Journal of Chromatography, 187 (1980) 399–408

CHROM. 12,330

SEPARATION OF FLUORESCENT COMPOUNDS IN MEMBERS OF THE GENUS *RHIZOPUS* BY THIN-LAYER CHROMATOGRAPHY

D. P. THOMPSON and A. W. SMALLEY
Department of Biology and Department of Chemistry, Southern University, Baton Rouge, La. (U.S.A.)
(First received May 14th, 1979; revised manuscript received August 22nd, 1979)

SUMMARY

Numerous fluorescent compounds were detected and separated by thin-layer chromatography in the chloroform extracts of species of *Rhizopus*. Qualitative similarities and differences existed among the species with respect to specific fluorescent compounds.

INTRODUCTION

The presence of secondary metabolites from mycelium of fungi has been well documented by numerous investigators[1–5]. These secondary metabolites, which serve no known function in the life cycle of many fungi[6], include antibiotics, pigments, mycotoxins and others. Chromatographic methods, which include gas–liquid chromatography[7], high-performance liquid chromatography[8], thin-layer chromatography (TLC)[9–13], and paper chromatography[14–20] have proved very useful in their separation.

This paper describes the use of TLC for the detection of UV-absorbing substances in *Rhizopus* species.

METHODS AND MATERIALS

Species of *Rhizopus* used in this work were obtained from the American Type Culture Collection (Rockville, Md., U.S.A.) and the Department of National Health and Welfare (Ottawa, Canada). Stock cultures were maintained at 5° on slants of potato dextrose agar (PDA) enriched with 0.2% yeast extract. Spores from heavily sporulated cultures, which had been previously grown on slants of PDA enriched with 0.2% yeast extract (PDAYE) for 7 days at 27°, were washed off the PDAYE slant with sterile distilled water. The spore suspension was adjusted to an optical density of 10.0 Klett Units at 450 nm with a blue filter (photoelectric colorimeter, Klett Sumnerson, New York, N.Y., U.S.A.) before being used to inoculate the medium. 50 ml of mycological broth enriched with 0.5% yeast extract (MYE) and 15% sucrose plus 2% yeast extract (YES) were inoculated with 1.0 ml of spore suspension and allowed to incubate for 7 and 14 days respectively at 27° (ref. 21).

The procedure for extraction of secondary metabolites was based on that of

Armbrecht *et al.*[22]. The mycelial mats were extracted with two volumes of warm (60°) chloroform in a Waring Blendor and then concentrated to dryness in a flash evaporator.

For the separation of the secondary metabolites, the chloroform extracts were dissolved in 0.2 ml of chloroform and 15 μl of each extract were spotted on 0.500 mm TLC plates (Redi-Coat 5-8172, Aflasil, Supelco, Bellefonte, Pa., U.S.A.). The plates were developed in two solvent systems (chloroform–methanol (99:1, v/v) (CM) and benzene–methanol–acetic acid (24:4:1, v/v) (BMA). After development, the plates were examined under longwave UV light. Fluorescent materials from the extracts and separated on TLC plates were compared with authentic mycotoxins and aflatoxins. To determine whether non-fluorescent materials were also present in the extracts, the plates were (1) sprayed with a freshly prepared mixture of 0.5 ml of *p*-anisaldehyde in 85 ml of methanol containing 10 ml of glacial acetic acid and 5 ml of concentrated sulfuric acid and then heated at 210° for 8–20 min[23] and (2) sprayed with 1% ethanolic ferric chloride[24].

