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The Effect of Cytochalasin A on the Lipid Composition of *Mucor mucedo*

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ABSTRACT. Cytochalasins are a group of fungal metabolites which inhibit a variety of cellular functions. Cytochalasin A (CA) is the most effective against fungi. After screening for the effect of CA, *Mucor mucedo* was chosen to study the influence of this drug on hyphal differenciation and metabolism. The effect of CA on the lipid composition of growing hyphae was studied. The reduction of total mycelial lipids was 31.33% in two days and 24.14% in four days. The enhancement in phospholipid content was 3.14% and 31.14% in two and four days-old mycelium respectively, while the neutral lipids were reduced by the same values. CA induced some modification in the proportions of : fatty acids; neutral lipids (sterol; diacylglycerides; free fatty acids; triacylglycerides; sterols esters) and in the constituents of phospholipids [decrease of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and increase of phosphatidylglycerol (PG)].

The cytochalasin secondary metabolites of microrganisms, particularly fungi, were first discovered in 1964 (Tanenbaum 1978). This term is used to describe the effect of these compounds on morphogenesis. One of the principle effects that has been established in the intracellular disorganisation of the microfilament system (Wessels *et al.* 1971). To date there are very few investigations on the activity of cytochalasins against fungi. Betina *et al.* (1972) and Betina and Micekova (1973) studied the morphogenetic effects of cytochalasin A and D on *Botrytis cinerea* and also showed that cytochalasin B was inactive. Patton and Marchant (1975) have studied the effect of cytochalasin B on hyphal morphogenesis of *Polyporus biennis*. El Mougith *et al.* (1984a) have studied the effects of cytochalasin A on spore germination, growth and ultrastructure of *Mucor mucedo* while effects of cytochalasin A on the chemical composition of the cell wall of *M. mucedo* have been studied by El Mougith *et al.* (1984b). The present investigation deals with the influence of CA on the lipid metabolism of *M. mucedo*.

Material and Methods

Organism

Mucor mucedo L. was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

Media

The fungus was maintained on potato-dextrose agar medium at 27°C and was subcultured at approximately two weeks intervals. Culture flasks containing 49 ml liquid medium [$(NH_4)_2SO_4$, 0.1 g; glucose, 1 g; citric acid, 0.3449 g; Na_2PO_4H $12H_2O$, 2.302 g; $MgSO_4$ $7H_2O$, 0.05 g; $ZnSO_4$ $7H_2O$, 0.005 g; $FeSO_4$ $7H_2O$, 0.003 g; $MnSO_4$ $5H_2O$, 0.002 g; $CuSO_4$ $5H_2O$, 0.001 g; KCl, 0.005 g; $CaCl_2$ $2H_2O$, 0.01 g and 100 ml distilled water; pH, 6.3)] were sterilized by autoclaving at 120°C for 20 min., and inoculated with 1 ml of a spore suspension prepared from 7 days-old cultures grown on potato dextrose-agar medium in the same sterile liquid medium. The stationary cultures were incubated at 27°C.

Antibiotic

Cytochalasin A was obtained from Sigma Chemical Co. (Saint-Louis, MO, USA). In quantitative tests cytochalasin A was dissolved in dimethylsulfoxide (DMSO) and dilued to a final stock concentration of 0.1% DMSO. The concentration of cytochalasin A used in this study was 1 μg/ml cold sterilized cytochalasin A solution dispensed into the sterile broth. In all experiments there were two controls: one with medium alone and the other with the same amount of DMSO as in the cytochalasin A treatments.

Analytical techniques

The mycelium was harvested after 2 and 4 days of growth, mycelial mats washed with distilled water and fixed by immersing in boiling distilled water for 15 min.

Lipids were extracted, phospholipids and fatty acids analysed as described previously by Bligh and Dyer (1959), McGee and Allen (1974) and Gerhardt and Gehrke (1977). Total lipids was determined gravimetrically. Neutral lipids were identified by thin layer chromatography (TLC) on silica gel plates using the solvent system : hexane/diethyl ether/acetic acid (72/20/4 v/v). Polar lipids were separated by two dimensional chromatography with chloroform/methanol/acetic acid/water (75/45/12/6 v/v) as the solvent in the first direction (Spanner 1973) and chloroform/methanol/acetic acid/NaCl so. 0.9% (100/15/16/4 v/v) as solvent in the second direction (Anderson *et al.* 1970). Spots were visualized by spraying with rhodamin B (Wagner *et al.* 1961) or charring with 20% H_2SO_4. Neutral lipids were visualized by spraying with rhodamine B recovered and were quantified on the

