Microbiology, Vol. 69, No. 5, 2000, pp. 509–515. Translated from Mikrobiologiya, Vol. 69, No. 5, 2000, pp 612–619.

EXPERIMENTAL ARTICLES

Different Mechanisms of the Biochemical Adaptation of Mycelial Fungi to Temperature Stress: Changes in the Lipid Composition

E. P. Feofilova, V. M. Tereshina, A. S. Memorskaya, and N. S. Khokhlova

Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia

Received December 29, 1999; in final form, April 11, 2000.

Abstract—A comparative study was conducted concerning the effect of temperature stress on the lipid composition of representatives of the subkingdoms *Eomycota* and *Neomycota*. Changes in the composition of lipid acyl chains (such as saturation and desaturation, isomerization, and changes in the length of fatty acid carbon chains), in the phospholipid composition, and in the contents of sterols and other neutral lipids were revealed. Hyperthermia resulted in (i) an increase in the phosphatidylcholine level, (ii) a decrease in the phosphatidylethanolamine level, (iii) a rise in the content of reserve lipids (triacylglycerols), and (iv) a decline in the free fatty acid level in the neutral lipids. An inverse pattern occurred under hypothermic conditions. The peculiarities in the patterns of the temperature adaptation–related changes in the lipid bilayer composition are considered in terms of the systematic position of the fungi.

Key words: mycelial fungi, temperature shock, lipids

All kinds of organisms respond to temperature stress (TS) by changing the composition of the membrane and reserve lipids. Presumably, the biochemical adaptation of the lipid bilayer is based on the following mechanisms.

A. Modification of the composition of lipid acyl chains including changes in

(i) the desaturation degree of the lipid bilayer. Two alternative theories have been suggested to explain temperature adaptation. The *induction theory* assumes that changes in the fatty-acid composition result from a de novo synthesis of respective desaturases. In terms of the other theory, desaturase activity increases due to a decrease in the fluidity of the lipid bilayer [1];

(ii) the length of lipid acyl chains; of paramount importance are the numbers of even- and odd-numbered carbon atoms;

(iii) the configuration of acyl chains (involving *cis–trans* isomerization); e.g., the introduction of one *cis* bond into the molecule of stearic acid lowers the melting point from 69.6 to 13.4°C;

(iv) the degree of cyclization of acyl chains. The methyl groups of phytanyl residues in the thermoacidophilic archaebacterium *Sulfolobus* sp. form cyclopentane rings as a result of internal cyclization [2]. Many gram-positive and some gram-negative bacteria contain cyclopropane-derived fatty-acids;

(v) the pattern of ramification of acyl chains (e.g., formation of *iso* and *anteiso* fatty acids in bacteria [2]; there is evidence that this process is also accomplished by some fungi [3]);

(vi) the contents of epoxy and hydroxy fatty acids.

B. Changes in the membrane lipid composition.

C. Changes in the ratio of phospholipids (PL) and sterols, as well as the ratio between sterols and sterol esters.

D. Changes in the qualitative and quantitative composition of neutral lipids (NL) due to the operation of mechanisms enabling cells to use fatty acids obtained by hydrolyzing reserve triacylglycerols (TAG) [4].

Cell adaptation to temperature changes involves the above biochemical mechanisms. For instance, cultivation at low temperatures typically results in an increase in Δ^{12}- and Δ^{15}-desaturases, which enhances the levels of γ- or α-linolenic acids. Raising the temperature results in the activation of Δ^{9}-desaturase, which is accompanied by an increase in the content of monodesaturated fatty acids. Palmitoleyl-CoA-desaturase activity is of paramount adaptive value for a number of organisms [4]. The fluidity of the lipid bilayer is also efficiently modified by changing the membrane sterol content [5].

Importantly, the lipid composition of an organism varies depending on its systematic position, and this provides for the selectivity of the above biochemical mechanisms of adaptation. For example, modulation of lipid membranes via the cyclization mechanism is characteristic of bacteria only [2]. A direct dependence between hypothermia and an increase in the lipid desat-

0026-2617/00/6905-0509$25.00 © 2000 MAIK "Nauka/Interperiodica"

uration degree is more pronounced in eubacteria [6]; it may not manifest itself in higher eukaryotes due to the peculiar composition of their lipid bilayer, which contains sphingomyelin and sterols.

Eubacteria are known to contain peculiar sterol analogs, the hopanoids (tricyclopolyprenoids) diplopterol and diplopton. The long alkyl-polysubstituted chain of hopanoids facilitates rapid modification of the fluidity of the lipid bilayer [7]. Epoxy acids (such as *cis*-9,10-epoxystearic acid) were detected only in *Puccinia graminis*, and hydroxy acids (D-13-hydroxy-Δ^9-octadecanoenic acid), only in yeast. These acids form part of TAG, and *Eomycota* and *Ascomycota* lack them.

