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EXPERIMENTAL ARTICLES

Lipid Composition of the Arthrospores, Yeastlike Cells, and Mycelium of the Fungus *Mucor hiemalis*

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Abstract—The fungus *Mucor hiemalis* F-1156, which is believed to be monomorphic, was found to be able to grow dimorphically in a liquid medium that is free of chemical agents influencing morphogenesis. The growing mycelium produced arthrospores in large amounts. The lipids of the mycelium, yeastlike budding cells, and arthrospores differed in the contents of saturated and unsaturated fatty acids and in the proportion of polar and neutral lipids. The arthrospores contained more monoenoic fatty acids in the total lipids, more triacylglycerides and sterol esters in the neutral lipids, and more phosphatidylcholine and phosphatidylethanolamine in the polar lipids than the yeastlike cells. These differences in the lipid composition of different types of fungal cells should be taken into account in the studies of the lipogenesis of *M. hiemalis.*

Key words: dimorphism, mycelial fungi, *Mucor*, lipids, sporangiospores, arthrospores, yeastlike cells.

Dimorphic growth is typical of many representatives of the genus *Mucor* [1–3]. In our previous works, we showed that two *M. hiemalis* strains grow in a liquid medium with 4-chloroaniline dimorphically and found some differences in the lipid composition of the yeastlike and mycelial cells of these strains [4, 5].

Moreover, the strain *M. hiemalis* F-1156 was found to be able to grow in the mycelial and yeastlike forms in a medium without 4-chloroaniline, provided that the sporangiospores of the mycelium grown on wheat bran for more than 7 days were used as the inoculum. In this case, numerous arthrospores detached from the mycelium underwent budding.

Arthrospores are very similar to oidia in that they both are formed on the hyphae of vegetative mycelia and differ only in their shape (the oidia are more spherical than the arthrospores) [6]. Some authors believe that the arthrospores (oidia) are yeastlike cells [2, 7], although Bartnicki-Garsia assumes that only budding cells are true yeastlike cells [1]. In any case, the physiology and metabolism of the arthrospores of *Mucor* fungi have been insufficiently investigated.

The lipids of dimorphic fungi were previously studied under stressful conditions: under anaerobic conditions, in the presence of antibiotics or respiratory inhibitors, and so on.

The aim of the present work was to compare the lipid and fatty acid compositions of the mycelium, yeastlike cells, and arthrospores of a culture of *M. hiemalis* F-1156 grown in the absence of stressful factors.

MATERIALS AND METHODS

The *Mucor hiemalis* F-1156 used in this study was obtained from the All-Russia Collection of Microorganisms (VKM).

The material for inoculation was prepared by washing off sporangiospores from the fungal mycelium grown on wheat bran for 10 days at 28°C. The cultivation medium contained (g/l) glucose, 60.0; urea, 1.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 1.0; $FeSO_4 \cdot 7H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.001; and yeast extract, 0.5. The fungus was grown at 28°C in 250-ml flasks with 50 ml of the cultivation medium on a shaker (130 rpm). Each flask was inoculated with 1 ml of a suspension containing 10^7 sporangiospores. Microscopic observations were performed after 1, 2, 3, 4, and 9 days of cultivation.

The mycelium was harvested by filtering the culture through a nylon gauze. The arthrospore chains were then separated from the culture filtrate by differential centrifugation. The arthrospores attached to the mycelium were detached by vigorously shaking the mycelium in distilled water. The suspension thus obtained was also filtered through the nylon gauze to remove the suspended fungal hyphae. The arthrospores present in the filtrate were harvested by centrifugation and combined with the arthrospore chains. The fraction of yeastlike budding cells contained a small amount of spherical cells without buds. At all steps of the separation of the morphologically different cells, the purity of the particular fractions was controlled microscopically. The dry mycelium biomass was evaluated gravimetrically.

Lipids were extracted by the Folch method [8]. The fatty acid composition of the lipids was determined

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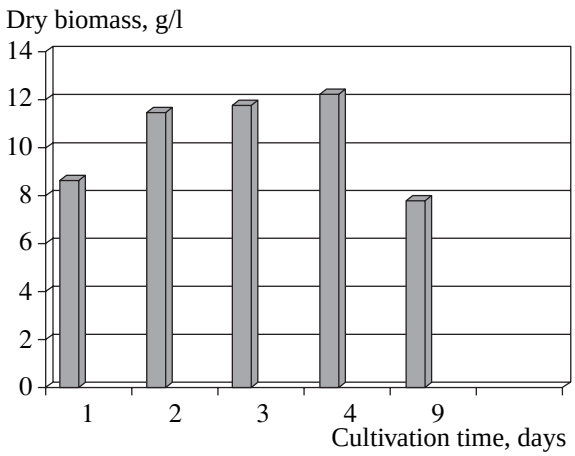

Accumulation of the mycelial biomass during the growth of *M. hiemalis* F-1156.

after their acid methanolysis. The methyl esters of fatty acids were analyzed by gas–liquid chromatography on a model 3700 chromatograph (Russia) equipped with a column packed with Chromosorb W containing 17% di(ethylene glycol) succinate. The carrier gas was helium at a flow rate of 40 ml/min; the column was kept at 170°C.

