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SHORT COMMUNICATIONS

Effect of Exogenous Lipids on Morphogenesis of the Fungus *Mucor lusitanicus* 12M

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The composition of spores and conditions of their formation significantly influence the development of the resulting cultures of mycelial fungi. The viability of spores of the fungus *Trichoderma harzianum* has been reported to depend on the duration of their maturation [1]. The germination of *Aspergillus niger* conidia is influenced by the intensity of the culture sporulation: the stimulation of sporulation with blue illumination led to lower germinability of the spores obtained [2].

Previously, we found that the sporangiospores formed by older cultures of the fungus *Mucor lusitanicus* 12 M grown on wheat bran or sunflower oil cake tend to produce yeast-like growth. With further ageing of the culture, the sporangiospores completely lost their viability [3, 4]. Microscopic examination demonstrated that the sporangiospores of the young and old cultures grown on bran differed in their volume and structure of their surfaces [5]. The spores of *M. lusitanicus* lost their germinability obviously due to autolysis of the mycelium and liberation of abundant biologically active organic substances and enzymes after the end of mycelium growth and spore formation. In the period of spore maturation in senescent cultures of the fungus, the lipase activity results in the degradation of lipids, phospholipids in particular. The composition of lipids of the spores from a 25-day-old culture, including phospholipids, significantly differed from that of young spores [3].

In light of recent publications on the multiple-factor influence of lipids on morphogenesis and differentiation of both micro- and macroorganisms, it may be assumed that fungal dimorphism is caused by lipid compounds and/or by changes in the properties of membranes of sporangiospores occurring due to change in the composition of phospholipids. It was reported previously that the products of phospholipid metabolism—diacylglycerols, phosphatidic acid, and lysophosphatidic acid—are messengers of morphological transitions [6–8]. Phosphatidic acid also participates, directly or indirectly, in the reorganization of the cytoskeleton during changes in cell morphology [9], and diacylglycerols may be sensors in the Golgi apparatus, which regulates the secretory function of membranes in the process of formation and transportation of vesicles during yeast budding [10]. It was shown that exogenously added phospholipase D causes dimorphism in the yeast *Candida albicans* [11]. Discontinuation of phospholipid synthesis caused by the addition of cerulenin caused dimorphism in *Mucor racemosus* [12].

Along with the products of the activity of phospholipases, other lipid compounds influence fungal morphogenesis. A paper has been published on the effect of prostaglandins, products of fatty acid metabolism, on the morphological development of microscopic fungi [13]. It has been noted that sterols play an important role in the dimorphism of pathogenic fungi [14].

Thus, it was of interest to investigate the effect of the addition to the growth medium of lipids of various origin (lipids isolated from old spores, other fungal lipids, and lipids of the nutrient substrate) on the germination morphology of sporangiospores formed in a young culture of the fungus *M. lusitanicus* 12 M.

The inoculum was sporangiospores washed off from a 4-day fungal culture grown on wheat bran. Liquid nutrient medium whose composition was described previously [3] was inoculated with a spore suspension to a final density of $5 \cdot 10^5$ cells/ml and supplemented with lipids extracted from (1) wheat bran, (2) sunflower oil cake, (3) wheat bran with a 20-day-old mycelium of the fungus investigated grown on it, (4) sporangiospores of a 4-day culture of the fungus grown on wheat bran, (5) sporangiospores of a 20-day culture of the fungus grown on wheat bran, and (6) 3-day-old mycelium grown on the mineral Czapek medium.

Fungal lipids were extracted and analyzed by thin-layer chromatography as described previously [3]. Extraction of lipids from the substrate was performed by the Folch method [15]. The composition of the extracted lipids is presented in Table 1. Lipids were added to the medium in a concentration 0.4 of g/l, without emulsification. Cultivation was carried out on a shaker at 180 rpm and 28°C. Lipids from different sources were similar qualitatively but differed in the ratios of the components (Table 1).

