ACTA CHEMICA SCANDINAVICA 24 (1970) 2355–2360

The Composition of Mycelium Lipids in *Blakeslea trispora* Grown with Various Lipid Sources

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The composition of lipids in *Blakeslea trispora* was studied using mycelium grown without lipid or with various lipids in the growth medium.

There were four predominant fatty acids in the mycelium grown without exogenous fat, *viz.* linoleic, oleic, palmitic, and stearic acids. Together, they constituted 75–90 % of all fatty acids, linoleic acid being the most abundant.

The fatty acid composition of mycelium grown in a lipid-containing medium reflected to a considerable extent the composition of the exogenously supplied lipid. It appears that the added lipid inhibits the endogenous fatty acid synthesis.

About 30 % of the exogenously supplied lipid was left in the medium after the mycelium had been grown for 5 days. The composition of this un-utilized lipid was markedly different from that of the originally supplied. This fact indicates the occurrence of extra-cellular lipases.

The biosynthesis of β-carotene in mated cultures of *B. trispora* is greatly stimulated by the addition of 4–5 % of a lipid source.[1,2] This effect is especially marked when using oils with a high content of oleic and linoleic acids.[1] Certain animal fats have also been reported as stimulating carotene formation.[2] According to White and coworkers [3,4] the fatty acid composition of another member of the family *Choanephoraceae*, namely *Choanephora cucurbitarum*, is influenced by environmental and culture conditions. The present work describes a close relationship between the composition of the exogenous lipid source and the composition of mycelium lipids in *B. trispora*.

EXPERIMENTAL

Organisms, growth media, and conditions of fermentation. Mated cultures of *B. trispora* NRRL 2456(+) and 2457(−) were used in all experiments. The strains were obtained through the courtesy of Dr. Hesseltine of the Northern Utilization Research and Development Division, Agricultural Research Service, Peoria, Ill. USA. The cultures were maintained on potato dextrose agar at room temperature with transfer to fresh media every 8−10 days. Preparation of inocula, growth media, and conditions of fermentation were essentially as those described elsewhere.[5]

The vegetable lipid sources were products from *AB Karlshamns Oljefabriker*. Animal fat was obtained from *Stockholms Benmjölsfabrik*. The various lipid materials are called by their commercial names.

Harvest and extraction of the mycelium. The mycelium was filtered under suction and dried *in vacuo* at room temperature. The dry mycelium cakes were milled in a grinding mill (A.H. Thomas Co., Pa., USA) and extracted with diethyl ether in a Soxhlet apparatus.

Determination of various lipid classes and fatty acid composition. The lipid samples were extracted with chloroform and separated into various lipid classes by preparative thin layer chromatography on silica gel G (Merck, Darmstadt) using as eluent a mixture of light petroleum (b.p. 40−60°C), diethyl ether and acetic acid (70:30:1). The fatty acid composition was determined by gas liquid chromatography of methyl esters using Varian Aerograph 660 and 6′ × 1/8″ stainless steel column with 10 % BDS on Anachrom ABS 70−80 mesh (Analabs Inc., Conn. USA). The injection temperature was set at 220°C, column oven at 190°C, and flame ionisation detector at 240°C. Quantitative values were calculated by means of a disc integrator.

RESULTS AND DISCUSSION

Table 1. The fat content of mycelium grown without an exogenous lipid source. The values for mycelium grown for 5 days with 5 % bone tallow or crude cotton oil were, per litre culture, 50 g of total solids, 30 g on fat-free basis.

Time of growth	Without exogenous lipid source		
	Total mycelium solids	Fat content of mycelium	Fat-free mycelium solids
Days	g/l	mg/g	g/l
2	21	56	20
3	30	28	29
5	37	23	36

Table 1 shows the variation in the fat content of mycelium during growth without exogenous lipid source. The fat content is seen to decrease markedly after two days of growth. There is a simultaneous shift in the fatty acid composition towards longer and more unsaturated acids (*cf.* Table 2). This picture is similar in the case of all the three predominant lipid classes, *viz.* triglycerides, diglycerides, and free fatty acids. Amongst these lipid classes the proportion of triglycerides increases steadily with the time of growth, whereas that of diglycerides markedly falls after a slight initial increase. The proportion of

Table 2. The fatty acid composition of predominant lipid classes from mycelium grown for varying lengths of time without an exogenous lipid source.

