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Cell Wall Composition of the Yeastlike and Mycelial Forms of *Blastomyces dermatitidis*

FUMINORI KANETSUNA AND LUIS M. CARBONELL

Center for Microbiology, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela

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The thermally induced changes in the cell wall polysaccharides of *Blastomyces dermatitidis* strain BD64, which produces a yeastlike form (Y form) at 37 C and a mycelial form (M form) at 20 C, were examined. The cell walls of the Y and M forms contained 36 and 51% of hexoses, respectively. The M-form cell wall contained glucose, galactose, and mannose in a molar ratio of 1:0.1:0.2. The Y-form cell wall contained mainly glucose and a very small amount of galactose and mannose. The glucans of the cell wall of the Y form consisted of about 95% α-glucan and 5% β-glucan, whereas those of the M-form cell wall consisted of about 60% α-glucan and 40% β-glucan.

Blastomyces dermatitidis and *Paracoccidioides brasiliensis* show thermal dimorphism: a yeastlike form (Y form) at 37 C and a mycelial form (M form) at 20 C (7). With the thermally induced change of the Y form to the M form of *P. brasiliensis*, the glycosidic linkage of the main cell wall glucan changes from the α-type to the β-type (5, 6). Because the cell wall composition and biological characteristics of *B. dermatitidis* are similar to those of *P. brasiliensis* (3, 5, 7), it is of interest to examine the cell wall glucans of both forms of *B. dermatitidis*.

MATERIALS AND METHODS

Preparation of the cell walls of the Y and M forms of *B. dermatitidis* (strain BD 64, Center for Disease Control, Atlanta, Ga.) was performed as described previously (5).

Fractionation of the cell walls. The cell walls of the Y and M form (2 g and 1 g, respectively) were treated with 100 ml of 1 N NaOH at 20 C for 1 hr. After centrifugation at 12,000 $\times$ *g* for 10 min, the precipitates were treated three times with 50 ml of 1 N NaOH for periods of 30 min. The alkali-insoluble residue was washed with water, ethyl alcohol, and diethyl ether, successively. The alkali extracts were neutralized with acetic acid, and the precipitated glucans were purified by repeated precipitations from an alkaline solution by neutralization with acetic acid (6). The supernatant solution obtained after removal of the glucans from the neutralized extracts was concentrated by lyophilization, dialyzed against water, and again lyophilized.

Snail digestive juice (Industrie Biologique Française, Gennevilliers, Seine, France) and exo-β-1,3-D-glucanase [β-1,3(4)-glucan glucanohydrolase, EC 3.2.1.6] of the *Basidiomycete* sp. QM 806 were used as described previously (5, 6), and the liberated glucose was determined by glucose oxidase (1). After hydrolysis of samples with 1 N HCl for 7 hr at 110 C for hexoses and with 6 N HCl for 16 hr at 110 C for amino sugars and amino acids, total amounts of hexoses, amino sugars, and amino acids were estimated by reactions with anthrone, Elson-Morgan reagent, and ninhydrin, with glucose, glucosamine hydrochloride, and alanine, respectively, used as standards (5, 6). Total nitrogen and phosphorus were determined by the methods of Johnson (4) and Chen et al. (2), respectively. Molar ratio of sugars was determined by the method of Wilson (8).

RESULTS

The gross chemical composition of the cell walls of the Y and M forms of *B. dermatitidis* is shown in Table 1. On paper chromatograms, the cell wall hydrolysate of the Y form revealed only glucose, whereas that of the M form showed small amounts of galactose and mannose in addition to glucose (molar ratios of glucose-galactose-mannose were 1:0.1:0.2). Snail digestive juice hydrolyzed 3 and 42% of the glucan in the cell walls of the Y and M forms, respectively, suggesting a different nature of glucans in the cell wall of the two forms.

