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EXPERIMENTAL ARTICLES

Lipid Composition of the Mucoraceous Fungus *Blakeslea trispora* under Lycopene Formation-Stimulating Conditions

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Abstract—Stimulation of lycopene synthesis in the mycelium of the mucoraceous fungus *Blakeslea trispora* was accompanied by major changes in its lipid composition. The phospholipid content in the membrane lipids doubled, whereas the sterol and sphingolipid levels changed insignificantly. The amounts of phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid in the phospholipids and the triacylglycerol content in the acylglycerols increased. The desaturation degree of fatty acids drastically increased against the background of a decrease in that of the neutral lipids. The data obtained are discussed in relation to the mechanism of action of the lycopene formation stimulator.

Key words: Blakeslea trispora, lycopene, lipids.

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Natural pigments of the carotenoid group possessing antioxidant activity have recently received increased attention. Of these isoprenoids, lycopene exhibits the maximum capacity to decelerate lipid oxidation processes. Lycopene is of particular interest, because it inhibits the development of tumors, including prostate cancer [1–3].

In the human organism, lycopene is present in small quantities in blood, the kidneys, and the lungs. It accumulates in the organs characterized by intense lipid metabolism, such as the adrenals, the liver, and the prostate [4]. It is presently believed that lipid peroxidation caused by reactive oxygen species underlies the development of a large number of malignant tumors, cardiovascular and neurodegenerative disorders, and aging processes. It is assumed that the antioxidant hypothesis accounts for the inhibitory effect of lycopene on tumors caused by DNA damage resulting from oxidative stress [5]. It was demonstrated that carotenoids lacking provitamin A activity (lycopene, lutein, canthaxanthin, and astaxanthin) enhance cell-mediated and humoral immune responses [6]. Presumably, this is due to the fact that immune cells are particularly sensitive to oxidative stress because their plasma membrane has a high content of polyunsaturated fatty acids. Lycopene is located in the membrane owing to a lack of hydrophilic groups in its molecules.

A biotechnological method of obtaining lycopene from the mucoraceous fungus *Blakeslea trispora* was developed at the Institute of Microbiology, Russian Academy of Sciences. This method involved a stimulator of lycopene formation, 6-methyl-2-aminopyridine (MAP), which increased the lycopene content of the mycelium from trace amounts to 3% of dry weight, while the lycopene percentage in the carotenoid fraction reached 90% [4].

We demonstrated earlier that plant oils stimulate carotene formation in *B. trispora* [7]. It is also known that the stimulatory effect of the sex hormone (trisporic acids) manifests itself only in the presence of oils in the medium. These data point to a close relationship between lipogenesis and carotenoid synthesis. An additional reason why research on this relationship is of special interest is that a large amount of lycopene, the β-carotene precursor exhibiting a significantly higher antioxidant activity, is accumulated in the mycelium. In addition, studies concerning the composition of fungal membrane lipids during intense lipid synthesis can contribute to our understanding of the mechanism of lycopene action in the cells of human organs characterized by intense lipid metabolism.

The goal of this work was to investigate the lipid composition of *B. trispora* under conditions stimulating lycopene formation.

MATERIALS AND METHODS

The studies were conducted with the (+)T and (–)T strains of *Blakeslea trispora* from the Collection of the Winogradsky Institute of Microbiology, Russian Academy of Sciences.

Two-day cultures of the (+) and (–) strains grown on hydrolyzed maize–soybean medium were used as inocula in our studies [8]. The inoculum (20%) with a 1 : 7 ratio of (+) strain and (–) strain was added to

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Table 1. Effect of MAP on the growth and carotenoid synthesis in *B. trispora*

Variant	Biomass, g/l	Carotenoids, % of dry weight		Carotenoids, g/l	
		β-Carotene	Lycopene	β-Carotene	Lycopene
Control	45.4	0.65	0.004	0.29	0.002
MAP	40.4	0.11	2.30	0.04	0.93

250-ml flasks containing 30 ml of flour medium [8]. It was supplemented with 0.005% of MAP on inoculation. The culture was grown for five days on a rotary shaker (200 rpm, 27–28°C). Biomass quantity was determined gravimetrically.

