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EXPERIMENTAL ARTICLES

Metabolic Characteristics and Lipid Composition of Yeastlike Cells and Mycelium of *Mucor circinelloides* var. *lusitanicus* INMI Grown at a High Glucose Content in the Medium

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Abstract—It is shown that the fungus *Mucor circinelloides* var. *lusitanicus* INMI grown under aerobic conditions in a medium with a high glucose concentration (20%) is capable of both yeastlike and mycelial growth. In the mycelium, the activity of NAD-dependent isocitrate dehydrogenase was more than twice as high as in yeastlike cells, whereas the isocitrate lyase activity was lower. A number of significant differences were found in the lipid composition of the cells of two different morphological variants. Yeastlike cells contained more polar lipids and free fatty acids and less principal reserve lipids (triacylglycerides) than mycelial cells; the content of γ-linolenic acid and the degree of lipid unsaturation were significantly lower in these cells than in the mycelium. In yeastlike cells, glycolipids composed the bulk of polar lipids; the proportion of phospholipids (primarily phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and cardiolipin) was lower. The relationship between cellular metabolism and the lipid composition of fungal cells of different morphotypes grown at high concentrations of glucose, one of the main inducers of dimorphic growth, is discussed.

Keywords: Mucor circinelloides, dimorphic growth, NAD-dependent isocitrate dehydrogenase, isocitrate lyase, lipids, γ-linolenic acid, glucose.

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It is well known that in dimorphic fungi, high glucose concentration in the growth medium, as well as other factors (anaerobiosis, various inhibitors, and high temperature), activate formation of yeastlike cells [1–3]. High glucose concentrations in the media inhibit the activity of the tricarboxylic acid cycle (TCA cycle) enzymes and caused a decrease in the respiratory activity of the cells [4–6].

We have previously demonstrated that a culture of *Mucor lusitanicus* grown under aerobic conditions on various media at elevated glucose concentrations was capable of yeastlike growth [7]. Yeastlike cells grown in the medium with 15% glucose differed from mycelial cells grown in the medium containing 6% glucose in their lower content of unsaturated fatty acids, including a twofold decrease in the γ-linolenic acid content in total lipids.

Synthesis of γ-linolenic acid is associated with the physiological state of the fungal mycelium; this is correlated with its respiratory activity and the functioning of the TCA cycle enzymes, which are required for both lipid synthesis and functioning of the desaturase system. According to the published data, there is a correlation between hyphal growth and the metabolic activity of many zygomycetes, members of the genera *Rhizopus, Cunninghamella, Absidia*, and *Mucor*, including *M. circinelloides* [8].

The goal of the present work was to investigate the activities of the key enzymes of the TCA and glyoxylate cycles (NAD-dependent isocitrate dehydrogenase and isocitrate lyase, respectively), as well as the composition of lipids in the mycelial and yeastlike cells of *Mucor circinelloides* var. *lusitanicus* INMI grown in the same culture on the medium with high glucose.

MATERIALS AND METHODS

Microorganism. The fungal strain *M. circinelloides* var. *lusitanicus* INMI (=VKM F-306 D) was obtained by the selection method at the Winogradsky Institute of Microbiology, Russian Academy of Sciences.

Cultivation conditions. The culture was grown until the end of the logarithmic growth phase at 27°C on a shaker (130 rpm) in two-liter flasks with 500 ml of medium (pH 7.0) containing the following (g/l): glucose, 200.0; urea, 2.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 1.0; $ZnSO_4 \cdot 7H_2O$, 0.05; $FeSO_4 \cdot 7H_2O$, 0.01; and yeast extract, 0.5. Both the mycelium and yeastlike cells developed simultaneously under these conditions. The mycelium suspended in the culture liquid was harvested by filtering through a nylon filter, whereas yeastlike cells were harvested by centrifugation.

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Table 1. Total lipid composition of mycelial and yeastlike cells of *M. circinelloides* var. *lusitanicus* INMI grown on media with high glucose content (20%)

Growth form	Lipids, % of total lipids								
	PL	DAG	FS	MS	FFA	Q	TAG	EFA	SE
Yeastlike	14.5	5.8	5.3	1.2	24.0	0.1	26.7	6.1	16.3
Mycelial	11.8	10.2	6.2	0.6	16.8	0.8	39.8	0.9	12.8

Note: PL, polar lipids; DAG, diacylglycerols; FS, free sterols, MS, methylated sterols; FFA, free fatty acids; Q, quinones; TAG, triacylglycerides; EFA, esters of fatty acids; SE, sterol esters.

