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Identification of phanerosporic acid in birch degraded by *Phanerochaete chrysosporium*

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Abstract

Extracts of *Phanerochaete chrysosporium* cultures grown on birch or on a malt extract-peptone-glucose agar medium were analysed by HPLC. A major component from the two sources appears to be identical by HPLC and UV-visible spectrometry. The product isolated from agar-grown cultures was purified to apparent homogeneity and structure analysis by NMR indicates that the metabolite is the beta-resorcylate phanerosporic acid, consistent with previous characterizations. The possible role for the metabolite in wood metabolism is discussed.

Key words: *Phanerochaete chrysosporium,* resorcylate, betulachrysoquinone, phanerosporic acid.

Introduction

In 1989, Arnone *et al.* (1) reported that the most abundant fungal metabolite in ethyl acetate extracts of *P. chrysosporium* CBS 481.83 cultures (cross references with *P. chrysosporium* BKM-F-1767, ATCC 24725) grown on a malt extract-peptone-glucose-agar (MPGA) medium is a β-resorcylate named phanerosporic acid (figure 1). We became interested in this metabolite primarily because 1) the structure suggests to us that if it is in wood during decay it might be involved in redox mediation of enzymatic reactions, 2) it might be involved in metal chelation and control non-enzymatic reactions, and 3) the metabolite is reported to be at surprisingly high concentrations in MPGA cultures and therefore possible implications should not be ignored if it were to be found in decayed wood.

Here we describe the isolation of phanerosporic acid with slight modification to the method previously described (1) and use the authentic material to identify phanerosporic acid from cultures of *P. chrysosporium* ME-446 grown on birch.

Materials and Methods

Organisms and culture conditions. P. chrysosporium BKM-F-1767 was grown on MPGA (malt-extract-peptone-glucose-agar) for three weeks as described (1) except that glass petri plates were used as growth vessels instead of Roux flasks. *P. chrysosporium* ME-446 was grown on yellow birch (*Betula lutea*) sawdust supplemented with nutrients essentially as described (2) including 0.18g NH_4NO_3, 0.12 g K_2HPO_4, 0.15g KH_2PO_4, 0.12g $MgSO_4 \cdot 7H20$ plus trace metals per 100g of wood. Initial moisture content was approximately 63% and cultures harvested after 12 weeks.

Isolation and purification of phanerosporic acid. Production, isolation and purification of phanerosporic acid was essentially as described by Arnone *et al.* (1). Phanerosporic acid was extracted from *P. chrysosporium* BKM-F-1767 cultures grown on MPGA using ethyl acetate containing 1% methanol (1) followed by flash chromatography and crystallization. Identity of the crystals was confirmed by ^{1}H and ^{13}C NMR. A Bruker DPX-250 spectrometer at 62.9 Mhz was used for NMR analyses. Acetone-d_6 was used as reference signal (29.83 ppm).

Extraction procedures for detection of metabolite in wood. Samples of *P. chrysosporium* ME-446 cultures grown on sawdust were air dried prior to extraction with ethanol.

HPLC. Analysis was with a Hewlett Packard series 1050 fitted with an Alltech Alltima C18 (5 micron, 250 mm x4.6 mm) column. Solvent was 85 % methanol with 0.5% acetic acid and a flow rate of 1 ml/min. Detection was with a Hewlett Packard diode array UV-visible detector.

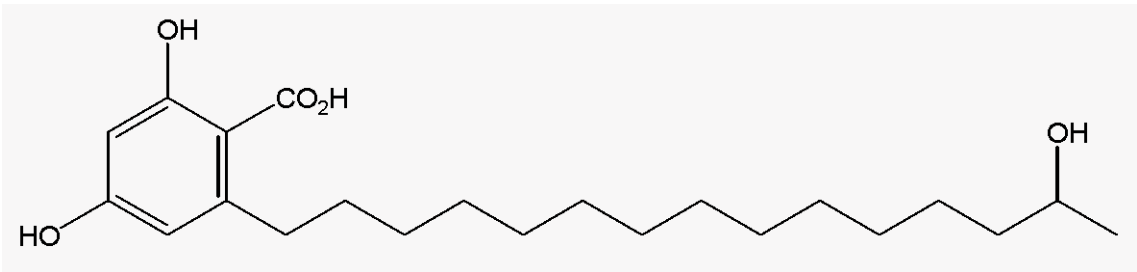

Fig. 1. Phanerosporic acid. Arnone *et al.* (1) described the isolation and purification of the major component of ethyl acetate extracts of *P. chrysosporium* CBS 481.83 grown on MPGA. They named the metabolite phanerosporic acid and assigned the structure as shown.