The goal of this work was to investigate the fungal mechanisms of temperature adaptation that involve lipid composition modifications. Our studies used fungi belonging to diverse systematic groups including the phyla *Basidiomycota*, *Ascomycota* (subkingdom *Neomycota*), and *Archemycota* (subkingdom *Eomycota*) (according to the classification presented in [8]). Importantly, few data exist on the thermoadaptation of *Basidiomycota*. Only the effect of cold shock on *L. edodes* is understood well [9].

MATERIALS AND METHODS

This study was conducted with mycelial fungi belonging to (i) *Archemycota* (class *Zygomycetes*): *Cunninghamella japonica* VKM F-1204 (–) and *Absidia coerulea* VKM F-859 (–); (ii) *Ascomycota*: *Aspergillus japonicus* VKM F-2145, *Penicillium lanosum* obtained from E.N. Bab'eva, and *P. lanosum* VKM F-1956; and (iii) *Basidiomycota*: *Pleurotus ostreatus*, a strain from the Institute of Microbiology, Russian Academy of Sciences, *Lentinus edodes*, and *Agaricus bisporus* from the collection of the Department of Mycology and Algology, Moscow State University.

The cultivation media used in this work were described by us earlier [10, 11], and the cultivation conditions of *P. ostreatus* are given below. In all experiments, we used mycelium grown to the middle of the trophophase.

Temperature shock conditions. *C. japonica* was cultivated for 24 h at 28°C, and, thereupon, some flasks were incubated at 28°C for 6 h, while other flasks were kept at 33 or 17°C. *A. japonicus* was incubated for 28 h at 28°C and thereupon for 8 h at 28, 34, and 15–16°C. Upon growing for 24 h at 29°C, a *P. lanosum* VKM F-1956 was cultivated for 8 h at 23 and 29°C. *P. lanosum* (obtained from E.N. Bab'eva) is a psychrophile, and it could grow at 6°C, the trophophase biomass being 25–30% of that under optimum conditions (23–24°C).

Following is the composition (g/l) of the soybean medium used for growing *P. osteratus*: soybean flour, 7.0; $MgSO_4 \cdot 7H_2O$, 0.38; KH_2PO_4, 3.5; sunflower oil, 10; and whey, 10% of the medium volume. The material used in our studies was grown in 250-ml flasks containing 50 ml of medium; a shaker (200–220 rpm) was used. The cultivation was carried out for 72 h at 26°C. Thereupon, the flasks were subdivided into four groups, incubated for 24 h at 26, 33, 7–10, and 2 °C.

Lipids were extracted by the Folch method [12] and separated into phospholipids (PL) and neutral lipids (NL) on a Silica Gel L column (100/160 mesh, Chemapol, Czechia) using solvents with different polarity degrees. Thin-layer chromatography of the lipids was carried out on glass plates with KSKG silica gel (Laene Kalur, Estonia). NL were separated employing a hexane–diethyl ether–acetic acid (85 : 15 : 1) system. PL were separated consecutively using (i) the hexane–diethyl ether–acetic acid (85 : 15 : 1) system and (ii) the chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2) system. 50–100 μg of lipids were applied onto the plates. The resulting chromatograms were developed with 5% sulfuric acid in ethanol and heated at 180°C until the spots became visible.

PL were identified using PL extracted from pig brain. Qualitative tests were conducted with ninhydrin (to test PL for amino groups) and the Dragendorf reagent (to detect choline-containing PL) [12]. The quantitative assessment of chromatograms was carried out using a Shimadzu CS-9000 dual-wavelength flying-spot scanner. The composition of methyl ethers of fatty acids (MEFA) was determined (1) on a Chrom-5 chromatograph (Czechia) equipped with a 2500 × 3 mm glass column containing 5% Silar-SCP on W-HP Chromosorb (100–120 mesh); the temperature was programmed to increase from 130 to 240°C at a rate of 2 deg/min; and (ii) on a Model 3700 gas-liquid chromatograph equipped with a 2-m-long glass column with 17% DEGS on the W-AWDMSE-HP Chromosorb carrier (80–10 mesh); the column temperature was 180°C. Fatty-acid isomers were identified by chromatographing MEFA on a column with Silar SCP and OV-101, using a Hewlett-Packard device (Model 5830).

MEFA (Serva, L-207) supplemented with the methyl ethers of linoleic and arachidonic acid and MEFA from natural sources containing the α-isomer of $C_{18:3}$ (from *Succus kalanhoes* leaves) and the γ-isomer of $C_{18:3}$ (from *Mucor* sp. mycelium) were used as standards. Di-, tri-, tetra-, and pentaenoic unsaturated fatty acids were identified using a Varian-Mat 311A chromatograph–mass spectrometer (USA). The temperature of the ion source was 120°C, the accelerating voltage was 3 kV, and the ionization energy was 70 cV. The relative amounts of fatty acids in samples were determined using an internal standard (behenic acid, 0.1 μg/ml sample). The $C_{20:4}$ acids were identified by chromatography–mass spectrometry using an LKB-9000A (Sweden) device.

