ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 4, pp. 455–460. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © V.M. Tereshina, A.S. Memorskaya, E.R. Kotlova, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 4, pp. 447–453.

EXPERIMENTAL ARTICLES

The Effect of Different Heat Influences on Composition of Membrane Lipids and Cytosol Carbohydrates in Mycelial Fungi

V. M. Tereshina[a, 1], A. S. Memorskaya[a], and E. R. Kotlova[b]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg*

Received September 22, 2010

Abstract—Comparison of the changes in the composition of the membrane lipids and soluble cytosol carbohydrates caused by two types of heat influence (within the tolerance zone and heat shock-level) revealed fundamental differences in the stress response of fungal cells. In three fungal species, *Aspergillus niger, Pleurotus ostreatus*, and *Cunninghamella japonica,* increased levels of trehalose and phosphatidic acids were observed under heat shock, while heat influences within the tolerance zone had no such effect. Under heat shock, the ratio of saturated fatty acids did not increase in any of the major phospholipids of all the studied species. This is in contradiction with the existing hypothesis and confirms the previously suggested the hypothesis of membrane stabilization by heat-protecting compounds.

Keywords: Aspergillus niger, heat shock, trehalose, phosphatidic acids, sphingolipids.

DOI: 10.1134/S0026261711040199

Heat treatment may have a varied effect on the organisms. While the optimal conditions promote maximal growth, suboptimal conditions (within the tolerance zone) result in decreased growth rate, heat shock (HS) inhibits growth processes, and the lethal shock causes death. Unlike the heat influences within the tolerance zone, HS switches on a protective system, resulting in heat resistance under conditions of the lethal heat shock. The main biochemical changes in fungi after HS are associated with protein synthesis: synthesis of the "household proteins" stops, heat shock proteins are produced extensively, antioxidant protective enzymes are produced, and trehalose is accumulated—a heat protector that preserves the membranes and macromolecules of the cell [1]. The preservation of the structure and functions of the membranes plays the most important role in the preservation of cell viability. Two hypotheses exist concerning membrane protection under heat influence. The hypothesis of homeoviscous adaptation [2] postulates preservation of the viscosity of the membranes by variations in the unsaturation degree of the acyl chains of the phospholipids. According to the hypothesis of homeophaseus adaptation [3], the balance between "bilayer" and "nonbilayer" lipids is of utmost importance.

These hypotheses, however, do not differentiate between HS and the heat influences within the tolerant zone. In contrast to the existing hypotheses [2, 3], we have demonstrated that, in mycelial fungus *Aspergillus niger*, HS conditions in the tropho- and idiophase did not result in an increased ratio of saturated fatty acids in the acyl changes of the major phospholipids or in an increased content of bilayer lipids [4]. On the contrary, heat shock resulted in an increased relative content of the "nonbilayer" phosphatidic acids and sphingolipids among the membrane lipids, as well as in accumulation of trehalose. We therefore proposed a hypothesis concerning another system of membrane protection under heat shock, viz., "membrane stabilization" by the heat-protecting compounds (carbohydrates, sphingolipids, and probably sterols). The universality of this mechanism for fungi and its differences from the adaptation mechanisms to heat influences within the tolerance zone remained unclear.

The goal of the present work was to compare the changes in the membrane lipids and cytosol carbohydrates for three mycelial fungi of different taxonomic positions under heat shock and heat influences within the tolerance zone.

MATERIALS AND METHODS

Three mycelial fungi of different phyla [5] were used in this work, namely *Aspergillus niger* VKM F-34 (*Ascomycota*), *Pleurotus ostreatus* strain INMI RAN (*Basidiomycota*), and *Cunninghamella japonica* VKM F-1204 (*Zygomycota*).

The fungi were grown on malt agar for 5–6 days at the optimal temperature. For inoculation of liquid

[1] Corresponding author; e-mail: V.M.Tereshina@inbox.ru

media, spore suspensions were added to the final concentration of 5×10^5–10^6 spores/ml. *A. niger* and *C. japonica* were grown in the Blumental–Roseman medium [6] and the Goodwin medium [7], respectively. In the case of *P. ostreatus*, washout of the mycelium were used, and the culture grown in 3.5*B* wort for 2 days was used as an inoculum for the experiments. Submerged cultures were grown in 250-ml flasks with 50 ml of the medium on a KE-12-250T electromagnetic shaker (Russia) at 150 rpm at the optimal temperature. For *A. niger* and *P. ostreatus*, the cultivation was carried out at 29–30°C (the control variant) for 24 h (the trophophase stage). For investigation of the effects of heat influences, the cultures were transferred to 34–35°C (tolerance zone) or 39–40°C (heat shock under the same aeration conditions) and cultivated for 3 h. The control variant was grown for the same time under optimal conditions. *C. japonica* was grown at 27–28, 33–34, and 37–38°C, respectively.

