Formation of Fusaric Acid by Fungi of the Genus *Fusarium*

W.-U. Mutert, H. Lütfring, and W. Barz

Lehrstuhl für Biochemie der Pflanzen der Universität Münster, Hindenburgplatz 55, D-4400 Münster

D. Strack

Botanisches Institut der Universität zu Köln

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Among various *Fusarium* strains tested *Gibberella fujikuroi* (SAW) WR was shown to be a high producer of the phytotoxin fusaric acid.

During studies on phytopathogenic *Fusarium* fungi [1, 2] and their effects on plant cell cultures [3, 4] it became necessary to determine in our experimental strains [1] the capacity for fusaric acid (5-*n*-butyl-pyridine-2-carboxylic acid) formation. This phytotoxin has repeatedly been isolated from various *Fusarium* species of both the *Elegans* and the *Martiella* group as well as of *Gibberella fujikuroi* [5, 6]. The involvement of fusaric acid in wilt diseases of various plant species has conclusively been shown [7]. Action mechanism and metabolism of fusaric acid in plant tissues warrants further investigations which have been started by using cell cultures of host plants [3, 4] and fusaric acid producing fungal cultures.

Strains were grown in shake culture [1] and at intervals between 1 and 28 days of fungal growth, aliquots of the culture fluid of two vials each were extracted with ethylacetate at pH 3.8 [6]. The phytotoxin was quantitatively determined in these aliquots by high performance liquid chromatography. This very accurate procedure (LiChrosorb RP-8 column) well separated fusaric acid from other UV absorbing metabolites and in contrast to other chromatographic procedures [3] allowed numerous analyses in a short period of time. In addition, fusaric acid was isolated, crystallized from *n*-hexane and identified by UV (λ_{max} 226 and 269 nm), ^{1}H-NMR (signals at 12.16 (s), 8.75 (s), 8.25 (d) and 7.82 (d), 2.84 (t), 1.58 (m) and 0.99 (t) ppm) and MS (*m/e* M^+ 179, 162, 135 (100), 119, 106, 91, 77, 65 and 27) spectroscopy. Evidence for the occurrence of dehydrofucaric acid has not been obtained in the shake cultures.

Strains III (*F. oxysporum* Schlecht ex Fr. f. *medicaginis*, CBS 179.29), XIV (*Gibberella sanbinetti*, CBS 265.54) and XVIII (*F. oxysporum* f. sp. *pisi*, recently isolated from infected "Picadir" pea plants) were shown (TLC, S_2 and S_3) not to synthesize fusaric acid. Strains X (*F. oxysporum* Schlecht ex Fr. f. *apii*, CBS 184.38) and XVII (*F. oxysporum* f. sp. *pisi*, also isolated from infected "Picadir" pea plants) produced moderate amounts of fusaric acid between 290 and 12 mg/l culture fluid (by HPLC). Strain XIII (*Gibberella fujikuroi* (Saw) WR, CBS 186.56) turned out to be one of the best producers of fusaric acid reported so far [8] because up to 2550 mg/l culture medium were measured. With te exception [8] of *G. fujikuroi* (Saw) Woll, strain ETH M 82, the yield of fusaric acid in most previous studies ranged between 100–1000 mg/l. As shown in Fig. 1 fusaric acid formation occurred parallel to growth with maximum production between days 3 and 8. In contrast to other reports [9] further metabolism of fusaric acid upon longer incubation periods could not be observed in our studies.

Attempts to induce fusaric acid formation in strain XVIII or improve its accumulation in strains X, XIII and XVII by growth at increased zinc levels (0.02–10 ppm) failed though earlier reports [10] had described the stimulatory role of zinc ions.

Our strain of *Gibberella fujikuroi* was also used to synthesize larger quantities of ^{14}C-labelled fusaric acid using [^{14}C]acetate [11, 12]. With reference to Fig. 1 and based on preliminary studies [4] using varied precursor concentrations, 8 flask (total volume 800 ml) were incubated with 800 µCi [1-^{14}C]-acetate (10^{-3} M) for 72 h between day 3 and day 6 of the growth curve. Radiochemically pure fusaric acid was recovered by ethylacetate extraction, repeated crystallisation from *n*-hexane and prep. TLC (yield: 1.064 g; spec. radioact.: 13.3 µCi/mmol; total precursor incorporation: 10%). In general, the data show the great diversity for phytotoxin formation among *Fusarium* fungi [6, 7] and its great variation in total yield.

