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EXPERIMENTAL ARTICLES

Formation of Diacylglyceryltrimethylhomoserines in the Surface Culture of the Basidiomycete *Flammulina velutipes*

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Abstract—Betaine-type lipids—diacylglyceryltrimethylhomoserines (DGTS)—were revealed in the mycelium of the basidial fungus *Flammulina velutipes* obtained by surface cultivation on agarized malt extract. DGTS accumulation was shown to occur at the late stages of culture development under deficiency of a complex of nutrients, including nitrogen, phosphorus, potassium, and trace elements. Induction of the synthesis of betaine lipids in *F. velutipes* occurred against the background of a decreased rate of growth of the vegetative mycelium, formation of monilioid hyphae, and inhibition of fructification. The relationship between DGTS formation and the environmental factors (temperature, illumination) was studied. It was established that the most active DGTS accumulation occurred at 15°C in the dark.

Keywords: *Flammulina velutipes,* basidiomycetes, surface culture, membrane lipids, betaine lipids, diacylglyceryltrimethylhomoserines

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Betaine lipids were first revealed in the representatives of *Chrysophyta* [1]. Later, the compounds of this group were identified in many species of green and brown algae, cryptogamic plants, and in certain species of α-proteobacteria [2–4]. In fungi, the betaine lipids represented by one class of compounds, diacylglyceryltrimethylhomoserines (DGTS), were revealed in the fruit bodies of wild asco- [5–7] and basidiomycetes [8–11].

Betaine lipids are the structural analogs of phosphatidylcholine (PC), one of the main membrane phosphoglycerolipids. Similar to PC, they contain the trimethylammonium group in the polar part of the molecule, but, as distinct from this phospholipid, they do not have the phosphoric acid residue. Synthesis of betaine lipids and PC occurs independently [12]. PC biosynthesis is carried out via two alternative pathways: in the reactions of CDP-choline condensation with diacylglycerol or by three-step *N*-methylation of phosphatidylethanolamine (PE) by methyltransferases utilizing *S*-adenosylmethionine as a donor of methyl groups. In fungal cells, PC formation predominantly occurs via PE methylation. The biosynthesis of the DGTS betaine lipids includes the addition of homoserine to diacylglycerol with the subsequent *N*-methylation of the diacylglycerylhomoserine formed with the participation of methyltransferases and *S*-adenosylmethionine. For *Rhodobacter sphaeroides* and *Chlamydomonas reinhardtii*, it was shown that the condensation and methylation reactions in bacteria were catalyzed by two separate enzymes, whereas in green algae, by one enzyme [13, 14]. The specific features of the synthesis of betaine lipids in fungal cells have not been studied in detail yet. However, the screening of bacterial and fungal genomes revealed the presence in *Candida albicans* and *Aspergillus fumigatus* of the sequences homologous to the *btaA* and *btaB* genes encoding the enzymes of DGTS biosynthesis in bacteria [4].

The ability to synthesize betaine lipids of a certain class, as well as the DGTS to PC ratio in algae and cryptogamic plants, are regarded as a taxonomic criterion [15]. Certain correlations between the DGTS/PC ratio and the taxonomic position of the species are also traced in the system of asco- and basidiomycetes. For example, in the basidial fungi of the order *Boletales*, DGTS and PC are the main membrane lipids; in *Agaricales* (mainly in the species of the family *Tricholomataceae*), DGTS are present only as a minor component, and in *Russulales*, betaine lipids are not synthesized at all [8, 10, 11]. However, the opinion about the taxonomic significance of betaine lipids in fungi is not shared by all authors. In contrast to photosynthesizing organisms, both DGTS-synthesizing and non-DGTS-synthesizing species can indeed be found within most orders of fungi. In particular, agaricoid basidiomycetes constitute such a group, which is heterogeneous by this trait [10, 11]. The reasons for the

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unstable formation of betaine lipids in the representatives of this taxonomic group are not known. It is possible that accumulation of DGTS in the fungi of the order *Agaricales* occurs only under stress conditions. Similar to other organisms, a whole complex of internal and external factors, such as phosphate limitation [16, 17] and dehydration [18], probably act as an inducer triggering the synthesis of betaine lipids.

