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POLAR LIPIDS AND FATTY ACID COMPOSITION OF PHYTOPATHOGENIC FUNGI

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Abstract—Polar lipids of three phytopathogenic fungi, *Alternaria dauci*, *Fusarium solani* f. *phaseoli*, and *Sclerotium rolfsii* were analyzed. Phosphatidyl ethanolamine and phosphatidyl inositol were absent in *Fusarium solani* f. *phaseoli*. The distribution of fatty acids ranged from C_{12} to C_{20} in all three fungi. The degree of unsaturation of the fatty acids was higher in polar lipids than in neutral lipids regardless of the organism. More short chain fatty acids were detected in *F. solani* f. *phaseoli* and *S. rolfsii* than in *A. dauci*.

INTRODUCTION

BECAUSE of their complex nature, polar lipids in fungi have not been studied extensively.[1-3] In preliminary studies, we found that polar lipids were predominant in the lipids of three plant pathogenic fungi, namely *Alternaria dauci* (Kuhn) Groves & Skolko, a leaf blight pathogen on tomato, *Fusarium solani* f. sp. *phaseoli* (Burk,) Synd & Hans., a bean wilt pathogen, and *Sclerotium rolfsii* Sacc., a root rot pathogen on various plants. As an extension of the previous work, the present investigation reports a detailed analysis of the polar lipids and the fatty acid composition of total, neutral and polar lipids of these fungi.

RESULTS

The lipid content and ratio of neutral to polar lipids of the three plant pathogenic fungi are shown in Table 1. In all three fungi the amount of neutral lipids was low; the ratio of neutral to polar lipids was higher in *S. rolfsii* than in the other two fungi. Analysis of polar lipids (Table 2) established the presence of lecithin and lysolecithin in all three fungi.

TABLE 1. LIPID CONTENT OF THREE PLANT PATHOGENIC FUNGI

Organism	Dried mycelial wt (g)	Total lipid wt (mg)	Neutral lipids (%)	Polar lipids (%)	Neutral polar ratio
A. dauci	5·5	127	24·2	75·8	0·3
F. solani f. *phaseoli*	4·2	86	23·8	76·2	0·3
S. rolfsii	4·1	113	34·7	65·3	0·5

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[2] R. B. HOLTZ and L. C. SCHISLER, *Lipids* **6**, 176 (1971).
[3] A. POULOS, W. M. STOURGEOU and G. A. THOMPSON, JR., *Lipids* **6**, 466 (1971).

Although the lysolecithin content varied between the three fungi, it was the major component. Phosphatidyl inositol and phosphatidyl serine were absent in *F. solani* f. *phaseoli*. Two unknown compounds (unknown *A* and *B*) were found in the polar lipids of each fungus. Unknown *A* was present in higher concentrations than unknown *B*.

TABLE 2. COMPOSITION OF POLAR LIPIDS OF THREE PLANT PATHOGENIC FUNGI

Compounds	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*
Lecithin	20·4	23·4	18·7
Lysolecithin	53·6	61·5	48·3
Phosphatidyl ethanolamine	13·9	—	18·1
Phosphatidyl inositol	4·4	—	9·4
Phosphatidyl serine	1·7	t	1·6
Unknown *A*	3·0	15·1	2·6
Unknown *B*	3·1	t	1·4

t = trace (less than 0·1 %).

The fatty acid composition of total lipids is shown in Table 3. The fatty acids of the total lipids from these fungi ranged from C_{12} to C_{20}. Palmitic acid was the major fatty acid in *A. dauci* and *F. solani* f. *phaseoli*, whereas linoleic acid (9:1) was predominant in *S. rolfsii*.