The oxygen consumption rate was determined by polarography using an LPF polarograph (Czech Republic).

Obtaining of cell-free extracts. The mycelium and yeastlike cells were frozen in liquid nitrogen, disrupted by extrusion in a high-pressure homogenizer analogous to a French press desintegrator, and resuspended in K-Na phosphate buffer (0.05 M, pH 7.2). Cell wall fragments were separated from the cell-free extract by centrifugation, the resultant lipid film was removed from the surface, and the supernatant was used for determination of enzymatic activity.

The enzymatic activities of NAD-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.41) and isocitrate lyase (ICL, EC 4.1.3.1) in the cell-free extracts were determined according to the previously described techniques [9–11] on a Specord UV-VIS spectrophotometer (Jena, Germany) at 340 and 324 nm, respectively. The enzymatic activity was expressed in μmol of the reaction product (NADH or its derivative, glyoxylate phenylhydrazone, respectively) formed during one minute per mg protein. The protein content was determined by the Lowry method. The presented results are averages of at least three independent measurements.

Lipids were extracted using the Folch method [12]; their amounts were determined gravimetrically.

The compositions of total and polar lipids was determined by thin-layer chromatography on TLC plates (Kieselgel 60 F_{254}, Merck, Germany), as described in [13].

Gas-liquid chromatography. To obtain methyl esters of fatty acids, the lipids were subjected to acid methanolysis at 80°C for 1.5 h in a methanol : acetyl chloride mixture. Methyl esters of fatty acids (MEFA) were extracted with hexane and analyzed by GLC in the isothermal mode at 170°C in a Model 3700 chromatograph (Russia), equipped with a column with 17% diethylene glycol succinate on Chromosorb W, with a carrier gas (helium) flow of 40 ml/min. The quantity of γ-linolenic acid was expressed as a percentage of total fatty acids. The unsaturation degree of the lipids was expressed as the number of double bonds per 100 molecules of fatty acids.

RESULTS AND DISCUSSION

During cultivation on the medium with 20% glucose, both the mycelium and yeastlike budding cells grew in the culture liquid. This enabled us to determine their lipid composition and metabolic activity in equivalent conditions, without considering the possible effects of various medium components, which had been used in our previous work [7].

In the total lipids of yeastlike and mycelial cells, significant differences were found in the content of some fatty acyl residues (Table 1). Yeastlike cells contained more polar lipids (PL), free fatty acids (FFA), and fatty acid or sterol esters than mycelial cells, whereas the content of principal reserve lipids (triacylglycerides, TAG) was 1.5 times lower than in the mycelium.

The polar lipids of yeastlike cells were represented mainly by glycolipids; the concentration of phosphatidic acid (PA) in yeastlike cells was two times higher than in mycelial cells. Low values of the phospholipid/glycolipid (PL/GL) ratio, as well as the low content of cardiolipin (CL) which contributes to the maintenance of the structural integrity of mitochondrial protein complexes, suggest that the lipid composition of membranes of yeastlike cells differs significantly from that of mycelial cells (Table 2); in mycelial cell lipids, the proportions of phospholipids, especially phosphatidyl serine (PS), phosphatidylcholine (PC), and phosphatidylethanolamine (PEA), were higher. Such differences in the polar lipid content indicate that, in addition to some changes in the membrane structures, there are certain differences in the membrane-related metabolic processes [14].

It was demonstrated that, at high glucose concentrations in the medium, the activities of the key enzymes of the TCA and glyoxylate cycles (NAD-dependent IDH and ICL, respectively) were low in both morphotypes (Table 3). The activity of NAD-dependent IDH in mycelial cells was twice as high, whereas the ICL activity was twice as low as in yeastlike cells. The protein content in the mycelium was almost twice as high as in yeastlike cells.

At high glucose concentrations (20%), the rates of oxygen consumption by fungal cells in the middle and

Table 2. Composition of polar lipids of the mycelial and yeastlike cells of *M. circinelloides* var. *lusitanicus* INMI grown on media with high glucose content (20%)

Growth form	Lipids, % of total lipids											PL/GL
	GL-1*	PA	PS	GL-2	PC	PE	CL	GL-3	GL-4	PL	GL-5	
Yeastlike	45.3	10.6	Traces	–	4.0	17.6	2.1	8.3	2.9	2.1	5.0	0.6
Mycelial	30.8	4.6	9.2	4.6	9.8	23.0	7.2	–	4.2	1.8	7.2	1.2

Note: GL-1–GL-5, glycolipids, PA, phosphatidic acid; PS, phosphatidyl serine; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; CL, cardiolipin, PL, unidentified phospholipid.

