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Exopolysaccharides of Some Medicinal Mushrooms: Production and Composition

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ABSTRACT: Production of exopolysaccharides of some medicinal mushrooms on different nutrient media was studied. It has been demonstrated that studied strains of *Coriolus hirsutus, C. versicolor, C. zonatus, Fomes fomentarius, Hirschioporus pergamenus, Pleurotus ostreatus,* and *P. sajor-caju* synthesized exopolysaccharides on glucose-peptone and beer wort media. It was found that the synthesis and the composition of exopolysaccharides by *C. hirsutus* and *P. ostreatus* depend on the available sources of carbon in the nutrient medium. Exopolysaccharides of both species consisted of galactose, mannose, and glucose, with a prevalence of the latter. The content of individual components is different.

KEY WORDS: Higher Basidiomycetes, medicinal mushrooms, exopolysaccharides, production, composition.

INTRODUCTION

Medicinal mushrooms produce several kinds of biologically active compounds—polysaccharides, lectins, terpenoids, phenols, etc. (Chang, 1999; Mizuno,1999; Wasser and Weis, 1999a,b). It was found that antitumor activity was connected with polysaccharides in most cases (Gunde-Cimerman, 1999; Mizuno, 1999; Wasser and Weis, 1999 b). The derivatives of polysaccharides and their partially hydrolyzed products were obtained from culture filtrates, by extracting the fruiting bodies or mycelia of mushrooms with different solvents (Jong and Donovick,1989; Karacsonyi and Kuniak, 1994; Gutierrez-Ana et al., 1996; Boldizsar et al., 1998).

The aim of our investigation was to study exopolysaccharide production in submerged culture by some higher Basidiomycetes mushrooms with medicinal properties, and the influence of different carbon sources on biosynthesis and composition of exopolysaccharides.

MATERIALS AND METHODS

The studied strains of *Coriolus versicolor* (L.: Fr.) Lloyd (= *Trametes versicolor*) 21, *C. hirsutus* (Willd.: Fr.) Quél. (= *Trametes hirsutum*) 25, *C. zonatus* (Fr.) Quél. 28, *Fomes fomentarius* (L.: Fr.) Fr. 15, *Hirschioporus pergamenus* (Fr.) Bond. et Sing. 31, *Pleurotus ostreatus* (Jacq.: Fr) Kumm. 9, 205, and *P. sajor-caju* Fr.: Fr. 16 were obtained from the culture collection of the Institute of Microbiology of the National Academy of Sciences of Byelorussia, Minsk.

Mycelia of these strains were grown in submerged conditions on glucose–peptone and beer wort (8°Balling) nutrient media. Effects of carbon sources on the synthesis of exopolysaccharides were studied on glucose–peptone nutrient medium in which glucose was replaced by arabinose, xylose, fructose, galactose, sucrose, maltose, or cellobiose. The composition of the glucose–peptone nutrient medium (g/l) was as follows: glucose–10, peptone–3, $KH_2PO_4 \cdot 3H_2O$–1, K_2HPO_4–1,

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$MgSO_4 \cdot 7H_2O$–0.25, corn extract–20 ml, deionized water–1000 ml, pH 5.5.

The dynamics of the biomass and exopolysaccharide production was studied on cellobiose–peptone nutrient medium. After preparation, the medium was sterilized by autoclaving for 20 min at 121°C. Mycelium was grown in 5-l flasks using the submerged cultivation technique. Inoculation material was produced in 0.5-l flasks containing homogenized mycelium from Petri dishes.

Exopolysaccharides were obtained in the following way. The culture liquid was treated with 96% ethanol at a volume ratio of 1:1. The precipitate (the fraction of the exopolysaccharides) was separated from the supernatant by centrifugation at 8000*g* for 15 min. The supernatant was removed, and the exopolysaccharides were dissolved in the minimal volume of distilled water and dialyzed against distilled water for 2 days. The dialyzed exopolysaccharides were precipitated with 96% ethanol at a volume ratio 1:2, then washed with ethanol, ether, and acetone and dried at 37°C. Exopolysaccharides were determined in glucose–peptone and beer wort media without mushroom mycelia also. Exopolysaccharides were absent in glucose–peptone medium. Traces of exopolysaccharides were determined in beer wort medium.

The homogeneity of the exopolysaccharides obtained was analyzed by gel filtration through Sephadex G-200 in phosphate buffer (pH 6.6) in a column (1 × 25 cm) at a flow rate of 3–5 ml/h. The carbohydrate composition of polysaccharides after hydrolysis was determined by gas–liquid chromatography (Khorlin, 1975).

RESULTS AND DISCUSSION

The data in Table 1 show that all studied species of medicinal mushrooms synthesized exopolysaccharides in the glucose–peptone and beer wort media. *C. hirsutus* 25 was the most active producer of exopolysaccharides as compared with studied species on both studied media. *P. ostreatus* 205 produced a considerable amount of exopolysaccharides on beer wort medium.

It was found that the synthesis of exopolysaccharides depends on the carbon sources in the nutrient medium (Ueda and Kono, 1965). Our results correspond to the data in the literature (Table 2). Both *C. hirsutus* 25 and *P. ostreatus* 205 synthesized the maximal quantity of exopolysaccharides on the medium containing cellobiose. It may be related to the ecology and the type of nutrition of the species investigated. *C. hirsutus* and *P. ostreatus* are saprotrophic xylotrophs. Cellobiose is the product of fermentative decomposition of cellulose, and the main substrate of the nutrition for these species in nature. Therefore, the maximal yield of exopolysaccharides is due to the natural adaptation of these mushrooms to this source of carbon. However, differences between *C. hirsutus* and *P. ostreatus* in the effectiveness of exopolysaccharide production on different carbon sources were considerable (Table 2). Minimal production of exopolysaccharides was noted for *C. hirsutus* on the media containing sucrose and maltose, and for *P. ostreatus* on media containing sucrose and galactose.