The proportion of different types of lipids was determined by TLC on Kieselgel 60 F_{254} plates (Merck, Germany) in a hexane–diethyl ether–acetic acid (80 : 20 : 1) mixture to determine neutral lipids and in a chloroform–methanol–25% ammonia (65 : 25 : 4) mixture to determine polar lipids. The lipid spots were visualized with a 10% solution of phosphomolybdic acid in ethanol. The lipids were identified using the respective color reaction tests [9] and comparing their R_f values with those of the authentic samples and those available in the literature. The amount of the different lipid classes was densitometrically evaluated as described earlier [5].

RESULTS AND DISCUSSION

In our earlier studies, the material for inoculation was prepared by washing off the sporangiospores from the *M. hiemalis* mycelium grown on agar slants for 4–5 days. When cultivated in a submerged culture, such sporangiospores germinated with the formation of germ tubes, thus giving rise to hyphal growth. However, when the inoculum was prepared using the *M. hiemalis* mycelium grown on wheat bran for 10 days, it gave rise mainly to arthrospores, which, after their detachment from the mycelium, underwent budding. Therefore, not only various physical and chemical factors but also the cultivation mode of the sporogenous mycelium may influence the ability of the fungus to grow dimorphically [10].

The belief that the fungus *M. hiemalis* can grow only monomorphically [1, 2] seems to be refuted by the experimental data showing that some *Mucor* strains can grow dimorphically under certain conditions.

After one day of cultivation, the fungal culture contained not only the mycelium but also spherical cells, cells with short germ tubes, and short chains made up of 2–3 cells. After 2–4 days of cultivation, the nonmycelial portion of the culture contained chains made up of 4–8 arthrospores and a small amount of yeastlike cells. The arthrospores began to generate as early as on the second day of cultivation, however, they remained bound to the mycelium. On the third day of cultivation, the amount of the arthrospore and budding yeastlike cells became sufficiently large to be isolated and investigated. By the ninth day of cultivation, the number of chains made up of 6–10 arthrospores considerably increased.

The dynamics of the mycelium biomass of *M. hiemalis* in the course of its cultivation is shown in the figure. The maximum yield of the mycelium was observed on the fourth day of the cultivation.

Table 1 summarizes the data on the fatty acid composition of the arthrospores, mycelium, and yeastlike cells of *M. hiemalis*. The total lipids of these fungal structures contained the same fatty acids (although in different amounts), among which palmitic, palmitoleic, stearic, oleic, linoleic, and γ-linolenic acids were dominant.

After 3–4 days of cultivation, the lipids of the arthrospores and mycelium were similar in the fatty acid composition. In 9-day arthrospores, however, the content of saturated and monounsaturated fatty acids was higher and that of polyunsaturated fatty acids was lower than in the mycelium of the same age. Throughout the cultivation period, the lipids of yeastlike cells contained more saturated and less unsaturated fatty acids than the mycelial lipids. Similar data were obtained earlier in the studies of the dimorphic fungal species *Mucor genevensis* [11] and *Candida albicans* [12, 13] and the strain *M. hiemalis* F-1156, which were grown dimorphically in the presence of 4-chloroaniline [5]. As was suggested by Vanden Bossche, the decrease in the activity of Δ9-desaturase, which shifts the equilibrium in the cells toward the prevalence of saturated fatty acids, may be caused by changes in the membrane fluidity due to the elevated content of 14-methylated sterols [14].

The lipids of the arthrospores contained less saturated and more monounsaturated fatty acids than the lipids of the budding yeastlike cells, whereas the content of polyunsaturated fatty acids in these two types of cells was almost the same.

Neutral lipids were found to contain diacylglycerides (DAGs), free desmethyl- and methylsterols, higher alcohols, free fatty acids (FFAs), triacylglycerides (TAGs), sterol esters (SEs), minor amounts of quinones, and some unidentified lipid compounds.