Results

Detection of metabolites from P. chrysosporium grown on MPGA and birch. To isolate phanerosporic acid, *P. chrysosporium* BKM-F-1767 cultures grown on MPGA were extracted with ethyl acetate containing 1% methanol (see Materials and Methods). This extract was taken to dryness by roto-evaporation and a small sample dissolved in methanol for HPLC analysis. Figure 2 shows that the major peak absorbing at 300 nm has the same retention time as the authentic phanerosporic acid. This is consistent with the previous report that this metabolite is the most abundant product in the ethyl acetate extracts of *P. chrysosporium* (1). The spectrum of the major metabolite is identical to that of the isolated and purified phanerosporic acid (data not shown).

Birch decayed with *P. chrysosporium* ME-446 was extracted with ethanol and analysed by HPLC for the determination of phanerosporic acid. As expected, polar components were detected that eluted with early retention times (figure 2). However, a major metabolite detected in the decayed birch has the same retention time and spectrum as authentic phanerosporic acid (figure 3). Quantitative analysis indicates that there are 26 mg of phanerosporic acid/100g of dried decayed birch.

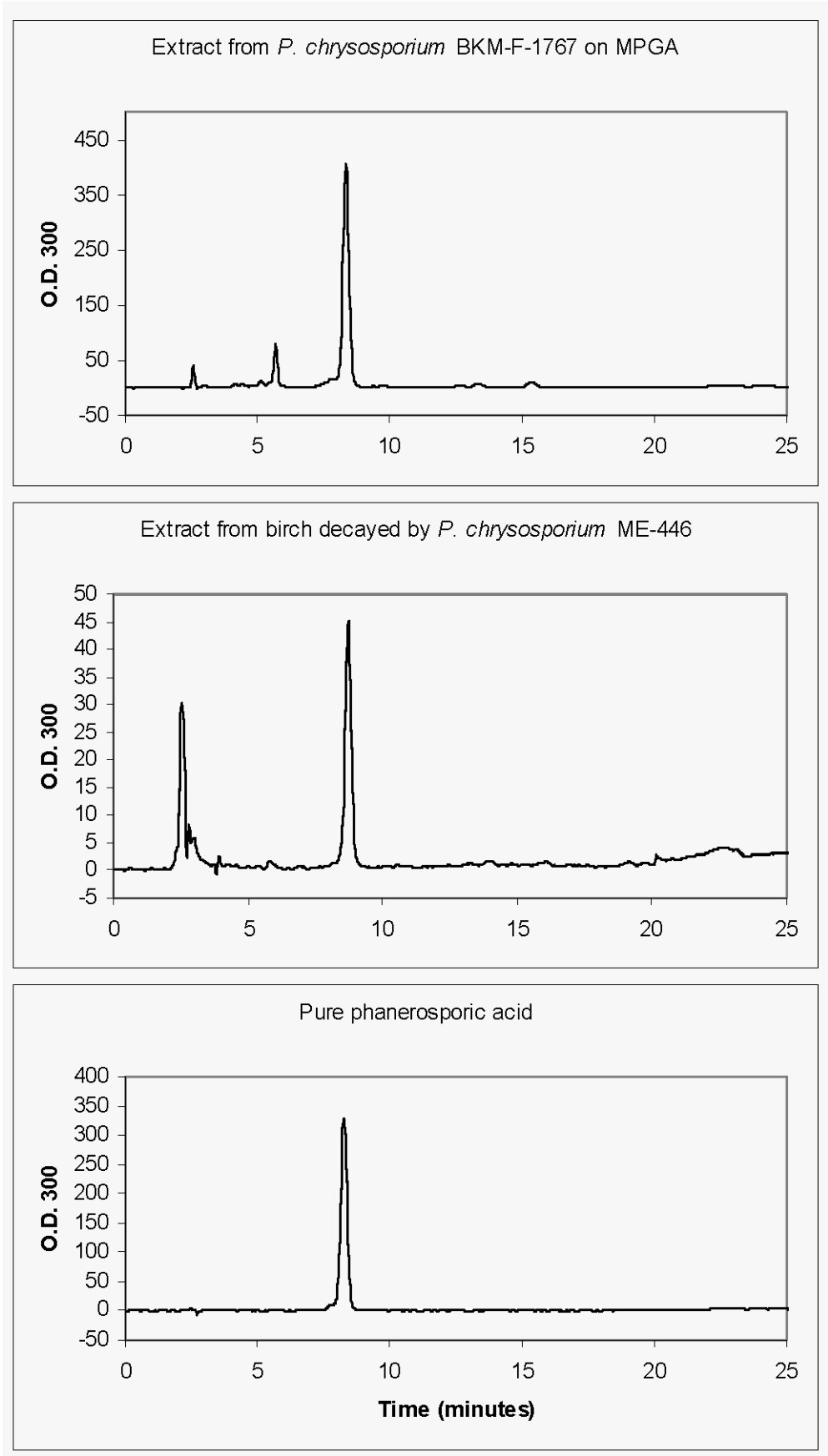

Fig. 2. Detection of phanerosporic acid. HPLC analysis of metabolites derived from *P. chrysosporium* BKM-F-1767 grown on MPGA (top panel), and *P. chrysosporium* ME-446 grown on birch (middle panel) shows the presence of phanerosporic acid.

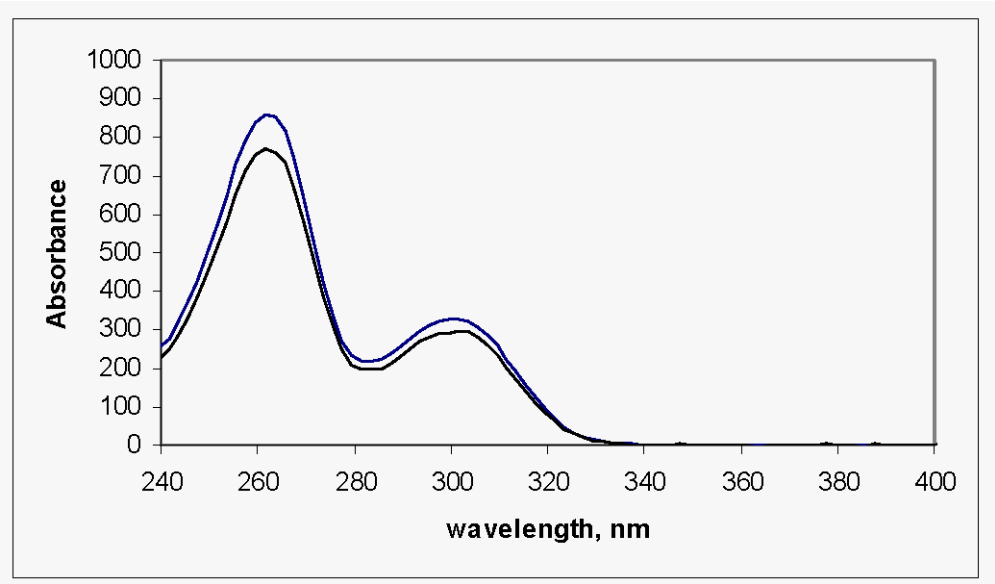

Fig. 3. Spectra of phanerosporic acid and the major metabolite from decayed birch. Spectra of peaks eluting at 8.3 minutes with decayed birch samples and authentic phanerosporic acid (figure 2) are superimposable.

Discussion

Our results on the isolation of phanerosporic acid from MPGA confirm that this metabolite is the major component in the ethyl acetate extracts of cultures grown on MPGA (1). Furthermore, we have shown by HPLC that phanerosporic acid is a major non-polar component of ethanol extracts of birch decayed by *P. chrysosporium.* The levels of this metabolite found in both growth conditions are impressive. So far, we have not been able to detect the metabolite in *P. chrysosporium* cultures grown in liquid cultures (data not shown) indicating that the synthesis of phanerosporic acid is regulated and it is not a constitutive component of the mycelia.