The yield and chemical composition of the three fractions (alkali-insoluble residue; alkali-soluble, precipitable glucan fraction; and alkali-soluble, non-precipitable fraction) obtained from the cell walls of both forms are also shown in Table 1. The alkali-insoluble residue of the Y form contained small amounts of hexoses (glucose-galactose-mannose, 1:0.4:0.3), and about 54% of the glucose in the residue was solubilized by snail digestive juice. On the other hand, the alkali-insoluble residue of the M form contained

large amounts of hexoses (glucose-galactose-mannose, 1:0.0:0.1), and snail digestive juice hydrolyzed 89% of the glucan in the residue.

The alkali-soluble, precipitable glucan fraction of the Y and M forms showed high dextrorotation ($[\alpha]_D$ = +210° and +218° in 1 N NaOH, respectively), indicating a predominance of α-glycosidic linkages. However, these same fractions contained 4 to 5% of glucan which was susceptible to snail digestive juice. After removal of this glucan by snail digestive juice, unhydrolyzed glucans of the Y and M forms had a specific optical rotation of +239° and +245° in 1 N NaOH, respectively, and their infrared spectra were identical to the α-glucan (presumably α-1,3-glucan) of *P. brasiliensis* (6).

The non-precipitable fractions obtained after neutralization of the alkali-extracts of both forms were obtained in small yields and contained polysaccharides composed mainly of galactose and mannose (glucose-galactose-mannose, 1:5.2:6.8 in the Y form and 1:1.8:3.7 in the M form).

The glucan in the alkali-insoluble residue of the M-form cell wall was solubilized completely, as estimated by the anthrone reaction, by β-1,3-D-glucanase of *Basidiomycete* sp. QM 806. However, only about 38% of the solubilized glucan was estimated as free glucose.

To isolate glucan, the alkali-insoluble residue (500 mg) of the M form was treated with chitinase (Calbiochem, Los Angeles, Calif.; 1 mg/ml) in 50 ml of 0.05 M acetate buffer, *p*H 5.0, at 37 C for 3 days. About 77% chitin and 6% glucan in the residue were solubilized by this treatment, as estimated by the release of glucosamine and glucose. After chitinase treatment, 42 mg of alkali-soluble glucan was isolated from the insoluble residue by treatment with alkali as described previously (6). This glucan was insoluble in water and had a specific rotation of +13° in 1 N NaOH, and its infrared spectrum was identical to that of the alkali-soluble β-glucan of *P. brasiliensis* (6). Since the infrared spectrum showed an absorption band at 885 cm^{-1} due to β-glycosidic linkages and no absorption band at 845 cm^{-1} due to α-glycosidic linkages, the isolated glucan was designated as β-glucan. The β-1,3-glucanase preparation solubilized the isolated β-glucan completely, although only 41.6% of the hydrolyzed glucan was estimated as free glucose. The final alkali-insoluble residue (200 mg) obtained after the isolation of β-glucan from the chitinase-treated cell wall contained 46.8% glucan and 20.7% chitin, and its infrared spectrum showed an absorption band at 890 cm^{-1} due to β-glycosidic linkages and no absorption bands at 845 and 820 cm^{-1}, which are produced by α-1,3-glucan (6). These results indicate that only β-glucan remained in the alkali-insoluble residue of the M-form cell wall.

TABLE 1. *Chemical composition of the various fractions of the cell walls of the yeastlike and mycelial forms of Blastomyces dermatitidis*

Determination	Yeastlike form	Mycelial form
Cell wall		
Total phosphorus (%)	0.12	0.08
Total nitrogen	4.30	4.21
Hexoses	36.2	51.0
Amino sugar	37.0	22.8
Amino acids	7.8	10.9
Alkali-insoluble residue		
Yield[a] (g)	1.17	0.56
Total phosphorus (%)	0.04	0.04
Total nitrogen	7.43	5.03
Hexoses	2.3	34.3
Amino sugar	66.6	36.3
Amino acids	8.0	13.0
Alkali-soluble, precipitable glucan fraction		
Yield[a] (g)	0.69	0.27
Total phosphorus (%)	0.00	0.00
Total nitrogen	0.15	0.03
Hexoses	101.2	96.3
Alkali-soluble, non-precipitable fraction		
Yield[a] (g)	0.01	0.04
Total phosphorus (%)		0.12
Total nitrogen		2.06
Hexoses (%)	32.3	38.7
Amino sugar		0.6
Amino acids		14.1

[a] Amounts obtained from 2 g and 1 g of the cell walls of the yeastlike and mycelial forms, respectively.