* Glycolipid with a free amino group.

end of the logarithmic phase were 275 and 130 mg O_2 l^{-1} h^{-1} (g biomass)$^{-1}$, respectively, (in the medium with 6% glucose, the rates of oxygen consumption were 378 and 194 mg O_2 l^{-1} h^{-1} (g biomass)$^{-1}$, respectively); i.e., the respiratory activity of the cells decreased by one third.

The fatty acid composition of total lipids in the mycelial and yeastlike cells of *M. circinelloides* var. *lusitanicus* INMI at the late logarithmic growth phase is shown in Table 4. Yeastlike cells contained less γ-linolenic (γ-$C_{18:3}$) and palmitoleic ($C_{16:1}$) acids than the mycelium, in which the proportion of γ-linolenic acid was 35% of total fatty acids. Correspondingly, the unsaturation degree of the lipids was significantly higher in mycelial cells than in yeastlike cells.

In our previous work [15] we demonstrated that, in *M. circinelloides* var. *lusitanicus* INMI cells that changed their morphology (mycelium deformation, swelling, and arthrospore formation) during batch cultivation, a decrease in the activities of the key enzymes of the TCA and glyoxylate cycles was observed. The content of unsaturated fatty acids (including γ-linolenic acid) in their lipids was low as well.

Table 3. Enzymatic activity of NAD-dependent isocitrate dehydrogenase and isocitrate lyase in the mycelial and yeastlike cells of *M. circinelloides* var. *lusitanicus* INMI grown on media with high glucose content (20%)

Growth form	Enzymatic activity, 10^{-2} μmol/(min mg protein)		Protein, mg/ml
	NAD-dependent IDH	ICL	
Yeastlike	1.0	0.4	10.8
Mycelial	2.4	0.2	19.2

The results presented in the papers [16] and [17] indicate that, in the yeast and mycelial cells of dimorphic fungi *Paracoccidioides brasiliensis* and *M. circinelloides*, certain differences in the gene expression and activities of some enzymes, including those involved in the central metabolism, were observed depending on the cultivation conditions (medium composition, aeration, temperature, and glucose concentrations).

It is well-known that excessive glucose content induces the repression of NAD-dependent enzymes, including NAD-dependent IDH [5, 18], as well as the catabolite inactivation of isocitrate lyase [19, 20]. This was the obvious reason why the enzymatic activities of both morphotypes of the studied *M. circinelloides* strain were low in all our experiments. In the course of catabolite repression, the activity of the TCA cycle in yeastlike cells decreased; this correlated with increased activity of the glyoxylate cycle enzymes. There is evidence in the literature regarding the anaplerotic function of the glyoxylate cycle, which contributes to the maintenance of the TCA cycle by supplying metabolites (e.g., oxaloacetate); the synthesis of these metabolites is hindered due to the low activity of the TCA cycle enzymes [21]. In addition, glucose affects both the activity of the TCA cycle enzymes [4, 5, 18] and the cAMP level (through regulation of the phosphodiesterase activity, which activates nuclear division and septum formation) [22, 23], and also controls a great number of chitin synthase genes that determine the shape of fungal cells, including those of dimorphic zygomycetes [3].

Thus, under the cultivation conditions favorable for yeastlike growth (high initial glucose concentrations, exhaustion of the available pool of nitrogen, and low respiratory activity), changes in the metabolic activity of the cells were observed. These changes resulted from changes in the expression and activity of the genes (including chitin synthase ones), which are due to changes in the cell morphology and switching from mycelial to yeastlike growth. A correlation between the low rate of metabolic processes in yeastlike cells of

Table 4. Fatty acid composition of the mycelial and yeastlike cells of *M. circinelloides* var. *lusitanicus* INMI grown on media with high glucose content (20%)

Growth form	Biomass, g/l	Fatty acids, % of total fatty acids										
		$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	γ-$C_{18:3}$	$C_{20:0}$	Δ/100 molecules
Yeastlike	1.1	1.3	1.8	18.1	2.3	Traces	7.6	26.3	30.6	8.8	3.2	116.2
Mycelial	9.9	3.9	0.8	14.0	5.3	Traces	5.5	16.2	16.9	35.5	2.0	161.7

M. circinelloides var. *lusitanicus* INMI and the lipid composition of these cells, which contained fewer phospholipids, triacylglycerides, and unsaturated fatty acids (including γ-linolenic) than mycelial cells, was observed.

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