In the agaricoid basidiomycete *Flammulina velutipes* (Curt.: Fr.) Sing., strain 1483, used as a model subject in this work, the formation of DGTS occurred under surface cultivation conditions on agarized media prepared on the basis of Biokar Diagnostics and Fluka malt extracts. The betaine lipids were absent in the mycelium and the fruit bodies of this strain grown on wort agar [19], as well as in the fruit bodies of wild *F. velutipes* [11].

The aim of the present work was to reveal the factors determining the accumulation of betaine lipids in the agaricoid basidiomycete *F. velutipes* in the course of culture development and to establish a relationship between this process and the morphology of its mycelium.

MATERIALS AND METHODS

Strain 1483 of the basidial fungus *Flammulina velutipes* (Curt.: Fr.) Sing. from the Collection of Basidiomycete Cultures of the Komarov Botanical Institute, Russian Academy of Sciences, was used as the study subject. The formation of fruit bodies in this strain occurs at ≤21°C in the light and depends on the medium composition. In the collection stock, the strain was maintained using the subculture method on wort agar slants at 4°C. For the experiments, the cultures were grown in petri dishes on the agarized medium prepared on the basis of malt extract (Biokar Diagnostics, France) (malt extract, 15 g/L; agar, 20 g/L). Inoculation was carried out at the center of the petri dish with a mycelial disk (mycelium down) taken from the growing margin of a nine-day colony. The cultivation was carried out for nine days in the thermostat at 25°C; the dishes with mycelium were then transferred to the environmental chamber (Sanyo MLR-351H, Japan) with a temperature of 10, 15, or 20°C.

Cultivation in the chamber was carried out in the dark or at 2000 lx (light regime 12 : 12). The 9-, 15-, 21-, and 39-day vegetative mycelium was used for lipid analysis. The cultures aged 7, 21, and 39 days grown in a similar way on agarized media with the Fluka malt extract (Germany) (malt extract, 15 g/L; agar, 20 g/L), the Senson wort malt concentrate (Finland) (Maltax10 malt extract diluted with distilled water to 4°Bx, 1 L; agar, 15 g), and the native hops-free wort produced by the Vasileostrovskii brewery (Russia) (wort diluted with distilled water to 4°Bx, 1 L; agar, 15 g) were also analyzed for comparison.

The mycelium micromorphology was studied with light microscopy using Carl Zeiss Axio Scope A1 differential interference contrasting (DIC). The state of the mycelium from different colony sites (the central and marginal zones) was analyzed. The colony macromorphology was described according to the standard method [20].

Lipid extraction was carried out according to the Nichols method [21] with modifications [19]. For this purpose, the mycelium was homogenized with isopropanol, heated for 30 min at 70°C, and centrifuged. The supernatant fluid was discarded, and the remaining lipids were extracted from the pellet with isopropanol and the isopropanol–chloroform mixture (1 : 1). The pooled extracts were evaporated in a rotary evaporator, dissolved in 3 mL of the chloroform–methanol (1 : 1) mixture, supplemented with 4 mL of 2.5% aqueous NaCl, and centrifuged. The lipid-containing chloroform layer was sampled from the two-phase system formed.

Individual classes of phospho-, betaine, and sphingolipids were analyzed using thin-layer chromatography (TLC) on 10 × 10 cm plates (Merck, Germany) in the system of solvents: chloroform–methanol–water (65 : 25 : 4) in the first direction and chloroform–acetone–methanol–acetic acid–water (50 : 20 : 10 : 10 : 5) in the second direction) [16]. The chromatographic zones corresponding to the lipid compounds were visualized, depending on the aims of the study, with 5% H_2SO_4 solution in methanol or with iodine vapors. The lipids were identified using the standard reference spots and specific reagents for individual functional groups [22]. The amounts of glycero- and sphingolipids were determined densitometrically using a Denscan device (Lenchrom, Russia). The content of separate classes of lipids on the chromatograms was calculated using the DENS-14-12-03 software in the linear approximation mode with the calibration curves constructed according to the standard PC and cerebrosides solutions (Sigma, Germany).

