ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 6, pp. 676–683. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © I.S. Mysiakina, Ya.E. Sergeeva, A.A. Ivashechkin, E.P. Feofilova, 2012, published in Mikrobiologiya, 2012, Vol. 81, No. 6, pp. 733–740.

EXPERIMENTAL ARTICLES

Impact of Morphogenetic Effectors on the Growth Pattern and the Lipid Composition of the Mycelium and the Yeastlike Cells of the Fungus *Mucor hiemalis*

I. S. Mysiakina[1], Ya. E. Sergeeva, A. A. Ivashechkin, and E. P. Feofilova

Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow

Received January 31, 2012

Abstract—We investigated the growth and the cell lipid composition of the mycelium and of the yeast-like form of *Mucor hiemalis* VKMF-1431 obtained under aerobic conditions by treatment with the morphogenetic agents itraconazole, exogenous triacylglycerols (TAGs), and trehalose. The sporangiospores of a 20-day culture were inoculated on the medium with glucose. Under these conditions, the fungus produced both mycelium and yeast-like cells. It was established that, upon the germination of old (20-day) sporangiospores, the fungus predominantly used the mycelium development strategy in the presence of trehalose and TAGs. It was characterized by a low ratio between the two bulk membrane lipids (PEA/PC) and increased levels of PC and polyunsaturated fatty acids (FA). Compared to the mycelium, the yeast cell morphotype obtained on the medium with glucose was distinguished by an elevated PEA/PC ratio, lowered TAG, free sterol (FS) and esterified sterol (ES) levels, a decreased ES/FS ratio that correlated with the reserve sterol pool size, and a lowered content of unsaturated fatty acids (the linoleic and the γ-linolenic acid). These peculiarities of the lipid composition of yeastlike cells correlated with the intensity of yeastlike growth. Light and electron microscopy revealed differences between the above cell morphotypes. With itraconazole, yeast-like cells were characterized by the destruction of the endoplasmic reticulum membranes and formation of a large number of vacuoles. The suggestion was confirmed that the state/age of inoculum sporangiospores exerts an influence on the capacity for dimorphism in mucorous fungi such as *M. hiemalis*. The data obtained testify to an involvement of lipids in the process of adaptation to environmental factors and to their regulatory role in morphogenetic processes associated with the formation of alternative morphotypes of the mucorous fungus.

Keywords: Mucor hiemalis, lipids, yeastlike cells, mycelium, trehalose, triacylglycerols, itraconazole

DOI: 10.1134/S0026261712060100

In our earlier works on the dimorphism of mucorous fungi under aerobic conditions, we revealed the ability of *Mucor hiemalis* F-1156 and *M. circinelloides* var. *lusitanicus* to grow in submerged culture in the mycelial and the yeastlike form, with sporangiospores from a wheat bran-grown culture used as inoculum [1–5]. The capacity for formation of arthrospores and budding cells in submerged culture was enhanced if the cultivation period of the sporogenic culture was increased to 10 or more days. The sporangiospores of young cultures produced only mycelium. It was also noted that the lipid composition of the sporangiospores formed by a sporogenic culture changed during its development [1]. We put forward the suggestion that the capacity for dimorphism in mucorous fungi including *M. hiemalis* is influenced by the state/age of the sporangiospores used as the inoculum. In our previous work that dealt with the lipid composition of *M. hiemalis* F-1431 in the presence of morphogenetic effectors [6], the sporangiospores of a 6-day culture (arbitrarily termed "the young spores") were used as the inoculum. They germinated and produced mycelium. One of the objectives of this work was to investigate the growth and lipid formation patterns in *M. hiemalis* F-1431 under the influence of the compounds that exert a morphogenetic effects in the case of the culture obtained using 20-day sporangiospores as the inoculum. For this purpose, we supplemented the medium with lipid compounds (triacylglycerols, TAGs), the antibiotic itraconazole that inhibits ergosterol biosynthesis in fungi, or the disaccharide trehalose that sustains mycelial growth, according to the data presented in the literature [7]. The nutrient medium components and additives used in this study are morphogenetic effectors that have been reported to influence growth and dimorphism in fungi [7–13]. The involvement of various phospholipases cleaving the membrane phospholipids in the morphogenesis and reproduction of a number of representatives of basidiomycete fungi was also established [14].

The goal of this work was to elucidate the lipid composition of the yeastlike cells and mycelium of the

[1] Corresponding author; e-mail: myssiakina@inmi.ru

fungus *M. hiemalis* F-1431 cultivated with the following morphogenetic effectors: exogenous TAGs, the antibiotic itraconazole, and trehalose. The 20-day sporangiospores were used as the inoculum.

