ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 1, pp. 40–46. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © V.M. Tereshina, A.S. Memorskaya, E.R. Kotlova, E.P. Feofilova, 2010, published in Mikrobiologiya, 2010, Vol. 79, No. 1, pp. 39–44.

EXPERIMENTAL ARTICLES

Membrane Lipid and Cytosol Carbohydrate Composition in *Aspergillus niger* under Heat Shock

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

[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 December 25, 2008

Abstract—A submerged *Aspergillus niger* culture exposed to heat shock (40–41°C) for 1, 3, and 6 h acquires resistance to a more severe, lethal heat shock (55°C, 20 min). A general trend characteristic of a heat shock occurring during the trophophase or idiophase (regardless of its duration) is an increase in the trehalose level in the cytosol carbohydrate fraction and in the content of sphingolipids and phosphatidic acid in the membrane lipid fraction. Contrary to generally accepted views, no increase in the content of unsaturated fatty acid in the main phospholipid fraction, sterol level, and share of "bilayer" phospholipids was detected. The results obtained are discussed in terms of the current hypotheses concerning membrane protection under heat shock and our own suggestion on this subject.

Key words: Aspergillus niger, heat shock, trehalose, phosphatidic acids, sphingolipids.

DOI: 10.1134/S0026261710010066

The term heat shock (HS) refers to the effect of high temperatures exceeding the growth optimum by 8–12°C [1]. Under these conditions, the expression of the house-keeping genes and the translation of their preexisting mRNA are arrested. Against this background, the protective system, which in fungi includes synthesis of heat shock proteins, trehalose accumulation, synthesis of catalase and superoxide dismutase for detoxication of reactive oxygen species, water redistribution in cytosol compartments, changes in membrane composition, and intracellular pH maintenance, is activated [2]. An HS results in a deceleration of growth processes and the acquisition of resistance to a lethal HS caused by the temperatures exceeding those of the original HS by 10–15°C.

The functional state of membranes under HS is a prerequisite for survival and acquisition of heat resistance. However, the two known hypotheses that account for the changes in membrane lipids at high temperatures deal not with HS conditions, but with temperature changes within the tolerance range that only result in growth deceleration; under such conditions, regulation of metabolism involves preexisting cellular biochemical mechanisms. The "homeoviscous adaptation" hypothesis [3] postulates the maintenance of membrane viscosity by altering the composition of phospholipid acyl chains. The "homeophasic adaptation" hypothesis [4] ascribes the greatest importance to maintaining of a certain balance between the "bilayer" and "nonbilayer" lipids.

Nevertheless, such changes in the membrane lipids may be difficult to accomplish under HS owing to (i) the activation of the protective system and a metabolic transition and (ii) the peculiarities of the fatty acid synthesis in eukaryotes, when saturated fatty acids are initially synthesized and then unsaturated ones are formed via desaturase activity [5, 6]. Converting saturated fatty acids into their unsaturated counterparts can be performed directly on the phospholipid involved, while the reverse process requires the operation of a whole enzyme complex including fatty acid synthases, phospholipases, and acyl hydrolases that are responsible for the formation of new phospholipids. In eukaryotes, the lack of saturases that could theoretically be HS-inducible and cause the accumulation of saturated acyl chains in their phospholipids, challenges the suggestion that the desaturation degree of fatty acids can quickly decrease. In addition, there is evidence of a lack of HS-induced changes in the fatty acids in yeast [7] and in the neutral, glyco-, and phospholipid fractions of the mycelial fungus *Aspergillus niger* [8]. These results, in conjunction with modern concepts on the protective role of trehalose in fungi at high temperatures [9, 10], enabled us to suggest a new "membrane stabilization" hypothesis [8] postulating that membrane protection under HS involves stabilizing compounds such as trehalose, sterols, etc.

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

The goal of this work was to investigate the composition of membrane lipids and cytosol carbohydrates in two different growth stages of a submerged *A. niger* culture under HS of different durations.

