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PROFILES OF SECONDARY METABOLITES IN *FUSARIUM SOLANI*

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Abstract

Cultures of *Fusarium solani* extracted in toluene/ethyl acetate/90% formic acid (5/4/1, v/v) showed consistent profiles of secondary metabolites on thin layer chromatographic plates. These profiles, alongwith macromorphological characters, can be used as taxonomic criteria for identification of *F. solani*..

Introduction

During a study of seed-borne mycoflora of *Coriandrum sativum* several species of *Fusarium* viz., *F. moniliforme, F. equiseti, F. oxysporum, F. pallidoroseum* and *F. solani* were isolated (Hashmi & Ghaffar, 1991). Since *F. solani* has been reported to be pathogenic on fenugreek and coriander seedlings (Hashmi, 1988; Hashmi & Ghaffar, 1991), its isolates were subsequently used to elaborate their profiles of secondary metabolites. The profiles of secondary metabolites have been used in the identification of species of *Aspergillus* (Frisvad, 1985) and *Penicillium* (Filtenborg .*et al*., 1983; Frisvad & Filtenborg, 1983; 1990). Efforts have also been made to correlate the profiles of secondary metabolites in the identification of species of *Fusarium*, especially those which have pathogenic and toxigenic importance (Thrane, 1989; Hashmi & Thrane, 1990; Singh *et al.,* 1991). The profiles of secondary metabolites of *F. solani* as criteria for the identification are reported here.

Materials and Methods

A total of 215 isolates of *F. solani* isolated from coriander were used. Cultures of *F. solani* were grown and maintained on spezieller nährstoffarmer agar (Nirenberg, 1976). Media for elaboration of secondary metabolites and thin layer chromatographic techniques were used as described by Hashmi & Thrane (1990). All retardation factors (R_f) were recorded relative to griseofulvin (relative $R_f = 1.00$) which was used as an external standard in all analyses (Frisvad & Filtenborg, 1983).

Results and Discussion

From 215 isolates of *F. solani*, the profiles of secondary metabolites were identified in 3 different patterns (Table 1). The 6 pigments of pattern I totalling 12.1% isolates fluoresced blue or orange under longwave UV light. A pigment at R_f 1.62 was identified as bostrycoidin by comparing with its standard reference sample (arrows in Fig.1). Another metabolite fluorescing light orange to orange at R_f 1.43 after treating with 20% $AlCl_3$ was identified as fusarubin. Identity of this metabolite was confirmed by compar-

Table 1. Chromatographical patterns of pigments of *Fusarium solani*. Symbols +, ++ and +++ indicate intensity of spots. Data based on 215 isolates.

Patterns of pigments /No. of isolates	Relative R_f value in TEF system	Colour of pigments on TLC plates/intensity of spots: In visible light	Under UV light	Under UV light after treating with 20% $AlCl_3$
Pattern I/26 (12.1%)	1.62^B	Pink/++	Orange Yellow/++	Orange/++
	1.48	Pink/+	Light orange/++	Orange/++
	1.43^F	Pink/+	Light orange/++	Orange/++
	1.10	ND	Blue green/+	Blue/+
	1.00	ND	Blue green/+	Blue/+
	0.84	ND	Blue green/+	Blue/+
Pattern II/42 (19.5%)	1.62^B	Pink/++	Orange yellow/++	Orange/+++
	1.43^F	Rose Pink/+	Light orange/+	Light orange/++
	1.10	ND	Blue green/+	Blue/+
	1.00	ND	Blue green/+	Blue/+
	0.84	ND	Blue green/+	Blue/+
Pattern III/147 (68.4%)	1.48	Pink/++	Orange yellow/++	Orange/+++
	1.42	ND	Blue green/++	Blue/++
	1.10	ND	Blue green/++	Blue/+

B: Bostrycoidin, F: Fusarubin, ND: not detected

ing with its standard (arrowheads in Fig.1). Four other pigments, one fluorescing orange at R_f 1.48, and three fluorescing blue at R_f's 1.10, 1.00 and 0.84 could not be identified. Pattern II consisting of 5 pigments was elaborated by 19.5% isolates of *F. solani*. It was identical to pattern I except for an orange pigment which was consistently observed in majority of isolates at R_f 1.48. The profile of pattern III totalling 68.4% isolates showed 3 pigments under longwave UV light. An orange pigment at Rf 1.48 was similar to the pigment observed in pattern I at the same R_f value and the blue pigment at R_f 1.10 was identifiable with profiles of a blue pigment in patterns II and III. However, a significant difference in pattern III was noted at R_f 1.42 where a pigment fluoresced blue green with moderate intensity. It fluoresced solid blue after spraying with 20% $AlCl_3$. This pigment alongwith the one fluorescing orange at R_f 1.48 has also been elaborated in profiles of a number of isolates of *F. solani* obtained from capsicum and fenugreek (Hashmi & Thrane, 1990) and seems to be species specific. Two naphthazarin toxins, bostrycoidin and fusarubin, detected in 31.6% isolates during this study have been reported earlier in *F. solani* (Hashmi & Thrane, 1990; Tatum & Baker, 1983; Ammar *et al.*, 1979). Bostrycoidin was also isolated by Kurobane *et al.*, (1980) who found that it was only "produced under conditions where fusarubin accumulated". During this study bostrycoidin was more often found on TLC plates together with fusarubin.

Independent culturing of isolates of *F. solani* followed by extraction for analysis by TLC using TEF system (Filtenborg *et al.*, 1983) showed consistent profiles of extracellular pigments and other secondary metabolites. The TAM developing solvent (Kamimura *et al.*, 1981) was used as an adjunct only because more often it produced trailing reddish-pink or blue streaks that interfered with the evaluation of R_f values. Likewise, combinations of chloroform or ethyl ether with ethanol, methanol or acetone were also compared for efficiency in extraction of secondary metabolites. Of the various substrates used in optimizing pigment production, yeast extract sucrose agar (Frisvad & Filtenborg, 1983) appeared most suitable for all isolates of *F. solani*, whereas cultures

Fig.1. Patterns of secondary metabolites of *Fusarium solani* as observed on a TLC plate and photographed under UV_{366} after treating with 20% $AlCl_3$. Bostrycoidin: arrows, Fusarubin: arrowheads, Griseofulvin: double arrows.

grown on grain-based media (Thrane, 1986) showed only weak responses on the TLC plate. In the latter case, the amount of pigment applied to the plate was increased by superimposing three or four plugs, whereas extended extaction time did not improve the result.

New approaches like profiles of secondary metabolites and isozymes (Frisvad & Filtenborg, 1983, 1990) and computer assisted keying (Bridge, 1990; Williams, 1990; Pitt, 1990a,b) have greatly improved and established the taxonomy of *Aspergillus* and *Penicillium*. However, in case of *Fusarium*, a general consensus has not yet emerged and there is a need for international collaboration to stabilize the taxonomy of this genus. Thrane (1990) developed FUSKEY, a computer key for 17 common species of *Fusarium* based on a data matrix of about 1200 *Fusarium* isolates using the DELTA system software. Computer keys can be helpful for those researchers who do not carry out mycological identification on a regular routine basis. Where specialized gadgets may not be readily available the macromorphlogical characters and profiles of secondary metabolites have their advantages. All known *Fusarium* species should, therefore, be explored for their profiles of secondary metabolites as well as any other character that could be species specific.

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