The results obtained were statistically processed using the sign criterion; median values were calculated at $n = 3$–4 [11].

Table 1. Changes in the fatty acid composition of fungal phospholipids under temperature shock

Species	Cultivation temperature, °C	Fatty acids, % of total								Desaturation degree
		$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
*C. japonica**	28	20.2	2.7	–	–	7.0	43.5	11.8	14.0	1.12
	33	20.6	2.4	–	–	8.7	35.6	19.2	11.3	1.10
	17	16.9	4.1	–	–	8.0	36.1	14.3	20.1	1.29
*A. japonicus**	26	19.7	Traces	–	–	20.8	18.9	37.9	2.5	1.02
	32	31.7	Traces	–	–	13.0	24.4	28.4	2.5	0.89
	14	9.5	5.4	–	–	11.5	20.6	40.2	12.8	1.45
P. lanosum	23	18.7	Traces	–	–	7.9	16.3	40.9	10.6	1.30
	29	20.6	1.7	–	–	5.8	28.2	42.3	Traces	1.15
	6	13.5	10.6	1.5	8.5	7.3	14.6	25.4	15.6	1.31
P. lanosum VKM F-1956	29	20.4	1.4	–	–	11.6	31.7	29.4	3.2	1.00
	23	20.5	Traces	–	–	9.0	12.5	37.0	12.9	1.25
P. ostreatus	26	16.3	Traces	4.4	–	3.4	15.2	59.3	1.4	1.38
	33	29.1	Traces	32.9	–	7.8	8.2	19.6	2.5	0.55
	12	13.0	5.0	4.3	–	3.2	13.3	60.4	2.1	1.45
	6	14.4	Traces	0.8	–	5.2	19.9	57.5	2.2	1.41

Note: The fatty acid content in *C. japonica*, *P. lanosum* and *A. japonicus* is given without taking into account $C_{12:0}$, $C_{14:0}$, and long-chain acids. "–" means that the acid was not detected.

* The $C_{18:2}$ and $C_{18:3}$ ratio is discussed in the text.

RESULTS

The tested representatives of some systematic groups of fungi differed in the composition of lipid acyl chains (Table 1). Two acids, $C_{16:0}$ and $C_{18:1}$, prevailed in the *Eomycota* (*Zygomycota*) representatives. $C_{16:0}$ and $C_{18:2}$ acids dominated the lipids of the *Neomycota* representatives (*Aspergillum*, *Penicillium*, and basidial fungi). The $C_{18:2}/C_{18:1}$ ratios in the PL of representatives of various systematic groups were significantly different: this ratio was 0.27–0.31 in *Mucorales* (*C. japonica* and *A. coerulea*), 2.03 in *Aspergillus*, 1.52–0.94 in *Penicillium*, and 3.4–3.9 in basidiomycetes (*L. edodes* and *P. ostreatus*). Hence, the regularity is as follows: the higher the position of a fungus in the hierarchical "tree," the higher the $C_{18:2}/C_{18:1}$ ratio. Interestingly, the pseudofungus *Phytophthora cryptogea* (*Oomycota*) is characterized by a ratio of 2.69 [13], which is similar to that of ascomycetous fungi.

1. Changes in the Composition of Lipid Acyl Chains

From the data of Table 1,[1] it is evident that all of the fungi tested possess a common biochemical mechanism of adaptation that operates under TS and is based on changing the desaturation degree of lipid acyl chains. In both *Eomycota* and *Neomycota*, this control mechanism is characterized by two typical features: (i) Lipid acyl chains become more desaturated under hypothermic and more saturated under hyperthermic conditions in most fungi tested; (ii) Apart from PL, this pattern is also typical of (reserve) NL; it is even more pronounced in NL (Table 2). However, the fatty acids involved in the regulation of the lipid bilayer fluidity vary depending on the species; these fatty acids can form de novo under stress. This particularly manifests itself under extreme conditions. Incubating ***C. japonica*** mycelium (grown at 33°C) for 3 h at 10°C results in the formation of unusual quantities of $C_{20:4}$ that amount to 2.0–3.5% of the total fatty-acid content.[2] The mass spectrum of the pyrrolidide of 5,8,11,14-eicosatetraenoic (arachidonic) acid provides evidence of the presence of this acid in ***C. japonica***. Under TS (described in the "Materials and Methods" section), PL of ***C. japonica*** display changes in the content of palmitoleic acid, whose quantity almost doubles upon decreasing the temperature (Table 1). Interestingly, NL of ***C. japonica*** virtually lack this acid. Different acyl chains are involved in the changes in the lipid bilayer fluidity in PL and NL. A number of long-chain fatty acids form in ***C. japonica*** PL under hyperthermic conditions, including $C_{20:0}$, $C_{20:1}$, $C_{22:0}$, $C_{24:0}$, and $C_{26:0}$.

