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CARBOHYDRATE COMPOSITIONS OF PREPARATIONS OF FUNGAL ORIGIN*

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The fractional composition of the polysaccharides formed by some local strains of basidial fungi (Panus tigrinus, Pleurotus ostreatus, Fomes fomentarius, *and* Phanarechaeta chrysosporium) *in a submerged medium on various plant wastes (spent cottonseed pulp and cottonplant stems) has been investigated. Water-soluble polysaccharides, pectin substances, and hemicelluloses have been isolated from the products synthesized by the fungi, and their qualitative and quantitative monosaccharide compositions have been determined.*

Polysaccharides are widely used in the food and medical industries, in view of which a search is being made for new sources of them. In recent years, particular attention has been devoted to the microbial synthesis of polysaccharides. The most diverse substrates have been used as nutrient media for the microorganisms, including wastes of plant material the bulk of which consists of cellulose, hemicellulose, and lignin [1].

The leading position among microorganisms is occupied by basidial fungi, which contain hydrolytic and oxidative enzyme systems favoring the rapid growth and high penetrating capacity of the mycelium into an insoluble substrate [2]. However, problems of the growth of basidiomycetes have not yet been solved, and the conditions for the degradation of lignocellulose wastes with the aim of obtaining biologically active substances, especially carbohydrates, are still being studied.

Our task was to obtain readily water-soluble biologically active polysaccharides by the deep cultivation of fungi on lignocellulose wastes — spent cottonseed pulp (I) and cottonplant stems (II) — and to study the fractional composition of the carbohydrates.

We have studied the bioconversion of lignocellulose wastes by basidiomycetes isolated from various plant wastes and from the soils of cotton-planting regions of Uzbekistan and by the fungi *Panus tigrinuis* UzBI-I13, *Pleurotus ostreatus* UzBI-I105, and *Fomes fomentarius* UzBI-Ya55, and also *Phanarechaeta chrysosporium* obtained from the museum of the Institute of the Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino). The results showed that during the growth of the fungi the substrates are degraded to different extents, while the pH of the culture medium falls from 5.6-6.0 to 2.3-1.5 through the formation under the action of ligninases not only of carbohydrates but also of organic acids, including phenolcarboxylic acids and phenylacetic acid [3].

Both in the culture liquid (CL) and in a cell-free homogenate of these fungi we determined a total carbohydrate content of from 26.5 to 41.4 mg/ml (Fig. 1). According to PC and TLC it consisted of mono-, oligo-, and polysaccharides. With respect to their capacity for biosynthesizing carbohydrates, the fungi formed the following sequence: *P. tigrinus* > *P. ostreatus* > *F. fomentarius* > *Ph. chrysosporium.*

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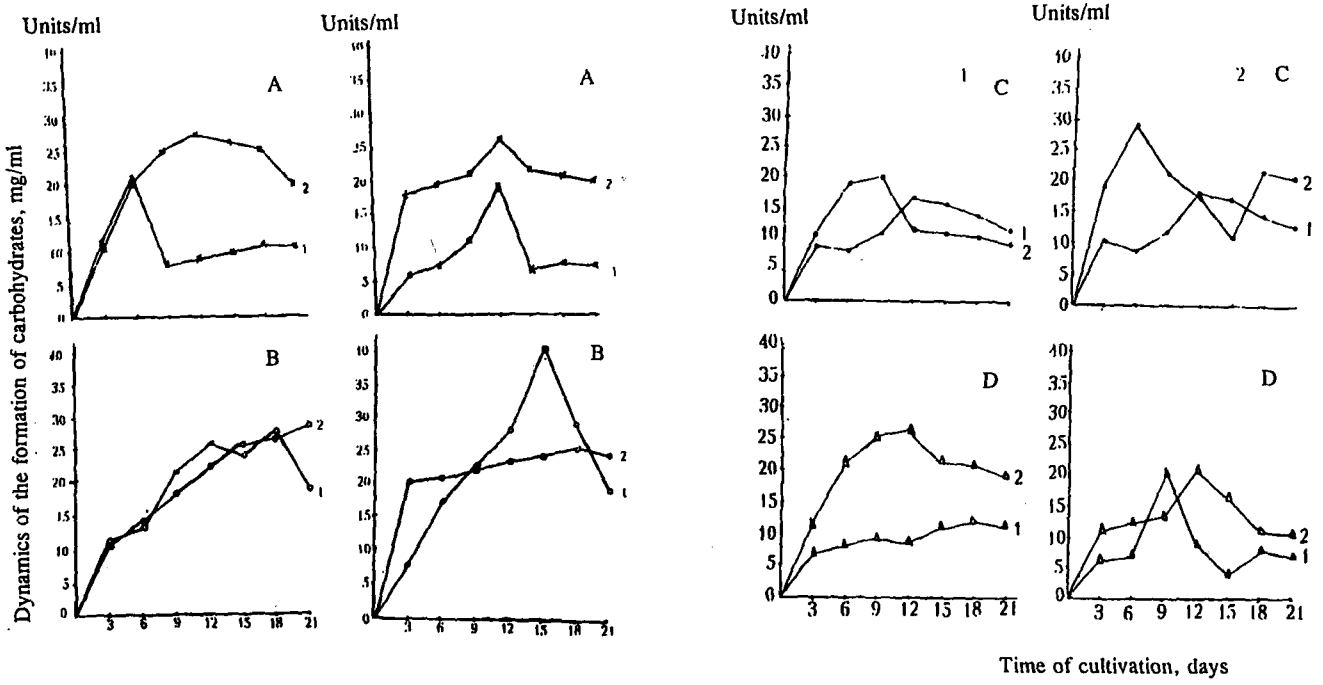