MATERIALS AND METHODS

The sporangiospores of a 20-day culture of *M. hiemalis* Wermer VKM F-1431 grown on wheat bran were washed off the surface of the sporogenic mycelium with sterile distilled water and used as the inoculum. The cultivation was carried out in the basic liquid medium described in our earlier publication [6]. In some series of experiments, we used trehalose (60 g/L) as the carbon source instead of glucose (60 g/L) or achieved a morphogenetic effect by supplementing the basic glucose-containing medium with (i) the antibiotic itraconazole (as DMSO solution yielding a final itraconazole concentration of 2 μg/mL in the medium) and (ii) sunflower oil TAGs with the unsaturated acyl chains $C_{18:1}$ and $C_{18:2}$ (30 mg per 50 mL of medium). In this work, we employed both sterilized (0.5 atm, 110°C, 30 min) and native TAG preparations, referred to as sTAGs and nTAGs, respectively. Their fatty acid composition was given in the previous work [6]. To prevent bacterial contamination, a mixture of the antibiotics penicillin and streptomycin (10000 U and 10 mg/mL, respectively; Sigma, United States) was applied.

The spore suspension with a density of 10^6 cells/mL was used for inoculation. The cultivation was carried out in 250-mL flasks with 50 mL of the medium on a shaker (130 rpm) at 27°C for 72 h (to reach the late trophophase). Yeastlike cells and the mycelium were separated by passing them through a Nylon filter (pore diameter 30 μm); the yeastlike cells were precipitated by centrifugation.

Cell morphology and the spore germination pattern were monitored with an Axio Imager.DI light microscope (Carl Zeiss, Germany) at 400× magnification in a phase contrast system. The spores were prepared for electron microscopy as follows: they were fixed in 1.5% $KMnO_4$ solution for 2 days at 4°C, twice washed in phosphate buffer (pH 7.0), and fixed in 1% OsO_4 solution in phosphate buffer (pH 7.0) for 12 h at 4°C. Upon dehydration, the material was embedded in Epon 812 epoxide resin. Ultrathin sections were prepared with an LKB-4800 A ultramicrotome, contrasted with 3% uranyl acetate solution for 15 min at 37°C, and stained with lead citrate according to Reynolds at 37°C for 15 min. The sections were examined in a JEM-100C electron microscope (Jeol, Japan) at magnifications of 8000 and 14000.

Lipids were extracted from the biomass using the modified Folch method [15], subjected to acidic methanolysis, and analyzed by GLC with a Chromatek Kristall-5000.1 chromatographer (Russia) on an Optima-240 60 m × 0.25 mm × 0.25 μm capillary column (Macheray-Nagel GmbH & Co, Germany). The stationary phase was 33% cyanopropyl–methyl and 67% dimethylpolysiloxane; the system operated in a programmed mode. The column temperature was 130 to 280°C, the carrier gas (helium) consumption rate was 30 mL/min.

The composition of each of the lipid classes present in the samples was determined by TLC on Kieselgel 60 F_{254} plates (Merck, Germany) as described earlier [6]. Densitometric analysis was performed using the Dens and Sorbfil software packages (Russia). Standard PC, TAG, and free fatty acid solutions were used as the standards for plotting the calibration curves.

The data presented in this work are based on the results of three independent experiments. The results were treated statistically using the median method and the Microsoft® Office Excel® 2007 software package.

RESULTS AND DISCUSSION

The following features were revealed in the cultures developing from 20-day *M. hiemalis* sporangiospores arbitrarily termed "the old spores": (i) sporangiospores germinated with a delay (of up to 1 day in the presence of trehalose); (ii) the cultures represented a mixture of mycelium and yeastlike cells; and (iii) arthrospore chains occurred in the mycelium and the culture liquid. On Fig. 1, the growth of the tested strain in various experimental systems at the end of the first day of cultivation is shown. With glucose, apart from mycelial growth, spore germination occurred also according to the yeastlike strategy with the formation of solitary cells undergoing mono- and multipolar budding (Figs. 1a, 1b). With trehalose, the spores used the mycelium strategy with the formation of growth tubes (Fig. 1c). Itraconazole caused a delay in germination. Thereafter, yeastlike cells were initially formed and a deformed mycelium developed (Figs. 1d, 1e). With TAGs added to the glucose-containing medium, sporangiospore germination proceeded in conformity with the mycelium strategy (Figs. 1f, 1g). However, in the presence of sTAGs, the mycelium had arthrospores and was more deformed than in the case of nTAGs. A large number of spores failed to germinate in all tested systems. The cultures obtained from such spores reached the late trophophase only by the end of the third day of cultivation.

Using transmission electron microscopy (Figs. 2, 3), we conducted comparative studies and revealed the structural and morphological differences between the alternative cell morphotypes developing from 20-day spores in the presence of itraconazole (an inhibitor of ergosterol synthesis) and in the control system (without additions).