MATERIALS AND METHODS

The study was conducted with the ascomycete fungus *Aspergillus niger* VKM F-34 that was grown on wort agar for 5–6 days at 29°C. The spore suspension added to the medium to a final concentration of 5×10^5–10^6 spores per 1 ml of medium was used as inoculum. The fungus was grown in submerged culture in 250-ml flasks with 50 ml of Blumental–Roseman medium [11] on a KE-12-250T electromagnetic shaker (150 rpm) at the optimum temperature (29–30°C, control variant) for 24 h (the trophophase) and 48 h (the idiophase). To study the heat shock effect, the cultivation temperature was raised, under the same aeration conditions, to 40–41°C and the cultivation was continued for 1, 3, or 6 h. The control variant was grown under optimum conditions.

The biomass was separated using a nylon filter, washed with distilled water heated to the respective temperature, immediately fixed with 5 ml of hot (70°C) isopropanol, and incubated at this temperature for 30 min. Thereupon, the biomass was extracted using the method described in [12] which involved extraction with isopropanol and the isopropanol–chloroform mixture (1 : 1) at 70°C, evaporation in a rotary evaporator, and extraction of the residue with a chloroform–methanol mixture (1 : 1) supplemented with 5% sodium chloride solution and water to remove water-soluble substances. After separating the mixture by allowing it to stay overnight or by centrifugation, the chloroform layer was dried by passing it through water-free sodium sulfate, evaporated, and desiccated with a vacuum pump. The resulting pellet was dissolved in a small amount of chloroform–methanol mixture (1 : 1) and stored at –21°C.

The composition of neutral lipids (NLs) was assessed using ascending thin-layer chromatography on glass plates with silica gel 60 (Merck, Germany). To separate NL, we used the hexane : diethyl ether : acetic acid (85 : 15 : 1) system [13]. Phospholipids (PL) and glycolipids (GL) were separated with the Benning system [14] for two-dimensional TLC. The lipids (150–200 μg) were applied on a plate. The chromatograms were sprayed with 5% sulfuric acid in ethanol with subsequent heating to 180°C for developing the stains. PL were identified using individual markers and qualitative tests for amino groups (with ninhydrin), choline-containing PL (with the Dragendorff reagent), and glycolipids (with α-naphthol). Neutral lipids were identified with individual markers for mono-, di-, and triglycerides, sterols (ergosterol), free fatty acids, and hydrocarbons (Sigma, United States). Sphingolipids were detected in the glycolipid fraction by the saponification method [13]. Quantitative analysis of the lipids was performed using the Dens software (Lenkhrom, Russia). Lipid quantities were determined using the following standards: phosphatidylcholine (Sigma, United States) for phospholipids, a glycoceramide mixture (Larodan, Sweden) for sphingolipids, and stigmasterol (Sigma, United States) for sterines.

The fatty acid composition of NL, PL, and GL was determined with a Kristall 5000.1 gas–liquid chromatograph (Khromatek, Russia) on an Optima-240 0.25-μm, 60-m, 0.25-mm capillary column (Macherey-Nagel GmbH & Co., Germany). During the chromatography, the temperature was gradually raised from 130 to 240°C. Fatty acids were identified using the Supelco 37 Component FAME Mix mixture of fatty acid methyl esters (United States) and gas–liquid chromatography–mass spectrometry.

To determine the carbohydrate composition of the mycelium, sugars were extracted from it with boiling water for 20 min; the extraction was done four times. Proteins were removed from the resulting extract [15]. The carbohydrate extract was further purified from charged compounds using a combined column with the Dowex-1 (acetate form) and Dowex 50W (H+) ion-exchange resins. Carbohydrate composition was determined by GLC, using trimethylsilyl sugar derivatives obtained from the lyophilized extract [16]. α-methyl-D-mannoside (Merck) was employed as the internal standard. Chromatography was carried out with a Kristall 5000.1 gas–liquid chromatographer (Khromatek, Russia) on a ZB-5 30 m, 0.32 mm, 0.25 μm capillary column (Phenomenex, United States). The temperature was raised from 130 to 270°C at a rate of 5–6 °C/min. Glucose, mannitol, arabitol, inositol, and trehalose (Sigma, United States) were used as markers.