The composition of the PC and DGTS molecular species was determined from the m/z value using the high-performance time-of-flight mass spectrometer with the orthogonal inlet and the electrospray ionization ion source (ESI-TOF) MX5310 developed at the Institute for Analytical Instrumentation, Russian Academy of Sciences. All the spectra were acquired in the positive ion mode, in the 150–1500 m/z range. The nebulizing potential on the capillary was 3.3 kV; the desolvation gas temperature was 50°C. The samples for ESI^+-MS analysis were dissolved in the methanol–acetonitrile–formic acid mixture (49 : 49 : 2). The sample volume was 10–50 μL; the sample supply rate, 1–5 μL/min.

Fatty acid (FA) methyl ethers were analyzed using GLC–MS on an Agilent 6850 chromatograph with a 5975C quadrupole mass-spectrometer as a detector and a Supelco Omega Wax 250 30-m capillary column

Table 1. Macro- and trace element content in the organic substrates* of the nutrient media used for cultivation of *F. velutipes*

Nutrient medium	Macroelements, mg/L					Trace elements, μg/L	
	N	P	K	Mg	Fe	Zn	Cu
Malt agar 1	81	52	55	0.25	0.06	21	3.2
Malt agar 2	62	40	87	0.15	0.09	74	2.1
Wort agar 1	140	98	224	2.03	0.53	119	82.4
Wort agar 2	166	104	120	1.60	1.45	109	28.2

Note: * The macro- and trace element content values in 1 L of a liquid nutrient medium (without agar) are given. Malt agar 1 is the medium based on the Biokar Diagnostics malt extract (France); malt agar 2 is the medium based on the Fluka malt extract (Germany); wort agar 1 is the medium based on the Maltax 10 Senson wort concentrate (Finland); wort agar 2 is the medium based on the hops-free beer wort (Vasileostrovskii brewery, Russia).

with an inner diameter of 0.25 mm and the immobile phase film thickness of 0.25 μm. The mass-selective detector was used in the mode of recording the total ion current; the scan range varied between 50 and 500 *m/z*; the scanning rate was 2 scans/s. FA methyl ethers were obtained by lipid hydrolysis with 2.5% H_2SO_4 in methanol at 70°C for 2 h [22]. To carry out chromatographic analysis of the FA derivatives, the following regime was used: the injector temperature, 250°C; split, 1 : 20; the carrier gas (He) rate, 1.0 mL/min; the oven temperature, 170°C for 3 min followed by a linear increase to 220°C at a rate of 4°C/min. The standard NIST mass-spectrometry library, as well as the retention time values, which were compared with the references available (Sigma, United States), were used for compound identification.

The amount of K, Mg, and trace elements in the organic substrates of the nutrient media (malt extracts the wort concentrates) was determined with the atomic-adsorptive method on a KVANT-AFA spectrometer (Russia). For this purpose, the dried extracts were subjected to dry combustion in a muffle furnace at 450°C for 12 h. Macro- and trace elements were determined in the ash dissolved in 2 N HCl and adjusted with double-distilled water to the specified volume [23].

The data obtained after statistical processing of the experiment carried out in 3–4-fold biological replicates were represented as $M \pm m_M$, where M is the arithmetic mean and m_M is the arithmetic mean error. The significance of the differences in the lipid composition and content in the cultures at different stages of their life cycle or under different cultivation conditions was assessed using the Student's *t*-test at the confidence level of significance $P_l = 95\%$. Only statistically significant differences are discussed in the article.

RESULTS AND DISCUSSION

Comparative analysis of the organic substrates present in the nutrient media used for the cultivation of *F. velutipes* (including the media stimulating the formation of the betaine-type lipids—DGTS, untypical of this species) revealed substantial differences in the macro- and trace element content (Table 1). According to the results obtained, the nutrient media, malt agar 1 and malt agar 2, compared to the wort media, contained half as much nitrogen and phosphorus and were much more inferior in terms of the amounts of potassium, magnesium, iron, and trace elements. In addition, taking into account the specific features of medium dilution (the malt extracts, 15 g/L; the wort concentrates, 4°Bx), they had a smaller total carbohydrate content. The *F. velutipes* cultures grown on depleted media exhibited lower biomass accumulation rates and a lesser density of the aerial mycelium. They were also characterized by emergence of monilioid hyphae at the late stages of development and the absence of basidiomes when they were grown under the temperature and light conditions stimulating fruiting (Table 2). These changes were accompanied by induction of DGTS synthesis. Note that, on the malt agar 1 medium, the accumulation of betaine lipids appeared to be more substantial and occurred at the later developmental stages. This medium was used for detailed investigation of the changes in the DGTS composition and content in the culture ontogenesis, as well as for the establishment of a relationship between accumulation of the betaine lipids and temperature and illumination.