basis of fatty acid content. Free sterols of total lipids were estimated by the Lieberman-Burchard method after precipitation with digitonine (Stadtman 1957). The spots of phospholipids were visualized by exposure to iodine vapours while phosphorus was quantitified by the Bartlett method (Kates 1972). Lipids were tentatively identified by comparing the Rf values of the unknown lipids with the Rf values of standard lipids (Supelco, Inc.).

Results

I - Estimation of Lipids

Total lipid, phospholipid and neutral lipid contents of the mycelia grown in the presence and absence of cytochalasin A in the culture medium for two and four days was determined and are shown in Table 1.

Table 1. Influence of cytochalasin A on total lipid, phospholipid and neutral lipid contents of the mycelium of *Mucor mucedo*

Experiment	Total lipids (% of dry mycelium)		Phospholipids (% of total lipids)		Neutral lipids (% of total lipids)	
	2 days	4 days	2 days	4 days	2 days	4 days
Control	8.23	7.45	44.65	49.92	55.35	50.08
DMSO	8.27	7.01	46.88	45.12	53.13	54.88
CA	5.68	5.32	48.36	68.17	51.64	31.64

Cytochalasin A addition to the culture medium reduced total lipid content in the mycelium. The decrease in total lipid was estimated to be 31.32% and 24.12% respectively after 2 and 4 days of growth. There was no significant difference in the lipid content of mycelium in both controls indicating that dimethylsulfoxide (DMSO) had no effect on the total lipid content of the mycelia. The total lipid content was higher in 2 days-old mycelium than in 4 days.

Total phospholipids were estimated and presented in Table 1. The phospholipid content of mycelium grown in the presence of cytochalasin A increased in comparison with the controls and the increase was most evident in four days-old mycelium (31.14%).

The neutral lipid content of mycelial mats was obtained by deducting the phospholipid fraction from the total lipids and the results are illustrated in Table 1. Cytochalasin A caused a decrease in the neutral lipid fraction in particular in 4 days old cultures.

The influence of cytochalasin A on the fatty acid composition of the total lipids of the mycelium is illustrated in Table 2.

Table 2. The effect of CA on the total fatty acids of *Mucor mucedo*

	2 Days			4 Days		
	Composition (%)					
	Control	**DMSO**	**CA**	**Control**	**DMSO**	**CA**
$C_{14:0}$	8.31	10.74	14.31	9.68	12.67	13.71
$C_{14:1}$	2.99	1.87	—	—	—	—
$C_{16:0}$	9.86	12.77	11.87	12.40	12.66	15.23
$C_{16:1}$	2.92	3.47	3.62	1.90	2.21	2.91
$C_{18:0}$	4.44	2.83	3.48	4.05	4.64	5.32
$C_{18:1}$	12.22	13.07	13.31	14.32	14.85	12.96
$C_{18:2}$	16.91	21.62	18.69	19.40	18.93	18.03
$\alpha C_{18:3}$	19.27	28.00	31.28	18.28	23.53	19.79
$\gamma C_{18:3}$	11.89	3.60	3.44	11.38	6.48	8.10
$C_{19:0}$	5.19	2.22	—	3.81	—	3.56
$C_{20:0}$	6.01	0.09	—	4.78	4.04	0.41

Myristic (14:0); palmitic (16:0); oleic (18:1); linoleic (18:2) and α and β linolenic (18:3) acids were the most abundant fatty acids. There was no major qualitative difference in the fatty acid composition of mycelium grown in the presence of cytochalasin A when compared to the controls.

When compared with the DMSO control, cytochalasin A induced the following quantitative variations in fatty acid levels:

— After two days of growth the amount of myristic (14:0) and α—linolenic (18:3) acids increased whereas both palmitic (16:0) and linoleic (18:2) acids decreased.

The presence of DMSO in the culture medium resulted in the enhancement of myristic (14:0); palmitic (16:0); palmitoleic (16:1); oleic (18:1); linoleic (18:2) and α-linolenic (18:3) acids and a decrease of myristoleic (14:1), stearic (18:0), γ-linolenic (18:3), nonadecanoic (19:0) and arachidic (20:0) acids in 2 days-old mycelium. An increase in the quantities of all the fatty acids except linoleic (18:2), γ linolenic (18:3) and arachidic (20:0) acids occured in 4 days-old mycelium. Nonadecanoic acid (19:0) was not detected.

