrates, 2-methoxy-3-phenylbenzoic acid (M1) and methyl-2-methoxy-3-phenyl benzoic acid (M4) were prepared according to Huynh & Crawford (1985). Units of demethylase activity were expressed as an increase in A_{310} (M1) or A_{320} (M4) min^{-1}.

For calculation of specific enzyme activities in *Streptomyces* sp. EC1, protein was determined by the Kjeldahl procedure (N × 6·25) on cultures harvested by centrifugation at 27000 *g* for 15 min.

Gel electrophoresis. Peroxidase activities were identified in concentrated culture supernatants by isoelectric focusing and native polyacrylamide gel electrophoresis. The Phast system (Pharmacia) was used and separation of proteins achieved under the conditions recommended by the manufacturer. Peroxidase bands were detected using a solution of L-DOPA/aminoantipyrine/H_2O_2 as described by Adhi *et al.* (1989).

Results

Degradation of lignin model compounds by actinomycetes

Six actinomycete strains of physiological and taxonomic diversity, and for which there is evidence of activity against lignin-related compounds (Ball *et al.*, 1989), were grown in shake-flask cultures containing the non-phenolic model compound I (Fig. 1) as the primary carbon source. Determination of growth yields revealed that only three strains could be considered as having substantially degraded the substrate (Table 1). *Streptomyces badius* 252 and *Thermomonospora mesophila* DSM 43048 produced a three- to fourfold-increase in yield when compound I was included in the basal medium and these were also the only two cultures in which monomeric products (vanillic acid and protocatechuic acid) were detected, demonstrating that cleavage of the Cα-Cβ bond had occurred (Table 1). In all cultures, a large amount of the substrate, compound I, remained undegraded and all of the strains, whether capable of growing on this substrate or not, were able to demethylate compound I to produce detectable amounts of compound III (Table 1).

In addition to demethylation, oxidation of Cα on the phenylpropane chain would be an important preliminary reaction in lignin degradation and evidence for this was examined by growing strains on basal medium containing compound II (Fig. 1). In all of the cultures, again irrespective of the strain's ability to grow on compound I, both compound I and compound III were detected by TLC in culture supernatants.

The products of actinomycete growth on compound I were further identified by reverse-phase HPLC analysis of culture supernatant extracts. Results similar to those produced by TLC analysis were observed with *T. mesophila* and *S. badius*, although compounds I and III could not be differentiated from one another. The presence of small amounts of unidentified compounds, possibly including guaiacol, was also revealed. The greater sensitivity of HPLC showed that *A. autotrophica* and *Streptomyces* sp. EC22 were also capable of Cα-Cβ bond cleavage, a fact which measurement of growth yields and product analysis by TLC had failed to demonstrate. There was also some evidence that monomeric products had been generated by *S. cyaneus*; degradation products produced by *Streptomyces* sp. EC1 were not analysed by HPLC. Adequate controls were always included to ensure that small amounts of product were not the result of chemical oxidations.

Aromatic products of straw degradation by Streptomyces sp. EC1

This strain was cultured in shake-flasks of medium containing ball-milled wheat straw as substrate, to determine whether any low-molecular-mass aromatic compounds resulting from degradation of the lignin component could be detected in the culture supernatant. The application of both TLC and HPLC, with identification of compounds by UV spectroscopy and comparison with appropriate standards, revealed that *Streptomyces* sp. EC1 produced four major lignin-derived products: syringic acid, ferulic acid, vanillin and vanillic acid (data not shown). These were first detected after 36 h growth on straw and were present in the culture supernatant, together with small amounts of other unidentified aromatic compounds, throughout the 7 d culture period.

Identification of enzyme activities possibly involved in lignin degradation

Extracellular peroxidase and catalase activity were detected in all of the strains studied here, and veratryl alcohol oxidase only in those strains previously shown to produce veratraldehyde in culture (Ball *et al.*, 1989). The concentrated straw culture supernatants of *S. badius* and *Streptomyces* sp. EC1 were also loaded onto mini isoelectric focussing gels (Pharmacia Phast system) developed as zymograms to detect bands of peroxidase activity. Three isoforms of peroxidase were observed for each strain, but the poor gel quality and band resolution preclude conclusions beyond this basic observation. Similar peroxidase isoform patterns have been reported previously for both *S. viridosporus* T7A and the *S. badius* strain 252 included in this study (Adhi *et al.*, 1989).