In the control, the culture grew in the form of mycelium and yeast cells; arthrospore chains occurred in the mycelium and the culture liquid. Mycelial hyphae had a dense thin cell wall (CW)

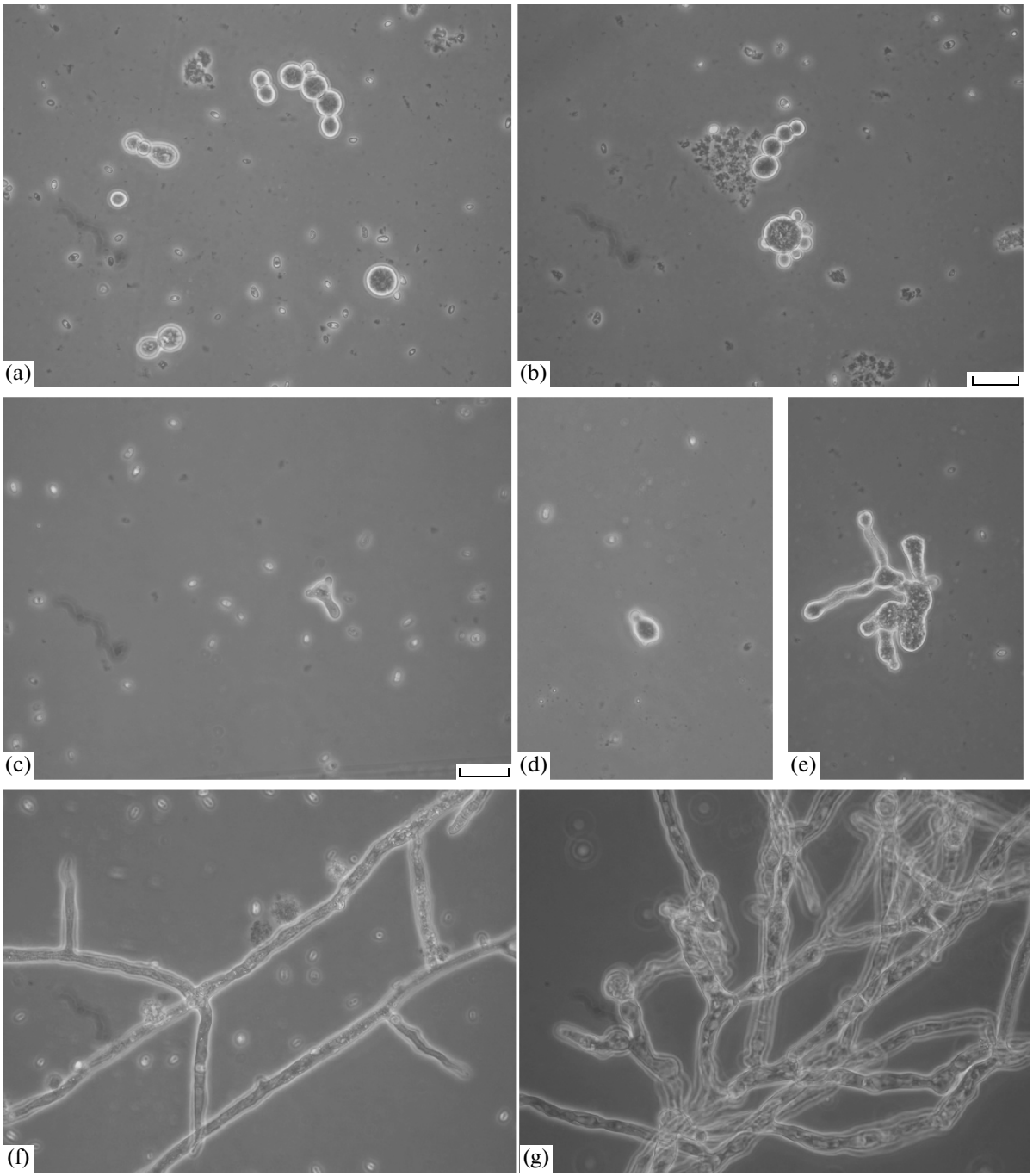

Fig. 1. Growth of *M. hiemalis* F-1431 (a 1-day culture) in the presence of morphogenetic effectors with 20-day spores as the inoculum: glucose (60 g/L) (a, b); trehalose (60 g/L) (c); itraconazole (2 μg/mL in the glucose-containing medium) (d, e); nTAG (0.6 g/L in the glucose-containing medium) (f); and sTAG (0.6 g/L in the glucose-containing medium) (g). Phase contrast system. Bar, 15 μm.

(Fig. 2a); mycelium cell wall-enclosed arthrospores also had their own CW. It was similar to the CW of the maternal hypha in its structure and thickness. Compared to the mycelium, the yeastlike cells possessed a thickened loose cell wall (Fig. 2b). In the presence of itraconazole (Fig. 3), the yeastlike cells were also characterized by a thickened cell wall. In addition, the degradation of the endoplasmic reticulum membranes (Fig. 3a) and formation of numerous vacuoles (Fig. 3b) occurred in the yeastlike cells. The data obtained suggest that ergosterol performs an important role in the operation of cell membranes and influences the synthesis of the components of fungal cell walls.