The biomass was obtained by filtration through a Nylon mesh and washed with distilled water of the relevant temperature. For inactivation of lipases, the biomass samples were immediately ground with isopropanol and incubated for 30 min at 70°C. The precipitate was then extracted twice with the isopropanol–chloroform mixture (1 : 1) at 70°C and dried on a rotor evaporator. The lipids were dissolved with a chloroform–methanol mixture (1 : 1), and the water-soluble compounds were removed by sequential addition of water and 5% NaCl solution. The mixture was vortexed, and the chloroform layer was separated, dehydrated by passing through anhydrous sodium sulfate, evaporated, and vacuum-dried to a constant mass. The precipitate was dissolved in a small amount of the chloroform–methanol mixture (1 : 1) and stored at –21°C.

The composition of neutral lipids (NLs) was analyzed by ascending thin-layer chromatography (TLC) on glass plates with silica gel 60 (Merck, Germany). The separation of NL was carried out in a hexane–dimethyl ether–acetic acid (85 : 15 : 1) solvent system [9]. For separation of phospholipids (PLs) and glycolipids (GLs), the Benning system for two-dimensional TLC was used [10]. The amount of lipids applied to a plate was 150–200 μg. The chromatograms were developed by spraying with 5% sulfuric acid in ethanol with subsequent heating to 180°C. The PLs were identified using individual markers and qualitative reactions with ninhydrin (for the presence of amino groups), Dragendorff reagent (for choline-containing PLs), and α-naphthol (for GLs) [9]. Neutral lipids were identified using individual markers of mono-, di-, and triacylglycerols; free fatty acids; sterols (ergosterol); and hydrocarbons (Sigma, United States). Saponifiaction was used to determine the sphingolipid nature of phospholipids [9]. Quantitative analysis of the lipids was carried out using the Dens software package (Lenkhrom, Russia). Phosphatidylcholine (Sigma, United States), glycoceramide mixture (Larodan, Sweden), and stigmasterol (Sigma, United States) were used as standards for phospholipids, sphingolipids, and sterols, respectively.

The composition of lipid fatty acids was determined on a Kristall 5000.1 gas–liquid chromatograph (Khromatek, Russia) equipped with an Optima-240 0.25-μm × 60-m × 0.25-mm capillary column (Macherey-Nagel GmbH&Co, Germany). Fatty acid methyl ethers were obtained by incubating the lipids in 2.5% H_2SO_4 in methanol for 1 h at 80°C. The temperature program used for chromatography ranged from 130 to 240°C. Identification was carried out with the Supelco 37 Component FAME Mix marker mixture of individual fatty acid methyl ethers (United States) and by GLC–mass spectrometry.

For determination of the carbohydrate composition of the mycelium, the sugars were extracted with boiling water four times for 20 min. Proteins were removed from the extract [11]. The carbohydrate extract was further purified from charged compounds on a combined column with the Dowex-1 (acetate form) and Dowex 50W (H^+) ion-exchange resins. The carbohydrate composition was determined by GLC of the trimethylsilyl sugar derivatives obtained from the dry lyophilic extract [12]. The internal standard used was α-methyl-D-mannoside (Merck). Chromatographic analysis was carried out on a Kristall 5000.1 gas–liquid chromatograph (Khromatek, Russia) equipped with a ZB-5 30-m × 0.32-mm × 0.25-μm capillary column (Phenomenex, United States) at 130–270°C (5–6°C/min). Glucose, mannitol, arabitol, inositol, and trehalose (Sigma, United States) were used as markers.

The experiments were carried out in triplicate. The results of a typical experiment are presented. The scatter of readings did not exceed 10%, and all the patterns were the same.

RESULTS AND DISCUSSION

Two variants of heat influence (within the tolerance zone and heat shock) caused fundamentally different results. For example, after 1 h of HS, acquired heat resistance (resistance to lethal HS of 20 min at 55°C) was already observed, while heat influences within the tolerance zone did not cause this effect even after 6 h.