A more detailed analysis of enzyme profiles and regulation of their production was undertaken in *Streptomyces* sp. EC1. In addition to the extracellular activities outlined above, cell-associated enzymes of the β-ketoadipate pathway (protocatechuate dioxygenase and β-carboxymuconate decarboxylase) were identified. Interestingly, a demethylating activity strictly dependent on Mn^{2+} and H_2O_2 was also discovered in this organism. 2-Methoxy-3-phenylbenzoate and methyl-2-methoxy-3-phenylbenzoate were used as substrates for demethylase because the reaction produced 3-phenylsalicylate which accumulated and could be quantitatively determined. Vanillic acid, the most oxidized and methylated compound that we detected in culture supernatants, is a possible substrate for the demethylase. Indeed, UV optical activity of vanillic acid virtually disappeared after incubation with a cellular extract, but only in the presence of Mn^{2+} and H_2O_2 and for cells grown on substrates which appeared to induce demethylase production (Table 2). The disappearance of UV optical activity is most probably attributable to the conjugate actions of protocatechuate dioxygenase and β-carboxymuconate decarboxylase (see Table 2).

To study the physiological regulation of enzyme production, *Streptomyces* sp. EC1 was grown in a mineral salts medium containing glycerol (0·2%) as sole carbon source (Godden *et al.*, 1989) and then transferred to media containing different carbon sources. Enzyme activities were determined after 24 h incubation, which corresponded to the lag phase preceding resumption of growth after transfer from glycerol medium, as previously determined (Godden *et al.*, 1989). The results are presented in Table 2 and demonstrate the extracellular and inducible nature of peroxidase activity in this organism. This activity was found to be strictly H_2O_2 dependent, and its production was induced by growth on straw, Indulin AT (Kraft lignin), syringic acid, 3,4-dimethoxycinnamic acid and vanillic acid. These compounds contain lignin or are lignin-related, but other such substrates (APPL, ferulic acid and 3,5-dimethoxy-4-hydroxycinnamic acid) did not appear to induce peroxidase production. Xylan and carboxymethylcellulose, which can be regarded here as representing the polysaccharides of lignocellulose, had no detectable inducing effects. The other three cell-associated enzymes were not significantly induced by lignocellulose and

Table 2. *Enzyme induction in Streptomyces strain EC1*

Growth substrate*	Specific activity [U (mg cellular protein)$^{-1}$]†			
	Peroxidase (E)	Demethylase (CB)	Protocatechuate 3,4-dioxygenase (CB)	β-Carboxymuconate decarboxylase (CB)
Straw	0·11	0·43	0·50	+
Straw + glycerol	ND	0·19	ND	ND
Indulin AT	0·10	0·44	0·50	+
Ferulic acid	ND	0·49	5·80	+
Syringic acid	0·10	ND	ND	ND
3,4-Dimethoxycinnamic acid	0·05	ND	0·45	+
3,5-Dimethoxy-4-hydroxycinnamic acid	ND	ND	0·62	+
Vanillic acid	0·05	0·45	4·50	+
Protocatechuic acid	ND	ND	6·20	+

* All substrates were added to the basal medium (Godden *et al.*, 1989) to a final concentration of 0·2%.

† Experiments were done in triplicate (standard deviations were all within 10% of the mean value presented). E, extracellular; CB, cell-bound. ND, Not detected; +, detected but not quantified (see Methods).

related substrates, but as expected were induced by various low-molecular-mass aromatic compounds (Table 2).

Possible catabolic pathway for lignin and related compounds

The pathway for the utilization of dimeric β-aryl ether linked methoxylated aromatic compounds by actinomycetes can be deduced from the results reported here and is similar to that found in *S. viridosporus* (Crawford, 1986). This forms the core of a proposed scheme by which actinomycete attack of the lignin component of plant biomass can be envisaged. This scheme is presented in Fig. 2 and further discussed below.

Discussion

Many actinomycetes can solubilize lignocarbohydrate from substrates such as straw and there is also some evidence for depolymerization of the lignin fraction (Ball *et al.*, 1990). Early steps in actinomycete-mediated attack on lignin are largely unknown but may involve extracellular peroxidases as has been established for white-rot fungi (see Kirk & Farrell, 1987) and subsequently suggested for streptomycetes (Ramachandra *et al.*, 1987, 1988). Studies directed at elucidating the mechanisms of lignin degradation by actinomycetes have tended to centre on the solubilization of lignocarbohydrate as an acid-precipitable complex, APPL (see Zimmermann, 1990). Our recent work has specifically addressed the relationship between lignin degradation and APPL production, and has led us to conclude that an ability to attack complex or even simple lignin-related compounds is not required for the latter (Ball *et al.*, 1989) although the generation of APPL is an interesting and exploitable phenomenon (Crawford *et al.*, 1984; Ball *et al.*, 1990). Here, we have concentrated on the production of biochemical evidence for reactions, by analysis of end-products, and for oxidative enzymes, by assaying for their presence in both extracellular and cell-bound fractions, that are likely to contribute to lignin degradation *per se*.

