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SHORT COMMUNICATION

Sterols of the Fungus *Mucor hiemalis* Sporangiospores

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We earlier reported that the viability and lipid composition of *Mucor hiemalis* F-1156 sporangiospores, as well as their capacity to develop yeast-like cells, depend on the age of the spore-forming culture [1]. With an increase in the time of culture growth, sporangiospores exhibit a decrease in the levels of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, cardiolipin, and reserve lipids, as well as in the degree of fatty acid unsaturation, whereas the level of glycolipids increases. However, sterol composition has not been studied. Sterols are involved in morphogenetic processes [2, 3] and, therefore, we suggested that sporangiospores from senescent cultures, which, upon germination, develop mycelium and yeast-like cells, may exhibit a distinctive sterol pattern, along with the changed composition of fatty acids and polar and neutral lipids.

The aim of this work was to study the sterol composition of sporangiospores present in *M. hiemalis* cultures of different ages.

M. hiemalis F-1156 was grown on wheat bran for 7, 10, 14, or 20 days. Sporangiospores were collected as described previously [1]. The experiments were conducted in three–four replicates. Lipid extraction, alkaline hydrolysis, and sterol extraction were conducted as described by Kates [4]. Individual sterols were identified using the methods of chromatography–mass spectrometry and quantified by gas–liquid chromatography [5].

Unsaponifiable lipid fraction in sporangiospores of different age comprised nine sterols. The relative levels of the sterols are presented in the table.

The major sterol in sporangiospores was ergosterol. Its level decreased significantly with the age of the spore-forming culture. However, we observed a concomitant increase in the levels of intermediates of ergosterol biosynthesis—demethylated fecosterol and episterol—and in the level of methylated sterols, primarily 24-methylene-4α-methyl-cholest-8-ene-3β-ol, eburicol, and 4,4-dimethylfecosterol. The ration between demethylated and methylated sterols decreased in the spores of senescent culture, which indicates certain changes in membrane structure and functioning. It is 4,14-desmethylsterols that are crucial for the synthesis and functioning of cell membranes [6], ergosterol being the major type of these compounds in most fungi. Lipid and sterol compositions are also influenced by secondary metabolites, which often inhibit P 450-lanosterol-C14-demethylase [7].

After 14 days of cultivation, the levels of 5-dihydroergosterol and 4,4-dimethylfecosterol increased significantly in sporangiospores. Some researchers consider them to be degradation products of intermediates of ergosterol biosynthesis [8]. Ergosterol deficiency results in the impairment of cytochrome synthesis and deceleration of the cytochrome oxidase and succinate dehydrogenase syntheses [9]. Such changes indicate a decrease in the cell energy potential, which influences

Sterol composition (% of total sterols) in sporangiospores of the fungus *M. hiemalis* F-1156 as dependent on the time of culture growth

No.	Sterols	7 days	10 days	14 days	20 days
1	24-methyl-cholesta-5,7,22-triene-3β-ol (ergosterol)	94.92	91.50	85.53	51.38
2	24-methyl-cholesta-7,22-diene-3β-ol (5-dihydroergosterol)	–	–	traces	3.19
3	24-methyl-cholesta-5,22-diene-3β-ol (7-dihydroergosterol)	2.54	2.78	traces	1.77
4	24-methylene-cholest-8-ene-3β-ol (fecosterol)	traces	traces	traces	1.42
5	24-methylene-cholest-7-ene-3β-ol (episterol)	traces	traces	traces	2.12
6	24-methylene-4α-methyl-cholest-8-ene-3β-ol (4-methylfecosterol)	0.42	0.93	6.99	6.73
7	24-methylene-4,14-dimethyl-cholest-5-ene-3β-ol	traces	2.32	2.10	0.79
8	24-methylene-lanost-8-ene-3β-ol (eburicol)	traces	0.62	4.40	12.13
9	14-nor-24-methylene-lanost-8-ene-3β-ol (4,4-dimethylfecosterol)	2.12	1.85	0.98	20.47
	Demethylated/methylated sterols	38.40	16.48	5.91	1.49

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spore viability and the processes of germination and further growth.

We have earlier reported that sporangiospores from a 20-day-old culture differ from sporangiospores of a 4–7-day-old culture by a decrease in germinating ability and by a predominance of yeast-like cells upon germination [1]. It is evident that the ability of *M. hiemalis* F-1156 to develop yeast-like cells is, to a great extent, determined by changes in the composition of membrane sterols, along with changes in the polar lipid pattern.

REFERENCES

1. Mysyakina, I.S. and Funtikova, N.S., Changes in the Lipid Composition of *Mucor hiemalis* Sporangiospores Related to the Age of the Spore-Forming Culture, *Mikrobiologiya*, 2003, vol. 72, no. 4, pp. 516–520.
2. Van den Bossche, H., Biochemical Targets for Anitfungal Azole Derivatives: Hypothesis on the Mode of Action, *Current Topics in Medical Mycology*, McGinnis, M.R., Ed., New York: Springer, 1985, vol. 1, pp. 313–351.
3. Farby, S., Gaborova, S., Bujdakova, H., Klobuš icky, M., Vollekova, A., and Kuchta, T., Inhibition of Germ Tube Formation, Filamentation and Ergosterol Biosynthesis in *Candida albicans* Treated with 6-Amino-2-*n*-Pentylthiobenzothiazole, *Folia Microbiol.*, 1999, vol. 44, no. 5, pp. 523–526.
4. Kates, M., *Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. 2nd Revised Edition*, Amsterdam: Elsevier, 1986.
5. Mysyakina, I.S., Funtikova, N.S., and Medvedev, F.A., Sterol Composition of Arthrospores and Mycelium of the Fungus *Mucor hiemalis*, *Mikrobiologiya*, 2002, vol. 71, no. 4, pp. 475–481.
6. Weete, J.D., Sancholle, M.S., and Montant, C., Effects of Tirazoles on Fungi: Lipids Composition of *Taphrina deformans*, *Biochim. Biophys. Acta*, 1983, vol. 752, pp. 19–29.
7. Trenin, A.S., Terekhova, L.P., Tsvigun, E.A., Tolstykh, I.V., Zenkova, V.A., and Katrukha, G.S., Sterol Biosynthesis Inhibitors of Fungal Origin, *Uspekhi meditsinskoi mikologii. Materialy I Vserossiiskogo kongressa po meditsinskoi mikologii, Moskva, 20–21 fevralya 2003 g.* (Advances in Medical Microbiology. Proceedings of the 1st All-Russia Congress on Medical Microbiology, Moscow, February 20–21, 2003), Moscow: Natsional'naya akademiya mikologii, vol. 1, pp. 257–258.
8. Coulon, J., Hakkou, A., Mpona-Minga, M., and Bonaly, R., Action de l'amphotericine B sur la composition en sterols de *Kluyveromyces bulgaricus* et *Kluyveromyces lactis*, *Can. J. Microbiol.*, 1986, vol. 32, no. 9, pp. 738–742.
9. Akhrem, A.A. and Titov, Yu.Ya., *Steroidy i mikroorganizmy* (Steroids and Microorganisms), Moscow: Nauka, 1970.