Lipids were extracted for 1 h with a chloroform–methanol mixture (2 : 1) at room temperature according to Folch et al. [9]. The extraction was repeated three times; the system was stirred with a magnetic mixer. The total lipids were separated into phospho-, glyco-, and neutral lipid fractions on a column with silica gel L (100/160 mesh, Chemapol, Czech Republic) using solvents with different polarity degrees [10]. The composition of neutral lipids (NLs) was analyzed by ascending thin-layer chromatography on glass plates with silica gel 60 (Merck, Germany). NL separation was performed using the hexane–diethyl ether–acetic acid (85 : 15 : 1) solvent system. The analysis of phospholipids (PL) and glycolipids (GL) was performed by the Nichols method [11], which excludes enzymatic degradation of lipids. To separate polar lipids, we used the Benning system [12] for two-dimensional TLC. The lipids (150–200 μg) were applied to an HPTLC plate with silica gel 60 (Merck, Germany). The chromatograms were sprayed with 5% sulfuric acid in ethanol, followed by heating to 180°C to develop the stains. PL identification was based on individual markers and qualitative tests for amino groups (with ninhydrin), choline-containing phospholipids (with the Dragendorff reagent), and carbohydrates (with α-naphthol) [10]. NL were determined with individual markers, such as mono-, di-, and triacylglycerols; free fatty acids; sterols (ergosterol); and hydrocarbons (Sigma, United States). Sphingolipids were identified using the saponification method [10]. Quantitative lipid analysis was based on the Dens software package (Lenkhrom, Russia). PL 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 sterols. The NL ratio was determined with a Sorbfil-M densitometer (Sorbpolimer, Russia).

The fatty acid composition of NL, PL, and GL was determined with a Kristall 5000.1 gas–liquid chromatographer (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).

Carotenoid composition was determined by a method we developed [13].

The statistical treatment of the results was carried out using the median (Me) method with $n = 2–3$ [14].

RESULTS AND DISCUSSION

The mucoraceous fungus *B. trispora* is used as an industrial carotenoid producer. Considering the composition of the carotenoids produced by *B. trispora*, it is a more advantageous producer of β-carotene and lycopene than algae, plants, and recombinant microorganisms [15, 16]. The lipophilic properties of carotenoids provide for their location, apart from membranes, in lipid bodies. The final product yield may therefore depend on the composition and quantity of fungal lipids.

The addition of MAP, the lycopene cyclase inhibitor, insignificantly influenced the biomass yield of *B. trispora*, stimulated carotenoid synthesis, and increased the β-carotene–lycopene ratio to 1 : 20, favoring lycopene formation (Table 1).

The NLs of the fungus were chiefly represented by triacylglycerols and carotenoids. Diacylglycerols, free fatty acids, sterols, and esters thereof were also detected. Since TLC fails to separate carotenoids, sterol esters, and hydrocarbons, the precise ratio between the individual classes of NL could not be determined. We only established that the triacylglycerol share in the acylglycerol fraction increased under the conditions stimulating carotene formation (Table 2).

The membrane lipids of *B. trispora* include phospholipids, sphingolipids, and sterols. The sterol and sphingolipid contents remained virtually unchanged under the conditions stimulating lycopene formation, while the PL level almost doubled (figure). No changes occurred in the qualitative composition of the PL, and phosphatidylethanolamine (PE) and phosphatidylcholine (PC) remained the main species. Phosphatidylserine (PS), lysophosphatidylcholine (LPC), phosphatidic acid (PA), and cardiolipin (CL) were detected as minor components. The percentages of PE, PC, and PA were markedly increased under the conditions stimulating lycopene formation.

Table 2. Lipid synthesis under the conditions stimulating lycopene synthesis

Variant	Lipids, % of dry biomass	NL : GL : PL, % of the total lipids	Neutral lipids, % of the total lipids				
			DG	ST	FFA	TAG	SE + HC + carotenoids
Control	40.1	83.0 : 12.0 : 5.0	6.9	8.4	7.5	45.3	31.9
MAP	35.6	84.1 : 7.5 : 8.4	3.4	7.3	6.4	30.0	52.8