At the early stages (up to 9–15 days), the *F. velutipes* cultures grown on the malt agar 1 medium were represented by the vegetative mycelium consisting mostly of fine, moderately branching undifferentiated hyphae 1.3–2.7 μm in diameter. The content of differentiated hyphae, including conidiogenic ones, and hyphae with thickened cell walls (3.0–5.0 μm in diameter) and granular contents (1.5–5.0 μm), was insignificant. Further culture development was accompanied by increased differentiation of mycelial cells (the proportion of thick-walled hyphae, as well as of the hyphae with various types of inclusions, increased). Simultaneously, the accumulation of DGTS occurred. At the later stages of development (39 days), monilioid hyphae appeared in great numbers; fruit bodies did not

Table 2. Morphological and biochemical features of *F. velutipes* cultures

Nutrient medium	Colony diameter (6 days), cm	Dry weight* of mycelium (6 days), mg	Aerial mycelium density (21 days)	Presence of monilioid hyphae (39 days)	Formation of fruit bodies (21–39 days)	DGTS formation
Malt agar 1	7.8 ± 0.2	0.8 ± 0.1	+	++	–	++
Malt agar 2	7.7 ± 0.1	0.8 ± 0.2	+	++	–	+
Wort agar 1	6.4 ± 0.4	1.7 ± 0.2	++	–	++	–
Wort agar 2	7.2 ± 0.2	2.1 ± 0.4	++	–	++	–

Note: * The average biomass values of the colony developing in one petri dish are shown. Cultivation conditions: 9 days in the dark at 25°C followed by 30 days in the illumination regime (12 : 12) at 15°C.

form. In mycological literature, simple or branching hyaline or pigmented hyphae consisting of short, sometimes barrel- or pear-shaped cells with a length to width ratio of 1–3 : 1 are referred to as monilioid [24, 25]. It is suggested that the formation of monilioid hyphae precedes their fragmentation and is the initial stage of one of the conidiogenesis variants [25]. The presence of numerous globules and granules in monilioid cells may indicate their storage function. The *Agaricus bisporus* model showed that emergence of the monilioid type of hyphae and the subsequent mycelium fragmentation were concomitant with the culture aging process, which may be considerably enhanced upon growth on agarized nitrogen-depleted medium, unbalanced liquid medium, as well as at extremely high temperatures [26]. Apparently, a similar state accompanied by the accumulation of betaine lipids also developed in *F. velutipes* upon long-term cultivation on malt agar 1.

As shown in Fig. 1, PC and PE were the main membrane glycerolipids of *F. velutipes* grown on malt agar 1. Phosphatidic acids (PA), phosphatidylserines (PS), phosphatidylinositols (PI), and diphosphatidylglycerols (DPG) were present in a lesser amount. Glycoceramides (GlCer) were revealed in the sphingolipid composition. DGTS appeared in trace amounts on the 15th cultivation day and constituted 9–17% of the total membrane lipids by days 21–39. Changes in the phospholipid content occurred parallel to DGTS accumulation. For example, on the 21st cultivation day, the amount of PA was increased and the PS content decreased. However, the most significant changes were noted in the PC fraction. The concentration of these lipids decreased twofold in the period from day 9 to day 39.