In the presence of cytochalasin A myristoleic (14:1), nonadecanoic (19:0) and arachidic acids (20:0) could not be detected.

— After four days of growth an increase in the quantities of myristic (14:0), palmitic (16:0), stearic (18:0) and γ-linolenic (18:3) acids, and a decline in oleic (18:1) and α-linolenic (18:3) acids were observed.

There was no significant difference in fatty acid composition of the mycelium of the two intervals.

II - The Different Components of Lipids

1. The Constituents of Neutral Lipids

The different fractions of neutral lipids (N.L.) were separated by thin layer chromatography (TLC). The free sterols (FS), diacylglycerides (DG), free fatty acids (FFA), triacylglycerides (TG) and sterol esters (SE) were the main constituents of the neutral lipid fraction in all mycelia. The different constituents of neutral lipids were determined quantitatively and are presented in Table 3.

Table 3. Effect of cytochalasin A on the neutral lipid composition of *Mucor mucedo*

Experiment	% of neutral lipid fractions									
	2 days					4 days				
	FS	DG	FFA	TG	SE	FS	DG	FFA	TG	SE
Control	2.17	20.88	36.20	28.20	12.55	4.90	17.86	9.94	60.53	6.86
DMSO	3.77	23.91	29.91	33.15	9.24	5.00	11.60	13.94	65.91	3.55
CA	1.52	18.13	39.21	37.89	3.24	1.20	12.65	13.89	67.54	4.72

The free fatty acids (FFA) are the major constituents in the 2 days-old mycelium with the free fatty acids (FFA) > triacylglycerides (TG) > diacylglycerides DG > sterols esters (S.E.) > free sterols (FS). After 4 days of growth the triacylglycerides (TG) were the major constituents with triacylglycerides (TG) > diacylglycerides (DG) > free fatty acids (FFA) sterols esters (SE) > free sterols (FS).

The presence of cytochalasin A in the culture medium increased the amount of sterol esters (SE) and resulted in a reduction of diacylglycerides (DG) and a strong inhibition of free sterols (FS).

In 4 days-old mycelium there was no significant effect of cytochalasin A on the composition of neutral lipids except a strong inhibition of free sterols (FS).

2. The constituents of Phospholipids

The main phospholipids of *Mucor mucedo* were phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI). In the presence of cytochalasin A phosphatidylglycerol (PG) appeared. Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were the most abundant phospholipids (Table 4).

Table 4. The effect of cytochalasin A on the phospholipid composition of *Mucor mucedo*

Experiment	% of total phospholipids									
	2 days					4 days				
	PE	PC	PS	PI	PG	PE	PC	PS	PI	PG
Control	54.02	28.12	12.02	6.59	—	58.33	27.47	10.07	4.16	—
DMSO	42.20	25.65	11.39	6.66	10.13	55.03	27.18	12.08	5.70	—
CA	38.97	29.43	8.96	7.06	15.58	48.03	18.57	12.81	7.05	12.94

The quantitative relationship of the different components of phospholipids was PE > PC > PS > PI. Cytochalasin A resulted in an increase in the quantities of PC, PI and PG and a decrease in the values of PE and PS after 2 days of incubation; after 4 days of mycelial growth, PG appeared and both PE and PC decreased in concentration.

Discussion

The lipid composition of *Mucor mucedo* reported in the present investigation is consistent with that of other studies (Sumner and Morgan 1969, Chavant 1980). The α and γ linolenic acids detected in *M. mucedo* have previously been identified in the Phycomycetes (Shaw 1966). Nonadecanoic (19:0) acid found in the present studies has also been detected in the lipids of *Taphrina deformans* (Sancholle 1984), in the plasma membrane lipids of *Hypomyces chlorinus* Tul., and in the spores of various species of fungi (Rami *et al.* 1978).