As can be seen from Table 2, 3- and 4-day arthrospores contained more sterol esters than the

Table 1. The fatty acid composition of the total lipids of the mycelium, arthrospores, and budding yeastlike cells of *M. hiemalis* F-1156

FFA, % of the total	Mycelium			Arthrospores			Budding cells		
	3 days	4 days	9 days	3 days	4 days	9 days	3 days	4 days	9 days
C 14 : 0	4.29	3.00	5.71	3.51	3.36	3.52	4.79	3.13	4.02
C 15 : 0	0.69	0.69	0.70	0.50	0.47	0.67	2.69	1.33	1.53
C 16 : 0	29.45	29.06	22.85	30.83	27.68	24.87	33.74	32.81	32.40
C 16 : 1	4.49	4.26	5.82	7.21	8.41	6.28	5.34	5.21	7.03
C 18 : 0	8.95	7.89	6.23	8.39	7.21	8.45	12.60	10.63	10.78
C 18 : 1	27.82	30.71	32.56	30.86	30.48	39.86	22.90	24.38	23.77
C 18 : 2	15.80	16.37	15.05	12.52	14.23	10.69	12.48	13.69	12.27
C 18 : 3	8.51	8.02	11.08	6.18	8.15	5.66	5.46	8.82	8.20
Δ/100 molecules	89.44	91.77	101.72	81.65	91.80	84.50	69.58	83.43	79.94

Note: The unsaturation index of fatty acids was calculated by the formula: Δ/100 molecules = 1 × % monoenoic FFA + 2 × % dienoic FFA + 3 × % trienoic FFA.

Table 2. The lipid composition of the mycelium, arthrospores, and budding yeastlike cells of *M. hiemalis* F-1156

Lipid class, % of the total	Mycelium			Arthrospores			Budding cells		
	3 days	4 days	9 days	3 days	4 days	9 days	3 days	4 days	9 days
PL	10.99	10.66	14.89	14.63	9.20	17.02	20.03	30.36	38.42
DAG	5.46	5.52	4.99	4.28	4.69	9.05	10.70	12.73	12.23
Sterols	8.46	7.23	6.87	3.67	6.27	8.08	7.21	8.32	5.53
Methylated sterols*	2.98	2.88	2.57	3.12	3.20	5.73	7.66	5.75	5.57
FFA	2.91	2.70	6.46	4.72	2.72	7.66	16.61	12.47	12.52
*X***	Traces	2.03	2.72	Traces	Traces	2.01	Traces	Traces	Traces
Quinones	4.49	2.10	3.17	2.92	3.19	2.10	Traces	Traces	Traces
TAG	53.86	51.22	43.31	50.47	52.54	38.13	30.07	24.76	21.28
SE	10.85	15.65	15.00	16.48	18.19	10.21	7.71	5.61	4.45

* The fraction of methylated sterols also contained higher alcohols.
** *X* stands for the fraction of unidentified lipids.

mycelium of the same age. In the total lipids of 9-day arthrospores, the relative content of DAGs, demethylated sterols, FFAs, methylated sterols, and higher alcohols increased and that of the TAGs and SEs decreased in comparison with the 3- and 4-day arthrospores. The content of storage lipids in the mycelium grown for nine days was lower than in the mycelium grown for 3 and 4 days, respectively.

The total lipids of the yeastlike cells contained more polar lipids, DAGs, FFAs, methylsterols, and higher alcohols and less TAGs and SEs than the mycelium. These data agree with the data of Ghannoum *et al.* [13], who studied the dimorphism-associated changes in the lipid composition of *C. albicans*. In the course of cultivation, the relative content of neutral lipids decreased and that of polar lipids increased. The high relative content of phospholipids (up to 35%) in the total lipids of yeastlike cells was found earlier in the fungus *Mucor rouxii* grown at a low partial pressure of oxygen [15]. As was assumed by Weete, the increased content of polar lipids and short-chain saturated fatty acids and the low level of storage lipids in the fungi and other organisms may be related to the inhibition of their oxidative metabolism [16].

The yeastlike cells contained less TAGs and SEs and more DAGs, FFAs, membrane lipids, and methylated sterols than the arthrospores. According to the data of Vanden Bossche, the accumulation of 14-methylated sterols in the fungus *C. albicans* is associated with the suppression of its mycelial growth [14]. The differences in the contents of particular lipids and in their fatty acid composition suggest a metabolic dissimilarity of the different types of fungal cells and provide evi-

Table 3. The polar lipids of the mycelium, arthrospores, and budding yeastlike cells of *M. hiemalis* F-1156