Experimental

Fungi

Cultivation and growth (100 ml batches) of fungal strains were as previously described [1].

Reprint requests to Prof. Dr. W. Barz.

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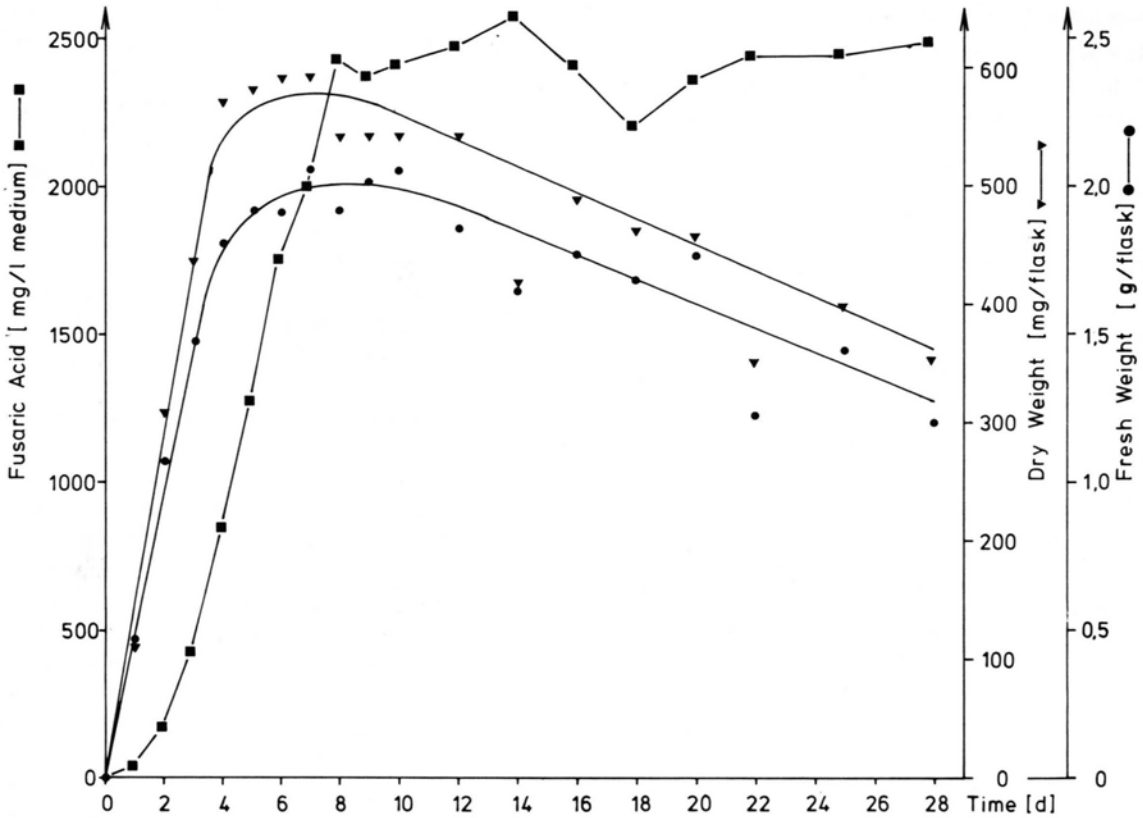

Fig. 1. Growth of *Gibberella fujikuroi* (strain XIII, CBS 186.56) in shake culture and accumulation of fusaric acid.

Chromatography

TLC on silica gel plates was performed with solvent systems S_1: CH_3OH: $CHCl_3$: H_2O 3:2:1; S_2: iso-PrOH: H_2O 85:15 and S_3: *n*-But-OH:HOAc: H_2O 4:1:1. Fusaric acid was detected with Dragendorff-reagent or UV absorption.

HPLC

Spectra-Physics chromatograph with a Spectro Flow SF 770 UV detector (270 nm), a LiChrosorb RP-8 (5 µm) column (250 × 4 mm). Solvents A: 1.5% H_3PO_4 in H_2O; B: 1.5% H_3PO_4 + 20% CH_3CO_2H + 25% CH_3CN in H_2O; column development isocratic with 50% B in A. Flow 1 ml/min and injection volume 20 µl. Alternatively, a LiChrosorb-NH_2 (5 µm) column (125 × 4 mm) with solvent 0.5% H_3PO_4 + 2.5% H_2O in CH_3CN was used. The analytical procedure was quantitated with authentic fusaric acid (Sigma).

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