Fig. 1. Dynamics of the formation of total carbohydrates on media with spent cottonseed pulp (I) and with cottonplant stems (II). *1*) ECL; 2) ICL. *A*) *Panus tigrinus*; *B*) *Pleurotus ostreatus*; *C*) *Fomes fomentarius*; *D*) *Phanarechaeta chrysosporium*.

The accumulation of carbohydrates by the fungus *P. tigrinus* (*A*) on the medium with cottonseed pulp was highest on the 6th day of growth in the extracellular homogenate (ECL) and on the 12th day in the intracellular homogenate (ICL). For the fungus *P. ostreatus* (*B*) the maximum accumulation of carbohydrates was observed on the 18th day of growth in the ECL and on the 21st day in the ICL.

A different pattern was observed for the *F. fomentarius* culture: the concentration of carbohydrates in the ECL of the fungi rose gradually and reached a maximum on the 12th day of growth: the maximum accumulation of carbohydrates in the ICL was observed on the 9th day of growth.

The capacity for synthesizing carbohydrates of the known wood-destroying basidial fungus *Ph. chrysosporium* (*D*) was shown in the culture medium in the latest periods of cultivation: in the ECL on the 15th and 18th days of growth, and in the ICL on the 12th day.

A study of the carbohydrate-forming capacity of these fungi on a medium with cottonplant stems showed a different pattern of the accumulation of carbohydrates, both in the CL and in the fungal mycelium: *P. tigrinus* exhibited its carbohydrate-biosynthesizing activity far later than on the medium with cottonseed pulp — on the 12th day of cultivation — while *P. ostreatus* and *F. fomentarius* did so on the 15th and 12th days, respectively, and *Ph. chrysosporium* on the 9th day. Almost all the fungi actively utilized this lignocellulose substrate, forming considerable amounts both of extracellular and intracellular carbohydrates (see Fig. 1).

After the end of growth, the fungal mycelium was separated from the CL and was homogenized. The supernatant liquid (SPL) and the homogenate yielded polysaccharide fractions: a water-soluble polysaccharide (WSPS), pectin substances (PcSs), and hemicelluloses (HMCs A and B). To determine their monosaccharide compositions, these fractions were hydrolyzed and the products were analyzed by PC and GLC (Tables 1 and 2).

In the CL obtained on medium I the WSPS content amounted to 0.47-1.67%. The main product in the hydrolysates of the CLs from the fungi *Panus tigrinus, P. ostreatus*, and *F. fomentarius* was glucose. Galactose predominated in the polysaccharides from the fungi *P. ostreatus* and *Ph. chrysosporium* grown on medium II. Glucose again predominated in hydrolysates of the SPLs obtained from all the species of fungi. For medium II the main component found in the products of the hydrolysis of the polysaccharide from the SPL was glucose in the case of the fungi *P. tigrinus* and *F. fomentarius*, while in the case of *P. ostreatus* the main components were rhamnose and glucose, and in that of *Ph. chrysosporium* they were galactose and mannose.

The PcSs predominated quantitatively in substrate I. In the products of the hydrolysis of the PcSs GLC showed the presence of rhamnose, arabinose, xylose, mannose, glucose, and galactose in various ratios. Galacturonic acid was identified by PC.

The HMC content was also higher in the case of substrate I. Glucose and rhamnose predominated among the monosaccharides of the HMCA from substrate I, and glucose and xylose among those from substrate II.

The CL from the fungus *F. fomentarius* yielded a glucan in a hydrolysate of which only glucose was detected. The glucan had $[\alpha]_D^{20}$ +20°C (*c* 1.0: H_2O). M_r = 38,300 Da; degree of polymerization (DP) 230. The polysaccharide gave a negative reaction with iodine, showing the absence of glucans of the starch type. The IR spectrum of the glucan showed absorption bands at 1050, 1100, 1350, 1460, 1650, 2900, and 3600 cm^{-1}.

Thus, agricultural plant wastes containing various amounts of lignocellulose are actively utilized by the fungi mentioned above, with the formation of a number of biologically valuable carbohydrates. Cottonplant stems have yielded a glucan of interest for biological study as an antitumoral drug.