Lipid, % of the total	R_f	Mycelium			Arthrospores			Budding cells		
		3 days	4 days	9 days	3 days	4 days	9 days	3 days	4 days	9 days
GL	0.0	3.25	0.41	2.51	1.04	4.69	5.81	17.47	10.84	10.86
PS + PA + PL*	0.05	19.28	25.98	11.89	5.00	11.25	8.22	Traces	6.05	5.08
LPEA + GL**	0.08	5.45	13.09	4.51	12.59	17.33	5.41	5.32	9.85	6.23
Unidentified lipid	0.20	3.78	0.63	–	Traces	0.19	0.85	11.29	1.22	–
PC	0.27	32.97	27.25	32.50	39.75	40.74	35.11	22.85	28.34	27.83
PEA	0.34	27.17	24.13	30.30	23.68	20.36	25.64	21.26	19.04	21.68
CL	0.41	5.84	4.72	10.34	8.16	5.19	13.59	11.67	14.11	12.45
GL	0.45	2.15	0.11	2.8	5.48	0.24	1.58	–	0.95	1.14
Unidentified PL	0.64	Traces	3.68	4.0	Traces	Traces	0.42	10.14	9.01	14.05
GL	0.77	0.11	Traces	–	4.20	Traces	4.41	–	0.39	0.67
PC/PEA		1.21	1.13	1.07	1.68	2.00	1.37	1.07	1.48	1.28

* Phospholipid with a free amino group.
** Clycolipid with a free amino group.

dence that arthrospores are a separate ontogenetic stage of fungi.

This suggestion was confirmed by the results of an investigation of the polar lipids of *M. hiemalis* (Table 3), among which we detected phosphatidylcholine (PC), phosphatidylethanolamine (PEA), cardiolipin (CL), glycolipids (GLs), a phospholipid (PL) with $R_f = 0.64$, and unidentified lipids with $R_f = 0.20$. Some lipid fractions also contained phosphatidylserine (PS), phosphatidic acid (PA), a phospholipid with a free amino group ($R_f = 0.05$), lysophosphatidylethanolamine (LPEA), and a ninhydrin-positive GL with $R_f = 0.08$. The CL fraction sometimes contained an amount of a GL with $R_f = 0.41$.

In the present work, we did not study the respiration of the fungus; however, according to the data of Brennan and Losel [17], the high content of CL is indicative of a high degree of development of mitochondrial membranes in all types of fungal cells. CL was found to be involved in the regulation of the cytochrome oxidase activity and the translocation of proteins through the cell membrane of *Saccharomyces cerevisiae* [18]. In mammal mitochondria, CL serves as an effector of a *P*-450$_{SCC}$-dependent enzyme cleaving a side chain in the cholesterol molecule [19].

The polar lipids of the arthrospores and yeastlike cells contained less PS and more CL than the mycelial polar lipids. The budding cells contained a considerable amount of the phospholipid with $R_f = 0.64$, which was present in the arthrospores and mycelium only in trace amounts. The arthrospores contained more PC, PEA, and LPEA than the yeastlike cells.

The PC/PEA ratio in the mycelium was maximum on the third day of cultivation, when the content of TAGs was also at a maximum, and then gradually decreased. The PC/PEA ratio in the arthrospores was greater than in the mycelium and yeastlike cells and reached a maximum on the fourth day of cultivation. According to the data of Feofilova and Pisarevskaya, high levels of PEA are typical of actively growing fungal mycelia [20]. The high PC/PEA ratio in the *M. hiemalis* F-1156 arthrospores is an indication of the diminished de novo synthesis of the membranes and the preferential accumulation of storage PC. The smaller PC/PEA ratio in the yeastlike cells in comparison with the mycelium is likely due to a decreased level of PC rather than to an elevated level of PEA.

According to the data of Barrera, the formation of arthrospores by fungi of the genus *Mucor* is induced by low pH values of the medium, high glucose concentrations and fermentation-promoting conditions [21]. Under such conditions, the fungus *M. hiemalis* does not form yeastlike cells [1, 3] and oidia on solid media [2]. The present work shows that the strain *M. hiemalis* F-1156 produces arthrospores and budding yeastlike cells in liquid media, provided that they were inoculated with the sporangiospores washed off from the mycelium grown on wheat bran for a relatively long time. This implies that the ability to form yeastlike cells is associated with the physiological state of the sporangiospores and the mycelium on which they are produced. In the course of the further cultivation of the fungus on wheat bran, some sporangiospores lose the ability to germinate, whereas the others lose the ability to form hyphae (unpublished data). It is likely that the formation of yeastlike cells and arthrospores by fungi serves to help them better survival.

The data presented allow two inferences to be made. First, at least some strains of the species *M. hiemalis* are able to grow as yeastlike cells under certain conditions. Second, the lipids of arthrospores differ from the lipids of mycelial and yeastlike cells, suggesting that the arthrospores represent a distinct ontogenetic stage of dimorphic *Mucor* fungi. When studying fungal lipo-

genesis, the arthrospores should be separated from the mycelial and yeastlike cells.

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