Maintenance of the physiological state of the membranes plays a key role in the acquisition of heat resistance. According to the present-day concepts, it may be associated with the composition of the membrane lipids and their fatty acids, as well as with the protective effect of the soluble carbohydrates of the cytosol [3, 13].

Prior to investigation of the effects of heat influences, the temperature characteristics of fungal growth were determined for all three species. The zone of optimal conditions, the zone of retarded growth

Table 1. Membrane lipids (% of the total) of mycelial fungi under different heat influences

Lipids	*A. niger*			*C. japonica*			*P. ostreatus*		
	C	TZ	HS	C	TZ	HS	C	TZ	HS
PE	11.4	12.6	11.5	24.7	19.0	16.5	22.7	25.4	20.3
PC	13.3	15.0	10.0	21.5	21.2	9.5	31.8	27.4	20.9
CL	12.5	10.3	12.6	1.6	1.8	1.8	4.2	3.5	4.1
PA	11.2	11.0	23.4	14.4	11.6	27.8	7.9	10.0	22.5
PS	6.1	5.6	4.7	2.6	1.5	Tr	1.7	2.1	1.6
PI	6.0	5.2	5.7	–	–	–	–	–	–
LPE	6.3	5.7	4.4	1.0	0.1	–	0.2	0.5	0.6
LPC	6.0	5.5	3.4	–	–	–	0.9	0.2	0.7
PG	4.7	4.6	3.4	–	–	–	–	–	–
St	–	–	–	0.9	1.3	2.3	–	–	–
ΣPL	77.5	75.5	79.1	66.7	56.5	57.8	69.4	69.2	70.8
SL-1	3.7	3.2	3.4	3.0	2.3	2.5	3.2	1.8	2.7
SL-2	0.3	0.4	1.7	–	–	–	1.2	0.2	0.4
SL-3	0.2	0.5	0.4	–	–	–	2.4	4.9	7.6
ΣSL	4.2	4.1	5.5	3.0	2.3	2.5	6.9	6.9	10.8
St	18.3	20.2	15.6	30.2	41.3	39.7	23.7	23.9	18.4
Σ membrane lipids, μg/g dry mass	9002.1	8635.2	10229.5	24907.3	42282.3	53416.3	8419.5	12560.9	26019.7

Note: PC, phosphatidylcholines; PE, phosphatidylethanolamines; CL, cardiolipins; PA, phosphatidic acids; PS, phosphatidylserines; PI, phosphatidylinositols; LPE, lysophosphatidylethanolamines; LPC, lysophosphatidylcholines; SL, sphingolipids; St, sterols; C, control; TZ, heat influence within the tolerance zone; HS, heat shock; and Tr, traces.

(tolerance zone), and the zone of inhibition of the growth processes (heat shock) were determined.

Both *A. niger* and *C. japonica* grew in submerged culture as small pellets (1–3 mm in diameter), while *P. ostreatus* formed a loose mycelium. No observable changes in morphology were detected in the experiments with 3-h heat influences.

The fungal membrane lipids were PLs, sphingolipids (SLs), and sterols (Sts) (Table 1). In *A. niger*, among the nine classes of PLs, phosphatidylethanolamine (PEs), phosphatidylcholines (PCs), cardiolipins (CLs), and phosphatidic acids (PAs) were major components, while phosphatidylserines (PSs), phosphatidylinositols (PIs), lysophosphatidylethanolamines (LPEs), lysophosphatidylcholines (LPCs), and phosphatidylglycerols (PGs) were minor components. This fungus also contained two classes of sphingolipids (SL-1 and SL-2). Compared to *A. niger, C. japonica* and *P. ostreatus* have lower levels of CLs and the minor phospholipids. No SL-2 was present in *C. japonica*. All the species had a high content of sterols (18–41% of the total membrane lipids).

Individual differences also existed, for instance, in *C. japonica*: the ratio of sterols increased under both types of heat influence. The PE/PC ratio decreased under heat influences within the tolerance zone and increased significantly (compared to the control) under heat shock. In the basidiomycete *P. ostreatus*, the PE/PC ratio increased under both types of heat influence. Moreover, no changes in the relative content of sterols were found in *A. niger* and *P. ostreatus*.