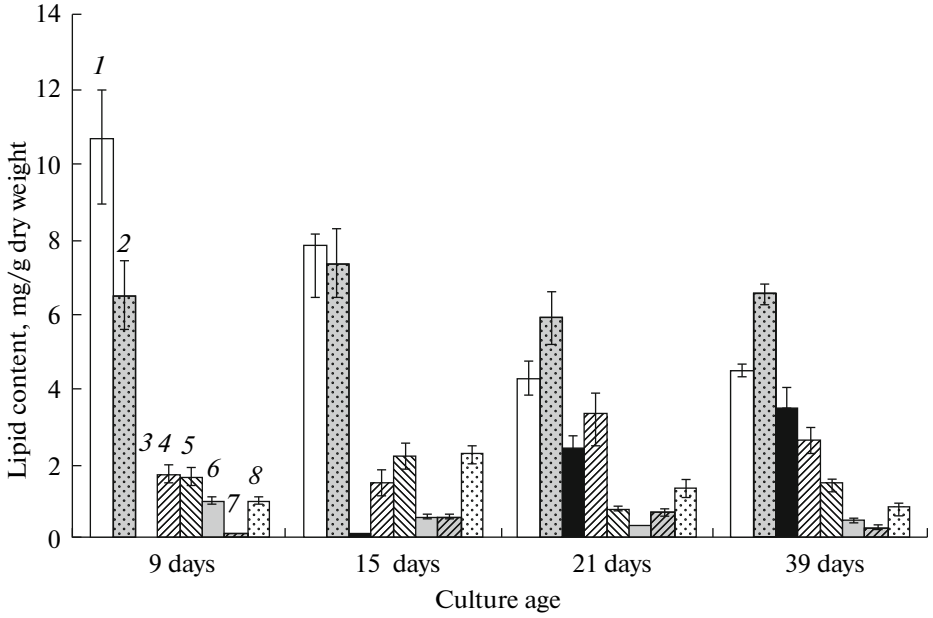

Fig. 1. Changes in the membrane glycero- and sphingolipid content in the process of development of *F. velutipes* surface culture. The cultivation conditions were: malt agar 1 as the nutrient medium; 15°C, darkness. Phosphatidylcholines (*1*), phosphatidyethanolamines (*2*), diacylglyceryltrimethylhomoserines (*3*), phosphatidic acids (*4*), phosphatidylserines (*5*), phosphatidylinositols (*6*), diphosphatidylglycerins (*7*), and glycoceramides (*8*).

Table 3. Changes in the fatty acid composition of the membrane lipids in *F. velutipes* mycelium depending on temperature and illumination

	T, °C	Illumination	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
PC	10	–	0.7 ± 0.1	0.6 ± 0.1	7.9 ± 0.8	1.6 ± 0.1	0.1 ± 0.1	1.8 ± 0.0	6.6 ± 1.2	71.3 ± 1.8	9.0 ± 0.5
	15	+	1.7 ± 0.1	1.8 ± 1.1	22.7 ± 7.9	1.7 ± 0.4	0.1 ± 0.1	5.1 ± 2.0	6.7 ± 2.1	54.4 ± 9.9	7.1 ± 3.4
		–	1.1 ± 0.1	0.8 ± 0.1	11.0 ± 0.5	1.6 ± 0.1	0.2 ± 0.0	3.0 ± 0.5	7.2 ± 0.2	69.8 ± 0.9	4.0 ± 0.1
	20	–	0.9 ± 0.0	0.9 ± 0.1	8.6 ± 0.5	1.2 ± 0.1	0.2 ± 0.0	1.4 ± 0.1	5.9 ± 1.0	77.4 ± 0.9	2.8 ± 0.1
PE	10	–	1.3 ± 0.3	1.4 ± 0.2	17.4 ± 1.9	0.8 ± 0.2	0.2 ± 0.0	0.9 ± 0.2	6.1 ± 0.4	65.5 ± 2.3	5.0 ± 0.3
	15	+	1.7 ± 0.2	0.1 ± 0.0	19.4 ± 0.3	1.6 ± 0.3	0.1 ± 0.1	2.8 ± 0.4	11.6 ± 2.2	55.0 ± 0.7	4.9 ± 2.0
		–	1.9 ± 0.2	1.9 ± 0.2	23.4 ± 2.3	1.1 ± 0.2	0.2 ± 0.0	2.3 ± 0.8	7.5 ± 0.6	58.1 ± 3.3	1.9 ± 0.1
	20	–	2.0 ± 0.1	1.9 ± 0.1	15.9 ± 0.6	1.6 ± 0.6	0.4 ± 0.1	1.7 ± 0.3	6.5 ± 0.7	66.9 ± 1.4	1.4 ± 0.0
DGTS	10	–	2.3 ± 0.6	1.5 ± 0.2	26.5 ± 5.7	2.6 ± 0.5	0.7 ± 0.1	7.0 ± 2.7	9.1 ± 0.9	45.4 ± 9.7	3.1 ± 0.8
	15	+	2.1 ± 0.0	1.7 ± 0.2	38.3 ± 3.0	5.4 ± 0.2	0.8 ± 0.3	12.7 ± 2.0	18.4 ± 3.6	19.3 ± 3.1	1.8 ± 0.6
		–	1.9 ± 0.1	1.5 ± 0.0	31.3 ± 2.3	3.8 ± 1.8	0.8 ± 0.0	6.7 ± 0.1	10.8 ± 1.3	41.0 ± 0.4	1.6 ± 0.5
	20	–	1.0 ± 0.1	1.3 ± 0.0	26.4 ± 0.6	1.0 ± 0.1	0.7 ± 0.0	5.4 ± 0.3	6.6 ± 0.4	55.7 ± 0.6	1.2 ± 0.1

