## SHORT COMMUNICATIONS

## The Dependence of Dimorphism in the Fungus *Mucor lusitanicus* 12M on the Preparation Conditions of Sporangiospores

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Our earlier studies showed a correlation between the age of wheat bran-grown sporogenous cultures of the fungi Mucor lusitanicus and Mucor hiemalis and the viability of their sporangiospores [1, 2]. The sporangiospores of middle-aged cultures inoculated into liquid media gave rise to yeastlike cells, whereas the sporangiospores of old cultures lost the ability to germinate. The long-term cultivation of the fungi on wheat bran decreased their lipid pools and the content of storage lipids and the major membrane phospholipids but augmented the relative content of glycolipids and sterols. The sporangiospores of 4- to 6-day-old fungal cultures induced mycelial growth alone. The long-term storage of such sporangiospores did not appreciably affect their viability and lipid composition but did affect both of these parameters in the case of older sporangiospores. There is no certainty as to the direct cause of the rapid degradation of sporangiospores in aging fungal cultures incubated on wheat bran, although the specific metabolism of the fungi on this agricultural substrate may be responsible for this process.

The aim of this work was to study the dimorphism and the viability of the sporangiospores of the fungus *M. lusitanicus* 12M cultivated on different nutrient media as a function of the culture age.

To obtain sporangiospores, the fungus *M. lusitani*cus 12M was cultivated over different time periods in a surface mode on media containing the agricultural wastes wheat bran and sunflower cake, as well as on agar media (malt extract agar and a modified Czapek agar with or without glucose). Sporangiospores were washed off from the media with water. The lipid composition of the sporangiospores was studied by routine methods [3]. Microscopic studies were performed by using a JSM T-300 scanning electron microscope (Jeol, Japan).

The sporangiospores of 4- to 6-day-old fungal cultures grown on wheat bran and sunflower cake germinated as hyphae, the sporangiospores of middle-aged (14-day-old) fungal cultures exhibited dimorphism, and the sporangiospores of 25- to 30-day-old sporogenous mycelia were unable to germinate at all. The long-



**Fig. 1.** Polar lipids in the 60-day-old *M. lusitanicus* 12M cultures grown on wheat bran and malt extract agar: (*1*) phosphatidylserine; (*2*) phosphatidylcholine; (*3*, *5*) glycolipids; (*4*) phosphatidylethanolamine; (*6*) free fatty acids.

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(c)

term cultivation of the fungus on sunflower cake induced changes in the lipid composition that were similar to those observed earlier during the cultivation of the fungus on wheat bran [1]. Namely, such cultivation diminished the lipid pool; decreased the content of triacylglycerides and sterol esters in neutral lipids and the content of phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine) in polar lipids; and increased the pool of free fatty acids, sterols, and diacylglycerides, as well as the content of glycolipids in polar lipids.

The sporangiospores of the fungus grown on agar media did not exhibit dimorphism. Namely, even at the age of six months, such sporangiospores remained viable and gave rise to mycelium alone. The lipid composition of these sporangiospores changed, but not so dramatically as in the case of fungal growth on the agricultural wastes. The sporangiospores of the 60-day-old culture grown on malt extract agar showed the presence of rather diverse polar lipids and the major membrane phospholipids phosphatidylcholine and phosphatidylethanolamine (Fig. 1).

In the case of cultivation on a mineral medium without glucose, fungal sporangiospores were monomorphic and germinated as hyphae. The poor mycelial growth observed in this case was due to the presence of 0.02% yeast extract in this medium. In spite of the minimal content of carbon sources in the medium, the sporangiospores of the 60-day-old fungal culture retained high viability.

Nonviable sporangiospores and those that were able to induce hyphal growth differed in morphology (Fig. 2). The sporangiospores of the 30-day-old fungal culture grown on wheat bran showed the presence of deep surface invaginations, whereas the viable sporangiospores of the 6-day-old culture grown on wheat bran or the 30-day-old culture grown on malt extract agar were large and had a flat smooth surface.

Thus, the cultivation of the fungus for 4-6 days on both agricultural substrates (wheat bran and sunflower cake) gave rise to functionally active sporangiospores. Further cultivation led to a rapid degradation of sporangiospores, which was accompanied by dimorphism during sporangiospore germination and correlated with the degradation of membrane lipids.

Lipids are known to influence fungal morphogenesis [4, 5]. The membrane-bound subcellular structures chitosomes are known to be involved in the synthesis of cell walls, which differ in yeastlike and hyphal cells [6]. Mucor fungi produce sporangiospores over the entire cultivation period, even when the mycelium does not develop [7]. Like Acrasiales, fungi synthesize various autoregulatory factors, including growth autoinhibitors [8, 9]. When grown on rice bran, mucor fungi may produce antibiotics, such as pramanicin [10]. The production of functionally active sporangiospores by the fungus M. lusitanicus 12M grown on wheat bran and sunflower cake for one week allows the suggestion to be

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Fig. 2. The sporangiospores of the fungus *M. lusitanicus* 12M grown on various media over different time periods: (a) 6-day-old culture grown on wheat bran (sporangiospores germinate as hyphae); (b) 30-day-old culture grown on wheat bran (sporangiospores are unable to germinate); (c) 30-day-old culture grown on malt extract agar (sporangiospores are able to germinate). Magnification, 2500×.

made that the observed changes in the chemical composition, morphological properties, and viability of sporangiospores in the course of further cultivation may be related to the effect of secondary fungal metabolites formed on these agricultural substrates.



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