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PHOSPHOLIPIDS OF THE THERMOPHILIC FUNGUS *Mucor miehei*

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The composition of phospholipids synthesized by the mycelial fungus Mucor miehei *at various cultivation temperatures is studied. It is demonstrated that the content of unsaturated fatty acids in the total increases at low temperatures (26 °C). The increased degree of unsaturation is accompanied by a change in the phosphatidylcholine and phosphatidylethanolamine content in the phospholipids.*

Key words: phospholipids, *Mucor miehei* fungus, unsaturated fatty acids.

One of the principal characteristics of lipogenase is the degree of fatty-acid (FA) unsaturation [1] caused by the type specificity of the producer, the activity of its enzyme systems, the nutritive medium, and the growth conditions of the microorganisms. It is known that the FA composition of phospholipids (PL) determines the microviscosity of the membrane and thereby facilitates enzyme functioning in the membranes, which includes desaturase. The change of membrane fluidity influences the desaturase activity in the synthesis of unsaturated FA [2, 3]. A deficit of FA with high biological activity can be relieved by using biotechnology methods to produce lipids with a high content of unsaturated FA from biomass of microscopic fungi.

The fungus *Mucor miehei*, strain UzLT-3 [4], grows over a wide temperature range, from 21 to 62 °C. The optimum temperature is 42-45 °C. We investigated the chemical composition of PL from the fungal mycelium grown at various temperatures (26 and 42 °C). We found that cultivation of this strain at low temperatures increases the synthesis of unsaturated FA in the PL.

At temperatures below 21 °C, the fungus practically does not grow whereas the growth is slow at 26 °C. For example, biomass accumulates to 2.3-2.5 g/l after 96 h cultivation (steady-state growth phase). Then, the growth stops. The lipid content in the biomass is 10-11%. Under optimum steady-state cultivation conditions (42 °C), fungus biomass development reaches 11.2 g/l whereas the lipid content in the biomass is 12.5-12.8%.

The PL level in the total lipids varies depending on the temperature from 35-60%. The qualitative and quantitative compositions of the PL components are listed in Table 1. Eight spots are observed after fractionation. The R_f values of these correspond to phosphatidylcholine (PC), phosphatidylinosite (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylglycerine (PG). Three of the spots were not identified. The content of the PL fraction and its composition were different for cultivation under different temperature regimes. The principal PL fractions are PC, PI, and PE. Minor components include some unidentified spots and PS and PG. The PL of fungus grown at 42 °C also contain minor cardiolipin and phosphatidic acids.

According to the data in Table 1, the content of PL components and their ratio in the biomass obtained at various cultivation temperatures change. Thus, the amount of PE increases at low temperature. This is accompanied by a decrease of PC content in the steady-state phase of culture development. Naturally, the ratio of PC and PE also changes: at 26 °C, it is 1.37 whereas at 42 °C, it is 1.86. The total content of these PL components at 26 °C is less than at higher growth temperature.

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TABLE 1. Phospholipid Composition of *Mucor miehei*, UzLT-3

Cultivation temperature, °C	PL content, % of total					
	PC	PI	PE	PS	PG	unid.
26	33.5	22.7	24.5	5.7	5.1	8.5
42	38.5	21.9	20.7	4.6	5.7	10.6

TABLE 2. Fatty-acid Composition of PL of *Mucor miehei* Grown at Various Temperatures (96 h Culture)

Cultivation temperature, °C	Fatty acids, % of total						Degree of unsaturation
	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
Polar lipid fraction							
26	23.2	0.7	1.6	21.1	22.3	31.1	159.7
42	26.1	0.9	4.9	26.7	21.6	18.8	127.2
Neutral lipid fraction							
26	20.3	2.1	0.2	27.5	23.6	26.3	155.7
42	22.6	4.1	2.9	41.2	18.1	11.1	114.8

Table 2 shows that the degree of lipid unsaturation is rather high at low cultivation temperature. It reaches 159.7 and 155.7 in the polar and neutral fractions, respectively. The lipid unsaturation at the optimum growth temperature is also higher in the polar fraction than in the neutral fraction. Nevertheless, the degree of lipid unsaturation in the neutral fraction at low cultivation temperature increases more significantly than in the polar fraction. The temperature change especially affects the content of the $C_{18:3}$ trienic acid. The reasons for such a high content (about 1/3 of the total FA) and the role of this acid in the vitality of the fungus will be determined.

Thus, the results indicate that the total lipid quantity decreases, the PE content increases, and the final product of PL lipogensis, PC, increases at low temperature compared with the optimum cultivation conditions. The content of linolenic acid in PL changes. At low temperature it is greater than at the optimum cultivation temperature. These data agree well with results obtained in living specimens [5].

EXPERIMENTAL

The fungus *M. miehei*, strain UzLT-3, from the collection of the Laboratory of Microorganism Enzymes of the Institute of Microbiology of the Academy of Sciences, Republic of Uzbekistan, was studied [4]. A culture was grown on an orbital shaker (180 rpm) at 26 and 42 °C in 750-ml conic flasks containing nutritive medium (100 ml) of the following composition (g/l): malt extract, 7.0; $(NH_4)_2SO_4$, 0.3; $CaCO_3$, 0.1; and cottonseed oil, 0.7. The initial pH of the nutritive medium was 6.5. The cultivation time was 96 h.

Biomass selected for chemical analysis was separated by centrifugation and lyophilized. The total lipids were extracted from the lyophilized preparations by the method of Bligh and Dyer [6] with subsequent purification from accompanying substances by gel chromatography through a column packed with Sephadex G-25.

Neutral and polar lipid fractions were separated by precipitation using cold acetone from a solution of total lipids or by TLC [7] on Silufol plates (Cavalier, Czech Republic) using the systems $C_6H_{14}(C_2H_5)_2O$—CH_3CO_2H (85:15:1) for separation of neutral lipids and $CHCl_3$—CH_3OH—H_2O (65:25:4) for separation of polar lipids.

Classes of compounds were identified by comparing their mobilities on chromatograms with those of standards. The FA content of the lipids was determined by GLC of the methyl esters of FA obtained by acid methanolysis [8]. A Chrom-4 (Czech Republic) gas—liquid chromatograph with a flame-ionization detector and polyethyleneglycoladipate packing was used.

The degree of unsaturation [1] was used to characterize the FA composition of lipids by their GLC data. This is the

sum of percent content of all unsaturated FA multiplied by the number of double bonds in them: Degree of unsaturation = [monenes + 2×(dienes) + 3×(trienes)].

A set of calibrated PL (Serva, Germany) was used as the standards.

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