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Degradation of a non-phenolic arylglycerol β-aryl ether by *Streptomyces cyaneus*

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A non-phenolic β-*O*-4 lignin substructure model compound, 1,3-dihydroxy-2-(2-methoxyphenoxy)-1-(4-ethoxy-3-methoxyphenyl)propane, was degraded by *Streptomyces cyaneus* in liquid shaken cultures. 4-Ethoxy-3-methoxybenzoic acid could be identified by TLC and GC/MS as the main product, indicating the cleavage of the Cα-Cβ bond of the aryl glycerol-β-aryl ether. In addition, 4-ethoxy-3-methoxybenzaldehyde and guaiacol were identified in culture extracts as further degradation products.

Lignin biodegradation; β-*O*-4 substructure; (*Streptomyces*)

1. INTRODUCTION

The biopolymer lignin is a water-insoluble recalcitrant aromatic macromolecule built from phenylpropane units and linked by several different types of carbon-carbon and aryl ether bonds.

The chemically complex structure of lignin and the difficulties in isolating it in native form are major obstacles in elucidating the mechanism of its microbial degradation and modification. Therefore one widely used approach for lignin degradation studies has been the use of synthetic low molecular mass lignin substructure model compounds. These dimeric or oligomeric compounds contain the linkages which are typical for polymeric lignin. The dilignol used in this study as substrate contains an arylglycerol-β-aryl ether bond, which is the most frequent type of linkage found in polymeric lignins [1].

Although most information on the mechanism of lignin degradation has come from research with the white-rot fungi *Phanerochaete chrysosporium* and *Coriolus versicolor* [2,3], lignin and various lignin model compounds are also degraded by several species of bacteria, including actinomycetes [3]. Many actinomycetes are able to utilize a range of aromatic substrates including lignin-related phenols [4–7]. In particular, nocardioform actinomycetes and some *Streptomyces* species are known to perform a large number of transformations with these substrates including hydroxylations, demethylations, and aromatic ring cleavages [5–9].

Although an arylglycerol-β-phenyl ether was readily degraded by various actinomycetes, the presence of a substituent on the phenyl ether moiety inhibited or completely prevented its breakdown [10]. The degradation of such a non-phenolic model compound by a strain of *Streptomyces* has been reported [11], but further studies could not confirm this result [12]. Previous work [13,14] has shown that *Streptomyces cyaneus* can effectively degrade graminaceous lignocellulose. In this paper, we report the degradation of a non-phenolic dimeric lignin model compound by this strain.

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2. MATERIALS AND METHODS

2.1. *Substrate and authentic compounds*

1,3-Dihydroxy-2-(2-methoxyphenoxy)-1-(4-ethoxy-3-methoxyphenyl)propane (**I**) and 4-ethoxy-3-methoxybenzaldehyde (**III**) were prepared as described previously [15,16]. 4-Ethoxy-3-methoxybenzoic acid (**II**) was prepared by oxidation of **III** with $AgNO_3$/NaOH. Compound **I** was purified prior to incubations by preparative TLC on Kieselgel $60F_{254}$ (Merck, Darmstadt, FRG) with ethylacetate/*n*-hexane (3:2, v/v) as eluent.

2.2. *Bacterial strain and culture conditions*

Streptomyces cyaneus NCIB 12383 (MT 813) [13] was maintained on 2% agar containing mannitol (20 g/l) and soybean meal (20 g/l). Liquid cultures were made in 100 ml Erlenmeyer flasks containing 20 ml of a mineral salt medium [13] supplemented with 0.1% yeast extract (pH 7.4). Some cultures also contained 0.1% glucose. 5–10 mg of **I** dissolved in *N,N*-dimethylformamide was added. The flasks were inoculated with an aqueous suspension of spores and hyphae and incubated on a rotary shaker with 190 rpm at 37°C. Control incubations consisted of culture medium containing only the bacteria or the substrate.

2.3. *Identification of degradation products*

After incubation for 72 h, the whole cultures were acidified and extracted with ethyl acetate. The extracts were washed with a saturated solution of NaCl, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Substrate degradation was monitored by thin layer chromatography (TLC) of extracts on Kieselgel $60F_{254}$ with CH_2Cl_2/CH_3OH (97:3, v/v) or ethyl acetate/*n*-hexane (1:2, v/v) as eluents.

