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Short communication

Metabolism of ferulic acid by a *Penicillium* sp.

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Abstract. Ferulic acid was metabolised by a *Penicillium* sp. (probably *P. rubrum*) by a novel route involving a preliminary demethylation to caffeic acid, followed by side-chain shortening to yield protocatechuic acid which was subsequently broken down via the *ortho*-fission pathway.

Key words: *Penicillium rubrum* – Ferulic acid – Metabolism – *Ortho*-fission pathway

Ferulic acid occurs free and in combined form in many plant constituents; for example, as diferulic and truxillic acids in plant cell walls (Fry 1988) and as a component of many flavonoids and of lignin (Robinson 1983). Thus its biodegradation is of biochemical and environmental interest.

Earlier workers have reported that the degradation of ferulic acid takes place by shortening of the side chain to yield vanilic acid, then demethylation to form protocatechuic acid followed by *ortho*- or *meta*- fission of the aromatic ring. Several alternative routes for side-chain shortening have been proposed, all of which lead to vanillic acid, and these are summarised below:

a. Oxidative: Toms and Woods (1970) proposed an oxidative route whereby a two-carbon unit was cleaved from ferulic acid to yield vanillin which was subsequently oxidised to vanillic acid. A similar route has been suggested for soil fungi (Henderson 1956).

b. Reductive: Enoki et al. (1981) found reduced rather than oxidised intermediates in their studies of the metabolism of ferulic acid by *Sporotrichum pulverulentum* and proposed reduction to the corresponding 4-hydroxy, 3-methoxy-phenylpropionic acid, → 4-hydroxy, 3-methoxy-phenylpropionyl alcohol → vanillin → vanillic acid → protocatechuic acid. Similarly Nishada and Fukuzumi (1978) reported that a white-rot fungus (*Trametes* sp) reduced ferulic acid to coniferyl alcohol which was subsequently converted to vanillin. *c. Non-oxidative:* Nazareth and Mavinkurve (1986) suggested that ferulic acid was broken down to vanillin via 4-vinylguaiacol by *Fusarium solani.*

In this paper we wish to report a novel route for the breakdown of ferulic acid, via an initial demethylation to caffeic acid, by *Penicillium rubrum.*

Materials and methods

The species of *Penicillium* used in these experiments was found growing as a contaminant on a stock solution of ferulic acid and produced a characteristic red pigment when grown on potato glucose agar plates, it was tentatively identified as *P. rubrum.* Stock cultures of *P. rubrum* were maintained on potato glucose agar whilst large crops of cells for metabolic experiments were grown on a basal mineral salt medium (2 g l^{-1} KH_2PO_4, 1 g l^{-1} $(NH_4)_2SO_4$, pH 5.5) containing 1 mM-ferulic acid as carbon source. Cultures were incubated at 28°C and harvested by filtration on a Buchner funnel. For replacement culture experiments the harvested mycelium was resuspended in basal medium containing 1 mM–ferulic acid but without N-source.

Analysis of phenolic compounds

Culture filtrates (10 ml) were acidified with 1 ml 2 M-HCl, extracted with ethyl acetate, and the organic layer dried over anhydrous Na_2SO_4 and concentrated in vacuo. This extract was then analysed by chromatography on paper or silica gel thin layers using the following solvent systems: *n*-butanol, acetic acid, water [BAW] (454:10:22); benzene, acetic acid, water [BzAc] (125:72:3) or benzene, dioxane, acetic acid [BzDA] (90:25:4). Following development chromatograms were examined under UV light ($\pm NH_3$ fumes) and then sprayed with diazotised sulphanilic acid (Walker and Taylor 1983).

Individual chromatogram spots were eluted with ethanol and their UV spectra compared to those of authentic standards.