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Table 1. Component content (% of total lipids) in lipids obtained from different sources

Lipid fractions	Source of lipids					
	3-day mycelium of *M. lusitanicus* 12M	Sporangiospores of a 4-day culture of *M. lusitanicus* 12M	Sporangiospores of a 20-day culture of *M. lusitanicus* 12M	Wheat bran with a 20-day mycelium of *M. lusitanicus* 12M after washing off of sporangiospores	Wheat bran	Sunflower oil cake
Polar lipids	9.3	25.3	16.2	20.7	10.5	10.7
Diacylglycerols	6.4	7.1	18.6	5.2	4.9	
Free sterols	6.5	6.6	14.5	27.1	7.8	14.9
Free dimethylsterols and alcohols	–	1.7	4.3	1.0	Traces	Traces
Free trimethylsterols	–	3.1	Traces	4.0	3.0	Traces
Free fatty acids	5.2	13.2	15.8	22.7	42.4	51.1
Quinones	2.3	–	–	1.3	–	–
Triacylglycerols (TAGs)	44.6	30.2	15.5	6.6	17.8	10.0
Alkyl esters of TAGs	–	–	–	–	0.7	–
Esters of fatty acids	0.7	–	–	2.1	1.8	Traces
Esters of sterols	20.0	6.5	5.2	–	11.1	13.3
Hydrocarbons, waxes, carotenoids	Traces	6.0	Traces	9.5	Traces	Traces

Table 2. Content of individual fatty acids (% of total fatty acids) in nutrient substrates used for growing the sporogenous culture of *M. lusitanicus* 12M

Nutrient substrate	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Sunflower oil cake	Traces	11.0	Traces	5.4	7.5	76.6	0.5
Wheat bran	Traces	31.3	Traces	0.8	28.1	38.8	1.0

The results that we obtained demonstrated significant differences between the exogenous lipids in their effect on the morphology of growing fungal cells. After 24 h of cultivation, visual and microscopic examinations revealed that, in variants with the addition of lipids extracted from wheat bran or sunflower oil cake, mycelial growth occurred, but the hyphae were shorter than in the control and exhibited intense branching, swellings, and numerous chlamydospores. The addition of lipids obtained from young spores (formed by a 4-day culture) caused the formation, in 2-day cultures, of arthrospore chains on the ends of the hyphae (usually, arthrospore chains are observed only on the third–fourth day of growth). In the experimental variants 3 and 6, similarly to the control, the mycelium was well developed; yeastlike cells and arthrospores were absent. In the case of the addition to the medium of lipids from old spores (from a 20-day culture), numerous yeastlike cells were present in the grown culture on the second day of growth, along with deformed mycelium. By the 72nd hour of growth, the yeastlike cells disappeared, and only mycelium was present in the culture liquid.

A specific feature of the lipids from wheat bran and sunflower oil cake was a high content of free unsaturated fatty acids, particularly, oleic and linoleic acids (Table 2), which may modify the development of mycelium. As to the effect of lipids from sporangiospores of a young culture, it should be noted that spores, being the final stage of the developmental cycle of the fungus, may contain signal lipid substances similar to the factor d formed at the end of development of microbial cultures [16]. Such autoregulators may be a signal for the beginning of formation of arthrospores on the hyphae of vegetative mycelium.

Of special interest are the data on the effect on the fungal cell morphogenesis of lipids from the spores of an old culture. These lipids caused yeastlike growth of the fungus. These results imply that, in a senescent culture of the fungus cultivated on wheat bran, sporangiospores contain signal lipid compounds that induce dimorphism in the fungus. It is probable that these compounds cause changes in the structure of cell membranes, which results in the production of yeastlike cells in the course of germination of sporangiospores of *M. lusitanicus* grown on wheat bran. The exact nature

of these signal compounds (autoregulators) remains unknown and requires further investigation.

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