RESULTS AND DISCUSSION

Seven members of the *Rhizopus* group were evaluated for their ability to produce metabolites in a chemically undefined culture medium and a semi-chemically defined culture medium. Visual examination of the TLC plates accomplished in the dark, with the aid of UV light, revealed numerous fluorescent compounds, as shown in Tables I–IV. The number of fluorescent compounds separated and detected in the two solvent systems CM and BMA, respectively, are as follows: (YES medium for 7 days) *R. arrhizus*, 6 and 5; *R. chinensis*, 8 and 8; *R. circinans*, 8 and 6; *R. kazanensis*, 4 and 6; *R. oryzae*, 6 and 6; *R. stolonifer*, 8 and 7; *R.* 66-81-2, 4 and 6; (YES medium for 14 days) *R. arrhizus*, 5 and 6; *R. chinensis*, 5 and 8; *R. circinans*, 7 and 6; *R. kazanensis*, 10 and 7; *R. oryzae*, 6 and 6; *R. stolonifer*, 6 and 5; *R.* 66-81-2, 5 and 7; (MYE medium for 7 days) *R. arrhizus*, 5 and 2; *R. chinensis*, 4 and 3; *R. circinans*, 2 and 1; *R. kazanensis*, 4 and 3; *R. oryzae*, 3 and 4; *R. stolonifer*, 4 and 4; *R.* 66-81-2; (MYE medium for 14 days) *R. arrhizus*, 8 and 4; *R. chinensis*, 6 and 4; *R. circinans*, 6 and 5; *R. kazanensis*, 7 and 6; *R. oryzae*, 7 and 5; *R. stolonifer*, 6 and 4; and *R.* 66-81-2, 4 and 5.

The data indicate that the seven fungi examined produced a number of fluorescent substances when incubated in chemically undefined and semi-chemically defined media. The fluorescent compounds were analyzed by their chromatographic behavior, reaction to spray reagents, and spectra. The data obtained from these analyses were then compared with those of known mycotoxins. The data on the fluorescent compounds did not reveal the presence of any of the known mycotoxins[25]. Qualitative differences were apparent in the number of detectable fluorescent substances with respect to (1) separation in the two solvent systems, (2) the media, and (3) period of incubation. It was of interest, however, to note the presence of a common fluorescent compound in all seven of the fungi which appeared green before spray treatment and purple after spray treatment with *p*-anisaldehyde in visible light. Caution must be exercised, however, in the interpretation of the significance of the differences and similarities of fluorescent compounds which were found in the fungi examined during this investigation. Studies on the structural elucidation and identi-

TABLE I

TLC OF THE CHLOROFORM EXTRACT OF *RHIZOPUS* SPECIES GROWN IN YES MEDIUM FOR 7 DAYS

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
R. arrhizus	1	0.23	CM	—	Blue	—	—		
	1b	0.38	CM	—	Green			—	Green
	2	0.48	CM	—	—			—	Pale Blue
	3	0.53	CM	—	Green	Purple	—		
	4	0.76	CM	—	Pale Blue	Green	—	—	Pale Blue
	5	0.97	CM	—	Bright Blue	Green	—		
	1	0.09	BMA	—	—	Green	—		
	2	0.31	BMA	—	Blue	—	—		
	2b	0.38	BMA	—	Green			—	Green
	3	0.48	BMA	—	Pale Blue			—	Pale Blue
	4	0.72	BMA	—	Pale Blue	Green	—	—	Pale Blue
R. chinensis	1	0.14	CM	—	Blue	—	—		
	2	0.29	CM	—	Blue	—	—		
	3	0.41	CM	—	Green	—		—	Green
	3b	0.48	CM	—	Bright Blue			—	Green
	4	0.64	CM	—	Green	Purple	—		
	5	0.73	CM	—	Dull Blue	—	—		
	6	0.85	CM	—	Blue	Green	—	—	Blue
	7	0.94	CM	—	Pale Blue	—	—	—	Pale Blue
	1	0.07	BMA	—	Blue	—	—	—	Blue
	2	0.15	BMA	—	Blue			—	Blue
	3	0.28	BMA	—	Blue			—	Blue
	4	0.34	BMA	—	Blue			—	Blue
	5	0.44	BMA	—	Blue	—	—		
	6	0.73	BMA	—	Green	Purple	—	—	Green
	7	0.84	BMA	—	Blue	Green	—	—	Blue
	8	0.91	BMA	—	Dull Blue	Green	—		
R.circinans	1	0.12	CM	—	Blue	—	—		
	2	0.28	CM	—	Blue	—	—		
	3	0.41	CM	—	Green			—	Green
	4	0.50	CM	—	Bright Blue			—	Bright Blue
	5	0.63	CM	—	Green	Purple	—		
	6	0.75	CM	—	Blue	Green	—		
	7	0.83	CM	—	Dull Blue	Green	—	—	Dull Blue
	8	0.97	CM	—	Bright Blue	—	—		
	1	0.09	BMA	—	—	Green	—		
	2	0.16	BMA	—	Blue			—	Blue
	3	0.333	BMA	—	Blue	—	—	—	Blue
	4	0.44	BMA	—	Blue	—	—		
	5	0.71	BMA	—	Green	Purple	—	—	Green
	6	0.82	BMA	—	Dull Blue	Green	—	—	Dull Blue