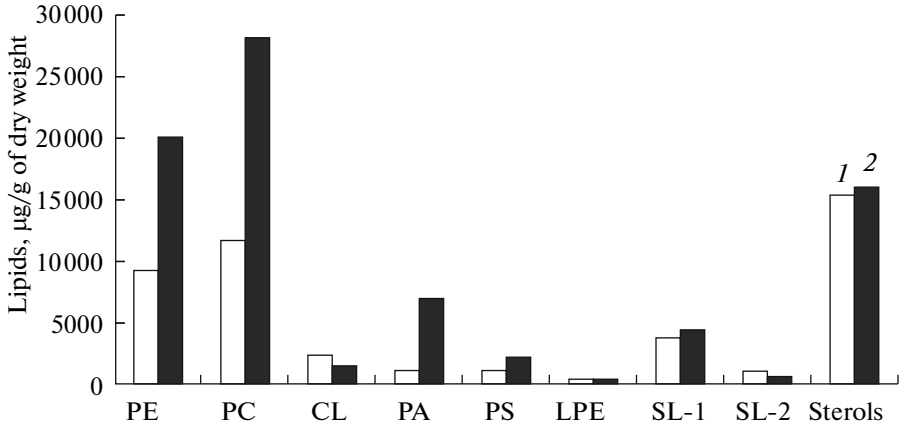
Designations: NL, neutral lipids; GL, glycolipids; PL, phospholipids; DG, diacylglycerols; ST, sterols; FFA, free fatty acids; TAG, triacylglycerols; SE, sterol esters; HC, hydrocarbons.

Analysis of the fatty acid composition of the main lipid fractions (neutral, glyco-, and phospholipids) revealed that the most unsaturated fatty acids were present in sphingolipids and neutral lipids, which was due to a high percentage of linoleic acid ($C_{18:2}$). Oleic acid ($C_{18:1}$) was the predominant fatty acid of the phospholipids, and an almost twofold decrease in their desaturation degree was observed (Table 3). In contrast, a decrease in the linoleic acid share in the neutral lipids and an increase in it in the PL occur under conditions stimulating lycopene formation, and the acyl chains of the PL become more unsaturated. This was confirmed by investigating of the fatty acid the composition of the main PL, PE, and PC, which were isolated by TLC (Table 4). In the presence of MAP, the contents of linoleic and oleic acid increased against a background of a decrease in the share of palmitic acid ($C_{16:0}$), resulting in a significant increase in the desaturation degree.

Therefore, our studies revealed that major changes in the lipid composition occurred under the conditions stimulating lycopene formation. Of paramount importance is a twofold increase in PL content and a rise in the unsaturated fatty acid share in the PL, which apparently reflects the intensification of formation of lipid body membranes under these conditions. A similar increase in PL content also occurs in the (–)4 strain of *Blakeslea trispora* if carotene formation is stimulated by trisporic acids [17].

Lipid storage and transport in fungi is carried out with the help of membrane-enclosed lipid bodies [18]. In *Saccharomyces cerevisiae*, these structures include triacylglycerols, diacylglycerols, sterol esters, and certain enzymes of lipid metabolism, suggesting that they have a regulatory role, apart from the reserve function. It is assumed that these structures also serve as carotenoid depositaries in carotene-forming fungi [16]. Triacylglycerols, carotenoids, and possibly sterol esters are the main components of the lipid granules of the tested fungus. The fact that the desaturation degree of fatty acids in PL is significantly higher for the mycelium accumulating large amounts of lycopene, indicates that lycopene-containing membranes of the lipid bodies possess peculiar properties and that lycopene produces an antioxidant effect.

The results obtained are of considerable interest in terms of the mechanism of action of MAP. It was demonstrated in this work that stimulation of lycopene synthesis was not accompanied by a decrease in the sterol content (figure). Since fungi only synthesize carotenoids via mevalonic acid [19], MAP is likely to



Membrane lipids of *B. trispora* under the conditions stimulating lycopene formation-stimulating conditions. Designations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin: PA, phosphatidic acid; PS, phosphatidylserine; LPE, lysophosphatidylethanolamine; SL, sphingolipids.