	Time of growth Days								
	2			3			5		
Lipid class [a]	TG	DG	FA	TG	DG	FA	TG	DG	FA
Lipid class % w/w	16	42	42	18	45	37	30	19	51
Fatty acid [b]	% w/w [c]								
12:0					2				
14:0	2	4	3	1	2				
15:0		3	3		1				
16:0	36	57	51	18	21	31	16	20	22
16:1				1	1	2	1		2
18:0	13	8	12	5	6	9	5	5	9
18:1	28	20	18	20	28	19	20	23	18
18:2	10	2	5	42	20	31	41	26	36
18:3									
19:1				8	5	2	11	5	4
20:0									
20:1									
21:0				1					
21:1	1								
22:0	2								
22:1	4			1					
24:0				2	3	2	2	9	5
24:1					2			4	

[a] TG, triglycerides; DG, diglycerides; FA, free fatty acids. Monoglycerides were present in minor amounts. Other lipid classes were not determined.
[b] Chain length: number of double bonds.
[c] Fatty acids present in amounts < 1 % are not indicated.

free fatty acids decreases somewhat between the 2nd and 3rd day of growth, after which there is a significant increase. This correlates fairly well with the data in Table 1. The results summarized in Tables 1 and 2 indicate that the endogenously synthesized lipids are extensively degraded upon prolonged growth.

White and Powell [4] have detected "substantial amounts" of sterol esters and phospholipids in mycelium of *Choanephora cucurbitarum* in addition to the predominant triglycerides. We were not able to detect any significant amounts of these two minor lipid classes in the present investigation of *B. trispora*. However, in other studies we have found that, *e.g.* ergosterol is present in amounts comparable to those of β-carotene, about 2 mg/g mycelium grown for 2 days with 3 % lipid in the medium (Björk and Neujahr, unpublished).

Thomas and Goodwin [7] report for *B. trispora* contents of about 4 mg ergosterol/g mycelium grown for 4–4.5 days without an exogenous lipid source.

There are four predominant fatty acids in the mycelium, *viz.* linoleic, oleic, palmitic, and stearic acids. They constitute about 75 – 90 % of all fatty acids detected (*cf.* Table 2).

B. trispora is usually grown with an exogenous lipid source. In this case the fat content of the mycelium is about 10 – 20 times higher than when no lipid is supplied during growth. The fatty acid composition of such lipid-rich mycelium reflects to a considerable extent the composition of the lipid supplied during growth. This is illustrated in Table 3. Thus, the longer fatty acids

Table 3. The fatty acid composition of various lipid sources in growth medium and the composition of lipids extracted from the corresponding mycelium of *B. trispora*. E, exogenous lipid source; M, mycelium lipids.

Fatty acid [a]	Lipid source															
	Crude cotton oil		Bone tallow		Coco-nut oil		Palm-kernel oil		Palm oil		Hydro-genated fishoil		Crude soybean oil		Crude rapeseed oil	
	% w/w [b]															
	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M
8:0					8	+	5	+								
10:0					8	2	5	2								
12:2					44	23	40	23	2	+						
14:0	1	1	2	2	19	20	17	19	4	2	11	6				
15:0			+	+							1	1				
16:0	20	29	25	29	10	18	12	12	37	31	25	32	11	12	3	3
16:1	1	3	4	6		1	1	2	3	5	12	12		3		1
18:0	2	3	14	9	3	6	4	5	5	3	8	7	4	1	1	2
18:1	22	25	45	40	7	15	15	22	35	27	13	19	23	27	12	17
18:2	54	36	5	8	2	14	3	15	9	19	1	3	54	53	14	6
18:3													7	2	9	
19:1																
20:0	+	+	+	+	+	1	+	+	+	1	3	2	+		1	1
20:1				1							8	4	+	+	8	21
22:1	+	+	+	+					3	9	4	3	1	+	49	42
24:0									+	+	+	1				1
24:1											+	2	+	+	1	3

[a] Chain length: number of double bonds.
[b] +: about 0.5 %, empty spaces: not detected or trace.

(C_{20}-C_{24}), that normally occur only in small or negligible amounts in the mycelium, seem to accumulate if supplied with the exogenous lipid source (*cf.* 20:1 and 22:1 from, *e.g.*, crude rapeseed oil, Table 3).