(Continued on p. 402)

TABLE I *(continued)*

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
R. kazanensis	1	0.12	CM	—	Blue	—	—		
	2	0.20	CM	—	Blue	—	—		
	3	0.50	CM	—	Green	Purple	—	—	Green
	4	0.82	CM	—	Pale Blue	Green	—	—	Pale Blue
	1	0.06	BMA	—	Blue			—	Blue
	2	0.14	BMA	—	Blue	Green	—	—	Blue
	3	0.31	BMA	—	Blue			—	Blue
	4	0.40	BMA	—	Blue	—	—	—	Blue
	5	0.74	BMA	—	Green	Purple	—	—	Green
	6	0.94	BMA	—	Pale Blue	—	—		
R. oryzae	1	0.09	CM	—	Blue	—	—		
	2	0.22	CM	—	Blue	—	—		
	3	0.43	CM	—	Green	—	—	—	Green
	4	0.53	CM	—	Bright Blue	Purple	—	—	Bright Blue
	5	0.66	CM	—	Blue	Green	—		
	6	0.80	CM	—	Pale Blue			—	Pale Blue
	1	0.05	BMA	—	Blue			—	Blue
	2	0.14	BMA	—	Blue	—	—	—	Blue
	3	0.28	BMA	—	Blue	—	—	—	Blue
	4	0.40	BMA	—	Blue	—	—	—	Blue
	5	0.68	BMA	—	Green	Purple	—	—	Green
	6	0.78	BMA	—	Blue			—	Blue
R. stolonifer	1	0.09	CM	—	Blue	—	—		
	2	0.22	CM	—	Blue	—	—		
	3	0.41	CM	—	Green			—	Green
	4	0.53	CM	—	Bright Blue			—	Bright Blue
	4b	0.56	CM	—	Green	Purple	—		
	5	0.69	CM	—	Blue	Green	—		
	6	0.81	CM	—	Dull Blue	Green	—		
	7	0.90	CM	—	Pale Blue			—	Pale Blue
	1	0.06	BMA	—	Blue			—	Blue
	2	0.14	BMA	—	Blue	Green	—	—	Blue
	3	0.28	BMA	—	Blue			—	Blue
	4	0.37	BMA	—	Blue			—	Blue
	5	0.42	BMA	—	Blue	—	—		
	6	0.68	BMA	—	Green	Purple	—	—	Green
	7	0.75	BMA	—	Blue			—	Blue
R. 66-81-2	1	0.21	CM	—	Blue	—	—		
	2	0.40	CM	—	Green			—	Green
	3	0.56	CM	—	Green	Purple	—		
	4	0.75	CM	—	Blue	Green	—	—	Blue
	1	0.06	BMA	—	Blue			—	Blue
	2	0.14	BMA	—	Blue	—	—	—	Blue
	3	0.26	BMA	—	Blue			—	Blue
	4	0.35	BMA	—	Blue	—	—	—	Blue
	5	0.65	BMA	—	Green	Purple	—	—	Green
	6	0.81	BMA	—	Pale Blue	Green	—	—	Pale Blue