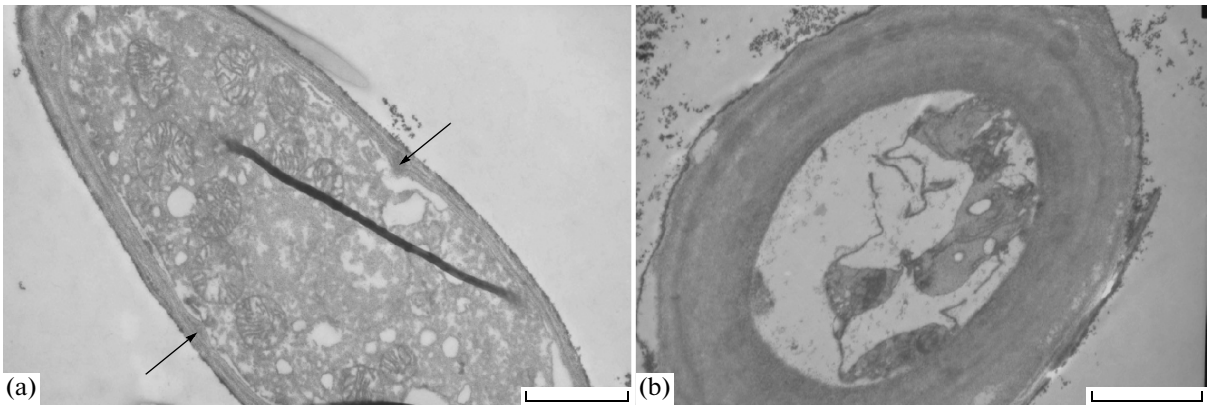

Fig. 2. Morphological features of the mycelium and the yeastlike cells of *M. hiemalis* F-1431 (control system): slanting section of a mycelium hypha at the initial stage of septum formation: the visible features include a thin mucilaginous cell wall, numerous mitochondria with developed cristae, two nuclei upon division in the center, the zones of synthesis and deposition of new septum material (arrows) at the two opposite CW points (a); and section of a yeastlike cell with a multilayer loose thickened cell wall (b). Bar, 2 μm.

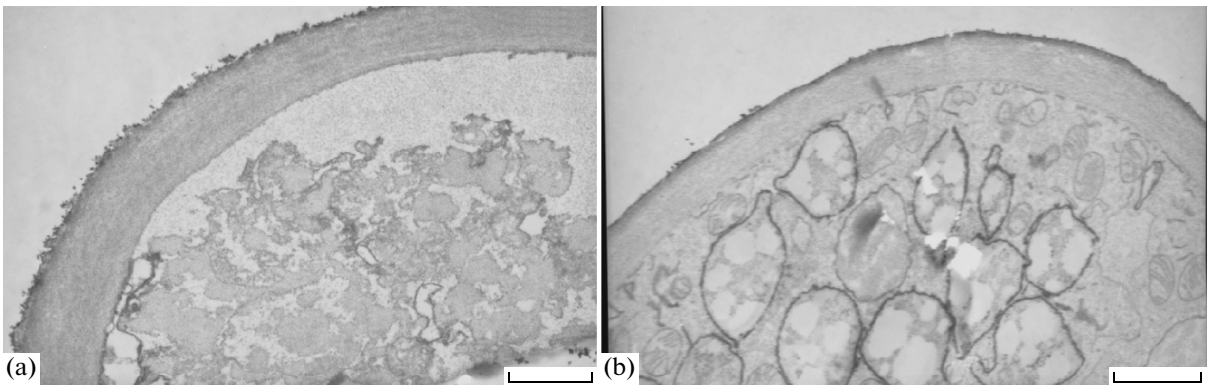

Fig. 3. Yeastlike cell of the *M. hiemalis* F-1431 culture grown with itraconazole: degradation of the endoplasmic reticulum membranes (a) and formation of a large number of vacuoles in the cell (b). Bar, 2 μm.

Biomass accumulation and lipid content dynamics are shown in Table 1. The most manifest yeastlike growth occurred in the control system (glucose-containing medium without additions) and accounted for up to 20–25% of the total fungal biomass, this percentage being only 2–5% in the other systems. In the control system the highest lipid content in the yeastlike cells was observed.

Studies on the fatty acid composition and lipid class distribution in the mycelium and the yeastlike cells of *M. hiemalis* F-1431 obtained using the sporangiospores of a 20-day culture as inocula revealed the differences between the lipids of morphologically different cells.

The lipid composition of the mycelium and the yeastlike cells is shown in Tables 2 and 4 and Tables 3 and 5, respectively. The composition of the bulk fatty acids is shown on Fig. 4.

No significant differences between the mycelium and the yeastlike cells were detected in the total lipid fraction in terms of the contents of individual lipid classes. However, the TAG level was 1.5–2 times higher in the mycelium than in the yeastlike cells (Table 2). The lipids of the yeastlike cells (Table 3) contained more MAGs and DAGs in all tested systems. We detected a large amount of an unidentified lipid (X-4) in the yeastlike cells in the control system but not in the mycelium. The free sterol level was lower in the yeast-like cells than in the mycelium, and this difference was more significant in the control system and the system with itraconazole. Importantly, the percentage of etherified sterols (ESs) in all tested systems except the control samples was higher in the yeastlike cells than in the mycelium. The ES level in the yeastlike cells was lower than in the mycelium in the control system where the most prominent yeastlike growth occurred. Accordingly, the control system exhibited the lowest ratio between the free and the esterified sterols (0.95). This provides evidence for an association between the decrease in the reserve sterol pool and the intensification of yeastlike growth in *M. hiemalis* F-1431.

