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EXPERIMENTAL ARTICLES

Alterations in the Composition of Membrane Glycero- and Sphingolipids in the Course of *Flammulina velutipes* Surface Culture Development

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Abstract—Qualitative and quantitative characteristics of the alterations in the lipid composition of the membrane of the basidial fungus *Flammulina velutipes* in the course of surface culture development were investigated. Modifications of the lipid composition were shown to be timed to specific ontogeny stages, such as changes in the growth rate of the colonies, the appearance of differentiated vegetative cells, and the formation of generative structures. A slowdown of growth correlated with an alteration in the ratio of major classes of phospholipids, namely, with a decrease of phosphatidylcholine relative content and an increase in phosphatidylethanolamines. The differentiation of vegetative cells of the mycelium proceeded along with modifications of molecular composition of glycoceramides. In the course of the first week of growth, the surface culture of *F. velutipes* produced monohexosylceramides with epoxidized methyl sphingadienine as a sphingoid base. Later on, along with culture growth and specialization of mycelium cells, molecular species with methyl sphingadienine, common for basidiomycetes, start to prevail among the fungal glycoceramides. The formation of fruit bodies is accompanied by enrichment of molecules of phospholipids, mainly, the phosphatidylcholines, with unsaturated fatty acids.

Key words: Flammulina velutipes, phospholipids, glycoceramides, growth, fructification.

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The life cycle of basidiomycetes is coordinated by various internal and external factors. For example, a slowdown of growth followed by initiation of fructification may be induced by specific light and temperature conditions, mechanical damage, or changes in aeration, humidity, and pH [1]. Various external organic compounds, such as glycosides of fatty acids and saponins synthesized by higher plants [2] and glycoceramides (GlCer) [3] may also induce transition to the generative stage.

One of the internal factors triggering the fructification program is activation of α-and/or β-subunits of a G-protein heterotrimeric complex responsible for signal transfer from membrane receptors to the enzymes of various signal systems of the cell [4, 5]. The leading role in transduction of the fructification signal belongs to the system employing adenylate cyclase and cAMP-dependent protein kinase [1]. In a number of cases, transcriptional regulation of genes responsible for the transition to the generative phase may be performed by the signal system of mitogen-activated protein kinase [5]. The relation between fructification and the intensification of the phosphoinositide metabolism is debated. In particular, certain meiosis stages were found to be accompanied by activation of type III phosphoinositide-4 kinases, performing phosphorylation of phosphatidylinositols to phosphatidylinositol 4-monophosphates [6].

Besides phosphatidylinositols, other lipid compounds may affect the growth and development of the fungus. There are data on the effect of sterols [7] and GlCer [8, 9] on the growth and reproductive function. Correlation between the rate of fruiting and accumulation of phospholipids esterified by unsaturated fatty acids was demonstrated [10, 11]. However, the data on relations between growth processes and the ratio of various classes of phospholipids are still rather controversial. The knowledge on the ability of certain molecular species of glycoceramides to initiate the particular program of morphogenesis is insufficient.

The aim of the present work was, by the example of the surface culture of a basidial fungus *Flammulina velutipes*, to elucidate the modifications of the lipid composition of the membrane related to the changes in

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the growth rate and those related to differentiation of the fungus cells and initiation of fructification.

MATERIALS AND METHODS

In this study we used a basidiomycete *Flammulina velutipes* (Curt.: Fr.) Sing. strain 1483 from the Collection of Basidiomycete Cultures of the Komarov Botanical Institute, Russian Academy of Sciences. The strain is characterized by the ability to form fruit bodies at ≤21°C and below and thus was chosen for the study to exclude the temperature effect on the lipid composition and content (for most of the isolates of this species, the temperature of transition to the generative phase is 10–16°C).

Cultures were grown in Petri dishes on wort agar (4° Balling, 1.5% agar) in a thermostat at 25°C. Mycelium disks from the growing edge of a 7-day colony were inoculated onto the center of the Petri dish (mycelium side down). To obtain fruit bodies, the dishes containing 14-day mycelia were moved to the room with the temperature of 21 ± 1°C and natural light (2000–4000 lx, light regime 12 : 12). The vegetative mycelium of 7, 14, 28, and 35 days grown in the dark as well as mycelium along with non-differentiated (28 days) and differentiated (35 days) fruit bodies obtained after incubation in the light were used in the experiment.