[1] In order to avoid cramming Table 1 with data, it does not contain the data obtained with all of the fungi tested; it only deals with typical representatives of systematic groups (these microbial species are in bold type in the text). The peculiarities of other tested fungi are discussed in the text.

[2] The data on the lipid composition of ***C. japonica*** were obtained in collaboration with L.S. Kuznetsova.

Table 2. Changes in the fatty acid composition of fungal neutral lipids under temperature shock

Species	Cultivation temperature, °C	Fatty acids, % of total							DD
		$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
*C. japonica**	28	15.4	Traces	–	12.5	48.8	13.3	8.5	1.00
	33	24.0	0.2	–	15.1	50.6	4.8	2.8	0.66
	17	30.1	0.5	–	4.2	35.1	15.1	15.0	0.96
*A. japonicus**	26	20.1	–	–	12.5	22.6	35.8	8.7	1.20
	32	25.3	–	–	15.5	27.5	28.9	2.8	0.94
	14	10.2	Traces	–	11.4	30.1	36.5	11.8	1.39
P. ostreatus	26	20.0	–	Traces	11.3	28.6	35.2	4.8	1.14
	33	21.9	–	Traces	11.3	34.9	27.3	4.6	1.03
	12	24.4	–	2.2	8.9	30.3	30.3	3.9	1.03
	2	17.0	–	Traces	10.8	28.4	37.9	5.9	1.22

Note: The fatty acid content in *C. japonica*, *P. lanosum*, and *A. japonicus* is given without taking into account $C_{12:0}$ and $C_{14:0}$. "–" means that the acid was not detected. DD is the desaturation degree.

* The $C_{18:2}/C_{18:3}$ ratio is discussed in the text.

The predominant species is $C_{24:0}$ (accounting for 2.6–3.3% of the fatty acid content). In addition, changes in the ratio between $C_{18:2}$ isomers occur in PL under TS. Hyperthermia results in the formation of two $C_{18:2}$ isomers, *cis-cis*-octadien-6,9-oic and *cis-cis*-octadien-9,12-oic acids, whose ratio is 2.4; i.e., $\Delta^{6,9}$-linoleic acid prevails under these conditions. $\Delta^{9,12}$-$C_{18:2}$ predominates under hypothermic conditions, and the ratio between the isomers is 0.05. The cultivation temperature influences the composition of the $C_{18:3}$ isomers. Cultivation at a low temperature promoted the formation of α-$C_{18:3}$ (whose amount did not exceed 1.5% of the total fatty acid content) in addition to the predominant γ-$C_{18:3}$ acids. Importantly, the desaturation degree (DD) of PL was higher than that of NL, and a clear-cut relationship between this value and the cultivation conditions was observed. The DD of PL and NL were higher under hypothermic and lower under hyperthermic conditions. An analogous relationship was revealed in another *Zygomycetes* representative, *A. coerulea*.

Hence, the lipid bilayer fluidity in typical *Zygomycetes* (*Eomycota*) representatives is under the control of biochemical mechanisms involving changes in the double bond number and the length of lipid acyl chains and isomer formation (chiefly in PL). Changes in the $C_{16:0}/C_{18:0}$ ratio are essential for the temperature-dependent regulation of NL homoviscosity, but not of PL homoviscosity. A common feature of PL and NL is that lipid fluidity alters at low temperatures due to the formation of the trienoic linolenic acid.

Almost no palmitoleic acid is contained in the PL and NL of *Ascomycota* representatives (***A. japonicus***) under normal conditions. However, its amount in PL reaches 5% of the total fatty acid content at a decreased temperature. $C_{20:4}$ and $C_{20:5}$ occur under these conditions; the contents of both acids do not exceed 4% of the total fatty-acid content. Importantly, ***A. japonicus*** PL also contain $C_{20:4}$ (1% or less) under normal conditions. In this respect, *Ascomycota* resemble oomycetous pseudofungi rather than *Zygomycota* [13] (see above). We detected the α- and γ-isomers of linolenic acid among the $C_{18:3}$ isomers of young (26-h-old) ***A. japonicus*** mycelium. More mature mycelium contained only the α-isomer. Of interest in this context is the fact that both isomers occur at low temperatures, but, in contrast to ***C. japonica***, α-$C_{18:3}$ is the predominant isomer (accounting for 80% of the total isomer content). α-$C_{18:3}$ is the only isomer detected under hyperthermic conditions. Adaptation to cold stress in *Zygomycetes* mainly involves desaturases, which alter the $C_{18:3}$ level in the acyl chains of PL. Desaturases actively regulate $C_{18:1}$ and $C_{18:2}$ synthesis in *Ascomycota*. Δ^{15}-Desaturase is more efficient in NL. Similar patterns occur in *Penicillium* representatives. Interestingly, a cold shock (at 6°C) in *P. lanosum* (from Bab'eva), a psychrophile, results in $C_{17:1}$ formation and an increase in the $C_{16:1}$ content; contrary to expectations, no significant increase in the $C_{18:3}$ content occurs. It should be emphasized that a cold shock does not result in an appreciable DD increase.