Table 1. Biomass and lipid accumulation in various *M. hiemalis* F-1431 morphotypes grown with morphogenetic agents. Inoculum, 20-day sporangiospores. Late trophophase

Morphotype	Characteristics	Control	Trehalose	Itraconazole	nTAGs	sTAGs
Mycelial	Biomass, g/L	8.52 ± 1.01	9.16 ± 0.45	7.92 ± 0.39	7.54 ± 1.93	7.81 ± 0.99
	Lipids, %	9.92 ± 0.70	9.19 ± 2.58	12.09 ± 1.58	14.46 ± 2.96	12.07 ± 2.48
Yeastlike	Biomass, g/L	2.04 ± 0.42	0.18 ± 0.02	0.22 ± 0.03	0.15 ± 0.01	0.24 ± 0.02
	Lipids, %	18.10 ± 1.20	6.23 ± 1.10	3.76 ± 1.27	6.66 ± 0.71	2.39 ± 0.30

Table 2. Composition of the total lipids of the mycelium of *M. hiemalis* F-1431 grown with morphogenetic agents. Inoculum, 20-day sporangiospores. Late trophophase

Lipids, % of total	Control	Trehalose	Itraconazole	nTAGs	sTAGs
PLs	8.33 ± 1.05	7.27 ± 0.21	7.85 ± 1.68	7.53 ± 1.01	7.77 ± 0.79
MAGs	Trace amounts	Trace amounts	Trace amounts	Trace amounts	Trace amounts
DAGs	1.08 ± 0.79	1.50 ± 0.98	1.35 ± 0.08	2.50 ± 1.71	0.63 ± 0.24
FSs	13.70 ± 2.55	13.40 ± 1.18	15.65 ± 0.18	11.53 ± 1.88	13.20 ± 1.09
X-1	9.27 ± 2.61	8.20 ± 0.41	5.60 ± 0.16	6.83 ± 1.26	7.80 ± 1.71
X-2	3.93 ± 0.69	1.80 ± 0.52	3.25 ± 0.72	4.83 ± 1.54	1.17 ± 0.17
Total fatty acids	14.43 ± 1.27	12.20 ± 0.68	11.9 ± 0.16	19.37 ± 7.22	14.17 ± 1.21
X-3	3.23 ± 1.02	2.70 ± 0.60	0.30 ± 0.48	1.40 ± 0.59	1.80 ± 0.85
TAGs	32.50 ± 3.70	40.60 ± 3.68	45.6 ± 3.84	32.30± 12.48	40.63 ± 3.13
X-4	–	3.50 ± 0.48	1.1 ± 1.12	7.47 ± 4.67	3.13 ± 0.17
ESs	10.60 ± 0.23	10.00 ± 0.78	7.45 ± 2.16	6.60 ± 2.61	9.37 ± 2.23

Phosphatidic acids (PAs), phosphatidylcholines (PCs), and phosphatidylethanolamines (PEAs) were the main polar lipids of the tested fungus. The ratio between the bulk membrane lipids (PEA/PC) was, in general, considerably higher in the yeastlike cells than in the mycelium (Tables 4 and 5), except for the system with itraconazole. The effect of itraconazole on the lipid composition should be addressed in special studies. The PEA/PC ratio changes reflect the shifts in the balance between the zwitterion (PC) and anionic (PEA) membrane phospholipids in favor of the negatively charged lipids and, as a result, an altered state of the cell membranes and their permeability. The PEA/PC ratio in the lipids of the mycelium grown on

Table 3. Composition of the total lipids of the yeastlike cells of *M. hiemalis* F-1431 grown with morphogenetic agents. Inoculum, 20-day sporangiospores. Late trophophase

