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EXPERIMENTAL ARTICLES

Differences in the Carbohydrate Composition between the Yeastlike and Mycelial Cells of *Mucor hiemalis*

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Abstract—The use of sporangiospores from a 20-day culture for inoculation enabled us to obtain yeastlike cells of strain *Mucor hiemalis* F-1431 (for which capacity for dimorphic growth was not previously studied) by cultivation in liquid medium under aerobic conditions. The carbohydrate composition of the fat-free biomass of the mycelial and yeastlike forms grown in the same culture under aerobic conditions was studied. In the fat-free biomass consisting of yeastlike cells, as compared to the mycelium, the contents of chitin and glucose decreased from 25 and 30.9% of dry biomass to 14 and 17.5% of dry biomass, respectively. We failed to detect any changes in the contents of fucose, galactose, mannose, and uronic acids among the heteropolysaccharide monomers of fungal cells of different morphotypes, which is probably due to the aerobic cultivation conditions. It was suggested that the composition of heteropolysaccharide monomers does not play such a significant role in the formation of yeastlike cells in the culture grown under aerobic conditions as the content of chitin, the main supporting biopolymer.

Keywords: Mucor hiemalis, cell wall, chitin, yeastlike cells, mycelium

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Mucoraceous fungi widely occur in nature, exhibit the proteolytic and saccharolytic activities, and are the common representatives of the saprotrophic microflora utilizing a broad spectrum of nutrients of plant and animal origin. Depending on the growth conditions (gas composition of the atmosphere, availability of fermentable glucose, pH, density of the spore inoculum, and presence of the chemical factors controlling morphogenesis), they are able to grow in the form of mycelium or yeastlike cells [1–4]. The formation of buds or germ tubes is the result of vectorial changes in the cell wall [5]. Deposition of the new cell wall material during spherical (yeastlike) growth occurs via diffuse intercalation all over the surface of the cell; during mycelial growth, apical deposition of the cell wall material is observed [6]. In mucoraceous fungi, transition from mycelial to yeastlike growth (dimorphism) under anaerobic conditions results in changes in the composition of the main cell wall biopolymers, as well as in the composition of their monosaccharides. Chitin and chitosan microfibrils in the matrix consisting of mucoran and mucoric acid polyuronides, glycoproteins, and glycopeptides are the main structural components of the cell walls of the hyphae and yeastlike cells of *Mucor rouxii* [7–10]. According to the published data, the main differences in the cell wall structure of the two morphological forms consist primarily in the contents of mannose and protein (the principal constituents of the mucoran matrix), which are higher in yeastlike cells [1], as well as of fucose- and galactose-containing polymers, the concentrations of which are higher in mycelial cell walls [8].

According to the published data, members of the species *M. hiemalis* are incapable of yeastlike growth [2]. During our previous studies of the capacity of some mucoraceous fungi (*M. circinelloides* var. *lusitanicus* and *M. hiemalis* F-1156) for dimorphic growth and its relation to the composition of cellular lipids, it was established that spore germination, germination behavior, morphology of the resultant mycelium, biomass yield, and the quantitative yield of the end product were largely dependent on the quality of sporangiospores used as inoculum [11–15]. Germination of sporangiospores of young (5- to 6-day) cultures of the model strains was accompanied by the development of the mycelium. When 20-day ("old") sporangiospores were used as inoculum, the morphogenesis of the resultant cultures had certain peculiarities: they grew as a mixture consisting of mycelium and yeastlike cells, while chains of arthrospores were detected in the mycelium and the culture liquid. This approach (used in order to isolate cultures in a liquid medium) enabled us to obtain yeastlike cells under aerobic conditions.

Since the capacity of the model strain *M. hiemalis* F-1431 used in this study for dimorphic growth has

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Table 1. The ratio of the cell morphotypes and the lipid content in the 3-day culture of *M. hiemalis* F-1431 obtained from 20-day-old sporangiospores

Morphotype	Dry biomass, g/L	Lipids, % of dry biomass
Mycelium	7.62	8.65
Yeastlike cells	2.04	26.46

not been previously studied, the goal of the present work was to obtain the mycelial and yeastlike forms of this fungus during growth under aerobic conditions, as well as to determine the composition of their carbohydrates.

MATERIALS AND METHODS

Obtaining of inoculum. The spore-forming mycelium of strain *Mucor hiemalis* VKM F-1431 was grown on wheat bran at 28°C for 20 days. Sporangiospores were washed off from the mycelium surface with sterile distilled water; after enumeration of the spores in a Goryaev count chamber, the suspension density was adjusted to 10^6 cells/mL.

Cultivation was carried out for 3 days at 28°C on a shaker in 250-mL flasks with liquid medium containing the following (g/L): glucose, 60; urea, 1; K_2HPO_4, 1; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; and $ZnSO_4$, 0.05.