Three repetitions of all experiments were conducted, and the data of one of the repeats are presented for each experiment.

RESULTS AND DISCUSSION

The main result of the operation of the protective system under HS is the acquisition of heat resistance. The main prerequisite for this capacity is maintenance of the functional state of the membranes, which may be conditional on the composition of the membrane lipids and their fatty acids and involve the protective action of the soluble carbohydrates of the cytosol, in terms of the relevant present-day concepts [4, 9].

It was revealed that growing the submerged culture of *A. niger* under HS (40–41°C) for 1 h resulted in its acquiring resistance to a lethal HS (55°C, 20 min), whereas the control sample loses its viability under these conditions.

The membrane lipids of *A. niger* include PL, sphingolipids (SL), and sterols (Tables 1, 2). Among the nine PL classes detected by us, phosphatidylethanolamines (PE), phosphatidylcholines (PC), cardiolipins (CL), and phosphatidic acids (PA) are the predomi-

Table 1. Membrane lipids of *A. niger* mycelium during a heat shock in the trophophase (% of total)

Lipids	C-1	HS-1	C-3	HS-3	C-6	HS-6
PE	32.1	31.9	30.3	26.9	27.8	22.8
PC	37.3	30.9	26.2	23.7	26.7	19.3
CL	5.9	9.9	15.5	10.9	12.6	8.7
PA	1.9	4.8	3.7	7.1	3.9	20.8
PS	3.6	1.8	1.9	2.7	1.0	1.0
PI	2.7	1.7	1.7	2.7	0.7	1.6
LPE	1.2	0.7	1.0	0.3	1.0	0.8
LPC	3.8	2.0	3.6	3.1	5.0	0.8
PG	–	–	0.9	–	0.6	–
Total PL	88.5	83.7	84.8	77.4	79.4	75.8
SL-1	4.0	4.5	3.5	4.4	3.7	5.2
SL-2	0.3	3.8	1.5	5.3	1.5	3.6
Total SL	4.3	8.3	5.0	9.7	5.2	8.8
Sterols	7.2	8.0	10.2	12.9	15.4	15.4
Total membrane lipids, μg/g of dry mass	15874.4	18118.8	13613.8	10863.2	14667.3	17708.8

Designations: PC, phosphatidylcholines; PE, phosphatidyletanolamines; CL, cardiolipins; PA, phosphatidic acids; PS, phosphatidylserines; LPE, lysophophatidylethanolamines; SL, sphingolipids; PG, phosphatidylglycerides, LPC, lysophosphatidylcholines.

Table 2. Membrane lipids of *A. niger* mycelium during a heat shock in the idiophophase (% of total)

Lipids	C-1	HS-1	C-3	HS-3	C-6	HS-6
PE	18.2	24.0	10.6	2.9	14.1	3.5
PC	21.3	25.0	15.7	3.1	22.0	5.7
CL	20.3	13.3	16.1	14.5	11.5	10.3
PA	12.8	14.0	11.4	29.7	7.7	23.1
PS	0.4	Traces	1.4	Traces	Traces	Traces
PI	0.9	Traces	0.7	Traces	3.1	–
LPE	0.7	–	–	–	–	–
LPC	0.2	–	–	–	3.1	–
PG	–	–	–	–	7.1	–
Total PL	74.8	76.3	55.9	50.2	67.8	42.6
SL-1	7.1	6.6	9.8	12.3	10.4	18.0
SL-2	4.4	3.8	3.0	14.0	0.2	18.5
Total SL	11.5	10.4	12.8	26.3	10.6	36.5
Sterols	13.7	13.3	31.3	23.4	21.6	20.9
Total membrane lipids, μg/g of dry mass	10477.7	10210.5	8242.8	10252.7	7737.7	13732.9