Table 3. Main fatty acids of *B. trispora* lipids (% of the total)

Fatty acids	NL		GL		PL	
	Control	MAP	Control	MAP	Control	MAP
$C_{12:0}$	–	0.9	0.1	–	–	0.2
$C_{14:0}$	–	1.1	0.3	–	1.2	0.3
$C_{15:0}$	0.6	1.9	0.2	–	–	0.3
$C_{16:0}$	9.8	16.4	9.9	11.6	23.6	7.4
$C_{16:1}$	0.2	–	0.2	–	–	–
$C_{17:0}$	1.7	–	1.5	–	2.0	–
$C_{18:0}$	4.1	7.4	4.1	2.2	11.8	0.8
$C_{18:1\ n9c}$	23.4	52.5	22.8	38.8	46.8	43.3
$C_{18:2\ n6c}$	55.8	12.8	55.6	44.9	8.8	46.9
$C_{18:3\ n3}$	–	–	0.6	–	–	–
$C_{20:0}$	0.3	–	0.3	–	0.8	–
$C_{20:1}$	0.4	1.2	0.5	–	0.9	–
$C_{20:2}$	–	1.6	0.2	2.5	–	–
$C_{20:4n6}$	2.0	–	2.0	–	2.6	–
$C_{22:0}$	0.7	–	0.6	–	1.0	–
$C_{22:1n9}$	0.6	4.2	0.7	–	–	0.7
$C_{24:0}$	0.4	–	0.2	–	0.6	–
Desaturation degree	1.44	0.87	1.45	1.43	0.76	1.38

act at a point located after the bifurcation between the pathways of carotenoid and sterol synthesis and, more precisely, posterior to the synthesis of the C_{15} precursor, farnesyl diphosphate, dimerization of which results in squalene formation. Squalene is the first specific compound of the sterol-formation pathway. It was shown earlier that CPTA (2-(4-chlorophenylthio)triethylammonium chloride), another inhibitor of lycopene cyclization, also did not affect sterol formation [30]. In addition, diphenylamine, an inhibitor of desaturation of phytoene, the colorless carotenoid precursor, did not change the sterol content in *Phycomyces blakesleeanus* mycelium [21]. Of special interest are the data that the squalene synthetase inhibitor did not affect carotenoid synthesis [20]. There was only one work that presented data militating against the above ideas. Its authors used fungicides that inhibit sterol synthesis and succeeded in stimulating lycopene formation [22]. A weak (23%) effect was achieved using terbinafine, which inhibits the initial stages of sterol synthesis (at the squalene oxidase level). Ketoconazole, which prevents the conversion of lanosterol to ergosterol, produced a significant (277%) effect. However, several objections can be raised. In the study conducted with ketoconazole, no control experiment with ketoconazole in the absence of nicotine, a stimulator of lycopene formation, was carried out. Since ketoconazole is an imidazole derivative, it can presumably inhibit lycopene cyclase, similarly to a large number of other imidazole derivatives [23]. Hence, in this system the stimulation of lycopene formation may be based on a different mechanism. It follows that MAP is unlikely to affect sterol synthesis. It seems to behave as a lycopene synthase inhibitor, similar to nicotine, which was shown to irreversibly bind to the active center of the enzyme [24].

In the light of the data that the sterol content was not affected by MAP, the increase in total carotenoid content we revealed is of particular interest. Presum-

Table 4. Fatty acid composition of the major PL during intense lycopene formation

Fatty acids	PE, % of the total		PC, % of the total	
	Control	MAP	Control	MAP
$C_{15:0}$	–	–	–	1.9
$C_{16:0}$	52.8	23.5	33.4	21.4
$C_{16:1}$	–	–	–	3.0
$C_{17:0}$	–	–	12.0	4.3
$C_{18:0}$	10.0	4.4	9.3	5.2
$C_{18:1n9c}$	22.2	46.1	29.2	38.1
$C_{18:2n6c}$	14.9	26.0	16.0	26.1
Desaturation degree	0.52	0.98	0.61	0.90

ably, the physiological regulation of overproduction of secondary metabolite involves several types of terminal product inhibition; regulation by carbon, nitrogen, and phosphorus sources; and the effects of autoregulators [25]. The fact that MAP causes carotenoid synthesis stimulation without inhibiting sterol synthesis suggests that terminal product regulation is based in this system on a feedback mechanism involving β-carotene. Its quantity significantly decreases under the conditions stimulating lycopene formation, which results in an increased activity of the enzymes of the carotenoid synthesis pathway.