Aliquots of the extracts were acetylated with acetic anhydride/pyridine (1:1, v/v) overnight at room temperature. A part of the acetylated extracts were fractionated by TLC on Kieselgel $60F_{254}$ with ethyl acetate/*n*-hexane (1:2, v/v) as solvent. Separated fractions were eluted from the gel with CH_2Cl_2/CH_3OH (80:20, v/v) and the solvent was evaporated under reduced pressure. Some samples were dissolved in methanol and ethylated by adding a solution of diazoethane in ether followed by evaporation of the solvents under reduced pressure.

The derivatized products were dissolved in acetone and analyzed by gas chromatography-mass spectrometry (GC-MS). A GCMS QP-1000 gas chromatograph-mass spectrometer (Shimadzu, Japan) (ionizing voltage, 70 eV) equipped with a chemically bonded fused silica capillary column HiCap CBP1 (Shimadzu, Japan) (25 m × 0.33 mm) was used. The column temperature was held at 100°C for 1 min and then increased to 240°C (5°C/min).

3. RESULTS AND DISCUSSION

S. cyaneus could be grown in liquid shaken culture with the lignin model compound in a salt medium supplemented with yeast extract. TLC analysis of extracts from supernatants of 3-day-old cultures showed the presence of 4-ethoxy-3-methoxybenzoic acid (**II**) as the main product, indicating the cleavage of the Cα-Cβ bond of the arylglycerol-β-guaiacyl ether (**I**). It was also present in similar amounts as estimated by TLC in cultures which had been supplemented with 0.1% glucose, indicating that the degradation of **I** was not suppressed by the presence of glucose in the medium. The identity of **II** could be verified by GLC-MS; it showed a retention time and mass spectra identical with those from the synthesized authentic compound.

The presence of **II** as the main degradation product suggests that it is not easily metabolised further, but is accumulating in the medium. Since the ethoxylated dimer is not a naturally occurring substrate, it is possible that the ethoxyl substituent in the *para* position of the compound prevented its further degradation.

Although **II** was the main product found in the extracts, small amounts of other degradation products were detected by GLC-MS. These compounds were separated by TLC and were identified by comparison of their mass spectra and retention times with those from synthesized authentic compounds. The presence of 4-ethoxy-3-methoxybenzaldehyde (**III**) in the extracts suggests that this aldehyde is the initial product of the Cα-Cβ cleavage, which is then oxidized to the corresponding acid (**II**). An aromatic aldehyde oxidase, which could presumably catalyze this reaction, has been reported from *S. viridosporus* [17]. The counterpart compound of the Cα-Cβ cleavage could not be detected in the extracts, possibly because it is immediately further metabolised. An aldehyde and the corresponding acid have also been found after degradation of an arylglycerol-β-phenyl ether by the actinomycete *Corynebacterium equi* [10], suggesting similar pathways for the degradation of these compounds.

Guaiacol (2-methoxyphenol, **IV**) could also be identified in the extracts. Its formation could result from the cleavage of the β-aryl ether bond of the dimeric lignin model compound. Similarly to the Cα-Cβ cleavage products, the corresponding counterpart compound could not be identified. Formation of **IV** from the lignin model compound veratrylglycerol-β-guaiacyl ether by *S. viridosporus* grown on corn stover lignocellulose has been reported, but the dimer was not degraded

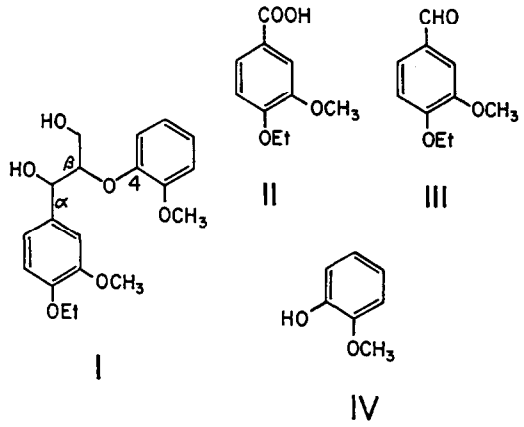

Fig.1. Structural formulae of the compounds.

when supplied as carbon source in a mineral salt medium supplemented with yeast [11].

No degradation products of **I** were detected in extracts from control incubations which contained only the substrate or the bacteria.

The results show that *Streptomyces cyaneus* can degrade low molecular weight dimeric lignin model compounds. In *P. chrysosporium*, Cα-Cβ-cleavage, β-aryl ether cleavage and aromatic ring cleavages are catalyzed by an extracellular peroxidase [2]. Peroxidases [18] and oxygenases [5,6], which have been reported from *Streptomyces* species, could be involved in the metabolism of these lignin model compounds.

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