TABLE 3. TOTAL FATTY ACID COMPOSITION IN THE LIPIDS OF THREE PLANT PATHOGENIC FUNGI

Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*	Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*
12·0	1·3	4·1	1·2	16:1	4·0	5·4	t
12:1	t	t	t	18:0	5·9	10·0	10·2
14:0	1·8	1·3	5·7	18:1	25·0	28·1	17·5
14:1	1·3	t	t	18:2	17·6	21·6	40·1
15:0	1·1	0·5	1·0	18:3	2·0	1·0	0·5
16:0	40·1	28·0	23·9	20:0	t	t	t

t = trace (less than 0·1 %).

The degree of unsaturation was low in *A. dauci* (49·9 %) compared to *F. solani* f. *phaseoli* (56·1 %) and *S. rolfsii* (58·1 %). The fatty acid pattern in the neutral lipids (Table 4) was similar to the total fatty acid composition in all the three fungi except that the palmitic acid was the major fatty acid in the neutral lipids of *S. rolfsii*. The distribution of fatty acids in polar lipids was very similar in *F. solani* f. *phaseoli* and *S. rolfsii*; whereas in *A. dauci*, the short chain fatty acids were essentially absent (Table 5). The degree of unsaturation was higher in polar lipids than in neutral lipids in all three organisms.

TABLE 4. FATTY ACID COMPOSITION OF NEUTRAL LIPIDS OF THREE PLANT PATHOGENIC FUNGI

Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*	Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*
12:0	1·0	t	0·8	16:1	t	4·1	t
12:1	—	—	—	18:0	2·8	0·8	4·5
14:0	1·2	1·6	1·3	18:1	9·0	15·0	6·6
14:1	t	t	—	18:2	23·9	30·5	31·5
15:0	—	—	0·8	18:3	—	2·9	t
16:0	62·0	45·2	51·5	20:0	t	—	t

t = trace (less than 0·1%).

TABLE 5. FATTY ACID COMPOSITION OF POLAR LIPIDS OF THREE PLANT PATHOGENIC FUNGI

Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*	Fatty acids	Composition (%) *A. dauci*	*F. solani* f. *phaseoli*	*S. rolfsii*
12:0	t	6·6	1·4	16:1	t	2·2	1·0
12:1	—	t	0·5	18:0	1·7	4·2	2·6
14:0	t	10·5	0·9	18:1	12·4	10·0	9·7
14:1	—	3·3	2·2	18:2	74·7	45·0	64·9
15:0	—	1·2	t	18:3	2·1	t	t
16:0	9·2	17·1	16·8	20:0	—	t	—

t = trace (less than 0·1%).

EXPERIMENTAL

Culture of the organisms. The organisms were maintained on potato dextrose agar (PDA) plates. Cultures were grown in 250 ml Erlenmeyer flasks containing 100 ml of potato dextrose broth. A 6-mm mycelial disc was used to inoculate the flasks and the cultures were incubated at 25° as a stationary culture for 15 days. After the incubation period, the mycelium was harvested by filtration through a Büchner funnel and dried. The method of lipid extraction and separation of polar lipids from neutral lipids has been previously described.[4] Polar and neutral lipids were removed from the thin layer plates and the polar lipids fractionated by TLC. The TLC plates were prepared according to the method of Bunn *et al.*,[5] which involved the use of Silica gel H and 0·02 M boric acid buffer. Plates were developed with $CHCl_3$–MeOH–H_2O (65:25:4, v/v/v) and individual compounds were identified by specific spray reagents[4] and by comparing their R_f values with known standards. The esters of the fatty acids of total, neutral and polar lipids were prepared by transesterification with 2·5% H_2SO_4 in MeOH at 70° for 3 hr. Fatty acid esters were extracted in light petrol. (b.p. 20–40°) and separated with a Packard gas chromatograph using a column packed with 10% glycol succinate (DEGS) coated on chromosorb-Q. The column was operated isothermally at 165° with a flow rate of 40 ml/min. The inlet and detector temperatures were set at 200°. The fatty acid methyl esters were identified from the retention times as compared to standards. Final characterization was made with the Varian Matt III gas chromatograph-mass spectrometer using the same column previously described. The percentage of each fatty acid was calculated from the peak area.

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