The lipids were extracted according to a modified Nichols procedure [12] developed particularly for the organisms with stable lipases. The mycelium was flooded with hot isopropanol, heated for 30 min at 70°C, then homogenized, and centrifuged. The supernatant was separated, the rest of lipids were extracted from the precipitate, first, by isopropanol, then by the isopropanol–chloroform 1 : 1 mixture. Every step of the extraction was performed for 30 min at 70°C. Combined extracts were evaporated on a rotary evaporator, dissolved in chloroform–methanol, 1 : 1, and centrifuged after the addition of water. The lipids-containing chloroform layer of the two-phase system was collected.

Individual phospho- and sphingolipids were analyzed by two-dimension TLC on plates covered with 5–20-μm silica gel (Laine Kalur, Estonia) according to a modified technique of Vaskovsky and Terekhova [13] using chloroform–methanol–toluene–28% ammonia (65 : 30 : 10 : 6) and chloroform–methanol–toluene–acetone–acetic acid–water (70 : 30 : 10 : 5 : 4 : 1) as solvents for the first and the second directions, respectively. Lipid spots were visualized by 5% H_2SO_4 solution in ethanol or in iodine vapors. The lipids were identified using chemical standards and reagents specific for definite functional groups [14].

Glycoceramides were analyzed using a 7-T LTQ FT mass spectrometer (Thermo, Germany) with electrospray ionization (ESI) equipped with a nanoelectrospray apparatus (Proxeon Biosistems, Denmark). The samples were dissolved in a mixture of methanol, acetonitrile, water, and formic acid, 44 : 44 : 10 : 2. The signal was registered in the positive ions mode. The capillary voltage was 1.6 kV. The temperature of the transforming capillary was 225°C. Mass spectra of the lipids were registered in the *m/z* range from 150 to 1500. Separate ions of GlCer were fragmented at approximately 30 eV in an ion trap of the apparatus. Fragmented ions were registered both in the ion trap and in the FT part of the mass spectrometer. Some of the fragmented ions were analyzed repeatedly by MS–MS. Mass spectra were processed by the Xcalibur software package and interpreted manually.

The contents of the total GlCer and of individual phospholipids were determined using a densitometer (DenSkan, Russia). Standard solutions of phosphatidylcholine and galactosylceramide (Sigma) were used to build the calibration curves. The content of total neutral lipids represented mainly by triglycerides and sterols was determined as the difference of mass between total lipids (by weight) and phospholipids together with GlCer.

Fatty acids were analyzed in the form of methyl esters obtained by methanolysis with 2.5% H_2SO_4 in methanol at 70°C for 2 h [14]. Methyl esters of fatty acids purified beforehand were separated on a gas–liquid chromatograph (Kristall 5000.1, Russia) using an HP-23 capillary column (Hewlett–Packard), 30 m × 0.32 mm × 0.25 μm; the temperature regime used: 3 min at 170°C followed by a linear increase up to 220°C at 4°C/min. The temperature of the injector was 230°C and of the detector, 250°C. Nitrogen was used as a carrier gas. Fatty acid identification was performed by comparison of retention times with standard samples (Sigma).

The results of two independent experiments with 3–4 recordings in each were statistically processed, and the data obtained were presented as $M \pm m_M$, M standing for means and m_M, their standard error. The significance of the differences was assessed using Student's criterion.

RESULTS AND DISCUSSION

Observation of the development of the *F. velutipes* surface culture revealed that under the experimental conditions the most active growth of the vegetative mycelium occurred during the first 14 days of cultivation (Table 1). Then, biomass accumulation slowed down and completely stopped after 28 days of cultivation.