Lipids, % of the total	Control	Trehalose	Itraconazole	nTAGs	sTAGs
PLs	7.0 ± 2.9	10.0 ± 0.2	7.5 ± 1.1	10.3 ± 2.9	6.4 ± 0.9
MAGs	3.8 ± 0.7	2.0 ± 0.1	2.0 ± 3.2	Trace amounts	Trace amounts
DAGs	4.5 ± 0.3	5.8 ± 0.3	5.5 ± 0.1	1.9	1.6
FSs	7.4 ± 0.2	8.4 ± 0.6	7.9 ± 0.9	9.3 ± 0.6	8.5 ± 0.5
X-1	5.7 ± 0.2	5.1 ± 0.5	5.5 ± 1.7	5.1 ± 0.5	5.2 ± 0.1
X-2	5.6 ± 1.4	1.8 ± 0.9	4.6 ± 0.2	5.1 ± 0.5	4.3 ± 0.3
Total fatty acids	15.0 ± 0.7	15.7 ± 3.0	13.6 ± 1.1	14.4 ± 1.8	13.8 ± 2.2
X-3	2.4 ± 0.8	3.7 ± 0.9	4.3 ± 0.2	2.8 ± 0.3	4.5 ± 0.6
TAGs	25.9 ± 0.2	22.4 ± 1.2	25.7 ± 2.2	23.4 ± 1.8	27.5 ± 0.1
X-4	13.7 ± 2.3	8.8 ± 2.0	11.2 ± 1.4	17.4 ± 0.9	13.1 ± 0.1
ESs	7.0 ± 1.8	16.6± 5.0	12.5 ± 4.0	10.5 ± 3.4	15.3 ± 5.6
ES/FS	0.95	1.98	1.58	1.13	1.8

Table 4. Composition of the polar lipids of the mycelium of *M. hiemalis* F-1431 grown with morphogenetic agents. Inoculum, 20-day sporangiospores. Late trophophase

PLs, % of the total	Control	Trehalose	Itraconazole	nTAGs	sTAGs
Gl-1	6.85 ± 1.15	8.14 ± 1.33	3.51 ± 1.43	3.35 ± 1.16	3.94 ± 1.33
Gl-2	2.08 ± 1.14	–	Trace amounts	1.85 ± 0.44	3.20 ± 0.82
PAs	19.03 ± 3.06	21.24 ± 7.49	25.52 ± 7.76	19.27 ± 2.05	31.32 ± 8.55
Gl-3	3.87 ± 0.92	4.36 ± 2.68	3.69 ± 2.51	3.02 ± 0.39	5.16 ± 1.14
DPGs	3.85 ± 0.95	2.31 ± 1.53	3.35 ± 1.80	Trace amounts	4.23 ± 1.00
PEAs	35.67 ± 1.34	33.92 ± 4.93	33.27 ± 8.47	33.12 ± 8.22	26.66 ± 2.63
X-1	–	–	–	Trace amounts	Trace amounts
PCs	24.90 ± 5.05	28.98 ± 8.52	23.65 ± 4.56	33.81 ± 5.83	21.10 ± 4.22
X-2	–	–	–	4.34 ± 1.57	–
PSs	Trace amounts	–	–	–	0.61 ± 0.19
PIs	Trace amounts	Trace amounts	–	–	–
PEA/PC	1.43	1.17	1.41	0.98	1.26

Note: Gl-1–Gl-3, glycolipids; PAs, phosphatidic acids; DPGs, diphosphatidylglycerols (cardiolipins); PEAs, phosphatidylethanolamines; PCs, phosphatidylcholines; PSs, phosphatidylserines; PIs, phosphatidylinositols; X, unidentified fractions.

Table 5. Composition of the polar lipids of the yeastlike cells of *M. hiemalis* F-1431 grown with morphogenetic agents. Inoculum, 20-day sporangiospores. Late trophophase

PLs, % of the total	Control	Trehalose	Itraconazole	nTAGs	sTAGs
Gl-1	5.54	3.85	0.98	0.43	3.34
Gl-2	–	14.41	17.32	4.13	–
PAs	19.06	24.30	22.42	27.22	33.62
Gl-3	10.67	9.74	8.16	11.03	13.06
DPGs	12.77	10.22	4.83	2.64	7.05
PEAs	33.62	20.73	23.91	29.52	27.68
X-1	1.40	–	–	–	–
PCs	13.71	16.76	20.22	23.83	15.25
X-2	3.23	–	–	–	–
X-3	–	–	2.16	1.2	–
PEA/PC	2.45	1.24	1.11	1.24	1.82

Note: See designations in Table 4.

the trehalose-containing medium was lower than in the control system (the glucose-containing medium). The data obtained concerning the lipid composition confirm the suggestion that trehalose, a membrane protector, promotes hyphal growth. Using it as the carbon source facilitates mycelium formation upon sporangiospore germination in both young [6] and old cultures of the tested fungus. Data are available in the literature concerning the effect of trehalose as a fatty acid protector in model systems [16]. This effect manifests itself in preventing (i) the denaturation of unsaturated linoleic and α-linolenic acid with the formation of aldehydes and (ii) the autooxidation of unsaturated fatty acids with the formation of peroxides. The author of the cited work draws the conclusion that trehalose directly interacts with acyl chains, stabilizing the fatty acid structure [16].