Table 3. Fatty acids of the major PL in *A. niger* under conditions of heat shock at the trophophase stage (% of total)

Fatty acids	PE		PC		CL		PA	
	C-6	HS-6	C-6	HS-6	C-6	HS-6	C-6	HS-6
$C_{16:0}$	27.9	29.4	21.8	23.2	24.9	23.5	41.4	25.6
$C_{17:0}$	15.6	18.8	12.4	16.5	14.4	21.3	16.7	8.7
$C_{18:0}$	6.3	4.3	7.4	7.0	10.9	6.6	12.1	11.3
$C_{18:1n9c}$	18.7	20.1	18.3	20.8	20.6	22.9	15.9	18.9
$C_{18:2n6c}$	25.8	27.4	36.3	32.5	20.6	25.7	13.9	20.9
$C_{21:1n9}$	5.8	Traces	3.8	Traces	8.6	Traces	Traces	14.7
Degree of unsaturation	0.76	0.75	0.90	0.86	0.70	0.74	0.44	0.75

Table 4. Fatty acids of the major PL in *A. niger* under conditions of heat shock at the idiophase stage (% of total)

Fatty acids	PE		PC		CL		PA	
	Control	HS	Control	HS	Control	HS	Control	HS
$C_{15:0}$	Traces	5.7	4.7	5.8	6.2	4.2	2.9	2.9
$C_{16:0}$	45.9	40.0	34.5	33.9	35.7	28.5	37.1	29.6
$C_{16:1}$	Traces	Traces	1.9	Traces	Traces	Traces	Traces	Traces
$C_{17:0}$	14.9	13.9	12.1	15.0	16.0	11.6	16.1	8.3
$C_{18:0}$	9.7	9.0	11.2	9.0	9.6	8.1	9.7	5.1
$C_{18:1n9c}$	16.2	18.1	18.4	18.8	20.7	24.0	25.9	23.7
$C_{18:2n6c}$	13.4	13.3	17.2	17.5	11.8	23.6	8.2	30.3
Degree of unsaturation	0.43	0.45	0.55	0.54	0.44	0.71	0.42	0.84

nant species. The rest of the lipids, such as phosphatidylserines (PS), phosphatidylinositols (PI), lysophosphatidylethanolamines (LPE), and phosphatidylglycerols (PG), can be considered minor phospholipid species.

During the trophophase, 1-h-long HS already resulted in two types of changes in PL composition (in contrast to the control variant): (i) a threefold increase in PA percentage and (ii) a tenfold decrease in SL-2 share (Table 1). If the HS continued for 6 h, the effect became more pronounced. The PA percentage reached 20.8% of the total membrane lipids, which is five times higher than its content in the control sample. Importantly, the total lipid amount and the ratio between the bulk PL species (the "bilayer" PC and the "nonbilayer" PE) remained virtually unchanged under prolonged HS. The SL-2 content significantly increased after 1 h and remained at this level during a 6-h HS. It was established that, in contrast to the control variant, the sterol content did not change in response to a HS.

The HS response in the idiophase differed from that in the trophophase: no significant changes in the composition of membrane lipids occurred after 1 h (Table 2). However, an increase in the PA and SL-2 percentages was detected after 3 h, in analogy to the trophophase-specific changes. In contrast to the trophophase, the amounts of PL, including PC and PE, decreased, while the SL (particularly the SL-2) content increased. Therefore, the membrane lipid profile

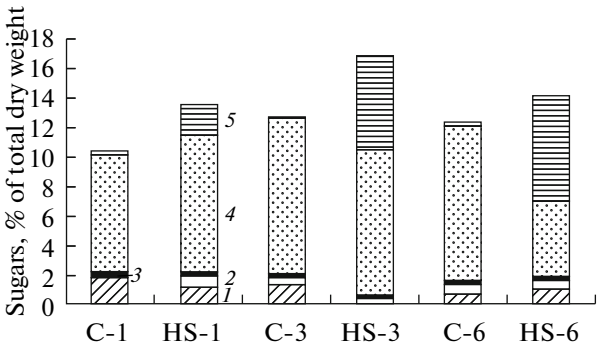

Fig. 1. Effect of heat shock on cytosol carbohydrate composition in the trophophase of *A. niger*: glycerol (*1*), erythritol (*2*), glucose (*3*), mannitol (*4*), and trehalose (*5*).