A number of peculiarities were revealed by morphological examination of the growing culture. Seven- and 14-day mycelia were represented mostly by thin-walled hyaline undifferentiated hyphae 1.0–5.0 μm in diameter with regular clamps and also with conidiegenous hyphae and arthroconidia. Diameter of the 28- and 35-day hyphae varied from 1.5 to 7.0 μm, multiple large differentiated cells with granular contents were pre-

Table 1. Biomass accumulation and content of the major lipid fractions in the course of development of the *F. velutipes* surface culture

Stage of development and culture age	Cultivation conditions	Dry weight[1], mg	Total lipid contents, % to dry weight	Major lipid fractions content[2], % to the total lipids		
				NL	GL	PL
Vegetative mycelium, 7 days	Dark, 25°C	8.0 ± 1.0	11.5 ± 0.9	81.3 ± 2.4	1.9 ± 0.1	16.8 ± 1.3
Vegetative mycelium, 14 days	Dark, 25°C	37.9 ± 2.6	9.3 ± 0.3	69.7 ± 1.2	2.7 ± 0.2	27.6 ± 1.1
Vegetative mycelium, 28 days	Dark, 25°C	99.7 ± 15.1	5.0 ± 0.1	64.1 ± 1.1	2.8 ± 0.2	33.1 ± 1.0
Mycelium with undifferentiated fruit bodies, 28 days	Light 12 : 12, 20°C	124.8 ± 33.1	6.4 ± 0.5	72.9 ± 3.2	2.7 ± 0.1	24.4 ± 2.0
Vegetative mycelium, 35 days	Dark, 25°C	116.8 ± 3.2	5.5 ± 0.2	62.2 ± 1.2	4.4 ± 0.2	33.4 ± 1.5
Mycelium with differentiated fruit bodies, 35 days	Light 12 : 12, 20°C	118.7 ± 21.0	9.3 ± 0.5	63.1 ± 2.9	5.6 ± 0.3	31.3 ± 2.2

[1] Average values for colony biomass developed in a single Petri dish are presented.

[2] Average values of the relative content of total phospholipids (PL), glycolipids (GL), represented by glycoceramides, and neutral lipids along with lipophilic pigments (NL).

sented, while the amount of conidiegenous hyphae and arthroconidia decreased significantly. The following modifications were registered upon exposure of the cultures to light and decrease of the temperature: the number of pigmented cells increased and the hyphae became more aggregated (1.5–5.0 μm thick). After 2 weeks under new conditions, multiple primordia appeared on the surface of the colony, part of them developed into mature fruit bodies. At this point, the 28-day cultures exposed to light were characterized by a predominance of thin-walled hyaline undifferentiated hyphae and continuing arthroconidiegenesis; differentiated cells with thick walls and granular contents constituted an insignificant part of the mycelium. Thirty five-day cultures appeared less homogeneous, the hyphae varied in thickness and color, and the number of arthroconidia decreased.

As was revealed by the analysis of total lipid content, the lipids were mostly abundant (per 1 g of dry biomass) in actively growing 7- and 14-day mycelia (Table 1). Further growth in the dark and exhaustion of substrate sources led to a decrease in the concentration of lipids. A greater amount of lipophilic compounds was found in the mycelium with fruit bodies grown in the light. The relative content of total lipids in the cultures with mature fruit bodies was 1.5 times higher than in those with primordia.

It should be pointed out that most of the lipids synthesized by young mycelium were deposited in the form of neutral triacylglycerides, which then were probably used for synthesis of other classes of lipids. According to the data presented in Table 1, the relative content of neutral lipids in 7-day mycelium was 81%, while in the 14-day, only 70%. Further growth and development of the culture led to an even greater decrease in neutral lipids, with the minimum content recorded for the 35-day mycelium.

Certain modifications were revealed in the fraction of phospholipids. In particular, the growth of the *F. velutipes* surface culture was accompanied by modifications in the ratio between major classes of individual phospholipids, phosphatidylcholines (PC) and phosphatidylethanolamines (PE) (Table 2). Phosphatidylcholine content in the 7-day culture was more than two times higher than that of PE, while for the 14-day mycelium the PC/PE ratio was 1.46. Growth slowdown observed for 28- and 35-day cultures in the dark was accompanied by a further decrease of the PC/PE ratio down to 0.83 and 0.98, respectively. An analogous correlation between this ratio and the culture's age was registered in the case of mycelia at the stage of fructification. Earlier, similar results were obtained for the surface culture of *Lentinus edodes* [15]. Young and actively growing mycelium of the fungus was characterized by the highest PC content, exceeding twofold the concen-