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Abbreviations used: BAW = *n*-butanol, acetic acid, water [BAW]-(454:10:22)
BzAc = benzene, acetic acid, water [BzAc](125:72:3)
BzDA = benzene, dioxane, acetic acid [BzDA](90:25:4)

Table 1. Identification of phenolic acids

Compound	R_f Value			UV fluorescence	λ max in EtOH [nm]	Diazotised sulphanilic acid
	BAW	BzAc	BzDA			
A	76	44	50	Blue	222, 256, 298	Pink
B[a]	–	–	88	–	–	–
C	–	47	46	Bright blue-green	220, 322 224, 322	Mauve-pink
Ferulic acid	90	95	77	Blue	223, 420	Pale brown
Vanillic acid	88	96	–	–	– –	Yellow-orange
Caffeic acid	–	47	46	Bright blue-green	220, 322	Mauve-pink
Protocatechuic acid	79	43	54	–	226, 256, 298	Pink

[a] Spot turned brown when chromatogram left in air

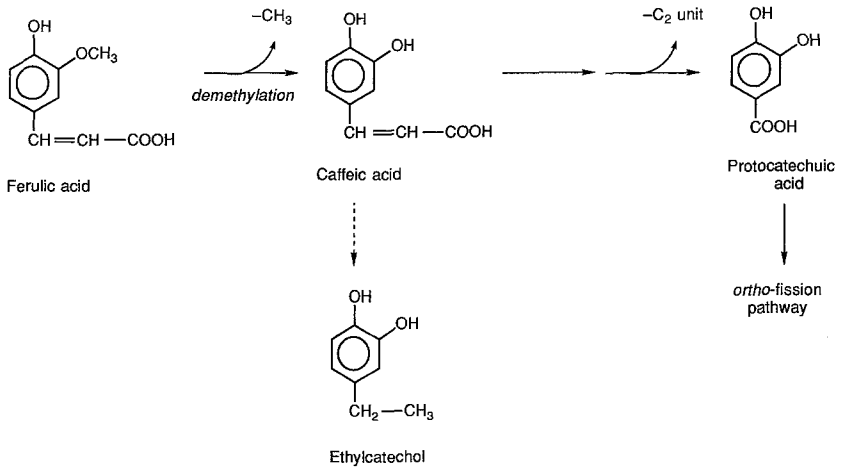

Fig. 1. Proposed route for the breakdown of ferulic acid by *Penicillium rubrum*

Analysis of keto acids

Culture filtrates were also tested for keto acids in order to try to establish the ring fission route. Keto acid intermediates were converted to their 2:4-dinitro-phenylhydrazone derivatives and analysed by TLC (Walker and Taylor 1983).

Results

Filtrates of growing cultures of *P. rubrum* were analysed by chromatography at regular (12 h) intervals and two compounds, A and B, appeared after 36 h; when cultures were grown on an orbital shaker at 40 rpm compound A did not appear until after 84 h whilst compound B was not detected. Replacement cultures yielded a third intermediate; compound C after 31 h.

Compounds A and C were identified as protocatechuic acid and caffeic acid respectively; these identifications were based on comparisons of R_f values, appearance under UV light, chromogenic spray reactions and UV spectra against those of authentic samples (Table 1). Compound B rapidly turned brown in air and, based on this evidence and its R_f value, it was tentatively identified as ethyl-catechol (Whiting and Carr 1957); its concentration was too low to permit UV spectroscopy.

Keto acid accumulation was monitored in the replacement culture experiments and after 31 h only 2-oxoglutaric acid was detected. To facilitate keto acid accumulation the replacement culture experiments were then repeated in the presence of 1 mM-Na arsenite (an inhibitor of oxidative decarboxylation) and in this case 3-oxoadipic acid, plus its breakdown product laevulinic acid, were detected after 6 h. These findings confirmed that *ortho* ring-fission was occuring.

Discussion

The results presented above suggest an alternative route for the breakdown of ferulic acid by *P. rubrum*. By contrast to earlier studies the presence of caffeic acid in the culture filtrates suggests that the first step in the alternative route appears to involve an initial demethylation followed by loss of a two-carbon unit to yield protocatechuic acid which is subsequently broken down via the *ortho*-fission pathway.

Conversion of caffeic acid to form protocatechuic acid by loss of a 2C unit from its side chain has been reported previously (Toms and Wood 1970). The appearance of ethyl catechol is thought to be as a result of some sort of side reaction rather than part of the main reaction route; Whiting and Carr (1957) reported this compound as one of the products of caffeic acid metabolism by *Lactobacillus pastorianus* var *quinicus*.

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