In submerged culture, β-carotene is the main carotenoid of the tested fungus, whereas the amount of lycopene, an intermediate precursor, is insignificant [26]. We earlier established that sexual reproduction is arrested by inhibiting β-carotene synthesis with MAP in the surface culture of the fungus [27], because β-carotene is the precursor of trisporic acids, the sex hormone, and sporopollenin, a component of the zygospore cell wall. Deceleration of the synthesis of the terminal product by an inhibitor and accumulation of a large amount of the precursor are currently interpreted in terms of metabolite stress, which manifests itself in accumulation of the disaccharide trehalose, a universal membrane protector, and a change in the composition of the main membrane lipids [28, 29]. We earlier demonstrated for the first time that an increase in trehalose content occurs in the presence of MAP, an inhibitor of β-carotene synthesis [8]. In this work, we revealed an increase in the PL content, the share of unsaturated fatty acids in the PL, and the PC level. It is currently believed that this enhances antioxidant protection and provides for stabilization of the membrane lipids [30].

It has not been established up to now whether (i) MAP directly influences the lipid composition or (ii) changes in the lipid composition result from the enhanced antioxidant activity of fungal cells associated with lycopene overproduction caused by MAP. The latter suggestion may be valid because it has been revealed that antioxidants, including trehalose, protect membrane lipids against free-radical oxidation and regulate cell metabolism under stress [31].

REFERENCES

1. Stahl, W. and Sies, H., Lycopene: A Biologically Important Carotenoid for Humans?, *Persp. Biochem. Biophys.*, 1996, vol. 236, no. 1, pp. 1–9.
2. Giovannucci, E. and Clinton, S.K., Tomatoes, Lycopene and Prostate Cancer, *Proc. Soc Exp. Biol. Med.*, 1998, vol. 218, no. 2, pp. 129–139.
3. Gerster, H., The Potential Role of Lycopene for Human Health, *J. Am. Coll. Nutr.*, 1997, vol. 16, no. 2, pp. 109–126.
4. Feofilova, E.P., Tereshina, V.M., Memorskaya, A.S, Burenin, I.S, Polyanskaya, N.I., Dul'kin, L.M., and Goncharov, N.G., Mycolycopene, an New Medicinal Agent with Immunomodulating and Antioxidant Activity for Treatment of Prostate Diseases, *Immunopatologiya, Allergologiya, Infektologiya*, 2006, no. 1, pp. 9–13.
5. Matos, H.R., Di Mascio, P., and Medeiros, M.H.G., Protective Effect of Lycopene on Lipid Peroxidation and Oxidative DNA Damage in Cell Culture, *Arch. Biochem. Biophys.*, 2000, vol. 383, no. 1, pp. 56–59.
6. Chew, B.P. and Park, J.S., Carotenoid Action on the Immune Response, *J. Nutr.*, 2004, vol. 134, p. 257S–261S.
7. Feofilova, E.P., *Pigmenty mikroorganizmov* (Pigments of Microorganisms), Moscow: Nauka, 1974.
8. Feofilova, E.P., Tereshina, V.M., and Memorskaya, A.S., Regulation of Lycopene Synthesis in a Mucor Fungus *Blakeslea trispora* by Pyridine Derivatives, *Mikrobiologiya*, 1995, vol. 64, no. 6, pp. 734–740.
9. Folch, J., Lees, M., and Sloane-Stanlet, G.H.S., A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem.*, 1957, vol. 226, no. 1, pp. 497–529.
10. Keits, M., *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Amsterdam: Elsevier, 1972 [Russ. Transl. Moscow: Mir, 1975].
11. Nichols, B.W., Separation of the Lipids of Photosynthetic Tissues: Improvement in Analysis by Thin-Layer Chromatography, *Biochem. Biophys. Acta*, 1963, vol. 70, pp. 417–422.
12. 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.
13. Tereshina, V.M., Memorskaya, A.S., and Feofilova, E.P., Express Method for Determination of Lycopene and β-Carotene Content, *Mikrobiologiya*, 1994, vol. 63, no. 6, pp. 1111–1116.
14. Ashmarin, I.P. and Vorob'ev, A.A., *Statisticheskie metody v mikrobiologicheskikh issledovaniyakh* (Statistical Methods in Microbiological Research), Leningrad: Gos. Izd-vo med. lit, 1962.
15. Bhosale, P., Environmental and Cultural Stimulants in the Production of Carotenoids from Microorganisms, *Appl. Microbiol. Biotechnol.*, 2004, vol. 63, pp. 351–361.
16. Mantzouridou, F. and Tsimidou, M.Z., Carotenoid Pattern in *Blakeslea trispora* Grown on Oil-Enriched Substrates with Regard to Triacylglycerol Species Accumulation, *Eur. J. Lipid Sci. Technol.*, 2007, vol. 109, pp. 3–10.
17. Dedyukhina, E.G., Lipid Biosynthesis in Cerotene-Producing Fungi *Blakeslea trispora, Cand. Sci. (Biol.) Dissertation*, Moscow: INMI AN SSSR, 1969.
18. Murphy, J. and Vance, J., Mechanism of Lipid-Body Formation, *Trends Biochem. Sci.*, 1999, vol. 24, pp. 109–115.
19. Saakov, V.S., Alternative Pathways of Carotenoid Biosynthesis in Prokaryotes and Eukaryotes, *Doklady Akademii Nauk*, 2003, vol. 392, no. 6, pp. 825–831 [*Doklady Biochem. Biophys.* (Engl. Transl.), vol. 392, pp. 294–300].
20. Fraser, P.D., Ruiz-Hidalgo, M.J., Lopez-Matas, M.A., Alvarez, M.I., Eslava, A.P., and Bramley, P.M., Caro-