Table 2. Major membrane glycero- and sphingolipid content in the course of development of a surface culture of *F. velutipes*

Stage of development	Lipid content, mg g^{-1} dry weight					
	PC	PE	PS	PA	DPG	GlCer
Vegetative mycelium, 7 days	11.42 ± 0.66	4.71 ± 0.60	2.02 ± 0.21	0.54 ± 0.09	0.70 ± 0.15	2.22 ± 0.28
Vegetative mycelium, 14 days	10.38 ± 0.78	7.10 ± 0.69	3.04 ± 0.07	4.18 ± 0.58	1.05 ± 0.10	2.96 ± 0.14
Vegetative mycelium, 28 days	6.06 ± 0.75	7.34 ± 0.64	1.39 ± 0.12	1.22 ± 0.11	0.39 ± 0.01	1.58 ± 0.08
Mycelium with undifferentiated fruit bodies, 28 days	5.56 ± 0.42	3.64 ± 0.05	1.65 ± 0.07	1.34 ± 0.09	0.42 ± 0.04	1.53 ± 0.12
Vegetative mycelium, 35 days	6.98 ± 0.24	7.15 ± 0.54	1.53 ± 0.09	1.58 ± 0.26	0.79 ± 0.03	2.16 ± 0.22
Mycelium with differentiated fruit bodies, 35 days	13.16 ± 0.56	12.24 ± 1.49	6.22 ± 0.47	2.45 ± 0.25	1.66 ± 1.13	6.27 ± 0.32

Abbreviations: PC, phosphatidylcholines; PE, phosphatidylethanolamines; PS, phosphatidylserines; PA, phosphatidic acids; DPG, diphosphatidylglycerols; and GlCer, glycoceramides.

tration of PE at the stage. Along with culture aging, the ratio changed to 1.4–1.5 depending on the exact age.

The data presented above argue against the accepted view of PC being rather inert compounds mostly abundant in resting cells [7]. Indeed, by the example of the mucoraceous mold *Blakeslea trispora*, membrane lipids of spores in the state of exogenous and endogenous rest were shown to have a much higher PC/PE ratio than the lipids of actively growing mycelium [16]. Similar data were obtained for the spores of *Agaricus bisporus* and fruit bodies of *Pleurotus ostreatus* [17]. Transition of a submerged culture of *Saccharomyces cerevisiae* from the logarithmic to the stationary growth phase was also accompanied by an increase in PC/PE ratio from 0.9 to 2.2 [18]. The physiological role of the PC portion increases at the stage of slowing down of growth processes is usually linked to increased stability of these compounds towards oxidation providing long-term maintenance of viability for the cells. On the other hand, as it has been demonstrated by the example of *Aspergillus nidulans*, increase in PC content may also be connected to the active growth of the colony through enlargement of certain cells and intensive proliferation [19]. Besides, enhanced PC synthesis in actively growing cultures may be due to the association of this process with the intracellular vesicular transport, reactions of signal reception and transduction, and synthesis of a number of long-chain unsaturated fatty acids requiring solely PC to proceed [20]. Finally, active PC metabolism may be in connection with the functioning of the phosphatidate signal system and accumulation of various phosphatidic acids necessary for particular phases of morphogenesis [21].

Besides the alterations in the PC/PE ratio, the development of the *F. velutipes* surface culture was also accompanied by modifications in the fatty acid composition of the lipids. In particular, fruit body formation correlated with an increase in the content of linolenic acid (Tables 3 and 4). Moreover, its maximum content was detected in the PC fraction which may be evidence of substrate specificity of the desaturases responsible for linolenic acid synthesis. Earlier, by the example of *L. edodes*, the increase in the degree of desaturation associated with fruiting was shown to be in connection with enhanced expression of the genes *Le-fad1* and *Le-fad2* coding for Δ9- and Δ12-desaturases, respectively. Interestingly, during fructification the *Le-fad1* expression level was several times higher than at adaptation to low temperature conditions [10], while increased expression of *Le-fad2* was only characteristic of the generative phase and was not related to the cold shock [11]. Increased unsaturated fatty acid content may be due to their involvement in the synthesis of oxylipins possessing hormonal activity. For example, in an ascomycete *A. nidulans* oxygenated derivatives of linoleic acid, 8-hydroxy-9,12-octadecadiene and 8,11-dihydroxy-9,12-octadecadiene acids, were demonstrated to induce the formation of ascospores and to inhibit conidia formation [22]. Recently, similar compounds were isolated from a basidial fungus *Agaricus bisporus* [23].