TABLE II

TLC OF THE CHLOROFORM EXTRACT OF *RHIZOPUS* SPECIES GROWN IN YES MEDIUM FOR 14 DAYS

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
				Visible light	*Ultra-violet*	*p-Anisaldehyde*		*Iron chloride*	
						Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*
R. arrhizus	1	0.08	CM	—	Bright Blue	—	—		
	2	0.41	CM	—	Green	Purple	—	—	Green
	3	0.72	CM	—	Blue			—	Blue
	4	0.76	CM	—	Dull Blue	Green	—	—	Dull Blue
	5	0.97	CM	—	Bright Blue	Green	—		
	1	0.09	BMA	—	Blue			—	Blue
	2	0.16	BMA	—	Blue			—	Blue
	3	0.41	BMA	—	Blue	—	—		
	4	0.63	BMA	—	Green	Purple	—	—	Green
	5	0.76	BMA	—	Blue	Green	—	—	Blue
	6	0.91	BMA	—	Bright Blue	—	—		
R. chinensis	1	0.12	CM	—	Blue			—	Blue
	2	0.25	CM	—	Blue			—	Blue
	3	0.46	CM	—	Green	Purple	—	—	Green
	4	0.76	CM	—	Blue	—	—	—	Blue
	5	0.94	CM	—	Dull Blue	Green	—		
	1	0.06	BMA	—	Blue			—	Blue
	2	0.13	BMA	—	Blue			—	Blue
	3	0.18	BMA	—	Blue			—	Blue
	4	0.41	BMA	—	Blue	—	—		
	5	0.65	BMA	—	Green	Purple	—	—	Green
	6	0.75	BMA	—	Blue			—	Blue
	7	0.88	BMA	—	Blue	—	—		
	8	0.95	BMA	—	Dull Blue	—	—	—	Dull Blue
R. circinans	1	0.08	CM	—	Blue	—	—		
	2	0.23	CM	—	Blue	—	—		
	3	0.45	CM	—	Green	Purple	—	—	Green
	4	0.67	CM	—	Blue			—	Blue
	5	0.76	CM	—	Blue			—	Blue
	6	0.84	CM	—	Dull Blue	Green	—		
	7	0.97	CM	—	Bright Blue	Green	—		
	1	0.07	BMA	—	Blue			—	Blue
	2	0.16	BMA	—	Blue			—	Blue
	3	0.28	BMA	—	Blue	—	—	—	Blue
	4	0.51	BMA	—	Blue	—	—	—	Blue
	5	0.81	BMA	—	Green	Purple	—		
	6	0.91	BMA	—	Dull Blue	Green	—	—	Dull Blue
R. kazanensis	1	0.03	CM	—	Blue			—	Blue
	2	0.06	CM	—	Blue	—	—	—	Blue
	3	0.19	CM	—	Blue	—	—		

(Continued on p. 404)

TABLE II *(continued)*

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
	4	0.26	CM	—	Green			—	Green
	5	0.38	CM	—	Dull Blue	—	—		
	6	0.50	CM	—	Blue	—	—		
	7	0.63	CM	—	Green	Purple	—		
	8	0.66	CM	—	Blue			—	Blue
	9	0.75	CM	—	Blue			—	Blue
	10	0.87	CM	—	Blue	Green	—	—	Blue
	1	0.15	BMA	—	Blue	—	—	—	Blue
	2	0.23	BMA	—	Green	—	—		
	3	0.35	BMA	—	Blue	—	—	—	Blue
	4	0.47	BMA	—	Blue	—	—	—	Blue
	5	0.61	BMA	—	Green	—	—	—	Green
	6	0.75	BMA	—	Green	Purple	—	—	Green
	7	0.90	BMA	—	Blue	Green	—	—	Blue
R. oryzae	1	0.07	CM	—	Blue	—	—	—	Blue
	2	0.19	CM	—	Blue	—	—		
	3	0.41	CM	—	Green	Purple	—	—	Green
	4	0.71	CM	—	Blue			—	Blue
	5	0.81	CM	—	Blue			—	Blue
	6	0.88	CM	—	Blue	Green	—	—	Blue
	1	0.01	BMA	—	—	Green	—		
	2	0.17	BMA	—	Blue	—	—	—	Blue
	3	0.31	BMA	—	Blue	—	—	—	Blue
	4	0.47	BMA	—	Dull Blue	—	—		
	5	0.64	BMA	—	Green	Purple	—	—	Green
	6	0.84	BMA	—	Dull Blue	Green	—	—	Dull Blue
R. stolonifer	1	0.08	CM	—	Blue	—	—		
	2	0.22	CM	—	Blue	—			
	3	0.45	CM	—	Green	Purple	—	—	Green
	4	0.68	CM	—	Blue			—	Blue
	5	0.78	CM	—	Blue			—	Blue
	6	0.93	CM	—	Blue	Green	—	—	Green
	1	0.15	BMA	—	Blue	—	—	—	Blue
	2	0.25	BMA	—	Blue	—	—	—	Blue
	3	0.34	BMA	—	Blue	—	—	—	Blue
	4	0.66	BMA	—	Green	Purple	—	—	Green
	5	0.94	BMA	—	Blue	Green	—	—	Blue
R. 66-81-2	1	0.12	CM	—	Blue	—	—	—	Blue
	2	0.39	CM	—	Green	Purple	—	—	Green
	3	0.56	CM	—	Blue			—	Blue
	4	0.65	CM	—	Blue			—	Blue
	5	0.86	CM	—	Blue	Green	—	—	Green
	1	0.10	BMA	—	—	Green	—		
	2	0.15	BMA	—	Blue	Green	—	—	Blue
	3	0.25	BMA	—	Blue	—	—	—	Blue
	4	0.33	BMA	—	Blue	—	—		
	5	0.43	BMA	—	Dull Blue	—	—		
	6	0.57	BMA	—	Green	Purple	—	—	Green
	7	0.86	BMA	—	Blue	Green	—	—	Blue