Note: *F. velutipes* mycelium was obtained by surface cultivation on the malt agar 1 nutrient medium. The culture age was 21 days.

The PC content decrease correlating with DGTS accumulation provided an indirect evidence of the compensatory relationships between these two zwitterionic lipids. The similarity between their structures and physicochemical properties, including close change-of-phase temperature and viscosity values, made it possible to propose the hypothesis that PC and DGTS were functionally interchangeable in the membrane [27]. It was shown on many species of bacteria, algae, and cryptogamic plants that, at certain stages of development or upon a drastic change in the environmental conditions, the betaine lipid concentration increased against the background of the PC content decrease [16, 17, 28]. The physiological sense of partial substitution of the betaine-type lipids for phospholipids may be connected with the stabilization of the membrane structures due to higher resistance of the betaine lipid molecules to hydrolytic processes [3], as well as with switching over to a more economical metabolism. In the latter case, the substitution of betaine phospholipids for the membrane phospholipids leads to a release of phosphates, which are used for building other necessary biological molecules, e.g., nucleic acids [17].

It should be noted that *F. velutipes* betaine lipids noticeably differed from PC and PE in the fatty acid (FA) composition (Table 3). For example, in the culture grown on malt agar 1 at 15°C in the dark, the PC content of the main FA, linoleic and palmitic, was 70 and 11% of the sum of FA, respectively; in the PE molecules, 58 and 23%. The DGTS content of linoleic acid did not exceed 41%. Saturated palmitic and stearic acids accounted for approximately the same share in the sum: 31 and 7%, respectively. According to the ESI^+-MS analysis data, the content of the 18:2/18:2 molecular species of PC was 30–50%, that of 16:0/18:1 was 12–26%. The 16:0/18:2 content did not exceed 9–14%. On the contrary, in DGTS, 16:0/18:2 was the predominant molecular species. Its content was 40–50%, whereas that of 18:2/18:2 did not exceed 7–8%.

In the course of culture development, the FA composition of phospho- and betaine lipids did not change fundamentally, except for a gradual decrease of the linoleic acid relative content in PC and PE (data not shown). It is interesting to note that, upon growth of *F. velutipes* on wort agar accompanied by the development of fruit bodies, we observed the opposite reaction, namely, the accumulation of the $C_{18:3}$ acid in PC and PE [19]. The fact that, under conditions which are unfavorable for basidiome formation, the amount of linoleic acid decreased, indirectly confirming the hypothesis that it was involved in the regulation of fructification in basidial fungi.

Despite the fact that the main cause of initiation of the synthesis of betaine lipids in *F. velutipes* was probably the limitation of certain nutrients, the rate of their accumulation was to be regulated by a whole complex of physicochemical factors. To determine the temperature dependence of DGTS accumulation, comparative analysis of the membrane lipid content in *F. velutipes* mycelium cultivated for nine days at 25°C and for the subsequent 30 days at a lower temperature (10, 15, or 20°C) (during the experiment the cultures were in the dark), was carried out. A change in temperature in the course of cultivation is required for a complete cycle of development of *F. velutipes*. As a rule,