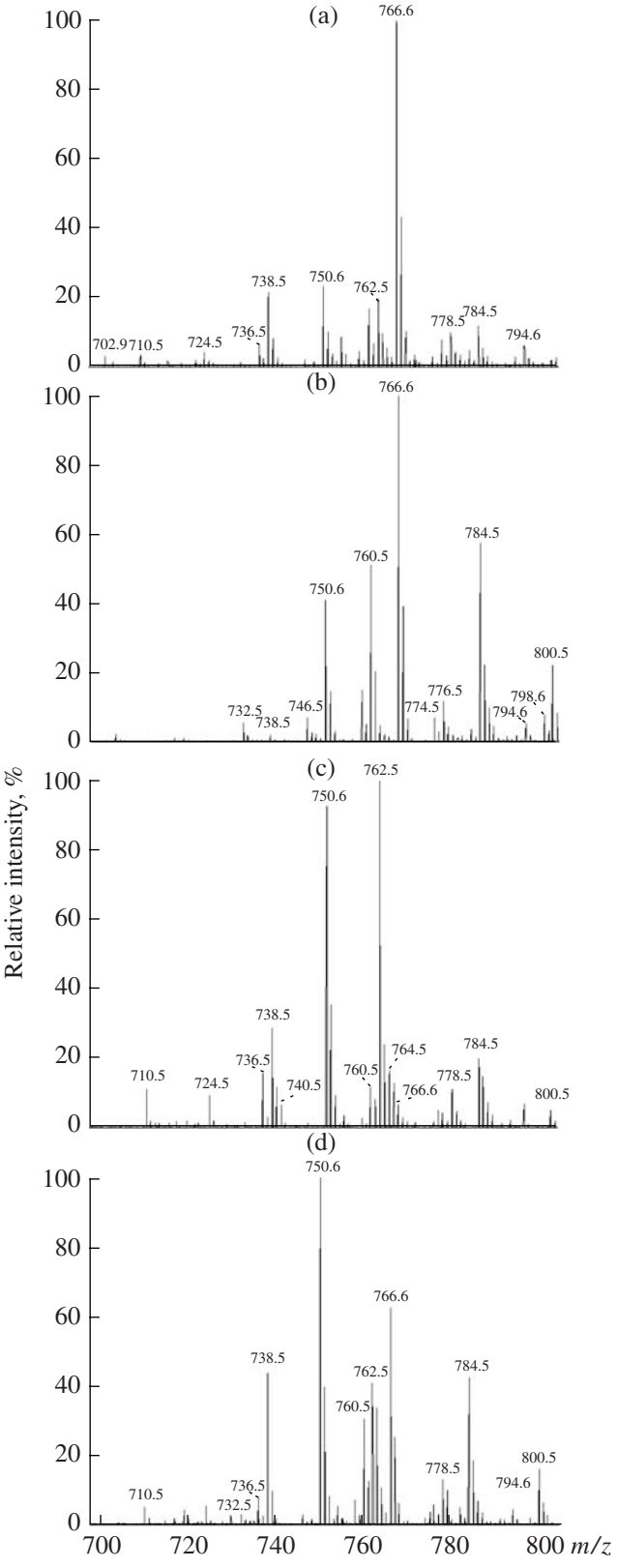
In the course of *F. velutipes* growth and development, the most significant changes were revealed in the GlCer fraction. The major modifications did not concern the total GlCer content (Table 2), yet altered the ratio between individual molecular species of the lipids. Mass spectra of cationized molecular ions $[M + Na]^+$ of the GlCer fraction obtained by electrospray ionization mass spectrometry (ESI–MS) are presented on the figure. In the spectrum, GlCer isolated from the 7-day mycelium were mainly represented by molecular ions with the *m/z* value 766. Ions with *m/z* 738, 750,