Analysis of data in Table 2 shows that the

TABLE 1
Production of Exopolysaccharides by Some Medicinal Mushrooms on Different Nutrient Media

Species, strain	Exopolysaccharide yield, g/l	
	Glucose–peptone medium	Beer wort medium
Coriolus versicolor 21	1.20	1.16
C. hirsutus 25	1.31	3.05
C. zonatus 28	0.90	1.34
Fomes fomentarius 15	0.34	0.68
Hirschioporus pergamenus 31	0.60	0.93
Pleurotus ostreatus 9	0.38	0.64
P. ostreatus 205	0.64	2.96
P. sajor-caju 16	0.50	0.76

TABLE 2
Effects of Carbon Sources on the Synthesis of Exopolysaccharides by *C. hirsutus* 25 and *P. ostreatus* 205

Carbon source	Exopolysaccharide yield			
	C. hirsutus		*P. ostreatus*	
	g/l	g/g of crude biomass	g/l	g/g of crude biomass
Arabinose	1.53	0.40	0.93	0.08
Xylose	1.84	0.40	0.91	0.23
Fructose	1.05	0.11	1.95	0.12
Galactose	2.81	0.46	0.69	0.05
Glucose	1.45	0.21	1.02	0.06
Sucrose	0.82	0.20	0.52	0.04
Maltose	0.77	0.20	1.15	0.16
Cellobiose	3.88	0.44	3.34	0.10

quantity of exopolysaccharides of *C. hirsutus*, expressed in relation to the volume of culture liquid and the weight of the biomass of mycelium on all carbon sources (with the exeption of fructose and maltose), was greater than that in *P. ostreatus*.

Investigation of the dynamics of biomass accumulation and exopolysaccharide synthesis by these mushrooms during various growth phases shows that the active synthesis of exopolymers occurs during the stationary growth phase (Fig. 1b) in *P. ostreatus*, and during the exponential phase in *C. hirsutus* (Fig. 1a).

The results obtained indicate that the exopolysaccharides of *C.hirsutus* and *P. ostreatus* consist of glucose, galactose, and mannose (Table 3). However, the content of each component changed along with the composition of nutrient medium and species. Glucose was the major component (88.42–100%) of the exopolysaccharides on all investigated sources of carbon for *P. ostreatus* and *C. hirsutus*. The quantitative composition of exopolymers of both species was practically identical on the medium with cellobiose only (Table 3).

The greatest differences in the quantitative composition of exopolysaccharides for the species investigated were demonstrated on the medium with galactose. It is interesting to note that the content of galactose and the mannose in the exopolysaccharides of *C. hirsutus* is highest in the case of its growth on the medium with galactose (Table 3). At the same time, the exopolymer of *P. ostreatus* on galactose–peptone medium consists of glucose only.

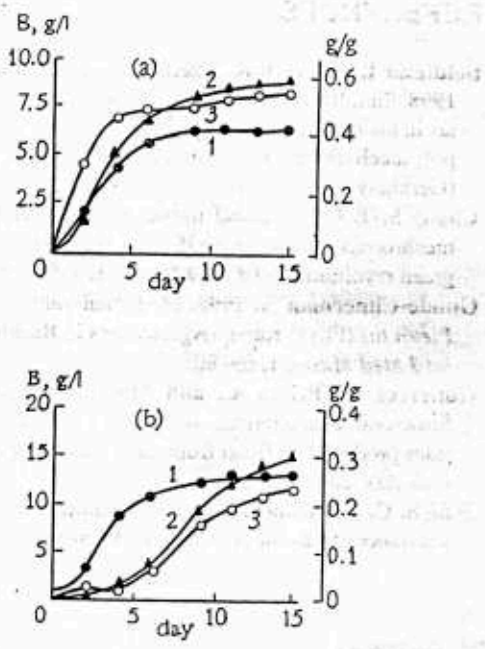

FIGURE 1. Active synthesis of exopolymers occurs during the exponential growth phase in *C. hirsutus* (**a**) and during the stationary growth phase in *P. ostreatus* (**b**).

It has been found that the molecular masses of exopolysaccharides of *C. hirsutus* 25 and *P. ostreatus* 205 were 300 and 200–220 kDA, respectively.

TABLE 3
Carbohydrate Content in Exopolysaccharides of *C. hirsutus* 25 and *P. ostreatus* 205 on Nutrient Media with Different Carbon Sources

	Carbohydrate content, % of total					
	C. hirsutus			*P. ostreatus*		
Carbon Source	Galactose	Mannose	Glucose	Galactose	Mannose	Glucose
Arabinose	3.82	1.47	94.71	5.87	1.40	92.73
Xylose	6.29	5.14	88.57	2.51	2.51	94.95
Fructose	5.37	4.26	90.37	trace	3.61	96.39
Galactose	12.95	7.05	80.00	trace	trace	100.00
Glucose	4.24	3.64	92.12	3.39	0.85	95.76
Sucrose	4.37	3.25	92.38	3.41	2.34	94.25
Maltose	7.34	4.24	88.42	2.55	trace	97.45
Cellobiose	3.35	2.56	94.09	3.41	2.34	94.25

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