Stress adaptation in basidial xylotrophic fungi (e.g., ***P. ostreatus***) is characterized by peculiar features. The main hyperthermia-induced changes occur in the $C_{18:2}\Delta^{9,12}$ content of both PL and NL. However, the saturation of PL acyl chains proceeds via a peculiar pathway involving hypersynthesis of $C_{17:0}$ and $C_{16:0}$ and the inhibition of oleic acid formation. Hence, both the saturation and the shortening of acyl chains contribute to the decrease in the lipid bilayer fluidity. The main changes occurring in PL and NL involve alterations in the $C_{18:2}$ and $C_{18:1}$ contents.

In contrast to the fungi considered above, the acyl chain composition of the PL of ***P. ostreatus*** significantly changes due to alterations in the $C_{17:0}$ content (resulting from acyl chain shortening and increased saturation of the lipid bilayer under hyperthermic conditions), while NL practically lack $C_{17:0}$, which, therefore, is not involved in the regulation of lipid fluidity. Of particular interest is the formation of $C_{16:1}$ in PL, but not in NL. Microviscosity changes in NL are mainly due to alterations in the $C_{18:2}$ and, particularly, $C_{18:1}$ contents. A more significant temperature decrease (to 0°C) causes no considerable changes in the acyl chains of PL and NL in ***P. ostreatus*** mycelium. Insignificant changes occur only in the $C_{18:3}$ content. These data indicate that such a temperature decrease creates sublethal conditions, and, therefore, the control mechanisms of the organism cannot operate. The data obtained also suggest that $C_{18:3}$ formation probably represents one of the initial stages of cold adaptation; further cooling, however, stops adaptation–related processes [14].

2. Changes in the Composition of PL and NL

The cultivation temperature influences the composition of PL and NL. In ***C. japonica*** (Fig. 1), hyperthermia results in an increase in the phosphatidylcholine (PC) and phosphatidylserine (PS) contents and a decrease in the phosphatidylethanolamine (PEA) and sphingomyelin (SM) levels. A cold shock causes the PEA and PS levels to increase and the PC and SM contents to decrease. As for NL, hyperthermia results in an increase in the triacylglycerol (TAG) content and a decrease in the free fatty acid (FFA) level. The TAG content increases and the FFA level decreases under hypothermic conditions (in contrast to hyperthermic conditions). In ***C. japonica***, the sterol (S) level decreases, and the sterol ester (SE) content increases.

The bulk PL of all tested representatives of ascomycetous fungi undergo virtually the same hypo-/hyperthermia-associated changes as those in *Zygomycetes*. The main changes in NL involve, apart from TAG and FFA, also S, whose level increases under hyperthermic conditions, while the SE content decreases.

The main changes in the PL of ***P. ostreatus***, a representative of basidial fungi, also involve PC and PEA. For example, raising the temperature results in an almost twofold increase in the PC level. The TAG content decreases and the FFA level enhances in ***P. ostreatus*** NL under hypothermic conditions (Fig. 2); an inverse pattern of changes occurs upon increasing the temperature. ***P. ostreatus*** is characterized by peculiar changes in the diacylglycerol (DAG) content, which increases more than twofold during a cold shock. Another peculiarity of ***P. ostreatus*** is that the S level slightly increases upon cooling and decreases upon heating.