tenoid Biosynthesis in Wild Type and Mutant Strains of *Mucor circinelloides, Biochim. Biophys. Acta*, 1996, vol. 1289, pp. 203–208.

21. Olson, J.A. and Knizley, H., The Effect of Diphenylamine on Carotenoid, Sterol and Fatty Acid Synthesis in *Phycomyces blakesleeanus, Arch. Biochem. Biophys.*, 1962, vol. 97, pp. 138–145.
22. Sun, Y., Yuan, Q.-P., and Vriesekoop, F., Effect of Two Ergosterol Biosynthesis Inhibitors on Lycopene Production by *Blakeslea trispora, Process Biochem.*, 2007, vol. 42, pp. 1460–1464.
23. Ninet, L., Renaut, J., and Tissier, R., Activation of the Biosyntesis of Carotenoids by *Blakeslea trispora, Biotech. Bioeng.,* 1969, vol. 11, pp. 1195–1210.
24. Bouvier, F., d'Harlingue, A., and Camara, B., Molecular Analysis of Carotenoid Cyclase Inhibition, *Arch. Biochem. Biophys.*, 1997, vol. 346, no. 1, pp. 53–64.
25. Spi žek, J. and Tichý, P., Some Aspects of Overproduction of Secondary Metabolites, *Folia Microbiol.*, 1995, vol. 40, no. 1, pp. 43–50.
26. Kalinina, N.V., Tereshina, V.M., Memorskaya, A.S., and Feofilova, E.P., The Correlation between the Synthesis of β-Carotene and Zygote Formation by *Blakeslea trispora* Heterothallic Strains, *Prikl. Biokhim. Mikrobiol.*, 2007, vol. 43, no. 1, pp. 77–81 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 43, no. 1, pp. 68–72].
27. Tereshina, V.M., Memorskaya, A.S., and Feofilova, E.P., Zygote Formation in *Blakeslea trispora*: Morphological Peculiarities and Relationship with Carotenoid Synthesis, *Mikrobiologiya*, 2003, vol. 72, no. 4, pp. 503–509 [*Microbiology* (Engl. Transl.), vol. 72, no. 4, pp. 448–454].
28. Tereshina, V.M., Mikhailova, M.V., and Feofilova, E.P., Physiological Role of Trehalose and Antioxidant in *Cunninghamella japonica* at Heat Stress, *Mikrobiologiya*, 1991, vol. 60, no. 5, pp. 781–789.
29. Mager, W.H. and De Kruijff, A.J.J., Stress-Induced Transcriptional Activation, *Microbiol. Rev.*, 1995, vol. 59, no. 3, pp. 506–531.
30. Oku, K., Watanabe, H., Kubota, M., Fukuda, F., Kurimoto, M., Tsujisaka, Y., Komori, M., Inoue, Y., and Sakurai, M., NMR and Quantum Chemical Study on the OH...π and CH...O Interaction between Trehalose and Unsaturated Fatty Acids: Implication for the Mechanism of Antioxidant Function of Trehalose, *J. Am. Chem. Soc.*, 2003, vol. 125, pp. 12739–12748.
31. Feofilova, E.P., Burlakova, E.B., and Kuznetsova, L.S., Role of Free Radical Oxidation Reactions in Eukaryotic Regulation of Growth and Lipid Production, *Prikl. Biokhim. Mikrobiol.*, 1987, vol. 23, no. 1, pp. 3–13.