The effect of heat influences on the fatty acid composition of phospholipids, which are the major structural components of the lipid bilayer, is of utmost importance. In order to investigate this issue, the major phospholipids of the three fungal species were isolated chromatographically and the structure of their acyl chains was determined. The results (Tables 2–4) demonstrate that in none of the major PL of these species the ratio of saturated fatty acid increased (the unsaturation degree UD did not decrease). Moreover, in such cases as PCs in *P. ostreatus* or PEs in *A. niger* and *C. japonica,* UD even increased. On the contrary, heat influences within the tolerance zone resulted in UD decrease in some phospholipids, such as PAs in *A. niger* or PEs in *P. ostreatus*. In the case of *C. japonica,* no type of heat influence resulted in decreased UD of the phospholipid acyl chains.

The general pattern in the composition of soluble carbohydrates in the cytosol of the three fungal species was an increase of trehalose levels under HS and the absence of such increases within the tolerance zone. For example, in the case of *A. niger* trehalose content under heat influences within the tolerance zone did not differ from the control values (0.2–0.4%), while under the HS conditions it increased to 5–6% (fig-

Table 2. Fatty acid composition of the major phospholipids of *C. japonica* (% of the total) under different heat influences

Fatty acids	PE			PC			PA		
	C	TZ	HS	C	TZ	HS	C	TZ	HS
$C_{14:0}$	2.5	2.1	–	3.0	2.9	2.6	–	3.0	2.7
$C_{16:0}$	28.2	23.0	23.4	24.5	25.7	23.2	25.3	30.0	26.7
$C_{16:1}$	0.3	0.5	0.4	0.7	0.7	0.4	0.5	0.5	–
$C_{17:0}$	1.7	1.1	1.8	1.9	1.9	1.3	4.7	2.1	1.7
$C_{16:1}$	1.2	0.7	0.8	0.8	0.9	0.3	2.1	1.0	1.6
$C_{18:0}$	16.9	10.8	14.6	14.7	11.8	14.1	16.9	16.3	16.8
$C_{18:1\,n9c}$	35.8	44.1	40.1	34.0	35.6	32.9	36.1	32.8	29.8
$C_{18:2\,n6c}$	9.5	9.6	10.3	15.2	13.0	22.1	12.1	10.3	16.6
$C_{18:3\,n6}$	4.0	8.1	7.4	5.2	7.5	3.1	2.3	4.1	4.0
$C_{24:0}$	–	–	1.1	–	–	–	–	–	–
Degree of unsaturation	0.68	0.89	0.84	0.82	0.86	0.87	0.70	0.67	0.77

Table 3. Fatty acid composition of the major phospholipids of *A. niger* (% of the total) under different heat influences

Fatty acids	PE			PC			CL			PA		
	C	TZ	HS	C	TZ	HS	C	TZ	HS	C	TZ	HS
$C_{16:0}$	41.1	34.1	35.1	27.6	23.3	28.9	27.4	24.4	26.6	31.6	38.7	33.3
$C_{18:0}$	9.5	6.2	5.3	8.1	8.3	12.0	10.1	11.3	7.0	9.7	12.6	10.0
$C_{18:1\,n9c}$	32.3	26.2	24.3	35.0	26.2	21.2	31.7	27.0	26.4	26.7	19.0	20.2
$C_{18:2\,n6c}$	17.1	33.5	35.3	29.3	42.2	37.8	30.8	37.3	40.0	32.0	29.7	36.5
Degree of unsaturation	0.67	0.93	0.95	0.94	1.11	0.97	0.93	1.02	1.06	0.91	0.78	0.93

Table 4. Fatty acid composition of the major phospholipids of *P. ostreatus* (% of the total) under different heat influences