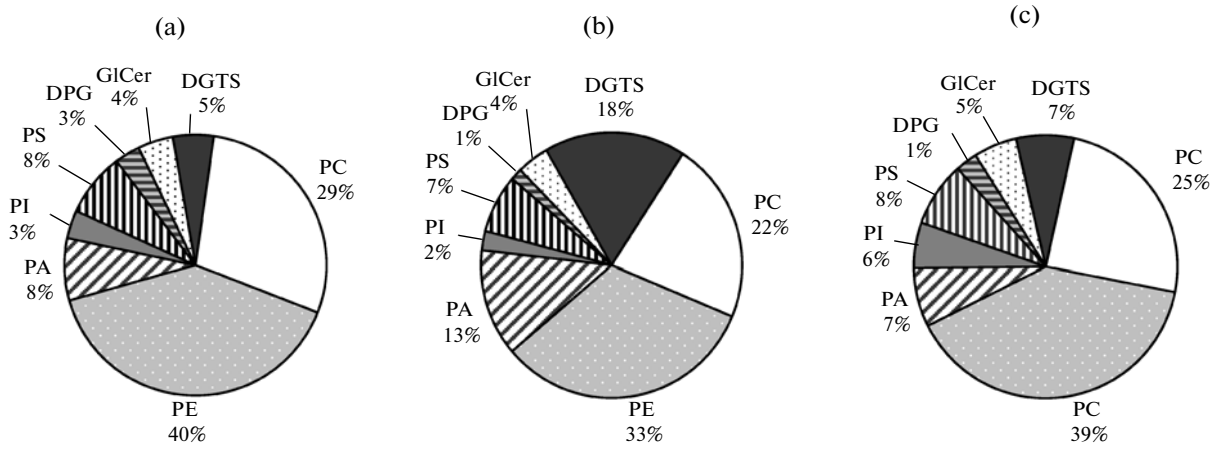

Fig. 2. Effect of temperature on the *F. velutipes* mycelium content of the main membrane glycero-and sphingolipids .The cultivation conditions: malt agar 1 as the nutrient medium; 10 (a), 15 (b), and 20°C (c); darkness. The culture age was 39 days.

with the observance of other compulsory conditions, including the maintenance of the humidity optimum, CO_2 content, the chemical composition of the nutrient medium and light regime, active mycelium growth occurred at 22–26°C, whereas a temperature decrease to 10–16°C was required for increasing the differentiation of mycelial cells and the formation of fruit bodies. Acceleration of the processes of differentiation of the vegetative cells and transition to the generative stage occurred in a sufficiently broad range of temperatures (5–20°C), although the optimal temperature was 15°C. According to the results obtained, the maximum accumulation of DGTS comparable with the accumulation of PC was observed at 15°C, i.e., at the optimal temperature for differentiation and fructification (Fig. 2). It should be noted that, irrespective of the temperature, the (PC + DGTS)/PE ratio in the membranes was always of an order of 1.1, which also confirms the presence of the mechanism of compensatory substitution of one class of trimethylammonium lipids for the other.

The influence of temperature conditions on the synthesis of betaine lipids has not been studied previously. However, the results obtained with *F. velutipes* suggest that, in contrast to algae and higher plants actively synthesizing the lipids of this group in a broad range of temperatures [28], in agaricoid basidiomycetes, intense DGTS formation under limitation of certain nutrients occurs in a more restricted range of temperatures.

The amount of DGTS in *F. velutipes* mycelium also varied depending on the light factor: 39-day cultures grown in the darkness contained several times more DGTS than the cultures grown under the illumination conditions (12 : 12) (Fig. 3). Interestingly, similar changes in the betaine lipid content, depending on illumination, are also characteristic of some photosynthesizing organisms. For example, in the culture of unicellular green alga *Pseudococcomyxa chodatii* grown under phosphorus limitation, the relative DGTS content was 1% of the total membrane lipids under autotrophic growth conditions (the light regime 12 : 12), whereas under heterotrophic conditions (in the dark), it was 16% [29]. A change in the light regime had practically no effect on the lipid fatty acid composition. Statistically significant differences were recorded only in the relative content of linoleic acid entering into the composition of DGTS (Table 3).