778, 784, 794 were far less abundant. In the GlCer fraction of 14-day mycelium, *m/z* 766 ions were still predominant, whereas the amount of *m/z* 750, 760, 762, and 784 ions increased significantly. In a 28-day culture grown in the dark and characterized by a low growth rate and by the appearance of thick-walled differentiated cells, *m/z* 766 ion was present in trace amounts. In this case, the ions with *m/z* of 750 and 762 were dominating. *F. velutipes* cultures grown in the light and possessing fruit bodies were similar to 28-day vegetative cultures.

Based on the data of tandem mass spectrometry analysis ($^+$ESI MS/MS) of the individual molecular species of GlCer, *m/z* 750 and 778 ions were established to be monohexosylceramides containing methyl sphingadienine (2-amino-1,3-dihydroxy-9-methyl-4,8-octadecadiene) as a sphingoid base and $C_{16:0(OH)}$ or $C_{18:0(OH)}$ fatty acids, respectively. Ions with *m/z* of 760 and 762 belong to the same group and contain $C_{18:1}$ and $C_{18:0}$ residues. The structure of another group of monohexosylceramides represented by *m/z* 738, 766, and 794 ions, is based upon an oxidized form of methyl sphingadienine (presumably, 2-amino-1,3-dihydroxy-4-epoxy-9-methyl-8-octadecene) and $C_{14:0(OH)}$, $C_{16:0(OH)}$, and $C_{18:0(OH)}$ fatty acids, respectively. The ions of *m/z* 740 and 784 were not identified.

Glycoceramides containing methyl sphingadienine as a sphingoid base and $C_{16–18}$ fatty acids or their hydroxy derivatives are rather typical of many fungi, including basidiomycetes. They were found in *Amanita muscaria*, *A. rubescens*, *Ganoderma lucidum*, *Lentinus edodes*, and *Polyporus ellisii* [24]. As for GlCer with epoxidized methyl sphingadienine, this is the first time a compound of this kind was isolated from fungi, although epoxidized forms of other sphingoid bases, particularly sphingosine, had already been reported [25]. To the best of our knowledge, there is a single study performed on a pathogenic organism *Fonsecaea pedrosoi* reporting the ability of the fungi to synthesize GlCer with oxidized methyl sphingadienine [26]. According to the authors of the study, the oxygen function is represented by an enol hydroxyl group at the C-4 double bond. The stable tautomer form in the case is a C-4 ketone. The possibility of the double bond oxidation to a ketone requires serious justification, which is absent in the cited work; meanwhile, epoxide group formation seems more reasonable in view of the available data on the distribution of similar compounds in nature [27].

In the recent years, new data appeared in literature, indicating that GlCer with methyl sphingadienine as a sphingoid base may have a significant effect on the growth and development of fungi. By the example of *Cryptococcus neoformans* using the immunofluorescence analysis, the compounds were shown to concentrate by the spots of contact of dividing cells. The addition of antibodies specifically binding GlCer to the cultivation medium led to partial inhibition of cell division



ESI-MS spectra of cationized molecular ions $[M + Na]^+$ of glycoceramides isolated from the surface culture of *F. velutipes*. Vegetative mycelium of 7 (a), 14 (b), and 28 days (c), respectively; 28-day old mycelium with fruit bodies (d).

