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CHANGE OF LIPID COMPOSITION OF *Mucor miehei* AS A FUNCTION OF CULTIVATION TEMPERATURE

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Lipids of the thermophilic fungus Mucor miehei, *strain UzLT-3, cultivated at 26 and 42 °C are studied. The lipids of this strain contain isomeric 18:2 (9,12) and (6,9) fatty acids and polyene acids α- and γ-18:3, which are uncharacteristic of thermophilic fungi. Cultivation at 26 °C increases the fraction of phospholipids (PL), glycolipids (GL), and isomeric 18:3 fatty acids in the total lipids. It is hypothesized that the liquid-crystalline state of the membranes of this strain at reduced temperature are due to activation of desaturases δ^{12} and δ^{15} and possibly δ^{6}, which are present in the fungus and participate in the biosynthesis of unsaturated acyl lipids.*

Key words: *Mucor miehei*, lipids, fatty acids, desaturases.

The thermophilic fungus *Mucor miehei*, strain UzLT-3 [1, 2], grows over wide temperature ranges (from 21 to 62°C). The optimal range is 42-45°C. These parameters indicate that the studied fungus is thermophilic, although this term has not yet been strictly defined. Apparently this is due to the facts that the nature of thermophilia remains unclear and the mechanism of thermophilia in general is poorly studied. Thermophilic micromycetes are interesting because of the great possibilities of practical application. More than 25 hypotheses explaining thermophilia have been discussed in the scientific literature [3-5]. Among these, the theory of "solubilization" of lipids is most interesting. This theory is interesting primarily because the majority of cell metabolic processes is regulated by membrane-bound enzymes, the effectors of which are membrane lipids. Therefore, the lipids may play an important role in thermophilia.

We studied the change of lipid and fatty-acid composition of *Mucor miehei*, strain UzLT-3, at cultivation temperatures of 26 and 42°C. Table 1 shows that unsaturated acids dominate the fatty acids of the total lipids. Of these, 18:1 (9) (42°C) and isomeric 18:3 (26°C) dominate. The acid γ-18:3 was previously found in *Mucor* 12M [6]. However, we first observed 18:2 (6,9) in addition to γ-18:3 in lipids of the present strain. These fatty acids are uncharacteristic of thermophilic fungi [7].

At 26°C the lipids contain noticeably less 18:1 and more 18:2 and 18:3. The ratios 18:1/18:2 (1.06) and 18:2/18:3 (0.8) are less than those at the optimal growth temperature (1.17 and 1.28, respectively). This suggests that the fungus possesses a mechanism for changing the fatty-acid composition of the lipids in response to a change of temperature. If the fungus is cultivated at 42°C, δ^{9}-desaturase is activated. This ensures the synthesis of primarily the monoene acid 18:1 (9) whereas δ^{12}- and δ^{15}-desaturases and possibly δ^{6}-desaturase; which are involved in the biosynthesis of 18:2 (9,12), 18:2 (6,9), 18:3 (9,12,15), and 18:3 (6,9,12); are activated at low temperature.

In order to obtain more complete information, we studied the fractional lipid composition of fungus grown at these temperatures before reaching steady-state. The results (Table 1) show that the ratio of PL, GL, and neutral lipids (NL) varies considerably depending on the temperature. At the optimal temperature, NL (52.3%) dominate in the lipids; at 26°C, PL (60.2%). The fraction of NL in the three fractions at low temperature is more than two times less; GL, 1.5 times greater. Thus, the fungus at 26°C synthesizes mainly metabolically active GL and PL; at 42°C, reserve lipids.

TLC in system 1 showed that the NL contain stearol esters, triacylglycerines, free fatty acids, diacylglycerines, and free stearols. The quantitative composition of the NL does not change noticeably if the cultivation temperature is changed.

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TABLE 1. Fractional and Fatty-Acid Composition of Lipids from *Mucor miehei*, Strain UzLT-3, at Various Cultivation Temperatures

Cultivation temperature °C	Lipids	Lipid content, % of total	Fatty-acid content, % (Trex)								DU value
			14:0	16:0	16:1	17:0	18:0	18:1(9)	18:2 (9, 12) + 18:2 (6, 9)	α-18:3 + γ-18:3	
26	Total	10.0	-	21.8	1.4	Tr.	0.8	24.3	22.9	28.8	157.9
	NL	22.3	-	20.3	2.1	1.0	0.2	27.5	23.6	26.3	
	PL	60.2	-	23.2	0.7	-	1.6	21.1	22.3	31.1	
	GL	17.5	-	16.9	0.8	0.1	8.0	18.2	49.9	4.6	
42	Total	12.5	Tr.	24.3	2.5	Tr.	3.9	34.0	19.8	15.5	122.6
	NL	52.3	1.5	22.6	4.1	0.7	2.9	41.2	18.1	11.1	
	PL	35.7	0.5	26.1	0.9	0.4	4.9	26.7	21.6	18.8	
	GL*	12.0	-	24.9	-	-	8.5	37.5	18.8	8.4	

*Acid 20:4 (5,8,11,14) is most probably also present.