Fatty acids	PE			PC		
	C	TZ	HS	C	TZ	HS
$C_{12:0}$	–	–	–	2.2	–	–
$C_{14:0}$	0.9	1.6	0.3	1.0	1.4	1.1
$C_{14:1}$	–	14.1	–	0.2	0.5	–
$C_{15:0}$	–	–	–	–	3.7	0.8
$C_{15:1}$	–	–	–	0.2	–	–
$C_{16:0}$	19.3	19.5	8.1	17.2	15.0	12.1
$C_{16:1}$	–	–	0.1	0.4	1.1	–
$C_{18:0}$	Tr	4.5	3.1	4.7	6.2	5.1
$C_{18:1\,n9c}$	11.9	14.3	24.7	35.6	28.8	14.0
$C_{18:2\,n6c}$	67.4	45.2	63.5	37.9	41.9	66.9
$C_{18:3\,n6}$	0.5	0.9	0.1	0.5	1.4	Tr
Degree of unsaturation	1.48	1.22	1.52	1.14	1.18	1.48

ure). In *C. japonica*, soluble carbohydrates (glucose, trehalose, and minor amounts of erythritol) constituted 6–7% of the dry mass. The carbohydrate content decreased to 3–4% under heat influences within the tolerance zone and increased to 9–10% under HS conditions. The relative content of trehalose increased significantly (up to 45% of the total carbohydrates) only under heat shock. In *P. ostreatus*, the carbohydrates in the control variant were mannitol (19% of the total), trehalose (41%), sucrose (33%), and glucose (6%), constituting 18% of the dry biomass. The carbohydrate levels changed insignificantly under heat influences. In the HS variant, mannitol content decreased significantly (to 9%) and sucrose content increased (47%), while the levels of trehalose and glucose remained practically unchanged.

Thus, increased levels of PA, trehalose, and the PE/PC ratio were common responses of the three fungal species to HS. Apart from that, some species exhibited elevated levels of sterols (*C.* japonica) and sphingolipids (*A. niger*). On the contrary, heat influences within the tolerance zone had practically no effect on PA and trehalose levels. In some cases, the following changes were observed for various species: decreased

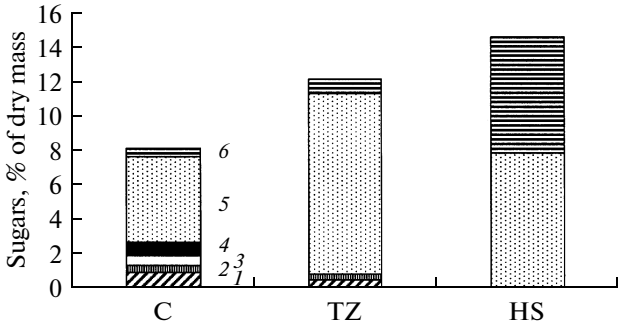
UD of the fatty acids of some phospholipids, decreased PE/PC ratio, and increased ratio of sterols.

Comparison of the responses of the fungal cells to two variants of heat influence revealed a fundamental difference between them in the variations in the level of trehalose and phosphatidic acids, of the PE/PC ratio, and of the unsaturation degree of the phospholipid fatty acids.

How to explain these results using the existing concepts? First of all, it should be noted that the modern concepts of heat adaptation at the membrane level do not differentiate between the types of heat influences, although the latter differ in the biological effect [1], since, unlike the influences within the tolerance zone, HS results in acquired resistance to the lethal shock.

Two hypotheses are known for the explanation of the variations in the structure of the membrane lipids under heat modulations of the environment. The first one, the homeoviscosity hypothesis [2], postulates the preservation of the membrane viscosity under temperature modulations by changing the degree of unsaturation for the cayl chains of the membrane lipids, not affecting their "heads." Since the author considers the ratio between the saturated and unsaturated fatty acids to be the main viscosity modulator, UD of the membrane lipids should decrease at hyperthermia and increase at hypothermia. The second hypothesis, of "dynamic phase behavior of the membranes" or the homeophase one [3], explains adaptation to temperature fluctuations within the tolerance zone mainly by the maintenance of the balance between the so-called bilayer lipids stabilizing the membrane (PC, PS, sphingomyelin, PI, digalactosyldiglyceride), which have cylinder-shaped molecules and are able to form a lipid bilayer, and the conical-shaped, so-called nonbilayer lipids destabilizing the membrane (PE with unsaturated fatty acids, PS at pH below 4, PA, CL, monogalactosyldiglyceride), which form an inverted phase instead of a bilayer.