Table 3. Fatty acid composition of phosphatidylcholines in the course of development of a surface culture of *F. velutipes*

Stage of development	Fatty acid composition, % of the total											
	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{16:2}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	$C_{22:0}$	$C_{22:x}$
Vegetative mycelium, 7 days	2.2 ± 0.3	1.4 ± 0.2	17.4 ± 0.9	4.6 ± 0.4	Traces	4.9 ± 0.8	11.4 ± 0.6	46.8 ± 5.3	4.0 ± 0.3	2.7 ± 0.7	1.4 ± 0.2	Traces
Vegetative mycelium, 14 days	1.3 ± 0.2	1.8 ± 0.1	20.0 ± 1.3	5.1 ± 0.4	Traces	4.0 ± 1.0	14.5 ± 1.2	29.8 ± 2.2	2.7 ± 0.1	7.6 ± 0.1	1.5 ± 0.2	5.7 ± 0.8
Vegetative mycelium, 28 days	0.7 ± 0.1	1.3 ± 0.1	16.7 ± 0.9	3.2 ± 0.8	0.8 ± 0.1	4.5 ± 0.2	15.7 ± 0.4	48.0 ± 1.3	1.4 ± 0.3	0.7 ± 0.1	2.9 ± 0.3	2.4 ± 0.5
Mycelium with undifferentiated fruit bodies, 28 days	1.0 ± 0.2	1.0 ± 0.1	15.8 ± 0.9	3.1 ± 0.0	Traces	3.2 ± 0.3	11.1 ± 0.7	51.2 ± 3.8	5.8 ± 0.5	1.4 ± 0.1	0.9 ± 0.2	2.2 ± 0.5
Vegetative mycelium, 35 days	0.9 ± 0.2	1.2 ± 0.5	16.8 ± 0.9	2.3 ± 0.4	1.1 ± 0.1	4.3 ± 1.1	21.4 ± 2.3	39.5 ± 2.6	2.4 ± 0.3	3.4 ± 0.4	1.4 ± 0.3	4.9 ± 1.0
Mycelium with differentiated fruit bodies, 35 days	1.2 ± 0.1	1.3 ± 0.1	12.8 ± 0.7	2.9 ± 0.3	0.7 ± 0.0	4.8 ± 0.4	11.5 ± 0.7	46.0 ± 1.6	14.4 ± 1.2	1.0 ± 0.1	1.7 ± 0.2	1.3 ± 0.3

Table 4. Fatty acid composition of phosphatidylethanolamines in the course of development of a surface culture of *F. velutipes*

Stage of development	Fatty acid composition, % to the total											
	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{16:2}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	$C_{22:0}$	$C_{22:x}$
Vegetative mycelium, 7 days	2.4 ± 0.4	2.6 ± 0.2	24.1 ± 1.2	9.6 ± 0.8	2.0 ± 0.1	4.7 ± 0.4	15.5 ± 1.0	27.2 ± 3.7	1.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.3	Traces
Vegetative mycelium, 14 days	2.0 ± 0.2	2.4 ± 0.2	20.2 ± 1.6	4.7 ± 0.5	1.8 ± 0.3	4.0 ± 0.4	11.9 ± 0.5	40.3 ± 2.2	1.7 ± 0.2	0.8 ± 0.1	1.9 ± 0.2	1.4 ± 0.5
Vegetative mycelium, 28 days	2.1 ± 1.1	2.4 ± 0.5	20.0 ± 0.8	2.5 ± 0.4	2.8 ± 0.2	3.3 ± 0.1	12.7 ± 1.1	47.4 ± 2.3	1.1 ± 0.2	1.5 ± 0.3	2.4 ± 0.4	1.6 ± 0.2
Mycelium with undifferentiated fruit bodies, 28 days	1.9 ± 0.1	2.0 ± 0.1	20.5 ± 0.6	3.4 ± 0.2	0.7 ± 0.1	3.4 ± 0.3	9.5 ± 0.1	44.4 ± 3.0	3.8 ± 0.6	1.5 ± 0.1	1.5 ± 0.3	0.9 ± 0.3
Vegetative mycelium, 35 days	2.5 ± 0.3	2.8 ± 0.3	22.7 ± 1.2	2.4 ± 0.4	3.5 ± 0.2	3.3 ± 0.3	15.0 ± 1.3	40.1 ± 4.3	0.7 ± 0.2	2.9 ± 0.7	1.6 ± 0.2	1.0 ± 0.1
Mycelium with differentiated fruit bodies, 35 days	1.3 ± 0.3	1.6 ± 0.2	17.2 ± 0.4	2.3 ± 0.3	1.3 ± 0.1	4.2 ± 0.2	13.2 ± 0.8	42.4 ± 1.1	9.2 ± 0.2	2.2 ± 0.4	1.2 ± 0.1	2.0 ± 0.1