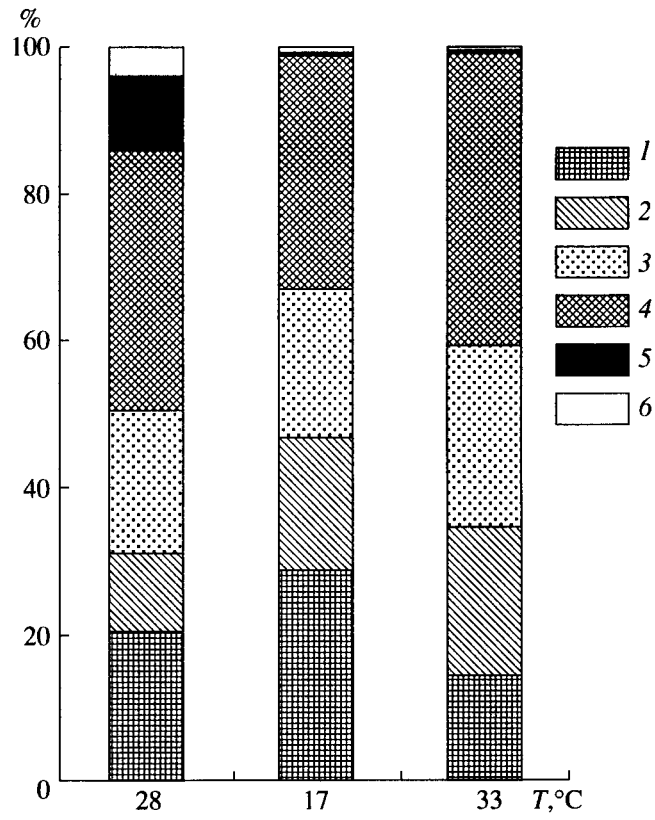


Fig. 1. Changes in the phospholipid composition of ***C. japonica*** under temperature stress. (*1*) PEA, (*2*) PS, (*3*) PI, (*4*) PC, (*5*) Sphingomyelin, (*6*) Unidentified minor phospholipids.

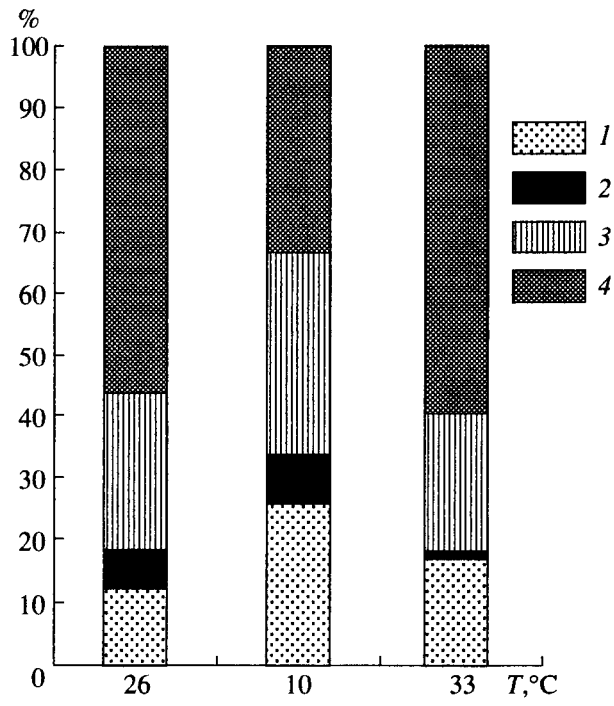


Fig. 2. Changes in the neutral lipid composition of ***C. japonica*** under temperature stress. (*1*) DAG, (*2*) S, (*3*) FFA, (*4*) TAG.

Table 3. Changes in the fatty acid composition (in % of the total fatty acid content) of lipids during cell differentiation process in *Agaricus bisporus*

Sample	Lipid fraction	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	γ-$C_{18:3}$	α-$C_{18:3}$	$C_{19:0}$	$C_{20:0}$	$C_{20:2}$	$C_{21:0}$	$C_{20:4}$	$C_{20:5}$	$C_{22:0}$	$C_{22:1}$	$C_{22:2}$	$C_{22:4}$	$C_{23:0}$	$C_{24:0}$
Fruiting body, cap	NL	1.6	0.7	13.2	1.8	2.8	–	5.6	12.4	12.5	1.4	1.7	1.7	1.8	1.9	2.3	2.6	2.6	2.9	3.3	3.5	3.9	4.3	4.7
	PL	1.1	0.3	10.6	1.6	0.7	0.2	6.3	4.1	53.5	1.5	2.7	0.2	–	–	0.6	3.9	–	5.4	1.5	–	9.9	–	–
Fruiting body, stem	NL	4.2	1.5	11.8	1.7	–	–	9.5	18.4	21.0	–	0.8	–	–	–	4.0	–	–	–	–	5.6	–	–	–
	PL	0.6	–	9.0	0.5	–	–	4.1	5.7	68.7	–	–	–	–	–	3.0	5.1	–	–	–	–	–	–	2.4
Mycelium	NL	3.0	2.0	6.7	4.1	4.0	7.1	5.5	8.8	10.1	–	6.1	–	5.7	–	4.7	–	–	10.6	–	5.5	–	1.5	–
	PL	0.8	2.0	1.8	2.1	–	–	9.9	8.4	20.7	–	0.4	–	–	3.4	0.6	–	3.9	–	0.2	–	–	0.7	–

Note: "–" means that the fatty acid was not detected.