Processing of the biomass and determination of the carbohydrate composition. Yeastlike cells and mycelium grown in the same culture were separated by filtration through a nylon filter; the process was monitored by light microscopy, and the yeastlike cells were harvested by centrifugation. The biomass was defatted with a series of ethanol : chloroform mixtures (2 : 1 and 1 : 2) and used for further processing. The dry, fat-free biomass was subjected to acid hydrolysis; the content of chitin in the obtained hydrolysates was assessed by the colorimetric method (Morgan–Elson assay) from the glucosamine content [16]. The composition of monomers was determined after the monosaccharides were transformed into polyol acetates; quantitative analysis was performed using gas–liquid chromatography (GLC) [17]. GLC of polyol acetates was carried out on a Hewlett-Packard 5890A chromatograph equipped with a flame ionization detector and a HP-1MS capillary column under nitrogen flow using the temperature gradient from 160 to 290°C with a ramping rate of 7°C/min. The results obtained were analyzed using the Multichrom 1.5 software package. The content of uronic acids was determined by the color reaction with 3,5-dimethyl phenol and concentrated sulfuric acid [18].

Microscopic investigations. Electron microscopic examination of ultrathin sections of the cell preparations stained with the Reynolds reagent was carried out under a JEM-100C electron microscope (Jeol, Japan) at the accelerating voltage of 80 kV and 8000–20000× magnification. The morphology of the fungus was examined under an Axio Imager.D1 light microscope (Carl Zeiss, Germany) at 400× magnification (phase contrast). The distribution of chitin in the cell

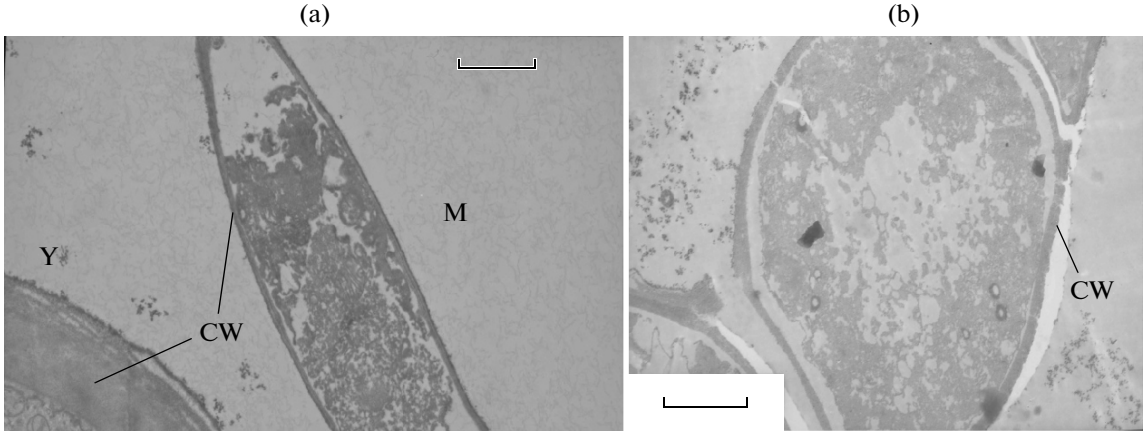

Fig. 1. Ultrathin sections showing the differences in the thickness of the cell walls of different *M. hiemalis* F-1431 morphotypes: yeastlike cells (Y) and mycelium (M) (a) and arthrospores (b). CW, cell wall. Scale bar, 2 μm.

walls of the fungus was determined by fluorescence microscopy. The mycelium and yeastlike cells were stained with Calcofluor White (Sigma). The calcofluor–chitin complex, especially in the segments where the new cell walls were synthesized, was visualized by bright apple-green fluorescence.

The experiments were performed in triplicate; the statistical analysis was performed by the median technique [19].

RESULTS AND DISCUSSION

Transmission electron microscopy revealed the structural and morphological properties of the cells representing two different morphotypes obtained from 20-day-old spores (Fig. 1). The cell walls of the hyphae of fungal mycelium were solid and thin, whereas the cell walls of yeastlike cells were thick, loose, and layered (Fig. 1a). Arthrospores surrounded by the cell walls of the mycelium were enclosed in their own cell walls similar to those of the maternal hyphae (Fig. 1b).

The fluorescence microscopic study of chitin distribution in the cell walls of *M. hiemalis* F-1431 mycelium, yeastlike cells, and arthrospores using Calcofluor White staining revealed that the fluorescence intensity of the mycelium, especially in the segments where the chitin of the new cell walls was deposited, was higher than that of the yeastlike cells (Figs. 2a, 2b). The intensity of fluorescence of the cell walls synthesized in the course of arthrospore formation indicated the intercalary mode of chitin deposition, similar to that of yeastlike cells (Figs. 2b, 2c).

Table 1 shows the data on the ratio between the morphological forms developing during cultivation and the content of lipids in the biomass. The content of yeastlike cells was about 20% of dry biomass; however, the content of total lipids in them was significantly higher than in the mycelium.