Degradation of the dimeric lignin model compound I was unequivocally demonstrated in *T. mesophila* and *S. badius*. More sensitive analysis, i.e. the use of reverse-phase HPLC, confirmed this and provided evidence that the other strains were capable of attack beyond simple demethylation. Furthermore, all of the strains studied could generate compounds I and/or III from compound II. In summary therefore, there are three elements to the degradation of β-O-4-linked dimeric lignin model compounds reported here: demethylation of aromatic ring structures; oxidation of Cα to introduce a carbonyl group; and α–β cleavage to monomeric products. These are also reactions that have been described in other actinomycetes and bacteria (see Vicuña, 1988) and in fungi (Betts & Dart, 1988). Cα oxidation in particular may have implications for *in situ* lignin degradation, beyond its role as a prerequisite for α–β cleavage. It has been suggested that detection of a strong carbonyl peak in ^{13}C NMR spectra of APPL produced by *T. mesophila* was due to Cα oxidation that would contribute to increased lignin solubility (McCarthy *et al.*, 1986). In the ligninolytic fungus *Phanerochaete chrysosporium*, Cα oxidation is a prominent reaction which Fenn & Kirk (1984) suggested would enhance the accessibilty of lignin to enzymic attack. Whether actinomycetes can utilize β-O-4 lignin model compounds *in vivo*, i.e. as carbon

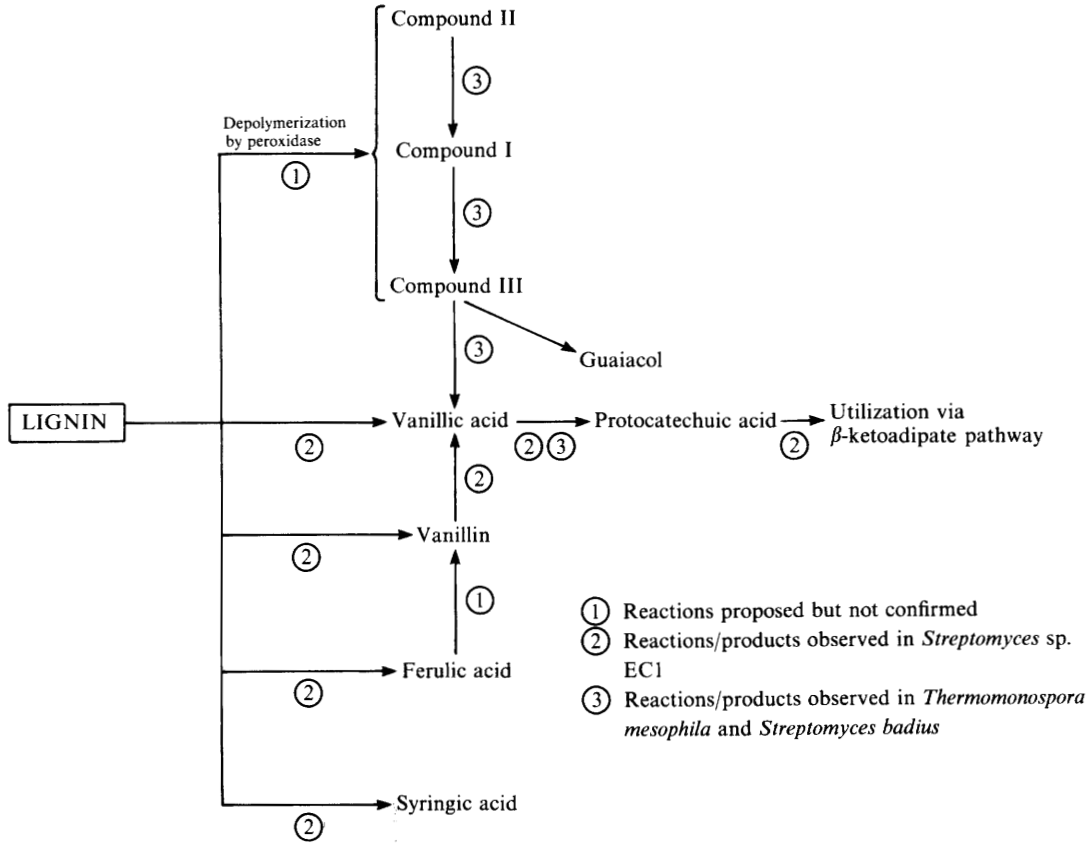

Fig. 2. Proposed scheme for degradation of lignin and related compounds by actinomycetes.