There are, however, minor changes towards the spectrum of fatty acids, which is characteristic of mycelium, grown without an exogenous lipid source. Thus, the shorter fatty acids (C_8-C_{12}), if present in the exogenous lipid source, appear to become extensively degraded in the mycelium (*cf.*, *e.g.*, coconut oil

and palmkernel oil, Table 3). Neither do such acids occur to any significant extent in the mycelium grown without a lipid source (*cf.* Table 2). It thus appears from Table 3 that the endogenous fatty acid synthesis is of minor importance when the organism is grown with an exogenous lipid source. This may be the consequence of the inhibition of fatty acid synthetase by acyl-CoA's derived from exogenously supplied fat. A similar regulation mechanism during fatty acid synthesis in yeast has been suggested by Lust and Lynen.[6]

Table 4. The influence of two exogenous lipid sources on mycelium growth (5 days) and lipid utilization in *B. trispora.*

	Lipid source during growth			
	Bone tallow		Crude cotton oil	
	g/l	%	g/l	%
Added lipids	44.2	100	45.0	100
Total mycelium lipids	20.1	45	20.4	45
Lipids left in growth medium after fermentation	13.7	31	13.5	30
Not recovered (degraded)	10.4	24	11.1	25
Yield of mycelium	49.2		50.0	

Table 4 shows the influence of two different lipid sources on mycelium growth and lipid utilization. It is seen that about 30 % of the added lipids are recovered in the medium after completed fermentation. About 25 % are not recovered, probably due to metabolic degradation. In reality, this fraction may be larger, depending on the extent of endogenous fat synthesis. Addition of animal or vegetable lipids gives practically identical results.

As seen in Table 4 about 30 % of the exogenously supplied lipid is left in the medium after the mycelium was grown for 5 days. Table 5 shows the composition of this un-utilized lipid when two different lipid sources, one animal and one vegetable, were supplied during growth. The lipids supplied were predominantly composed of triglycerides. However, the proportion of this lipid class in the un-utilized lipid is only about 50 %. It contains, in addition, about 30 % of diglycerides and about 20 % of free fatty acids. Thus, only about 15 % of the originally supplied triglycerides remain intact. This indicates the occurence of extracellular lipases. The observation correlates fairly well with the findings of Ciegler *et al.*,[1] according to which free fatty acids and esters are almost equally efficient in their effect on growth and β-carotene production in *B. trispora.*

Only 5 – 6 fatty acids (C_{14}-C_{18}) are present in the un-utilized lipid (Table 5). Oleic acid dominates the lipid derived from bone tallow. It is equally

Table 5. The distribution of fatty acids between the triglyceride (TG), diglyceride (DG), and the free fatty acid (FA) fraction in the lipid material, that is left in the medium after removal of the mycelium, grown for 5 days in the presence of two different lipid sources. The exogenously supplied lipids were predominantly composed of triglycerides.

	Lipid source					
	Bone tallow			Crude cotton oil		
Lipid class	TG	DG	FA	TG	DG	FA
Lipid class % w/w [a]	52	29	19	52	29	19
Fatty acid [b]	% w/w [c]					
14:0				1		
16:0	15	13	19	19	14	24
16:1	2	2	1	1		
18:0	9	6	8	3	4	4
18:1	65	61	61	23	40	37
18:2	6	10	5	52	33	33

[a] Monoglycerides were present in minor amounts. Other lipid classes were not determined.
[b] Chain length: number of double bonds.
[c] Fatty acids present in amounts < 1 % are not indicated.

distributed between the three lipid classes. Palmitic, oleic, and linoleic acids together constitute the major part of the lipid derived from crude cottonseed oil. Linoleic acid is comparatively most and oleic acid least abundant in the triglyceride fraction.

Acknowledgements. The authors are greatly indebted to dr. Ulf Persmark, Karlshamns Oljefabriker, for the gas chromatographic analyses.

This investigation was supported by graduate student fellowships from The Royal Institute of Technology to Lars Björk and Eva Cederberg.

REFERENCES

1. Ciegler, A., Arnold, M. and Anderson, R. F. *Appl. Microbiol.* **7** (1959) 98.
2. Ciegler, A., Nelson, G. E. N. and Hall, H. H. *Appl. Microbiol.* **10** (1962) 132.
3. White, H. B., Jr., Chu, F. S. and Quackenbush, F. W. *J. Am. Oil Chemists' Soc.* **39** (1962) 123.
4. White, H. B., Jr. and Powell, S. S. *Biochim. Biophys. Acta* **116** (1966) 388.
5. Cederberg, E. and Neujahr, H. Y. *Acta Chem. Scand.* **23** (1969) 957.
6. Lust, G. and Lynen, F. *European J. Biochem.* **7** (1968) 68.
7. Thomas, B. M. and Goodwin, T. W. *Phytochemistry* **6** (1967) 355.

Received January 14, 1970.