TABLE III

TLC OF THE CHLOROFORM EXTRACT OF *RHIZOPUS* SPECIES GROWN IN MYE MEDIUM FOR 7 DAYS

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
R. arrhizus	1	0.50	CM	—	Green			—	Green
	2	0.65	CM	—	Blue			—	Blue
	3	0.69	CM	—	Green	Pink	—		
	4	0.73	CM	—	Blue	Green	—		
	5	0.80	CM	—	Green	—			
	1	0.62	BMA	—	Green	Pink	—		
	2	0.68	BMA	—	Green			—	Green
R. chinenensis	1	0.20	CM	—	Blue	—	—	—	Blue
	2	0.60	CM	—	Green	Pink	—	—	Green
	3	0.76	CM	—	Blue	—	—	—	Blue
	4	0.91	CM	—	Blue	Green	—	—	Blue
	1	0.53	BMA	—	Blue	—	—		
	2	0.80	BMA	—	Green	—	—		
	3	0.89	BMA	—	Blue	Green	—	—	Blue
R. circinans	1	0.53	CM	—	Green	Pink	—	—	Green
	2	0.77	CM	—	Blue	Green	—	—	Blue
	1	0.61	BMA	—	Green	Pink	—	—	Green
R. kazanensis	1	0.05	CM	—	Blue			—	Blue
	2	0.58	CM	—	Green	Pink	—	—	Green
	3	0.73	CM	—	Blue	Green	—	—	Blue
	4	0.87	CM	—	Blue	—	—	—	Blue
	1	0.25	BMA	—	Blue	—	—		Blue
	2	0.68	BMA	—	Green	Pink	—		Green
	3	0.84	BMA	—	Blue	Green	—	—	Blue
R. oryzae	1	0.04	CM	—	Blue			—	Blue
	2	0.50	CM	—	Green	Pink	—	—	Green
	3	0.77	CM	—	Blue	Green	—	—	Blue
	1	0.09	BMA	—	Blue				Blue
	2	0.34	BMA	—	Blue	Pink	—	—	Blue
	3	0.62	BMA	—	Green	Pink	—	—	Blue
	4	0.85	BMA	—	Blue	Green	—		
R. stolonifer	1	0.20	CM	—	Blue	—	—	—	Blue
	2	0.55	CM	—	Green	Pink	—	—	Green
	3	0.78	CM	—	Pale Blue	Green	—	—	Pale Blue
	4	0.80	CM	—	Blue	Green	—		
	1	0.13	BMA	—	Blue			—	Blue
	2	0.40	BMA	—	Blue	Green	—	—	Blue
	3	0.67	BMA	—	Green	Pink	—	—	Green
	4	0.81	BMA	—	Blue	Green	—	—	Blue
R. 66-81-2	1	0.28	CM	—	Blue	—	—		
	2	0.59	CM	—	Green	Pink	—	—	Green
	3	0.87	CM	—	Blue	Green	—	—	Blue
	1	0.20	BMA	—	Blue	—	—	—	Blue
	2	0.47	BMA	—	Blue	—	—	—	Blue
	3	0.68	BMA	—	Green	Pink	—	—	Green
	4	0.83	BMA	—	Blue	—	—	—	Blue