TLC in systems 2 and 3 of PL from fungus grown at 26°C revealed primarily phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinosites accompanied by phosphatidylserines, phosphatidylglycerines, and unidentified components. PL of biomass grown at the optimal temperature contain not only these components but also cardiolipin and phosphatidic acids.

The composition of the GL was not studied.

The cultivation temperature substantially affects on the fatty-acid composition of the separate lipid fractions (Table 1). Whereas at 42°C the content of 18:1 is high in all fractions, at 26°C isomeric 18:3 acids dominate in the PL. In GL, up to 50% are isomeric 18:2. In NL, 18:1 and 18:3 dominate.

It is noteworthy that long-chain polyene arachidonic acid 20:4 (5,8,11,14) appears in the GL fraction at the optimal cultivation temperature. The results for the lipid composition of *M. miehei* do not correspond with certain ideas about the lipids of thermophilic fungi. The studied strain contains fatty acids 18:2 (6,9), 18:3 (9,12,15), and 18:3 (6,9,12); which indicates that the fungus contains δ^{15}-, δ^{12}-, and δ^{6}-desaturases. These data do not support the hypothesis [8] that thermophiles cannot live at low temperatures because they lack the principal desaturases (δ^{12} and δ^{15}), which are responsible for the change of microviscosity of the lipid bilayer upon cooling. Apparently the adaptation of the culture to the cultivation temperature is related to the fatty-acid composition of the total lipids.

It is commonly known that fungal membranes, regardless of the growth temperature, exhibit advantageous multiphasic properties, i.e., the ability to change an inactive thermotropic gel phase into an active liquid-crystalline phase by increasing the degree of unsaturation (DU) of lipids at lower cultivation temperatures [9]. In our experiments the DU value, which is the sum of the contents of unsaturated fatty acid (in percent) multiplied by the number of double bonds in it, increased from 122.6 at 42°C to 157.9 at 26°C. Nevertheless, an inverse correlation between the DU of lipids and their ability to grow at higher and lower temperatures was not found for thermophilic fungi. It is noteworthy that such a relationship is clearly found for yeast and certain single-celled microorganisms [10, 11].

Thus, the experiments suggest that the microviscosity of the lipid bilayer of the studied fungus strain is regulated mainly by changing the lipid composition and, in particular, their acyl chains.

EXPERIMENTAL

GLC analysis of the fatty-acid methyl esters was performed on a Chrom-5 (Czech Republic) instrument with a flame-ionization detector in a glass column (2500×3 mm) packed with Chromasorb W-HP (100-120 mesh, YM, USA) coated with 5% Silar 5 CP (ASL, USA), which resolves positional isomers of unsaturated fatty acids [12]. The thermostat temperature was

198°C. The carrier gas was He. Components were identified by the literature method [12, 13] using mixtures of the methyl esters; pure methyl esters of 18:2 (9,12), 18:2 (6,9), and 20:4 (5,8,11,14) (Serva, FRG); and a mixture of the methyl esters of fatty acids containing α-18:3 (leaves of *Succus kalanchoes*) and γ-18:3 (animal tissues) as standards.

TLC was performed on glass plates (5×10 cm) with a layer of Kieselgel 60_{F254} - 0.25 mm thick (Merck, FRG) using systems 1) C_6H_{14}—$C_2H_5OC_2H_5$—CH_3COOH (80:10:1) for NL and 2,3) $CHCl_3$—MeOH—H_2O (60:15:2; 85:25:4) for PL. Spots were developed using 30% H_2SO_4 and C_2H_5OH (NL) followed by heating, Vaskovskii reagent and 1% ethanolic solution of phosphomolybdic acid for PL, and a mixture of α-naphthone with phosphomolybdic acid for GL [14].

The mycelial thermophilic fungus *M. miehei* was cultivated at 26 and 42°C on an orbital rocker at 180 rpm and in nutrient medium containing malt extract (7%), $(NH_4)_2SO_4$ (0.3%), $CaCO_3$ (0.1%), and cottonseed oil (0.7%). The initial pH value of the nutrient medium was 6.5.

Total lipids from the biomass were washed repeatedly with distilled water and isolated by a modified Bligh-Dyer method [14] using $CHCl_3$—MeOH (2:1). Lipids were fractionated in cold acetone with subsequent separation on a silica-gel column [14].

The methyl esters of fatty acids were prepared by acid methanolysis according to the literature [14] with subsequent purification by column chromatography on silica gel L 40/100 (Czech Republic) using C_6H_6—$C_2H_5OC_2H_5$ (19:1).

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