In the case of heat influences within the tolerance zone, the adaptation is carried out by the biochemical mechanisms already present in the cell, while under HS, significant metabolic changes occur. The theory of HS response postulate the inhibition of expression of the so-called housekeeping genes and induction of a protective system, which includes synthesis of trehalose and heat shock proteins, elevated activity of catalase and superoxide dismutase, structuring and redistribution of water in the cytosol compartments, changes in the composition of the membranes, and preservation of the intracellular pH [1]. From the biophysical point of view, HS promotes transition of an organism from homeostasis to stress, another discrete stationary state [14]. We demonstrated the stability of this state in three fungi, which acquired resistance to both nonlethal and lethal heat shock already after 1 h of HS. Since eukaryotic organisms do not possess saturases, de novo synthesis of fatty acids is required in order to decrease the unsaturation degree, i.e., to increase the ratio of saturated fatty acids. Under experimental conditions, the capacity of an organism for such alterations of the membrane lipids is probably limited.



Effect of different heat influences on the composition of the cytosol carbohydrates in *A. niger*: glycerol (*1*), erythritol (*2*), arabitol (*3*), glucose (*4*), mannitol (*5*), and trehalose (*6*). C indicates control; TZ, heat influence within the tolerance zone; and HS, heat shock.

These considerations made it possible to suggest the hypothesis of membrane protection under HS conditions by the stabilizing compounds (trehalose, sphingolipids, sterols, etc.) [4]. The present work confirmed our suggestion, revealing no decrease in the UD of the acyl chains of the phospholipids and no increase in the content of bilayer lipids in three species of mycelial fungi, while the trehalose level was found to increase in all the cases. Moreover, a significant increase in the ratio of the nonbilayer lipids in the membranes was observed, resulting from an increase in PA content and a decreased PC level. We presently have no adequate explanation for this finding. The origin of PA is unclear, since this compound may be synthesized via three pathways—from PC (phospholipase D), from diacylglycerol (DAG kinase), and from lysophosphatidic acid (acyl transferase) [16]. Analysis of fatty acids in PA and PC revealed a certain similarity in their fatty acid composition (Tables 2 and 3), suggesting activation of phospholipase D under heat shock conditions. This conclusion is indirectly supported by decreased PC levels in the membrane lipids. Significant amounts of PA in the membrane lipids under HS conditions are probably associated with the structural, rather than signal, role of this phospholipid. According to the literature data [17, 18], PA is a unique phospholipid with a small negatively charged head, high affinity to bivalent ions, and capacity for formation of intramolecular hydrogen bonds. Under physiological conditions at neutral pH and in the absence of bivalent ions, PA acts as a bilayer lipid. However, under slightly acidic conditions and in the presence of ions (for example, in the Golgi apparatus) it forms type II micelles. Capacity for aggregation results in formation of PA microdomains that participate in development of the negative curving of the membrane. PA is believed to physically participate in formation of vesi-

cles, thus regulating transport from the Golgi apparatus, endo- and exocytosis. Phospholipase D, which is responsible for PA formation in the membranes, was shown to control such vitally important processes as cytoskeleton reconstruction, proliferation, and survival. The significant accumulation of PA under HS conditions reported in the present paper and the literature data on high PA content in the thermophilic fungus *Humicola grisea* var. *thermoidea* [19] suggest an important role of this phospholipid in modification of the structure of the membranes. Under HS conditions, PA probably promotes formation of the endosomes involved in the isolation and degradation of proteins.

The mechanism of stabilization of the lipid bilayer by trehalose is not known in detail. It has been suggested that formation of hydrogen bonds between this disaccharide and several phospholipid molecules results in stabilization of the structure of the membranes [20]. We have previously shown the presence of sucrose in xylotrophic fungi *P. ostreatus* and an increase of its level under HS [21]. Although this disaccharide also has membrane-stabilizing properties, it should be noted that, under optimal conditions, *P. ostreatus* contains high levels of trehalose (up to 7.8% of the dry mass), equal to its content in *A. niger* under HS conditions. This may explain the absence of trehalose increase in *P. ostreatus* under HS conditions. Unlike trehalose, the role of other compounds stabilizing the lipid bilayer (sterols and sphingolipids) is not universal, although it has been reported for some fungi, as, for example, increased sphingolipid content in *A. niger* and an increased ratio of sterols in *C.* japonica. Thus, the results of the present work support our hypothesis of membrane stabilization by heat-protective compounds.