DISCUSSION

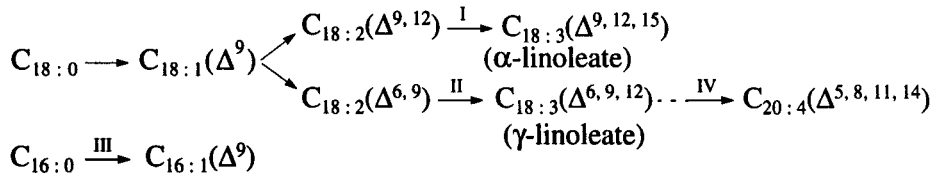
Following are the conclusions drawn from the data obtained. All tested *Eomycota* (*Archemycota*) and *Neomycota* (*Ascomycota*) display similar changes in the PL and NL composition under hyper-/hypothermic conditions. They can be schematically represented as follows:

Hypothermia: PEA↑PC↓TAG↓FFA↑ S↓

Hyperthermia: PEA↓PC↑TAG↑FFA↓S↑

However, minor PL and NL components also undergo certain changes. For instance, the phosphatidylinositol (PI) and PS contents increase in ***C. japonica*** under hyperthermic conditions, which stimulate PS formation in *Ascomycota*.

The pattern of the temperature-dependent changes in lipid acyl chains varies depending on the systematic position of fungi. In ***C. japonica***, these changes can be represented as follows:



Pathway I, which results in $C_{18:2}\Delta^{9,12}$ formation predominates in this system, and, to an insignificant extent, α-$C_{18:3}$ also forms. Desaturation can also proceed via pathway III. If rapid adaptation is necessary (under sublethal conditions, e.g. at 10°C), $C_{16:1}$ and $C_{20:4}$ synthesis is activated, indicative of the operation of the "catastrophic" mechanism first revealed in *Tetrahymena pyriformis* [14].

The same adaptive desaturases function in ***A. japonicus*** under hypothermic conditions, i.e., desaturation proceeds via pathways I and III. Depending on the extent to which the temperature is decreased, $C_{20:4}$, $C_{20:5}$, and, as a minor isomer, γ-$C_{18:3}$ may form *P. lanosum* (a psychrophile) displays a peculiar pattern of cold adaptation associated with a drastic increase in the $C_{17:1}$ and $C_{16:1}$ contents. Interestingly, a temperature decrease of 5–6°C results in an increase in the $C_{17:1}$ content (up to 17% of the total fatty acid content) in the fairy-ring mushroom *Kuhneromyces mutabilis* [11].

The modification of the DD of lipid acyl chains under hypothermic conditions is accomplished in basidiomycetes via pathways I and III. The mechanism based on acyl chain isomerization does not operate in this system. Of special importance is the following point: the $C_{18:2}$ content in the PL is almost two times higher than in the NL. The same pattern is also characteristic of ***L. edodes*** and *A. bisporus* and it is particularly manifest in their fruiting bodies (both in the stems and in the caps, Table 3). The pathway resulting in shortening of fatty acid chains under hypothermic conditions is more prominent in basidial fungi than in *Eomycota* representatives. *Basidiomycota* are characterized by a peculiar pattern of temperature regulation based on the "sterol mechanism." The sterol level is enhanced during a cold shock and markedly decreased under hyperthermic conditions. This may be due to a peculiar sterol composition of *Basidiomycota* [15].

Hyperthermia in the tested fungi is under the control of more uniform mechanisms based on the saturation of unsaturated fatty acids and the formation of saturated acids, including those with long chains. However, there are a number of differences among these species. For instance, the $C_{16:0}$ content predominantly increases in ***A. japonicus*** upon increasing the temperature, while an

unusual acid, $C_{17:0}$, forms along with $C_{16:0}$ in the basidiomycete ***P. ostreatus.***

Despite a number of major differences in the morphology, developmental cycle, and cell composition (e.g., the composition of cytosol carbohydrates [16]), all tested fungi use mechanisms of temperature adaptation involving the following membrane lipid (PL) changes: (i) saturation and desaturation, (ii) acyl chain reduction/elongation, and (iii) isomerization (synthesis of *cis* and *trans* forms). NL display corresponding changes in the TAG and FFA contents. TAG are used as reserve substances if acyl chains are restructured, and FFA are involved in changing the desaturation degree of the lipid bilayer [17]. Of particular interest is the presence of $C_{20:4}$ in *Mucorales* (*Eomycota*), which lack this acid under normal conditions. We did not detect $C_{20:4}$ in the mycelium of basidial fungi (***P. ostreatus*** and *A. bisporus*), but this acid was contained in the fruiting bodies of these fungi. The data for *A. bisporus* are shown in Table 3. The results obtained suggest that the formation of $C_{20:4}$ in basidioms is associated with a cold shock, which is a prerequisite for fruiting body formation [9]. Of interest in this context is the fact that nematodes, comparatively advanced organisms in terms of the level of organization, also enhance the synthesis of polyunsaturated fatty acids at low temperatures [18]. In light of this fact, the above mechanism of adaptation may be universal and arachidonic acid may play a crucial role in it.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (grant no. 98-04-49171).

