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C. FANELLI, A.A. FABBRI and M. SERAFINI (*)

Lipid composition and aflatoxin production of mycelium and conidia of Aspergillus flavus Link (**)

SUMMARY. — The mycelium and conidia of *Aspergillus flavus* show a different distribution of the lipid fractions grown on synthetic media with different carbohydrates. Also the aflatoxin production in mycelium and conidia is quantitatively different. The qualitative changes of aflatoxins in time are described.

RIASSUNTO. — Il micelio e i conidi dell'*Aspergillus flavus* presentano una differente distribuzione delle frazioni lipidiche quando si sviluppano su terreni sintetici con diversi carboidrati. Anche la produzione delle aflatossine nel micelio e nei conidi è quantitativamente differente. Sono descritti i cambiamenti nella composizione delle aflatossine nel tempo.

INTRODUCTION

Previously we studied the lipid metabolism of a strain of *Aspergillus flavus* isolated from wheat seeds in silos [1]. In our studies it was evident that different lipid sources in the culture medium stimulate the growth of *Aspergillus flavus*, but not the production of aflatoxins [2]. In our experiments it also appeared that when lipid sources were in the culture media as sole carbon source the production of conidia from the fungus was very inferior than in the media with carbohydrates.

This result could be correlated with the differences found in the composition of fatty acids of FFA and TG fractions of lipids of *Aspergillus flavus* when the fungus grew on different carbon sources. As there are few evidences that the medium of culture influences the lipid composition of the different fungal structures [3, 4], we have determined the lipid composition of conidia

(*) Cattedra di Micologia, Istituto dell'Orto Botanico, Università di Roma, Largo Cristina di Svezia 24, Roma.

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and mycelium of *A. flavus* with particular attention to the fatty acids of FFA and TG fractions. We have also analysed the aflatoxins produced in our experimental conditions inside conidia and mycelium.

Materials and methods

Vegetative growth of cultures and conidia production

Aspergillus flavus Link. isolated in our Institute from wheat seed harvested in silos was normally grown on Czapek Dox broth medium. As alternative carbon sources glucose and maltose were tested. The cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of liquid medium. Each flask was incubated with 8×10^5 15 days old conidia from culture grown on Czapek Dox medium. After 4-7-10-14 days in these conditions at 30°C the mycelium grown in each flask was washed three times in distilled water to remove the conidia. The mycelium so obtained was dried to constant weight at 80°C for 48 h.

The conidia were concentrated by centrifugation and dried to constant weight at 80°C for 48 h.

Chemical extraction and analysis

Mycelium and conidia were extracted in Soxhlet with 250 volumes of chloroform: methanol (2 : 1 v/v) for 3 h. Extracts were collected and filtered on a sintered glass and dried over anhydrous Na_2SO_4. The extracted lipids and aflatoxins were recovered by evaporation of the solvents under reduced pressure on a rotary evaporator below 40°C.

The total lipid extracts were separated into their various components by thin layer chromatography [5]. The lipids on the developed chromatograms were detected heating at 150°C with 30% v/v H_2SO_4 for photodensitometry or by staining with bromocresol green solution [6].

The esterification procedure and determination of the fatty acid composition of free fatty acids and triglycerides fractions were performed with the method previously described [6].

Aflatoxins

The aflatoxins extracted together with lipids in Soxhlet were purified and analysed by High Performance Liquid Chromatography according to Serafini and coll. [7].

Results and discussion

The lipid composition of mycelium and conidia of *Aspergillus flavus* after 10 days of growth (Tab. 1) is very different as regards the percentage

TABLE 1 — *Effect of different carbohydrates added in basal medium on lipid composition of mycelium and conidia of Aspergillus flavus after 15 days of growth. Total lipid content of mycelium varied from 22.0 to 27.7 mg/100 dry weight. Total lipid content of conidia varied from 37.4 to 43.0 mg/100 dry weight.*

Carbon source	g/100 ml	LIPID FRACTIONS (%)					
		PL	ST	DG	FFA	TG	Others
MYCELIUM							
GLUCOSE	1	45.0	2.0	0.7	16.1	35.0	0.5
SUCROSE	1	46.5	2.0	0.7	15.3	34.5	0.7
MALTOSE	1	44.5	2.0	0.8	18.1	33.2	1.4
CONIDIA							
GLUCOSE	1	75.2	1.3	trace	6.5	5.0	11.8
SUCROSE	1	78.2	1.8	trace	5.5	5.8	8.7
MALTOSE	1	75.0	2.0	trace	5.0	4.5	13.5

Abbreviations: PL: polar lipids; ST: sterols; DG: diglicerides; FFA: free fatty acids; TG: triglycerides.

of the lipid fractions analysed. The most evident difference is the higher percentage of polar lipids in the conidia as compared with the mycelium. Other evident differences are the lower percentage of free fatty acids and triglycerides in the conidia as compared with mycelium. The three carbohydrates used as carbon sources do not influence the lipid composition both of the mycelium and of the conidia.