and, consequently, to a decrease in the growth rate of the colony [24]. More numerous are the data on the role of methyl sphingadienine-based GlCer in differentiation of fungi cells. In *Pseudallescheria boydii* cultures binding of antibodies to GlCer was shown to inhibit the generation of specialized cells responsible for the pathogenic fungus pervasion into the host organism, not affecting the growth rate [28]. In a series of experiments carried out by Kawai et al. on asco- and basidiomycetes, the ability of methyl sphingadienine-containing GlCer to initiate or accelerate fructification was demonstrated [3, 8, 29].

The properties of GlCer as bioeffectors depend strongly upon their structure. For example, in antibody-binding reactions the presence of alpha-hydroxyl in the fatty acid residue of GlCer is important [24, 30]. The length of hydrocarbon chains of fatty acids influences their ability to stimulate the formation of fruit bodies [3, 8]. By the example of *Lentinus edodes*, GlCer with $C_{14–18}$ acids were found to induce fructification more actively than GlCer with $C_{20–22}$ acids [29]. Further increase of the chain length leads to a complete loss of biological activity of the compounds. Less is known about the physiological meaning of the modifications in sphingoid bases. In a study performed with *Pichia pastoris*, mutation of C9-methyltransferase, responsible for methylation of sphingadienine, was not shown to be lethal or to inhibit the growth of the fungus [31]. In the authors' opinion, sphingadienine methylation is of importance at definite stages of the life cycle, for example, at the transition to fruit body formation. Indeed, fungal GlCer containing methyl sphingadienine were shown to be better inductors of fructification than plant GlCer, comprising fatty acids and glycosides similar to fungal in combination with other sphingoid bases such as sphingosine and phytosphingosine [3].

There is good reason to believe the development of a fungus culture is accompanied by some rearrangements in the structure of GlCer. Moreover, as follows from the present study, these modifications may affect even the most conservative part of the molecule, i.e. its sphingoid base. In fact, the relative content of the GlCer molecular species containing the oxidized form of methyl sphingadienine decreases along with the development of the *F. velutipes* surface culture. In a 7-day vegetative mycelium, the total relative content of compounds of such (including *m/z* 738, 766, and 794) was 68%, while in a 14-day, only 47%. The subsequent development of the culture with formation of specialized cells and growth deceleration occurred on the background of further decrease in GlCer containing oxidized methyl sphingadienine. In a 28-day mycelium their content didn't exceed 16%, and in cultures with primordia and differentiated fruit bodies, 32 and 30%, respectively. Earlier, the attachment of an extra oxygen atom to methyl sphingadienine was registered in sclerotium cells of a pathogenic fungus *Fonsecaea pedrosoi* [26]. Interestingly, oxidized GlCer didn't react with the antibodies against methyl sphingadienine-containing GlCer. As a result, sclerotium cells were found to be more resistant against the host's immune system than the vegetative mycelia. Therefore, presumably, in the life cycle of a number of fungi, stages exist whose stability depends upon structural modifications of GlCer. Apparently, the modified GlCer ensure fungus resistance against the protective systems of surrounding organisms, which may be of importance not only for parasites, but also for saprotrophic fungi at the start of colony development.

Summarizing the results obtained, it may be concluded that the development of the surface culture of the basidial fungus *F. velutipes* is accompanied by certain rearrangements in the composition of membrane lipids. These modifications affect both the major matrix lipids made up of phospholipids and lipid rafts mainly based on GlCer. Modifications of the lipid composition are timed to various events of ontogeny including the transition from the active linear to the stationary growth phase, the appearance of differentiated vegetative cells, and the start of fructification. Decrease in the growth rate and intensification of differentiation processes correlate with alterations in the ratio between major classes of phospholipids (PE portion increases) and individual molecular species of GlCer (the concentration of molecular species with an additional oxygen atom in the sphingoid base decreases); fruit body formation is accompanied by an increase in the relative content of unsaturated fatty acids in phospholipid molecules, particularly, in PC.

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