Comparative analysis of the carbohydrate composition of fat-free cells with different morphologies demonstrated that fucose, mannose, glucose, galactose, and uronic acids were found among the heteropolysaccharide monomers; chitin was found to be the main supporting biopolymer (Table 2). However, despite published data [7–9], we failed to detect any considerable changes in the contents of fucose, galactose, mannose, and uronic acids in fungal cells of different morphotypes. It is quite possible that these peculiarities were due to the aerobic cultivation conditions, under which the yeastlike cells of *M. hiemalis* F-1431 were obtained. In the yeastlike cells, as compared to the mycelium, the contents of chitin and glucose decreased from 25 and 30.9% of dry biomass to 14 and 17.5% of dry biomass, respectively.

On the basis of the data obtained, it may be concluded that certain relationships exist between the composition of cellular polysaccharides, including components of the cell walls of *M. hiemalis* F-1431, and the morphology of this fungi. The composition of heteropolysaccharide monomers probably plays a less significant role in the formation of yeastlike cells in the

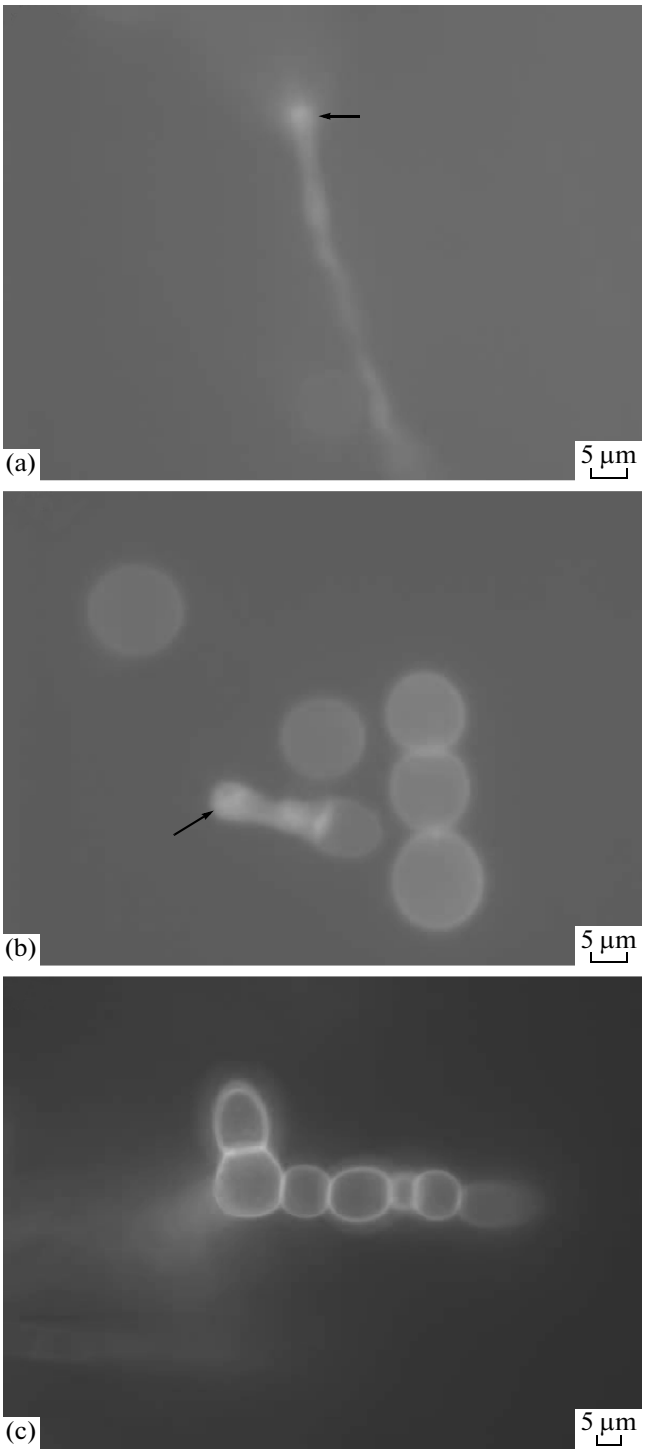

Fig. 2. Distribution of chitin in the cell walls of the mycelium (a), yeastlike cells (b), and arthrospores (b), (c) of *M. hiemalis* F-1431; Calcofluor White staining, phase contrast. The arrows point to chitin deposition in the new cell wall during apical growth. Scale bar, 5 μm.

Table 2. Carbohydrate composition of the mycelial and yeastlike cells of *M. hiemalis* F-1431, % of dry fat-free biomass

Morphotype	Fucose	Xylose	Mannose	Glucose	Galactose	Uronic acids	Chitin
Mycelium	1.89	–	3.65	17.53	1.18	5.3	30.9
Yeastlike cells	1.42	–	3.5	14.05	1.29	4.9	25.0

culture grown under aerobic conditions than the content of chitin, the main supporting biopolymer.

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