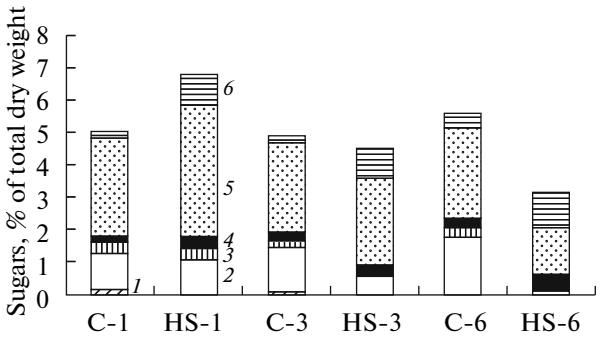

Fig. 2. Effect of heat shock on cytosol carbohydrate composition in the idiophase of *A. niger*: glycerol (*1*), erythritol (*2*), arabitol (*3*), glucose (*4*), mannitol (*5*), and trehalose (*6*).

under prolonged HS in the idiophase was substantially different from that in the trophophase.

In our studies on the fatty acid composition of the four main PL under HS (6 h) we established the following facts (Table 3, 4):

(i) the fatty acid composition in the control and experimental samples was similar;

(ii) the fatty acid desaturation degree was higher in the trophophase than in the idiophase; and

(iii) the acyl chain desaturation degree of the main PL did not decrease in either growth phase, regardless of the HS duration; the PC content was even increased.

As for the carbohydrate composition of the cytosol during an HS, a common trend occurring in both growth phases was a marked increase in trehalose level after 1 h of HS (Fig. 1, 2). The differences between the phases were that, in the trophophase, trehalose content additionally doubled under a prolonged HS, whereas in the idiophase it remained at the same level. Interestingly, the cytosol carbohydrate content in the idiophase was two times lower than in the trophophase. Increasing the HS time to 6 h resulted in a twofold decrease in carbohydrate content in the idiophase, which was not the case for the trophophase.

An increase in the trehalose, SL, and PA levels was therefore a general pattern, which is characteristic of the fungal HS response. Contrary to the hypotheses suggested by Sinensky [3] and Hazel [4], no increase in unsaturated fatty acid percentage, i.e., no decrease in desaturation degree, occurred in the bulk PL. In addition, a relatively constant PE/PC ratio and an increase in the amount of "nonbilayer" PA were characteristic of both growth phases under HS. This is at variance with Hazel's hypothesis, which assumes an increase in "bilayer" lipid amount upon elevating the growth temperature. Interestingly, no appreciable increase in sterol content was detected under HS. Therefore, the data obtained support the hypothesis that the membranes are stabilized under HS by trehalose and carbohydrate-containing sphingolipids.

Importantly, our data that saturated acyl chains fail to accumulate in fungal bulk PL do not rule out the involvement of alternative mechanisms of changing the microviscosity of the membrane lipid bilayer. Of special interest, therefore, are studies on the induction of the synthesis of a small (30 kDa), highly hydrophobic HS protein during HS [17]. Presumably, this protein can decrease the lipid bilayer fluidity that increases under HS.