REFERENCES

1. Kasei, R., Yamada, T., Hasegawa, J., Muto, Y., Yoshioka, S., Nakamura, T., and Nazawa, Y., Regulatory Mechanism of Desaturation Activity in Cold Acclimation of *Tetrahymena pyriformis*, with Special Reference to Quick Cryoadaptation, *Biochim. Biophys. Acta*, 1985, vol. 836, pp. 397–401.
2. Russel, N.J., Mechanism of Thermal Adaptation in Bacteria: Blueprints for Survival, *TJBS*, 1984, vol. 9, pp. 108–112.
3. Feofilova, E.P., Lipids of Mycelial Fungi and the Prospects of Microbial Biotechnology, *Biol. Nauki*, 1990, no. 11, pp. 5–26.
4. Spector, A.A. and Yorek, M.A., Membrane Lipid Composition and Cellular Function, *J. Lipid Res.*, 1985, vol. 26, pp. 1015–1055.
5. Watson, K., Temperature and Lipids, *The Yeasts*, Rose, A.H. and Harrison, Y.S., Eds., London: Academic, 1987, vol. 2.
6. Wada, M., Fukunaga, N., and Sasaki, S., Effect of Growth Temperature on Phospholipid and Fatty Acid Composition in Psychrotrophic Bacterium *Pseudomonas* sp. Strain E-3, *Plant Cell Physiol.*, 1987, vol. 28, no. 7, pp. 1209–1217.
7. Gay, Q., Mithel, R., and Karl, P., Prokaryotic Hopanoids and Other Polyprenoid Sterol Surrogates, *Annu. Rev. Microbiol.*, 1987, vol. 42, pp. 301–335.
8. Cavalier-Smith, T., A Revised Six-Kingdom System of Life, *Biol. Rev.*, 1998, vol. 73, pp. 203–266.
9. Song, C.Y. and Cho, K.Y., Effect of Low Temperature Shock on Sporophore Initiation, Lipid Profile and Nutrient Transport in *L. edodes*, *Mycologia*, 1991, vol. 83, no. 1, pp. 24–29.
10. Feofilova, E.P., Polotebnova, M.V., and Kogtev, L.S., The Use of Data on Fatty Acid Composition of Fungi To Elucidate Their Phylogenetic Affiliation, *Biol. Nauki*, 1989, no. 4, pp. 66–70.
11. Mikhailova, M.V., Feofilova, E.P., Shumskaya, G.G., and Shevtsov, V.K., Changes in the Lipid Composition during the Differentiation Process in the Fungi of the Order *Agaricales*, *Prikl. Biokhim. Mikrobiol.*, 1993, vol. 39, no. 2, pp. 315–319.
12. Kates, M., *Techniques of Lipidology: Isolation, Analysis and Identification of Lipids*, Amsterdam: Elsevier, 1972. Translated under the title *Tekhnika lipidologii*, Moscow: Mir, 1975.
13. Pan'kina, O.I. and Konova, I.V., Lipid Characteristics of *Phytophthora cryptogea*, *Mikrobiologiya*, 1996, vol. 65, no. 2, pp. 182–188.
14. Nozawa, Y. and Kasai, R., Mechanism of Thermal Adaptation of Membrane Lipids in *Tetrahymena pyriformis* NT-1, *Biochim. Biophys. Acta*, 1978, vol. 529, pp. 54–66.
15. Yaoita, Y., Amemiyi, K., Ohnuma, H., Furumura, K., Masaki, A., Matsuki, T., and Kikushi, M., Sterol Constituent from Five Edible Mushrooms, *Chem. Farm. Bull.*, 1998, vol. 46, no. 6, pp. 944–950.
16. Feofilova, E.P., Tereshina, V.M., Memorskaya, A.S., Zav'yalova, L.A., and Maryshova, N.S., Changes in the Cytosol Carbohydrate Composition during the Cytodifferentiation Process in Fungi of the Order *Agaricales*, *Mikrobiologiya*, 1999, vol. 68, no. 3, pp. 356–361.
17. Kates, M., Push, E.L., and Ferrante, C., Regulation of Membrane Fluidity by Lipid Desaturase, *Membrane Fluidity*, New York: Plenum, 1984, pp. 378–395.
18. Tanaka, T., Ikita, K., Ashida, T., Motoyama, Y., Yamaguchi, Y., and Saouchi, K., Effect of Growth Temperature on the Fatty Acid Composition of the Free-Living Nematode *Caenorhabditis elegans*, *Lipids*, 1996, vol. 31, no. 11, pp. 1173–1178.