Phytochemistry, 1977, Vol 16, pp. 545–546 Pergamon Press. Printed in England

THE DETOXIFICATION OF α-TOMATINE BY *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

J. E. FORD, D. J. MCCANCE* and R. B. DRYSDALE
Department of Microbiology, Birmingham University, Birmingham B15 2TT, U.K.

(*Received* 1 *October* 1976)

Key Word Index—*Fusarium oxysporum* f. sp. *lycopersici*; fungi; α-tomatine; tomatidine; lycotetraose.

Abstract—*Fusarium oxysporum* f. sp. *lycopersici* detoxifies α-tomatine by producing an inducible extra-cellular enzyme which cleaves the glycoalkaloid into the tetrasaccharide lycotetraose and tomatidine.

INTRODUCTION

While no convincing evidence is yet available that α-tomatine (Fig. 1) is involved in the resistance of tomato to Fusarium wilt [4, 6] Arneson and Durbin [1] have suggested that the ability to overcome the toxicity of α-tomatine may be necessary for the success of some fungi, including *Fusarium oxysporum* f. sp. *lycopersici*, as tomato parasites.

Preliminary experiments indicated that culture filtrates of *F. oxysporum* f. sp. *lycopersici* contained an enzyme able to detoxify α-tomatine [3]. The present paper describes the mode of detoxification of the glycoalkaloid by the fungus.

RESULTS

Examination of the incubation mixture by TLC revealed a spot corresponding to the aglycone tomatidine. Material corresponding to α-tomatine, glucose, galactose and xylose was not detected. The incubation mixture also failed to give a positive test for glucose with glucose oxidase although a positive test was obtained following acid hydrolysis of the incubated mixture. Controls in which α-tomatine was incubated with boiled enzyme yielded only an α-tomatine spot following TLC.

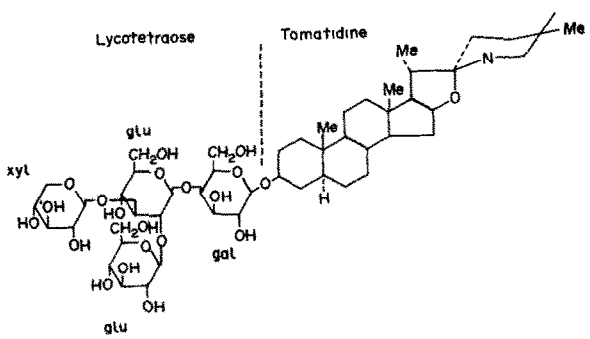

Fig. 1. Structure of α-tomatine. *O*-β-D-glucopyranosyl-(1 → 2 glu)-*O*-β-D-xylopyranosyl-(1 → 3 glu)-*O*-β-D-glucopyranosyl-(1 → 4 gal)-β-D-galactopyranosyl-tomatidine.

* Present address: Department of Microbiology, Guy's Hospital Medical School, London Bridge, London, SE 1.

Table 1. Monosaccharide content of incubation mixture following acid hydrolysis*

Sugar	μg/ml	Molarity (μM)	Molar ratio†
Glucose	22	122.1	2
Galactose	12	66.6	1.09
Xylose	11	73.3	1.2

* Monosaccharides were not detectable in incubation mixture prior to acid hydrolysis.
† Glucose taken as 2.

For quantitative experiments samples of the acid hydrolysed and the unhydrolysed material were assayed for glucose, galactose and xylose. These assays (Table 1) showed that no free monosaccharide was present in unhydrolysed samples whereas following hydrolysis the three individual sugars were present in the 2:1:1 ratio expected for the parent tetrasaccharide. This result was confirmed, qualitatively, by TLC examination of portions of the solutions.

While spots corresponding to the three monosaccharides were not detected following TLC of the unhydrolysed dialysate a band with a R_f of 0–0.2 was present. Material from the same region of replicate plates was recovered in water, a portion hydrolysed with HCl, the hydrolysed and unhydrolysed samples freeze-dried and the residues redissolved in water prior to assay for the three sugars. The results obtained were in close agreement with those in Table 1. Similarly, following fractionation of the incubation mixture on a Biogel P2 column (and treatment of the fractions as described in the Experimental) results similar to those in Table 1 were obtained.

DISCUSSION

The initial experiments suggested that *F. oxysporum* f. sp. *lycopersici* produces an enzyme that converts α-tomatine to tomatidine and the tetrasaccharide lycotetraose. Quantitative experiments carried out after the isolation of the presumptive tetrasaccharide by TLC or column chromatography confirm this conclusion. Thus

these experiments indicate that *F. oxysporum* f. sp. *lycopersici* detoxifies α-tomatine by removing the sugars, apparently as a tetrasaccharide, leaving the aglycone tomatidine which is much less toxic than the parent compound [2, 3]. This mechanism of detoxification is different from that of *Septoria lycopersici*, in which a constitutive glucosidase hydrolyses α-tomatine to yield β_2-tomatine and glucose [1], but may be similar to that which has been reported briefly for *Botrytis cinerea* [9].