Fascinating data were obtained on the accumulation of sphingolipids under HS in both growth phases. These carbohydrate-containing lipids, the quantity of which may amount to 30% of the total membrane lipid content [18], are currently believed to perform a large number of functions in fungal cells. It was demonstrated that disrupting SL synthesis results in the inhibition of the polarized growth of fungal spores [19]. These compounds are also involved in stimulating fruiting body formation in basidial fungi [20]. In addition, sphingolipids are the source of biologically active intermediates such as ceramides and sphingosines. They are implicated, as secondary messengers, in arresting the developmental cycle at the G_1 stage and in inducing apoptosis [21, 22]. Supposedly, such metabolites of the SL synthesis pathway as sphingoid bases and their phosphates perform a signaling function under HS. The level of these compounds increases five- to tenfold in eukaryotes, peaking after 10–15 min of HS and then decreasing to the baseline value [23]. The mechanism of HSP induction was revealed. For instance, under HS conditions, D,L-erythro-dihydrosphingosine induces the expression of the gene coding for the second trehalose synthetase subunit (TPS2) via the stress response element (STRE). It was also suggested that SL are involved, in combination with sterols, in the formation of specialized membrane domains (rafts) that enhance the HS resistance of the membranes [24]. In this work, it was established that an increase in SL content occurs in both growth phases, although it is more significant in the idiophase, in which the SL share among the membrane lipids may increase to 26%. In contrast to the SL

content, the trehalose level is considerably higher in the trophophase. Presumably, there is a certain relationship between the two compounds under HS, since the carbohydrate components of CL can form hydrogen bonds with the phospholipid head at the membrane surface and stabilize the membrane in a way similar to trehalose [25].

Of special interest is also the fact that the PA level increases during the development of the HS response in both growth phases. First, PA is the initial compound in the PL synthesis pathway. An increase in PA content may testify to PL degradation during a prolonged (6 h) HS, which results from phospholipase D activation. This is particularly manifest during the idiophase that is characterized by a significant PL content decrease. Second, PA is a biologically active compound performing signaling and regulatory functions [26]. PA accumulation may be linked to a special function of this "nonbilayer" PL under HS conditions, e.g., vesicle formation [27]. Of particular interest, therefore, are the data that a high relative PA content (up to 30% of the total PL) occurs in the thermophilic fungus *Humicola grizea* var. *thermoidea* [28]. In our further studies, we plan to focus on the issue of the cause of the PL level increase. It may be due to (i) *de novo* synthesis of PL or (ii) PL degradation under HS. Resolving this issue will improve our understanding of the PA involvement in membrane protection under stress.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, grant no. 07-04-01520.