The presence of DMSO (solvent of CA) in the culture medium resulted in some modification of lipid metabolism but was not sufficient to produce recognizable morphological variations similar to that produced by cytochalasin A during the germination and the growth of *M. mucedo* (El Mougith *et al.* 1984b). Dimethylsulfoxide has certain effects on metabolism and cellular permeability (Jacob *et al.* 1964, Carley *et al.* 1967, Ghajar and Harmon 1968, Bean *et al.* 1969, Tillman and Bean 1970, Gunasekaran *et al.* 1972, Lockhausen and Kristen 1983) and therefore is not an inert solvent. For this reason, it was necessary to compare the results obtained for CA plus DMSO with those of DMSO alone.

Cytochalasin A modified lipid metabolism of *Mucor mucedo* but the exact mode of action could not be determined. Cytochalasin A appeared to significantly modify the lipid composition of the fungus relative to the control. Treated mycelium contained less lipid but with a higher level of phospholipids when compared to the untreated mycelium.

It is evident that the cytochalasin A does affect the lipid metabolism of *M.*

mucedo but it is doubtful if this is the main site of actions of this drug. Quantitative modifications observed in the range of lipids are a part of modifications of hyphal growth and morphogenesis which we are studing.

References

Anderson, R.E., Feldman, L.S. and Feldman, G.L. (1970) Lipids of ocular tissue. II-The phospholipids of nature bovine and rabbit whole retina. *Biochem. Biophys. Acta.* **202**:: 367-373.

Bean, G.A., Rambo, G.W., and **Klarman, W.L.** (1969) Influence of dimethyl sulfoxide (DMSO) on conidial pigmentation and consequent U.V. light sensitivity of aflatoxin producing strains of *Aspergillus flavus. Phytopathology* **59**: 1555, Abstr.

Betina, V. and **Micekova, D.Z.** (1973) Morphogenetic activity of cytochalasin, cyanein and monorden in *Botrytis cinerea. Z. All. Mikrobiol.* **13**: 287-298.

Betina, V., Micekova, D.Z. and **Nemec, P.** (1972) Antimicrobial properties of cytochalasins and their alteration of fungal morphology. *J. Gen. Microbiol.* **71**: 343-349.

Bligh, E.G. and **Dyer, W.S.** (1959) A rapid method for total lipid extraction and purification. *Can. J. Biochem. Biophys.* **37**: 911-917.

Carley, H.E., Wastson, R.W. and **Huber, D.M.** (1967) Inhibition of pigmentation in *Aspergillus niger* by dimethyl sulfoxide. *Can. J. Bot.* **45**: 1451-1453.

Chavant. L. (1980) *Biosynthèse des acides gras et des phospholipides chez Mucor mucedo et Aspergillus.* Thèse Sciences, UPS Toulouse, France n° 916.

Dargent, R. (1977) *Cytodifférenciation et croissance apicale chez les champignons filamenteux.* Thèse Sciences, UPS, Toulouse France n°766.

El Mougith, A., Dargent, R., Touzé-Soulet, J.M. and **Montant, Ch.** (1984a) Effets de la cytochalasine A sur la composition chimique de parois de *Mucor mucedo* L. *Third Cell Wall Meeting Fribourg* 26-29 mars, p. 66.

El Mougith, A., Dargent, R., Touzé-Soulet, J.M. and **Montant, Ch.** (1984b) Effect of cytochalasin A on growth and ultrastructure of *Mucor mucedo* L. *Biol. Cell.* **52**: 181-190.

Gerhardt, K.O. and **Gehrke, Ch. W.** (1977) Rapid microdetermination of fatty acids in biological materials by gas-liquid chromatography. *J. Chromatography,* **143**: 335-344.

Ghajar, B.M. and **Harmon, S.A.** (1968) The effect of DMSO on permeability of *Staphilococcus aureus. Biochim. Biophys. Res. Comm.* **32**: 940-944.

Gunasekaran, M., Weber, D.J. and **Bushnell, J-L.** (1972) Effects of dimethyl sulfoxide on growth, sporulation, and spore germination of *Rhizopus arrhizus Fischer. Arch. Mikrobiol.* **82**: 184-188.

Jacob, S.W., Bischell, M. and **Herschler, R.J.** (1964) Dimethyl sulfoxide: effects on the permeability of biologic membranes (permeability report). *Current therapeutic Research,* **6**(3): 193-198.

Kates, M. (1972) *Techniques of lipidology: analysis and identification of lipids.* North Holland publishing Co., 610 p.