The relative fatty acid content (Fig. 4) in mycelium lipids was similar in all tested systems, except for the system with nTAGs where elevated linoleic acid content (39.19%) was observed. The lipid desaturation degree differed in the different morphotypes: the mycelium lipids were more desaturated in all tested systems, particularly with TAGs (Table 6). The yeastlike cells were characterized by an increased saturated fatty acid content and, accordingly, lowered linoleic and γ-linolenic acid levels, compared to the mycelium. These differences were particularly prominent in

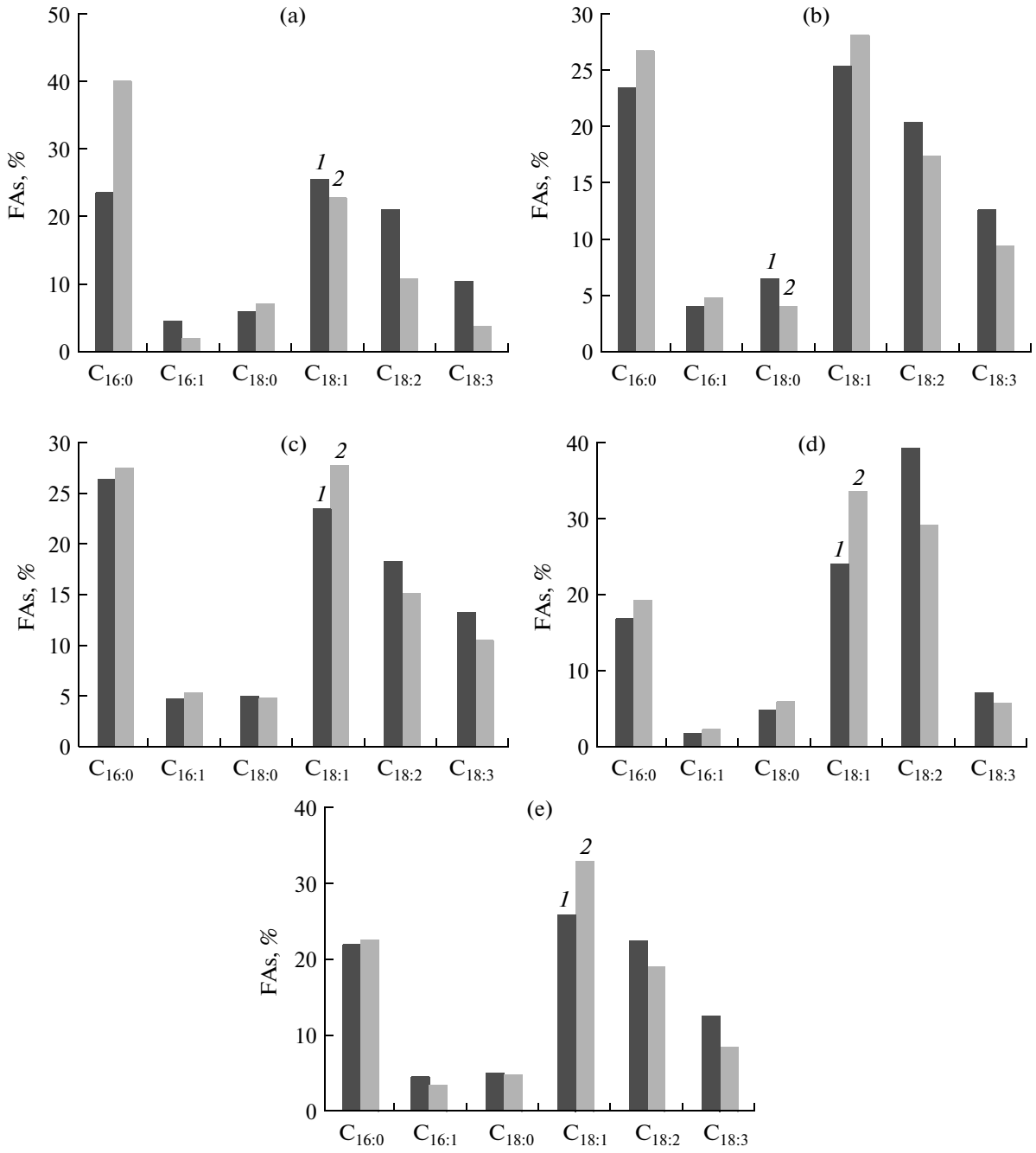

Fig. 4. Fatty acid composition of the lipids of the mycelium (*1*) and the yeastlike cells (*2*) of the fungus *M. hiemalis* F-1431 grown with morphogenetic agents: glucose (6%, control) (a); trehalose (6%) (b); itraconazole (2 μg/mL) (c); nTAG (0.6 mg/mL) (d); and sTAG (0.6 mg/mL) (e). Inoculum, 20-day sporangiospores. Late trophophase.

the control system (Fig. 4a) where the most pronounced yeastlike growth occurred.

Thus, using 20-day sporangiospores as inoculum made it possible to induce the formation of yeastlike cells in the fungus *M. hiemalis* F-1431 under aerobic conditions without adding toxic compounds (e.g., 4-chloroaniline) [17]. Using trehalose as the carbon source and adding TAGs to the glucose-containing medium makes it possible to implement the mycelium strategy of fungal development, i.e., it stimulates polarized growth and increases the mycelium contribution to the total biomass even if the sporangiospores of old cultures are used as inoculum. The data obtained on the lipid composition of various cell morphotypes not only provide evidence for the involvement of lipids in fungal adaptation to environmental factors but also point to their possible regulatory role in morphogenetic processes.

