Aust. J. Biol. Sci., 1978, **31**, 93–6

Production of Enniatin as a Criterion for Confirming the Identity of *Fusarium lateritium* Isolates

G. C. Bishop and A. H. Ilsley[A]

Waite Agricultural Research Institute, University of Adelaide,
Private Bag No. 1, Glen Osmond, S.A. 5064.
[A] Present address: Department of Anaesthesia and Intensive Care,
Flinders Medical Centre, Bedford Park, S.A. 5042.

Abstract

The use of an organic metabolite as an aid in confirming the identity of isolates of *Fusarium* thought to be *F. lateritium* has been investigated.

One hundred and thirty-six isolates of *Fusarium* (83 of which were *F. lateritium*) were tested for their ability to produce a cyclohexadepsipeptide called enniatin. All isolates identified morphologically as *F. lateritium*, except five identified as *F. lateritium* f. sp. *mori*, were found to produce enniatin, whilst only two of the other 53 fusaria tested did so. *F. lateritium* f. sp. *mori* should perhaps not be regarded as a subspecies of *F. lateritium*.

Introduction

Fusarium lateritium (Nees) emend. Snyder and Hansen has been demonstrated to be effective in protecting pruned apricot sapwood against invasion by *Eutypa armeniacae* Hansf. and Carter, the fungus that causes *Eutypa* dieback of apricot (Carter and Price 1974, 1975). The isolate of *F. lateritium* used in this work was found, *in vitro*, to produce a non-volatile, diffusible metabolite toxic to ascospores and mycelium of *E. armeniacae* (Carter and Price 1974). This *in vitro* inhibition of germination and growth was shown to be due to the production by *F. lateritium* of antibiotic substance(s) known by the general term 'enniatin(s)' (Carter and Ilsley, unpublished data). Enniatins are a bioactive mixture of homologous cyclohexadepsipeptides of the general structure shown in Fig. 1.

Studies on the possible use of *F. lateritium* as a biological control agent for *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar, the causal organism of silver leaf disease of stone and pome fruit trees, involved a survey of the occurrence of *F. lateritium* in an apple orchard in the Adelaide Hills. From this survey a large number of isolates designated as *F. lateritium* were obtained from apple (*Malus sylvestris*) and several other hosts. These isolates, together with 10 isolates received from the Commonwealth Mycological Institute (Herb. I.M.I.) designated as *F. lateritium*, were screened for their ability to produce enniatin.

In this paper, we evaluate the possibility of using the presence of enniatin as an additional criterion for confirming the identity of fungal isolates that have been classified on morphological characteristics as *F. lateritium*.

Materials and Methods

One hundred and thirty-six isolates designated as *F. lateritium* were tested for their ability to produce enniatin. The isolates used in the investigation and their host origin are listed in Table 1. All

solates were maintained on glucose peptone agar at full strength (GP) or at 1/20th strength of nutrients (GP/20). GP contained 10 g Difco–Bacto peptone, 10 g glucose, 5 g sodium chloride and 15 g Difco-Bacto agar in 1 litre of distilled water.

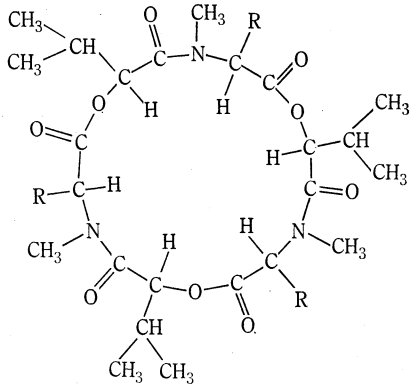

Fig. 1. General structure of enniatin. R = isopropyl or butyl.