REFERENCES

1. Piper, P.W., Molecular Events Associated with Acquisition of Heat Tolerance by the Yeast *Saccharomyces cerevisiae, FEMS Microbiol. Rev.*, 1993, vol. 11, pp. 339–356.
2. Sinensky, M., Homeoviscous Adaptation—a Homeostatic Process That Regulates the Viscosity of Membrane Lipids in *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1974, vol. 71, no. 2, pp. 522–525.
3. Hazel, J.R., Thermal Adaptation in Biological Membranes: Is Homoviscous Adaptation the Explanation?, *Annu. Rev. Physiol.*, 1995, vol. 57, pp. 19–42.
4. Tereshina, V.M., Memorskaya, A.S., and Kotlova, E.R., Membrane Lipid and Cytosol Carbohydrate Composition in *Aspergillus niger* under Heat Shock, *Mikrobiologiya*, 2010, vol. 79, no. 1, pp. 45–51 [*Microbiology* (Engl. Transl.), vol. 79, no. 1, pp. 40–46].
5. Ainsworth, J. and Bisby, H., Dictionary of the Fungi, Kirk, P., Cannon, P., David, J., and Stalpers, J., Eds., *CAB Int.*, 2001.
6. Blumental, N.J. and Roseman, S., Quantitative Estimation of Chitin in Fungi, *J. Bacteriol.*, 1967, vol. 74, pp. 222–225.
7. Garton, C.U., Goodwin, T.W., and Lijinsky, V., Studies in Carotenogenesis of *Phycomyces blakesleeanus, Biochem. J.*, 1961, vol. 48, no. 2, pp. 154–163.
8. Nichols, B.W., Separation of the Lipids of Photosynthetic Tissues; Improvement in Analysis by Thin-Layer Chromatography, *Biochim. Biophys. Acta*, 1963, vol. 4145, pp. 417–422.
9. Kates, M., *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Amsterdam: Elsevier, 1972 [Russ. Transl. Moscow: Mir, 1975]
10. Benning, C., Huang, Z.-H., and Gage, D.A., Accumulation of a Novel Glycolipid and a Betaine Lipid in Cells of *Rhodobacter sphaeroides* Grown Under Phosphate Limitation, *Arch. Biochem. Biophys.*, 1995, vol. 317, no. 1, pp. 103–111.
11. Somogui, M., Determination of Blood Sugar, *J. Biol. Chem.*, 1945, vol. 160, p. 69.
12. Brobst, K.M., Gas-Liquid Chromtography of Trimethysilil Sugar Derivatives, in *Metody issledovaniya uglevodov* (Methods of Carbohydrate Research), Horlin, F.J., Ed., Moscow: Mir, 1975, pp. 9–13.
13. Thevelein, J.M, Regulation of Trehalose Metabolism and Its Relevance to Cell Growth and Function, in *The Mycota*, Brambl, R. and Marzluf, G.A., Eds., Berlin: Springer, 1996, pp. 395–420.
14. Veselova, T.V., Veselovskii, V.A., and Chernavskii, D.S., *Stress u rastenii* (Stress in Plants), Moscow: Mosk. Gos. Univ., 1993.
15. Weete, J.D., *Lipid Biochemistry of Fungi and Other Organisms*, New York: Plenum, 1980.
16. Cazzolli, R., Shemon, A.N., Fang, M.Q., and Hughes, W.E., Phospholipid Signaling Trough Phospholipase D and Phosphatidic Acid, *IUBMB Life*, 2006, no. 8, pp. 457–461.
17. Kooijman, E.E., Chupin, V., de Kruif, B., and Burger, N.J., Modulation of Membrane Cutvature by Phosphatidic Acid and Lyso Phosphatidic Acid, *Traffic*, 2003, vol. 4, pp. 162–174.
18. McMahon, H.T. and Gallop, J.L., Membrane Curvature and Mechanisms of Dynamic Cell Membrane Remodeling, *Nature*, 2005, vol. 438, pp. 590–596.
19. Mumma, R.O., Sekura, R.D., and Fergus, C.L., Thermiphilic Fungi: III. The Lipids of *Humicola grisea* var. *thermoidea, Lipids*, 1971, vol. 6, no. 8, pp. 589–594.
20. Sum, A.K., Faller, L., and de Pablo, J.J., Molecular Simulation Study of Phospholipid Bilayers and Insights of the Interactions with Disaccharides, *Biophys. J.*, 2003, vol. 85, pp. 2830–2844.
21. Tereshina, V.M., Dormant Cells and Adaptation of Mycelial Fungi to Heat Shock, *Doctoral* (*Biol.*) *Dissertation,* Moscow: INMI RAN, 2006.