Thus, our studies revealed that the formation of the DGTS betaine-type lipids, which depends on the medium composition, as well as on the temperature and illumination, may occur in the mycelium of the agaricoid basidiomycete *F. velutipes* under certain conditions. DGTS accumulation was recorded at the late stages of development of the cultures grown on media with a low content of carbohydrates, nitrogen, phosphorus, potassium, and trace elements. The *F. velutipes* colonies developing on these media were characterized by slow biomass accumulation, low density of the aerial mycelium, the formation of monilioid hyphae, and the absence of fruit bodies.

Additional studies are required to elucidate the nature of the factor triggering the synthesis of betaine lipids in agaricoid basiodiomycetes. Nevertheless, at the present stage it may be already stated that DGTS synthesis is induced by a certain composition of the nutrient medium. Similarly to other organisms, phosphorus limitation may be the chemical stimulus initiating the synthesis of betaine lipids in fungi. Indeed, it was shown for many species of α-proteobacteria and green algae that phosphorus limitation induced the synthesis of a series of phosphorus-free lipids (including betaine lipids) capable of substituting membrane phospholipids, thereby aiding in the successful adaptation to the limitation of this highly important nutrient [4, 16, 17, 29]. Zinc limitation was probably

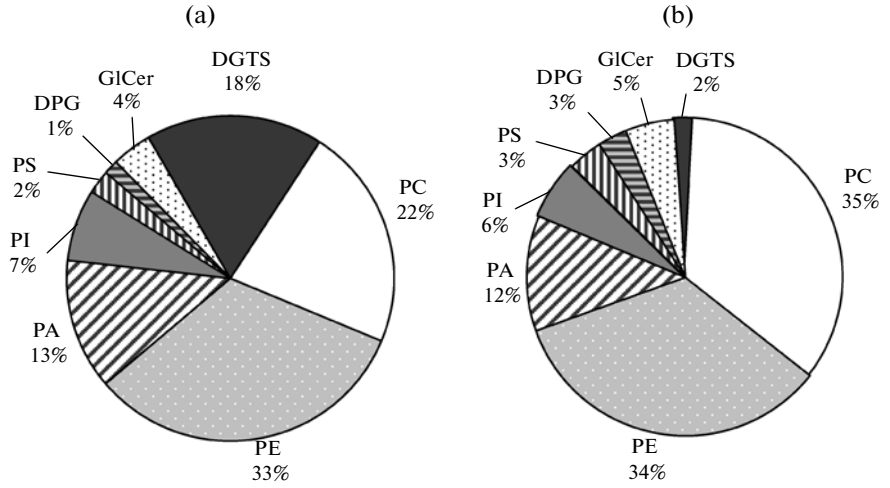

Fig. 3. Effect of the light regime on the *F. velutipes* mycelium content of the main membrane glycero-and sphingolipids. The growing conditions: malt agar 1 as the nutrient medium; 15°C, darkness (a) and illumination 2000 lx (the light regime 12 : 12) (b). The culture age was 39 days.

another critical factor for *F. velutipes* metabolism. It was shown in a series of experiments that this trace element is one of the most important for normal growth and development of fungi. For example, the absence of zinc resulted in a twofold decrease in the growth rate of the ascomycete *Cordyceps sinensis* [30]. On the contrary, addition of zinc to the cultivation medium of *Penicillum rubrum* increased the biomass yield three- or fourfold [31]. The growth rate of fungi in the culture, as well as their morphophysiological characteristics, including the fruit body formation, is known to depend much on the carbon to nitrogen ratio in the nutrient medium. Thus, for example, the 12 : 1 ratio proved to be optimal for the mycelial growth of *Cordyceps sinensis* [30] and *Agaricus campestris* [32] while 20 : 1–40 : 1 was optimal for *Pochonia chlamydosporia* and *Beauveria bassiana* [33]. The most suitable ratios for higher fungi were in the 5 : 1–12 : 1 range [34]. It is possible that the unbalanced carbon to nitrogen ratio became one of the most important causes that influenced morphogenesis and stimulated DGTS synthesis in the *F. velutipes* cultures grown on depleted media. Future experiments aimed at revealing the nature of the chemical stimulus triggering the synthesis of betaine lipids in agaricoid basidiomycetes will make it possible to determine which of the factors mentioned is the most important.

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