REFERENCES

1. Lindquist, S., The Heat-Shock Response, *Ann. Rev. Biochem.,* 1986, vol. 55, pp. 1151–1191.
2. 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.
3. Sinensky, M., Homeoviscous Adaptatio—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.
4. Hazel, J.R., Thermal Adaptation in Biological Membranes: Is Homoviscous Adaptation the Explanation?, *Annu. Rev. Physiol.*, 1995, vol. 57, pp. 19–42.
5. Weete, J.D., *Lipid Biochemistry of Fungi and Other Organisms*, New York: Plenum Press, 1982, pp. 301–312.
6. Carratu, L., Franceschelli, S., Pardini, C.L., Kobayashi, G.S., Horvath, I., Vigh, L., and Maressa, B., Membrane Lipid Perturbation Modifies the Set Point of the Temperature of Heat Shock Response in Yeast, *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, pp. 3870–3875.
7. Swan, T.M. and Watson, K., Membrane Fatty Acid Composition and Membrane Fluidity as Parameters of Stress Tolerance in Yeast, *Can. J. Microbiol.*, 1997, vol. 43, pp. 70–77.
8. Tereshina, V.M., Resting Cells and Adaptation of Mycelial Fungi to Heat Shock, *Doctoral (Biol.) Dissertation*, Moscow: INMI RAN, 2006.
9. 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.
10. Tereshina, V.M., Thermotolerance in Fungi: The Role of Heat Shock Proteins and Trehalose, *Mikrobiologiya*, 2005, vol. 74, no. 3, pp. 293–304 [*Microbiology* (Engl. Transl.), vol. 74, no. 3, pp. 247–257].
11. Blumental, N.J. and Roseman, S., Quantitative Estimation of Chitin in Fungi, *J. Bacteriol.*, 1967, vol. 74, pp. 222–225.
12. 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.
13. Keits, M., *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Amsterdam: Elsevier, 1972 [Russ. Transl. Moscow: Mir, 1975].
14. 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.
15. Somogui, M., Determination of Blood Sugar, *J. Biol. Chem.*, 1945, vol. 160, p. 69.
16. 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.
17. Seymour, I.J. and Piper, P.W., Stress Induction of HSP30, the Plasma Membrane Heat Shock Protein Gene of *Saccharomyces cerevisiae*, Appears not to Use Known Stress-Regulated Transcription Factors, *Microbiology* (UK), 1999, vol. 145, pp. 231–239.
18. Dickson, R.C. and Lester, R.L., Sphingolipid Functions in *Saccharomyces cerevisiae, Biochim. Biophys. Acta*, 2002, vol. 1583, pp. 13–25.
19. Cheng, J., Park, T.S., Fischl, A.S., and Ye, X.S., Cell Cycle Progression and Cell Polarity Require Sphingolipid Biosynthesis in *Aspergillus niger, Mol. Cell. Biol.*, 2001, vol. 21, no. 18, pp. 6198–6209.
20. Sakai, H. and Kajiwara, S., Membrane Lipid Profile of an Edible Basidiomycete *Lentinula edodes* during Growth and Cell Differentiation, *Lipids*, 2004, vol. 39, no. 1, pp. 67–73.
21. Levery, S.B., Momany, M., Lindsey, R., Toledo, M.S., Shayman, J.A., Fuller, M., Brooks, K., Doong, R.L., Straus, A., and Takahashi, H.K., Disruption of the Glycosylceramide Biosynthetic Pathway in *Aspergillus nidulans* and *Aspergillus fumigatus* by Inhibitors of UDP-Glc:Ceramide Glucosyltransferase Strongly Affects Spore Germination, Cell Cycle, and Hyphal Growth, *FEBS Lett.*, 2002, vol. 525, pp. 59–64.

22. Siskind, L.J., Mitochondrial Ceramide and Induction of Apoptosis, *J. Bioenerg. Biomembr.*, 2005, vol. 37, no. 3, pp. 143–153.
23. Jenkins, G.M., The Emerging Role for Sphingolipids in Eukaryotic Heat Shock Response, *CMLS Cell. Mol. Life Sci.*, 2003, vol. 60, pp. 701–710.
24. Beck, J.G., Mathieu, D., Loudet, C., Buchoux, S., and Dufours, E.J., Plant Sterol in "Rafts": A Better Way to Regulate Membrane Thermal Shocks, *FASEB J.*, 2007, vol. 21, pp. 1714–1723.
25. Yu, R.K., Koerner, A.W., Scarsdale, J.N., and Prestegard, J.H., Elucidation of Glycolipid Structure by Proton Nuclear Magnetic Resonance Spectroscopy, *Chem. Phys. Lipids*, 1986, vol. 42, pp. 27–48.
26. Wang, X., Devaiah, S.P., Zhang, W., and Welti, R., Signaling Functions of Phosphatidic Acids, *Prog. Lipid Res.,* 2006, vol. 45, pp. 250–278.
27. Cazzolli, R., Shemon, A.N., Fang, M.Q., and Hughes, W.E., Phospholipid Signaling through Phospholipase D and Phosphatidic Acid, *IUBMB Life*, 2006, vol. 58, no. 8, pp. 457–461.
28. Mumma, R.O., Sekura, R.D., and Fergus, S.L., Thermophilic Fungi: III. The Lipids of *Humicola grisea* var. *thermoidea*, Lipids, 1971, vol. 6, no. 8, pp. 589–594.