Ten 8-mm diameter discs cut from a 1-week-old culture of the particular fungal isolate growing on GP/20 agar were placed in a 1000-ml Erlenmeyer flask containing 300 ml of glucose peptone medium. The fungi were grown for 4 days at 25°C in shaken liquid culture under continuous fluorescent lighting (Audhya and Russell 1974; Carter and Ilsley, unpublished data). Each culture was then filtered through Whatman No. 4 filter paper in a Büchner funnel and subsequently through a membrane filter of pore size 0·2 μm (Sartorius).

Table 1. Source of *Fusarium* isolates used in the investigation

Isolate number		Origin
IMI 90139a	Amaryllidaceae	*Agave* sp.
B20, B21	Cruciferae	*Brassica oleracea* var. *botrytis*
B19	Cupressaceae	*Chamaecyparis lawsoniana*
B16		*Cupressus macrocarpa*
IMI 128749	Euphorbiaceae	*Manihot* sp.
B14, B15	Fagaceae	*Castanea sativa*
IMI 134593	Leguminosae	*Laburnum* sp.
B10, B11, B12		*Robinia pseudoacacia*
B27, B28, B29	Moraceae	*Morus nigra*
F1295, F1303, IMI 122838		*Morus* sp.
IMI 105481	Oleaceae	*Fraxinus excelsior*
B18	Pinaceae	*Cedrus atlantica aurea*
B17	Rosaceae	*Cydonia oblonga*
2, 3, 5, 11, 13		*Malus sylvestris* cv. Jonathon
10		cv. Red Delicious
14		cv. Granny Smith
21–60, 62–84, 86–117		cv. Jonared
B1		*Prunus armeniacae*
B6		*Prunus avium* cv. Early Rivers
B3, B7		cv. Lustre
B2, B5		cv. Smith's Black
B4		*Prunus cerasus*
IMI 116392		*Prunus domestica*
IMI 131457		*Rosa* sp.
B25	Rutaceae	*Citrus limon* × *sinensis* cv. Meyer Lemon
IMI 158154, IMI 94758		*Citrus* sp.

The clear filtrate (approximately 280 ml) was extracted twice with ethyl acetate, using 90 ml and then 50 ml of ethyl acetate. The combined organic phase was evaporated to dryness under reduced pressure at 35°C. The dried extract was dissolved in 5 ml methanol and 10-μl aliquots were spotted at the origin on precoated t.l.c. plates (silica gel 60 F_{254}; thickness 0·25 mm; Merck). The plates were developed with ethyl acetate : hexane : methanol : water (75 : 200 : 17 : 1) (Audhya and Russell 1973). The chromatographed compounds were detected by exposing the air-dried plates to iodine vapour. The enniatin spot had an R_F value of 0·20, which was the same as that of a crystalline enniatin isolated from a stock culture of *F. lateritium* (isolate B1 in Table 1) (Carter and Ilsley, unpublished data).

Table 2. Production of enniatin by *Fusarium* isolates

(*a*)	*F. lateritium*; enniatin produced 2, 3, 10, 11, 13, 14, 21–26, 32, 34, 35b–42, 44–48, 50, 52, 53, 55, 57, 59, 60, 65–70, 74, 77, 82, 84, 86, 87, 89–91, 97, 98, 100–102, 104–108, 110, 112, 116, 117, B1, B2, B3, B4, B5, B6, B7, B10, B11, B12, B14, B20, B21, IMI 126338, IMI 131457, IMI 134593
(*b*)	*F. lateritium*; no enniatin produced B27, B29, IMI 122838, F1295, F1303
(*c*)	Not *F. lateritium*; enniatin produced 99, 115
(*d*)	Not *F. lateritium*; no enniatin produced 5, 27–31, 33, 35a, 43, 49, 51, 54, 56, 58, 62–64, 71–73, 75, 76, 78–81, 83, 88, 92–96, 103, 109, 111, 113, 114, B15, B16, B17, B18, B19, B25, B28, IMI 90139a, IMI 94758, IMI 105481, IMI 116392, IMI 128749, IMI 158154

Results

A number of the isolates tested (including several from the Commonwealth Mycological Institute) did not produce enniatin. The identity of all isolates was subsequently reviewed and it was found that of the 136 fusaria tested, 83 were identified using morphological criteria as being *F. lateritium*, and 78 of the 83 were found to produce enniatin (Table 2). Of the members of the group identified as not being *F. lateritium*, only two isolates were found to produce enniatin whilst the remaining 51 isolates did not.