Extracts of stem segments of the tomato cultivars Homestead 24 and Red Top infiltrated with spores of *F. oxysporum* f. sp. *lycopersici* and incubated at 25° for 6 days were found to contain the enzyme [5] but the amount of enzyme present was not measured. Tomatidine was not detected in these extracts possibly because if produced, it was further metabolised. Attempts are continuing to assess the role of this enzyme in pathogenesis.

The Fusarium enzyme is probably inducible since it is not present in cultures grown in Vogel's medium but is present in similar cultures to which α-tomatine has been added. Information is not yet available about other inducers or substrates for the enzyme, nor is there any information about its function during the life cycle of the fungus. If α-tomatine is the sole inducer and/or substrate for the enzyme then it would seem likely that the enzyme is important in pathogenesis, otherwise lack of selection pressure would have resulted in the loss of this enzyme activity.

EXPERIMENTAL

Cultures of *F. oxysporum* f. sp. *lycopersici*, Race 1, were maintained on Si gel as described previously [4]. Working cultures were produced by transferring a few pieces of gel to the appropriate medium and incubating, with shaking, at 25°.

Media. (a) *Tomato leaf extract.* The filtrate obtained after boiling 250 g tomato leaves in 1 l. H_2O for 20 min was dispensed in 100 ml vols in 500 ml conical flasks and sterilised by autoclaving. After inoculation cultures were incubated for 5 days. (b) *Vogel's medium.* The mineral salts soln of Vogel [10] supplemented with 2% sucrose was used in expts to investigate the inducible vs constitutive nature of the enzyme. In these expts 50 ml of medium in 250 ml flasks was inoculated and incubated for 4 days. Filter-sterilised α-tomatine soln (20 μg/ml final concn) in citrate–Pi buffer (pH 5) or an equal vol. of sterile buffer was then added to the flasks and incubation continued for a further 4 days.

Enzyme preparation. Following incubation the cultures were filtered, the filtrate centrifuged (2600 *g*, 30 min, 4°) and $(NH_4)_2SO_4$ added to the supernatant to give 90% satn. After stirring at 4° for 1 hr the suspension was centrifuged (2600 *g*, 30 min, 4°), the residue dissolved in a small vol. of H_2O, dialysed 18 hr against citrate–Pi buffer (pH 5, 4°) and then stored in 1 ml vols at −20° until required. Following incubation (24 hr, 25°) of the enzyme prepn with α-tomatine (1 mg/ml final concn) the incubation mixture was used without further treatment in qualitative expts and for column chromatography. For quantitative expts the mixture was dialysed against H_2O, the dialysate freeze-dried, the residue dissolved in H_2O and a sample hydrolysed (1 M HCl, 100°, 1 hr). After hydrolysis the sample was again freeze-dried to remove the HCl and the residue redissolved in H_2O.

TLC. Effected on Si gel G developed in *n*-PrOH–H_2O (17:3) until the solvent front had moved 14 cm. After drying at room temp. spots were visualised with 50% H_2SO_4 and heating at 105° for 10 min. Tomatine, tomatidine, glucose, galactose and xylose standards were used.

Column chromatography. The incubation mixture (2.5 ml) was loaded onto a Bio-gel P2 column (20 × 2 cm), eluted with H_2O (16 ml/hr) and 2 ml fractions collected. A sample from each fraction was assayed for carbohydrate using anthrone [7], the remaining material from the main anthrone-positive peak pooled and a sample hydrolysed as before. The hydrolysed and unhydrolysed pools were then freeze-dried, each of the residues dissolved in a small vol. of H_2O and samples assayed for glucose, galactose and xylose.

Sugar assays. Enzymic assays for glucose and galactose, with glucose oxidase and galactose dehydrogenase respectively, were carried out using commercially available test kits (Boehringer). Xylose was assayed colorimetrically [8]. All assays were carried out in duplicate and each expt was repeated at least twice. Appropriate blanks and standards were run with each set of assays.

Acknowledgements—We wish to thank the Science Research Council for financial support (to D.J.M.) and Dr. R. D. Durbin for helpful discussion.

REFERENCES

1. Arneson, P. A. and Durbin, R. D. (1967) *Phytopath.* **57**, 1358.
2. Durbin, R. D. Personal communication.
3. Ford, J. E. Unpublished results.
4. Langcake, P., Drysdale, R. B. and Smith, H. (1972) *Physiol. Plant Path.* **2**, 17.
5. McCance, D. J. (1974) Ph.D. Thesis. University of Birmingham.
6. McCance, D. J. and Drysdale, R. B. (1975) *Physiol. Plant Path.* **7**, 221.
7. Morris, D. L. (1948) *Science* **107**, 254.
8. Tracey, M. V. (1950) *Biochem. J.* **47**, 433.
9. Verhoeff. K. and Liem, J. I. (1975) *Phytopath. Z.* **82**, 333.
10. Vogel, H. J. (1956) *Microbial Genetics Bulletin* **13**, 42.