DISCUSSION

The cell wall of the Y form revealed only glucose on paper chromatograms. However, fractionation of the cell wall showed the existence of a small amount of galactose and mannose, estimated to be about 1 to 2% of the amount of glucose, in the cell wall preparation. Such a small amount of galactose and mannose could not be detected clearly on paper chromatograms. On the other hand, the M-form cell wall contained measurable amounts of galactose and mannose.

In addition to the differences in the amounts of galactose and mannose in the cell walls of the Y and M forms, the present study indicates that the thermally induced change of *B. dermatitidis* induces alterations in the cell wall glucans as in

the case of *P. brasiliensis* (6). Whereas about 95% of the glucans of the Y-form cell wall were identified as an alkali-soluble α-glucan, the M-form cell wall contained large amounts of an alkali-insoluble glucan. The isolation of β-glucan from the alkali-insoluble residue of the M form of *B. dermatitidis* strongly suggests that the alkali-insoluble glucan may have mainly β-glycosidic linkages. Compared with the α- and β-glucans of *P. brasiliensis* (6), the isolated α- and β-glucans of *B. dermatitidis* may have, mainly, α-1,3- and β-1,3-glycosidic linkages, respectively.

Since the α-glucan of *P. brasiliensis* is not hydrolyzed by snail digestive juice or by the β-1,3-glucanase preparation (5, 6), we may assume that the glucose liberated by the enzyme preparations was derived from the β-glucan, although crude enzyme preparations could liberate glucose from other macromolecules. By the above assumption, the glucans of the Y-form cell wall of *B. dermatitidis* consisted of about 95% α-glucan and 5% β-glucan, and the glucans of the M-form cell wall consisted of about 60% α-glucan and 40% β-glucan.

The existence of an α-glucan in the M-form cell wall of *P. brasiliensis* could not always be demonstrated (5, 6). At present, the factor(s) responsible for the concentration of α-glucan in the M form of *B. dermatitidis* and *P. brasiliensis* is unknown. However, it appears obvious that the thermally induced change of *B. dermatitidis* from the Y form to the M form results in the synthesis of large amounts of β-glucan, as in *P. brasiliensis*.

LITERATURE CITED

1. Bergmeyer, H. U., and E. Bernt. 1963. D-Glucose: determination with glucose oxidase and peroxidase, p. 123–130. *In* H. U. Bergmeyer (ed), Methods of enzymatic analysis. Academic Press Inc., New York.
2. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
3. Gilardi, G. L. 1965. Nutrition of systemic and subcutaneous pathogenic fungi. Bacteriol. Rev. **29**:406–424.
4. Johnson, M. J. 1941. Isolation and properties of a pure yeast polypeptidase. J. Biol. Chem. **137**:575–586.
5. Kanetsuna, F., L. M. Carbonell, R. E. Moreno, and J. Rodriguez. 1969. Cell wall composition of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. J. Bacteriol. **97**:1036–1041.
6. Kanetsuna, F., and L. M. Carbonell. 1970. Cell wall glucans of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. J. Bacteriol. **101**:676–680.
7. Nickerson, W. J., and G. A. Edwards. 1949. Studies on the physiological bases of morphogenesis in fungi. 1. The respiratory metabolism of dimorphic pathogenic fungi. J. Gen. Physiol. **33**:41–55.
8. Wilson, C. W. 1959. Quantitative determination of sugars on paper chromatograms. Anal. Chem. **31**:1199–1201.