TABLE IV

TLC OF THE CHLOROFORM EXTRACT OF *RHIZOPUS* SPECIES GROWN IN MYE MEDIUM FOR 14 DAYS

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
R. arrhizus	1	0.04	CM	—	Blue			—	Blue
	2	0.11	CM	—	Blue	—	—	—	Blue
	3	0.29	CM	—	Blue	—	—	—	Blue
	4	0.49	CM	—	Green			—	Green
	5	0.69	CM	—	Blue			—	Blue
	6	0.75	CM	Yellow	—	—	—	—	—
	7	0.83	CM	—	Green	Pink	—		
	8	0.98	CM	—	Blue	Green	—		
	1	0.25	BMA	—	Blue	—	—	—	Blue
	2	0.47	BMA	—	Blue	—	—	—	Blue
	3	0.74	BMA	—	Green	Pink	—	—	Green
	4	0.87	BMA	—	Blue	Green	—	—	Blue
R. chinensis	1	0.10	CM	—	Blue	—	—	—	Blue
	2	0.25	CM	—	Blue	—	—		
	3	0.45	CM	—	Green			—	Green
	4	0.62	CM	—	Blue			—	Blue
	5	0.77	CM	—	Green	Pink	—		
	6	0.92	CM	—	Blue	Green	—	—	Blue
	1	0.29	BMA	—	Blue	—	—		
	2	0.45	BMA	—	Blue	—	—	—	Blue
	3	0.70	BMA	—	Green	Pink	—	—	Green
	4	0.86	BMA	—	Blue	Green	—		
R. circinans	1	0.09	CM	—	Blue	—	—	—	Blue
	2	0.25	CM	—	Blue	—	—		
	3	0.50	CM	—	Green			—	Green
	4	0.67	CM	—	Blue			—	Blue
	5	0.78	CM	—	Green	Pink	—		
	6	0.89	CM	—	Blue	Green	—	—	Blue
	1	0.27	BMA	—	Blue	—	—	—	Blue
	2	0.37	BMA	—	Blue			—	Blue
	3	0.44	BMA	—	Blue	—	—	—	Blue
	4	0.68	BMA	—	Green	Pink	—	—	Green
	5	0.82	BMA	—	Blue	Green	—	—	Blue
R. kazanensis	1	0.08	CM	—	Blue			—	Blue
	2	0.16	CM	Yellow	Dull Blue			—	Dull Blue
	3	0.26	CM	—	Blue	—	—	—	Blue
	4	0.60	CM	—	Green	Pink	—	—	Green
	5	0.71	CM	—	Blue			—	Blue
	6	0.78	CM	Orange	Dull Blue			—	Dull Blue
	7	0.90	CM	—	Blue	Green	—	—	Blue

TABLE IV *(continued)*

Organism	*Spot*	R_F	*Solvent system*	*Detection*					
				Before spray treatment		*After spray treatment*			
						p-Anisaldehyde		*Iron chloride*	
				Visible light	*Ultra-violet*	*Visible light*	*Ultra-violet*	*Visible light*	*Ultra-violet*
	1	0.25	BMA	—	Blue			—	Blue
	2	0.35	BMA	—	Blue			—	Blue
	3	0.49	BMA	—	Blue	—	—	—	Blue
	4	0.76	BMA	—	Green	Pink	—	—	Green
	5	0.87	BMA	—	Blue	—	—		Blue
	6	0.95	BMA	—	Blue	Green	—	—	Blue
R. oryzae	1	0.03	CM	—	Blue			—	Blue
	2	0.08	CM	—	Blue			—	Blue
	3	0.15	CM	—	Blue			—	Blue
	4	0.53	CM	—	Green	Pink	—		
	5	0.72	CM	—	Blue	Green	—	—	Blue
	6	0.90	CM	—	Blue			—	Blue
	7	0.95	CM	—	Blue	Green	—	—	Blue
	1	0.23	BMA	—	Blue			—	Blue
	2	0.45	BMA	—	Blue	—	—	—	Blue
	3	0.70	BMA	—	Green	Pink	—	—	Green
	4	0.82	BMA	—	Blue	Green	—	—	Blue
	5	0.95	BMA	—	Blue	Green	—	—	Blue
R. stolonifer	1	0.08	CM	—	Blue			—	Blue
	2	0.20	CM	—	Blue	—	—		
	3	0.56	CM	—	Green	Pink	—	—	Green
	4	0.66	CM	—	Blue			—	Blue
	5	0.84	CM	—	Blue	Green	—	—	Blue
	6	0.94	CM	—	Blue	Green	—	—	Blue
	1	0.46	BMA	—	Blue	—	—	—	Blue
	2	0.73	BMA	—	Green	Pink	—	—	Green
	3	0.84	BMA	—	Blue	—	—	—	—
	4	0.94	BMA	—	Blue	Green	—		
R. 66-81-2	1	0.14	CM	—	Blue	—	—	—	Blue
	2	0.60	CM	—	Green	Pink	—	—	Green
	3	0.77	CM	—	Blue	Green	—	—	Blue
	4	0.94	CM	—	Blue	Green	—	—	Blue
	1	0.43	BMA	—	Blue			—	Blue
	2	0.49	BMA	—	Blue	—	—	—	Blue
	3	0.75	BMA	—	Green	Pink	—	—	Green
	4	0.87	BMA	—	Blue	Green	—	—	Green
	5	0.93	BMA	—	Blue	Green	—	—	Green