The five isolates of *F. lateritium* that did not produce enniatin were all from mulberry (*Morus* sp.). Booth (1971) recognizes a physiological form which occurs only on mulberry, called *F. lateritium* (Nees) emend. Synd. and Hans. f. sp. *mori* (Desm.) Matuo and Sato.

Discussion

Biochemical criteria have been used for many years, in conjunction with morphological characteristics, in the classification of yeasts (Kreger-van Rij 1962). In more recent years chemical and physiological tests have come to assume considerable importance in the taxonomy of some higher fungi (Murray 1966; Watling 1966; Taylor 1974). Booth (1966) suggested that biochemical characteristics could well be utilized as an aid to *Fusarium* taxonomy.

Results from the present study suggest that the production of enniatin is a useful criterion for confirming the identity of an isolate thought to be *F. lateritium* on morphological grounds.

All isolates of *F. lateritium* that were tested were found to produce enniatin with the exception of the physiological form *F. lateritium* f. sp. *mori*. This suggests that *F. lateritium* f. sp. *mori* should not be regarded as a subspecies of *F. lateritium*.

It must be stressed, however, that a *Fusarium* isolate producing enniatin need not necessarily be *F. lateritium*. Audhya and Russell (1973) have shown that *F. sambucinum* Fuckel produces an enniatin which they call enniatin A. In fact, in this study two fusaria which were not *F. lateritium* were found to produce enniatin.

Acknowledgments

We are indebted to Dr M. V. Carter for advice and discussion throughout the work and for making available several of the isolates tested, and to Mrs Lea Wichman for preparation of the illustration. This work was carried out while the senior author was holding a Commonwealth Postgraduate Research Award.

References

Audhya, T. K., and Russell, D. W. (1973). Production of enniatin A. *Can. J. Microbiol.* **19**, 1051–4.

Audhya, T. K., and Russell, D. W. (1974). Production of enniatins by *Fusarium sambucinum*: selection of high-yield conditions from liquid surface cultures. *J. Gen. Microbiol.* **82**, 181–90.

Booth, C. (1966). Physical and biochemical techniques in the identification of Fusaria. *J. Gen. Microbiol.* **42**, vii–viii.

Booth, C. (1971). 'The Genus Fusarium'. (Commonwealth Mycological Institute: Kew, Surrey.)

Carter, M. V., and Price, T. V. (1974). Biological control of *Eutypa armeniacae*. II. Studies of the interaction between *E. armeniacae* and *Fusarium lateritium*, and their relative sensitivities to benzimidazole chemicals. *Aust. J. Agric. Res.* **25**, 105–19.

Carter, M. V., and Price, T. V. (1975). Biological control of *Eutypa armeniacae*. III. A comparison of chemical, biological and integrated control. *Aust. J. Agric. Res.* **26**, 537–43.

Kreger-van Rij, N. J. W. (1962). The use of biochemical criteria in the taxonomy of yeasts. *Symp. Soc. Gen. Microbiol.* **12**, 196–211.

Murray, I. G. (1966). Some aspects of the biochemical differentiation of pathogenic fungi. *J. Gen. Microbiol.* **42**, ii–iii.

Taylor, J. B. (1974). Biochemical tests for the identification of mycelial cultures of basidiomycetes. *Ann. Appl. Biol.* **78**, 113–23.

Watling, R. (1966). Chemical tests in Agaricology. *J. Gen. Microbiol.* **42**, iii–vi.

Manuscript received 9 August 1977