sources, has been equivocal (Ruttiman *et al.*, 1987; Zimmermann, 1990). Clearly, the conclusion that *S. badius* and *T. mesophila* can use dimeric model compound I as a source of carbon and energy (Table 1) is further supported by the fact that TLC analysis sufficed to detect degradation products in the culture supernatants of these, but not the other strains.

The generation of utilizable carbon sources from lignin requires the action of extracellular, or possibly surface-bound, enzymes. Peroxidases are the current prime candidates and these enzymes are secreted by actinomycetes in multiple forms. There is evidence that peroxidase isoforms are related, within and between *Streptomyces* strains (Magnuson *et al.*, 1991) and the purification of actinomycete extracellular peroxidases to homogeneity in sufficient quantities for detailed biochemical and molecular characterization is one of our research priorities. That actinomycete extracellular peroxidases attack and depolymerize lignin is possible but not yet established. While not detracting from the potential role of peroxidases as initial agents of attack on lignin, the intimate involvement of other actinomycete enzymes which disrupt lignocarbohydrate structure should not be ignored (Donnelly & Crawford, 1988; Bachmann & McCarthy, 1991; Faulds & Williamson, 1991). In particular, aromatic acids esterified to lignin can be released from lignocellulose by esterase activity (Donnelly & Crawford, 1988) and could be the source of at least some of the vanillic acid and ferulic acid produced during growth of *Streptomyces* sp. EC1 on straw.

The products of lignin fragmentation are available for the cellular carbon pool of actinomycetes during primary growth on lignocelluloses. However, estimates of the mineralization of the radiolabelled lignin fraction of straw by actinomycetes are low (McCarthy & Broda, 1984) and the extent to which lignin degradation can contribute to the energy metabolism of these organisms remains to be addressed. Indulin AT, a Kraft lignin essentially free of carbohydrate, can be utilized but is not a good growth substrate for actinomycetes (Giroux *et al.*, 1988; Ball *et al.*, 1989; Godden *et al.*, 1989). Both Indulin AT and wheat straw induced relatively high levels of extracellular peroxidase and cell-associated demethylase production by *Streptomyces* sp. EC1 but comparatively low amounts of dioxygenase and decarboxylase (Table 2). This supports the suggestion that lignin degradation does not contribute significantly to the carbon and

energy metabolism of actinomycetes. Previous studies on *Streptomyces* sp. EC1 have shown it to be capable of consuming approximately 20% of the hemicellulose fraction of straw in a 24 h batch culture, with a growth yield of 0·48 g dry weight per g xylan utilized (Godden *et al.*, 1989). In fact, hemicellulose is the most degradable fraction of straw and the main growth substrate for this strain, and probably most other straw-degraders. Mineralization of the lignin fraction is likely to be in the form of metabolic co-oxidation, and optimal concentrations of inducers for enzymes of the β-ketoadipate pathway would probably never be achieved when growing on lignocellulose. Lignin degradation products present in small amounts in culture supernatants, described here and previously (Crawford, 1981) may nevertheless have an important role in regulation of growth and enzyme production when lignocellulose is the substrate. In *Streptomyces* sp. EC1 at least, both cellulolysis and hemicellulolysis appear to be inducible by lignin-derived compounds in addition to the products of polysaccharide hydrolysis (Godden *et al.*, 1989).

In conclusion, the main function of the scheme presented in Fig. 2 is to participate in the disorganization of lignocellulose structure to increase the accessibility of hemicellulose and cellulose. The scheme is derived from the results of experiments reported here, considered together with those previously published by others (Crawford, 1981, 1986; Donelly & Crawford, 1988). Biodegradative actinomycetes are aerobic saprophytes whose primary substrate in soil is inevitably plant biomass. It is therefore not surprising that solubilization of lignocarbohydrate to produce a humic-acid-like complex (APPL), secretion of peroxidases and attack on aromatic ring compounds are common traits in this group of organisms.

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