Table 6. The desaturation degree (Δ/100 molecules) of the fatty acids of the lipids of the cell morphotypes of *M. hiemalis* F-1431. Inoculum, 20-day sporangiospores

Morphotype	Control	Trehalose	Itraconazole	nTAGs	sTAGs
Mycelium	105.02	107.92	104.83	127.85	114.18
Yeastlike cells	60.86	96.17	95.81	114.11	102.20

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 10-04-0659a).

REFERENCES

1. Mysyakina, I.S. and Funtikova, N.S., Changes in the Lipid Composition of *Mucor hiemalis* Sporangiospores Related to the Age of the Spore-Forming Culture, *Microbiology*, 2003, vol. 72, no. 4, pp. 461–465.
2. Funtikova, N.S. and Mysyakina, I.S., Sporangiospores of the Fungus *Mucor lusitanicus* 12M: Correlation between Lipid Composition, Viability, and Morphology of Growth upon Germination, *Microbiology*, 2003, vol. 72, no. 6, pp. 686–689.
3. Mysyakina, I.S. and Funtikova, N.S., Sterols of the Fungus *Mucor hiemalis* Sporangiospores, *Microbiology*, 2003, vol. 72, no. 6, pp. 762–763.
4. Funtikova, N.S. and Mysyakina, I.S., The Dependence of Dimorphism in the Fungus *Mucor lusitanicus* 12M on the Preparation Conditions of Sporangiospores, *Microbiology*, 2004, vol. 73, no. 6, pp. 734–736.
5. Funtikova, N.S. and Mysyakina, I.S., Effects of Conditions for Obtaining Sporangiospores of the Inoculum on the Morphology and Productivity of the Fungus *Mucor circinelloides* var. *lusitanicus* 12 M, a Producer of γ-Linolenic Acid, *Appl. Biochem. Microbiol.,* 2008, vol. 44, no. 4, pp. 411–415.
6. Mysyakina, I.S., Sergeeva, Ya.E., Ivashechkin, A.A., and Feofilova, E.P., Lipid Composition of the Mycelium of the Fungus *Mucor hiemalis* Cultivated with Trehalose, Triacylglycerols, and Itraconazole, *Microbiology*, 2012, vol. 81, no. 6, pp. 669–675.
7. Lucio, A.K.B., Polizeli, M.L.T.M., Jorge, J.A., and Terenzi, H.F., Stimulation of Hyphal Growth in Anaerobic Cultures of *Mucor rouxii* by Extracellular Trehalose. Relevance of Cell Wall-Bound Activity of Acid Trehalase for Trehalose Utilization, *FEMS Microbiol. Lett.*, 2000, vol. 182, pp. 9–13.
8. Vanden Bossche, H., Importance and Role of Sterols in Fungal Membranes, in *Biochemistry of Cell Walls and Membranes in Fungi*, Kuhn, P.J., Trinci, A.P.J., Jung, M.J., Goosey, M.W., and Copping, L.G., Eds., Berlin: Springer, 1990, pp. 135–157.
9. Klose, J., de Sa, M.M., and Kronstad, J.W., Lipid-Induced Filamentous Growth in *Ustilago maydis, Mol. Microbiol.*, 2004, vol. 52, no. 3, pp. 823–835.
10. Bartnicki-Garcia, S., Symposium on Biochemical Bases of Morphogenesis in Fungi. III. Mould-Yeast Dimorphism of *Mucor, Bacteriol. Rev.*, 1963, vol. 27, pp. 293–304.
11. Sypherd, P.S., Borgia, P., and Pasnokas, J.L., Biochemistry of Dimorphism in the Fungus *Mucor, Adv. Microb. Physiol.*, 1978, vol. 18, pp. 67–104.
12. Van Laere, A., Trehalose, Reserve and/or Stress Metabolite?, *FEMS Microbiol. Rev.*, 1989, vol. 63, pp. 201–210.
13. Wiemken, A., Trehalose in Yeast, Stress Protectant rather Than Reserve Carbohydrate, *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.*, 1990, vol. 59, pp. 209–217.
14. Dippe, M. and Ulbrich-Hofmann, R., Phospholipid Acylhydrolases Trigger Membrane Degradation during Fungal Sporogenesis, *Fungal Genet. Biol.*, 2011, vol. 48, pp. 921–927.
15. Folch, G., Lees, M., and Sloane-Stanley, G.H., A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem.*, 1957, vol. 226, no. 1, pp. 497–509.
16. Higashiyama, T., Novel Functions and Applications of Trehalose, *Pure Appl. Chem.*, 2002, vol. 74, pp. 1263–269.
17. Funtikova, N.S., Mysyakina, I.S., and Poglazova, M.N., Morphogenesis and Lipid Composition of *Mucor* Fungi Grown in the Presence of Chloroanilines in Submerged Culture, *Microbiology*, 1999, vol. 68, no. 4, pp. 406–411.