In 1977 Chen *et al.* (2) reported the characterization of the most abundant compound in the chloroform-soluble extractives from yellow birch wood that was decayed by *Phanerochaete chrysosporium* ME-446. They concluded that the compound was a fungal metabolite and named it betulachrysoquinone hemiketal with the chemical structure as depicted in figure 4. The growth conditions that we used to look for phanerosporic acid in decayed birch closely parallels their conditions for production of betulachrysoquinone hemiketal. It would be expected that our ethanol extraction method would extract betulachrysoquinone hemiketal from wood but the apparent major metabolite that we detect is phanerosporic acid. This apparent discrepancy is under further study.

Phanerosporic acid has antimicrobial activity against *Bacillus cereus, B. subtilus, Escherichia coli, Saccharomyces cerevisiae, Aspergillus niger, Ophiostoma ulmi, Ustilago maydis, Cladosporium cucumerinum, C. cladosporioides,* and *Botrytis cinerea* (1). This would suggest that the metabolite might have a role in helping *P. chrysosporium* colonize wood by limiting the growth of competing organisms.

Phenolics and in particular hydroxy-benzoic acids might have a role in wood decay (3). These have been studied with the brown rot *Gloeophyllum trabeum* and the

metabolites named Gt chelators (3). Experiments show that these metabolites have an affinity for ferric ion and also mediate the reduction of the metal in redox recycling that supports the generation of oxygen radical species. The Gt chelators are sufficiently small that they are expected to penetrate the plant cell wall where they would non-enzymatically degrade the plant polymers via the radicals generated during redox cycling. Whether phanerosporic acid may have a similar role is under investigation. The metabolite does appear to complex with ferric iron when using the modified Keller method (4) test for salicylates (data not shown).

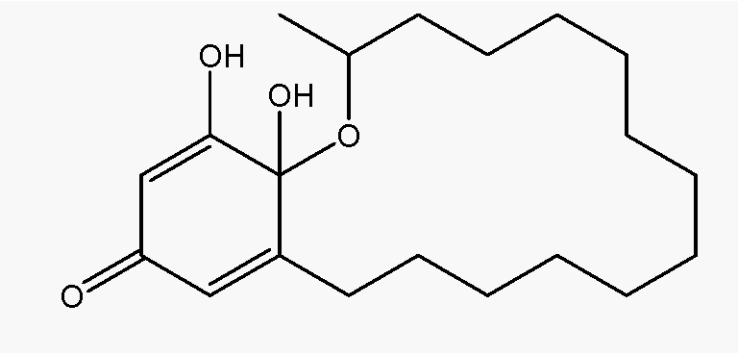

Fig. 4. Betulachrysoquinone hemiketal. Chen *et al.* (2) proposed this structure for the major metabolite isolated from birch decayed by *P. chrysosporium* ME-446.

Acknowledgments

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References

1. **Arnone, A., G. Assante, G. Nasini, and O. V. De Pava.** 1989. Phanerosporic acid, a beta-resorcylate obtained from *Phanerochaete chrysosporium.* Phytochemistry **28:**2803-2806.
2. **Chen, C. L., H. M. Chang, and T. K. Kirk.** 1977. Betulachrysoquinone hemiketal: a p-benzoquinone hemiketal macrocyclic compound produced by *Phanerochaete chrysosporium.* Phytochemistry **16:** 1983-1986.
3. **Goodell, B., J. Jellison, J. Liu, G. Daniel, A. Paszczynski, F. Fekete, S. Krishnamurthy, L. Jun, and G. Xu.** 1997. Low molecular weight chelators and phenolic compounds isolated from wood decay fungi and their role in the fungal biodegradation of wood. J. Biotech. **53:** 133-162.
4. **Jarvie, D. R., R. Heyworth, and D. Simpson.** 1987. Plasma salicylate analysis: a comparison of colorimetric HPLC and enzymatic techniques. Ann. Clin. Biochem **24:**364-373.