Lockhausen, J. and **Kristen, U.** (1983) Effect of cytochalasin B on the mitochondria and the golgi apparatus in ovary gland cells of *Aptenia cordifolia. Z. Pflanzenphysiol.* **110**: 151-199.

McGee, J. and **Allen, K.G.** (1974) Preparation of methyl esters from the saponifiable fatty acids in small biological specimens for gas liquid chromatographic analysis. *J. Chromatogr.* **10**: 35-42.

Patton, M.A. and **Marchant, R.** (1975) The effect of cytochalasin B on hyphal morphogenesis in *Polyporus biennis. J. Gen. Microbiol.* **86**: 301-310.

Rami, J., Sancholle, M., Montant, Ch. and **Touzé-Soulet, J.M.** (1978) Presence d'acide nonadécanoique et d'acide gras à longue chaîne carbonée dans le plasmalemme d'*Hypomyces chlorinus* Tul., carencé ou non en biotine, *C.R. Acad. Sci. Paris,* **286**, série D : 845.

Sancholle, M. (1984) *Etude des lipides de quelques champignons. Effet de propiconazole inhibiteur de synthèse des stérols.* Thèse Science, UPS, Toulouse France n° 1165.

Shaw, R. (1966) The polyunsaturated fatty acids of microorganisms, *in:* **Rodolfo Paoletti** and **David**

Krichevsky (eds.) *Advance in lipid research,* Academic Press, New-York, **4**: 107-174.

Spanner, S. (1973) Separation and analysis of phospholipids, *in:* **R.M.C.** and **Hawthorne, (eds.) J.N.** *form and Function of Phospholipids.* Elsevier, B.B.A. Library **3**: 43-62.

Stadman, T.C. (1957) Preparation and assay of cholesterol and ergosterol. *In:* **Colowick S.P.** and **Kaplan N.O. (eds.)** *Methods in Enzymology.* Academic Press **III**: 392.

Sumner, J.L. and **Morgan, E.D.** (1969) The fatty acid composition of sporangiophores and vegetative mycelium of temperature adapted fungi in the order mucorales. *J. Gen. Microbiol.* **59**: 215-221.

Tanenbaum, S.W. (1978) *Cytochalasins Biochemical and Cell Biological aspects.* North-Holland Publishing Company Amsterdam, New York, Oxford. 1-564.

Tillman, R.W. and **Bean, G.A.** (1970) Tentative identification and influence of sterols and dimethyl sulfoxide (DMSO) on growth and survival of *Fusarium roseum, Mycologia* (N.Y.) **62**: 428-436.

Wagner, H., Horamer, L. and **Wolf, F.P.** (1961) Dünnschicht chromatographie vor phosphatiden und glykolipiden. *Biochem Z,* 334-375.

Wessels, N.K., Spooner, B.S., Ash, I.F., Bradley, M.O., Luduena, M.A., Taylor, E.L., Wrenn, J.T. and **Yamada, K.M.** (1971) Microfilaments in cellular and developmental processes. *Science* **171**: 135-143.

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تـأثـير السيتـوكـلازين (أ) عـلى تـركيب الدهنيات عند فطرة ميوكر ميسيدو (ل)

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يهدف هذا البحث إلى دراسة تأثير مركب السيتوكلازين أ (وهو مضاد حيوي يخلق بواسطة بعض الفطريات) على تركيب الدهنيات عند فطرة ميوكر ميسيدو (ل)؛ وقد أوضحت نتائج هذه الدراسة على أن هذا المركب له تأثير قوي على الأيض الغذائي الدهني لهذه الفطرة حيث أن وجوده في الوسط الغذائي قد أدى إلى إنخفاض في المحتوى الدهني بنسبة ٣١,٣٢٪ في اليوم الثاني وبنسبة ٢٤,١٢٪ في اليوم الرابع للنمو.

بالنسبة لمحتوى الدهون الفسفورية فقد حدث لها زيادة طفيفة (٣,١٤٪) بعد اليوم الثاني من النمو وارتفعت هذه الزيادة لتصل إلى ٣١,١٤٪ في اليوم الرابع.

أوضحت أيضاً هذه الدراسة أن لهذا المضاد الحيوي بعض التأثير الكمي أو الكيفي أو كلاهما على كل من: محتوى الأحماض الدهنية والمكونات المختلفة للدهون المتعادلة وكذلك مكونات الدهون الفسفورية.