Table 2 shows the effect of varying carbohydrates added to basal medium on fatty acid composition of FFA and TG fractions of lipids of mycelium and conidia of *Aspergillus flavus*.

The fatty acid composition of FFA and TG fractions of mycelium and conidia appears different particularly as regards oleic acid and linoleic acid. The oleic acid is the fatty acid that appears in higher percentage in FFA fraction in the conidia, while in the mycelium it appears in very inferior percentage as compared with stearic acid. The linoleic acid instead is in the conidia in very lower percentage as compared with its presence in the mycelium. Not so evident differences appear among the fatty acids of the TG fraction as regards oleic acid and stearic acid, while the linoleic acid appears in this case also in percentage very inferior in the conidia with its presence in mycelium.

As regards the production of the aflatoxins the strain that we used is a producer of the four aflatoxins B_1, B_2, G_1, G_2. As Table 3 shows we have

TABLE 2 — *Effect of addition of different carbohydrates to basal medium on fatty acid composition of FFA and TG fractions of lipids of mycelium and conidia of Aspergillus flavus after 15 days of growth.*

Carbon sources	$C_{16:0}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$		$C_{20:0}$		Others	
	FFA*	TG*	FFA*	TG*	FFA*	TG*	FFA*	TG*	FFA*	TG*	FFA*	TG*
MYCELIUM												
Glucose	35.6	27.9	13.8	11.1	26.8	23.2	20.8	31.3	—	—	3.0	6.5
Sucrose	36.6	24.4	14.0	12.5	25.8	22.8	18.8	39.9	—	—	4.8	0.4
Maltose	41.8	28.2	8.8	13.6	24.2	21.0	13.8	32.8	—	—	9.4	2.4
CONIDIA												
Glucose	27.1	52.4	17.7	5.8	52.6	18.2	2.2	17.9	—	1.0	0.4	4.7
Sucrose	24.0	33.2	18.4	12.0	48.6	31.0	6.6	6.1	1.5	2.1	0.9	15.6
Maltose	24.6	33.3	15.6	10.1	44.8	32.1	3.4	11.3	0.8	1.6	10.8	11.6

Abbreviations: FFA: free fatty acids; TG: triglycerides.
* Data represent percentage of specific fatty acid in fractions.

TABLE 3 — *Effect of addition of different carbohydrates to basal medium on the production of aflatoxins ($B_1 + B_2 + G_1 + G_2$) in mycelium and conidia after 4, 7, 10 and 14 days of growth.*

Carbon source	g/100 ml	4		7		10		14	
		Dry weight (mg)	Aflatoxins (ng)	Dry weight (mg)	Aflatoxins (ng)	Dry weight (mg)	Aflatoxins (ng)	Dry weight (mg)	Aflatoxins (ng)
MYCELIUM									
Glucose	1	624	363	1840	4254	2748	7883	1988	1882
Sucrose	1	554	296	1468	3128	2222	6841	1683	1210
Maltose	1	468	257	1388	3044	2108	6438	1436	1113
CONIDIA									
Glucose	1	105	—	768	—	1386	20	1586	40
Sucrose	1	88	—	680	—	1228	trace	1310	26
Maltose	1	75	—	506	—	1044	trace	1338	28

Trace means less than 10 ng.

TABLE 4 — *Aflatoxins produced both in mycelium and in conidia grown in culture media with different carbohydrates added in basal medium after 4, 7, 10, 14 days at 30°C.*

DAYS OF INCUBATION		GLUCOSE B_1	B_2	G_1	G_2 (ng)	SUCROSE B_1	B_2	G_1	G_2 (ng)	MALTOSE B_1	B_2	G_1	G_2 (ng)
4	mycelium	112	—	226	15	90	—	206	—	59	—	198	—
	conidia	—	—	—	—	—	—	—	—	—	—	—	—
7	mycelium	1628	40	2198	188	968	25	2106	29	1192	32	1805	15
	conidia	—	—	—	—	—	—	—	—	—	—	—	—
10	mycelium	2343	320	3089	140	2228	190	4242	181	1887	177	4129	245
	conidia	—	—	20	—	—	—	trace	—	—	—	trace	—
14	mycelium	211	85	1496	90	166	58	888	98	102	44	832	137
	conidia	—	—	40	—	—	—	26	—	—	—	28	—

Trace means less than 10 ng.

measured the production of the four aflatoxins inside mycelium and conidia for 14 days.

The production of the aflatoxins is very low as those toxic metabolites are in very higher percentage in culture media [2]. Nevertheless the amount of aflatoxins produced and present in mycelium is higher as compared to those present in conidia. We have found only few ng in conidia at 10 and 14 days when the production of conidia was more abundant. Only few work have reported the presence of aflatoxins in conidia [8, 9] but the detection was clear only when the fungus was grown in organic media.

Our results show that when the aflatoxins are produced they are not however equally distributed in mycelium and conidia, and with time it appeared that the toxins present both in mycelium and in conidia change qualitatively (Table 4).

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