fication of the metabolites are presently under way and will be reported on in the near future.

ACKNOWLEDGEMENT

This work was supported by grant RR08025-08 from the National Institute of Health.

REFERENCES

1 H. J. Blumenthal, in G. C. Ainsworth and A. S. Sussman (Editors) *The Fungi, Vol. I, The Fungal Cell*, Academic Press, New York, 1965, p. 229.
2 D. J. Niederpruem, in G. C. Ainsworth and A. S. Sussman (Editors) *The Fungi, Vol. I, The Fungal Cell*, Academic Press, New York, 1965, p. 269.
3 J. H. Burnett, *Fundamentals of Mycology*, St. Martin's Press, New York, 1968, p. 261.
4 J. G. Heathcote and J. R. Hibbert, *J. Chromatogr.*, 108 (1975) 131.
5 C. O. Emeh and E. H. Marth, *Trans. Br. Mycol. Soc.*, 68 (1977) 112.
6 J. W. Bennett, F. A. Fernholz and L. S. Lee, *Mycologia*, (1978) 104.
7 R. F. Vesonder, A. Ciegler, R. F. Roger, K. A. Burbridge, R. J. Bothast and A. H. Jensen *Appl. Environ. Microbiol.*, 36 (1977) 885.
8 R. E. Peterson and A. Ciegler, *Appl. Environ. Microbiol.*, 36 (1978) 613.
9 H. Murakami, S. Takase and T. Ishii, *J. Gen. Appl. Microbiol., Tokyo*, 13 (1967) 323.
10 R. M. Eppley, *J. Ass. Offic. Anal. Chem.*, 51 (1968) 74.
11 K. K. Maggon, L. Viswanathan and T. A. Venkitasubramanian, *J. Gen. Microbiol.*, 59 (1969) 119.
12 C. N. Shih and E. H. Marth, *J. Milk Food Technol.*, 35 (1972) 585.
13 S. V. Thomson and M. C. Mehdy, *Phytopathology*, 68 (1978) 1112.
14 L. Reio, *J. Chromatogr.*, 1 (1958) 338.
15 L. Reio, J. *Chromatogr.*, 4 (1960) 458.
16 L. Reio, *J. Chromatogr.*, 13 (1964) 475.
17 L. Reio, *J. Chromatogr.*, 47 (1970) 60.
18 L. Reio, *J. Chromatogr.*, 47 (1970) 11.
19 L. Reio, *J. Chromatogr.*, 68 (1972) 183.
20 L. Reio, *J. Chromatogr.*, 88 (1974) 119.
21 W. van Walbeek, P. M. Scott and F. S. Tatcher, *Can. J. Microbiol.*, 14 (1968) 131.
22 B. H. Armbrecht, F. A. Hodges, H. R. Smith and A. A. Nelson, *J. Ass. Offic. Anal. Chem.*, 46 (1963) 805.
23 P. M. Scott, J. W. Lawrence and W. van Walbeek, *Appl. Microbiol.*, 20 (1970) 839.
24 P. S. Steyn, *J. Chromatogr.*, 45 (1969) 473.
25 Z. Wurečková, V. Betina and P. Nemec, *J. Chromatogr.*, 116 (1976) 141.