EXPERIMENTAL

General Observations. GLC analysis was conducted on a Chrom 5 chromatograph. Column 3 mm × 2.5 m with the phase 5% of XE-60 on N-AW-DMCS Chromaton 0.160-0.200 mm. Carrier gas nitrogen, rate 60 ml/min. Temperature of the column thermostat 210°C. IR spectra were taken on a Perkin-Elmer model 2000 spectrometer in tablets with KBr. Number of scans 100.

We tested cultures of the basidial fungi *Panus tigrinus* UzBI-I13, *Pleurotus ostreatus* UzBI-I105, and *Fomes fomentarius* UzBI-Ya55, and also *Phanarechaeta chrysosporium*, obtained from the museum of the Institute of the Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. The fungi harvested from a solid medium (wort agar) were sown in a sterilized nutrient medium containing 3% of brewers' wort and 1% of spent cottonseed pulp (a

TABLE 1. Amounts and Compositions of the Polysaccharides Obtained on Medium I (spent cottonseed pulp)

Fungus	Source of PSs	Type of PSs	Yield, %	Amounts of monosaccharides, %					
				Rham	Ara	Xyl	Man	Glc	Gal
P.tigrinus	CL	WSPS	0.47	–	1.4	1.9	1.7	95.0	–
	SPL	WSPS	1.27	1.9	5.7	5.9	16.7	69.8	–
		PcSs	0.50	Tr.	5.1	8.2	5.6	81.1	Tr.
		HMCA	0.64	–	Tr.	28.1	Tr.	71.9	–
		HMCB	4.06	4.1	72.8	–	3.4	19.7	–
P.ostreatus	CL	WSPS	1.39	–	3.0	2.2	15.2	79.6	–
	SPL	WSPS	1.11	2.4	1.7	2.1	5.1	88.7	Tr.
		PcSs	1.6	Tr.	1.2	1.8	5.7	91.3	–
		HMCA	1.6	1.9	0.8	33.6	8.0	55.7	–
		HMCB	5.45	Tr.	Tr.	10.4	18.7	60.5	–
F.fomentarius	CL	WSPS	1.67	3.9	4.1	12.3	11.7	68.0	–
	SPL	WSPS	0.57	2.7	4.9	6.2	16.0	70.2	–
		PcSs	0.77	Tr.	Tr.	11.9	58.4	11.9	17.8
		HMCA	4.16	51.0	28.6	Tr.	–	20.4	–
		HMCB	1.59	5.5	10.9	–	–	83.6	Tr.
Ph.chrysosporium	CL	WSPS	1.34	5.2	10.4	22.5	37.9	12.0	12.0
	SPL	WSPS	3.44	Tr.	7.7	7.7	7.2	59.0	18.4
		PcSs	2.8	3.2	3.2	3.2	3.2	87.2	–
		HMCA	11.8	32.3	Tr.	54.1	Tr.	13.6	–
		HMCB	1.25	25.0	4.0	42.0	4.0	–	25.0

TABLE 2. Amounts and Compositions of the Polysaccharides Obtained on Medium II (cottonplant stems)

Fungus	Source of PSs	Type of PSs	Yield, %	Amounts of monosaccharides, %					
				Rham	Ara	Xyl	Man	Glc	Gal
P.tigrinus	CL	WSPS	1.42	–	2.7	–	2.3	95.0	–
	SPL	WSPS	0.76	11.6	12.7	1.3	6.8	67.6	–
		PcSs	0.14	17.1	11.6	11.6	6.1	18.9	34.7
		HMCA	0.46	–	15.4	8.1	5.5	71.0	–
		HMCB	0.43	1.0	–	97.0	1.0	1.0	–
P.ostreatus	CL	WSPS	0.33	6.1	9.2	15.8	–	24.4	44.5
	SPL	WSPS	0.18	45.6	4.3	8.7	–	31.5	9.9
		PcSs	0.10	6.9	7.5	12.8	–	67.0	5.8
		HMCA	0.10	–	Tr.	98.3	–	1.7	–
		HMCB	0.91	–	1.0	98.0	–	–	1.0
F.fomentarius	CL	WSPS	3.34	–	–	–	1.3	98.7	–
	SPL	WSPS	0.07	–	–	–	8.5	84.4	7.1
		PcSs	0.07	46.8	10.4	10.4	–	22.0	10.4
		HMCA	1.08	–	3.7	48.8	7.9	39.6	–
		HMCB	0.75	1.8	–	68.4	1.8	28.0	–
Ph.chrysosporium	CL	WSPS	0.40	14.0	9.3	10.6	4.1	3.0	59.0
	SPL	WSPS	0.08	2.7	6.1	Tr.	27.8	16.2	47.2
		PcSs	0.07	–	Tr.	10.9	24.3	38.6	26.2
		HMCA	2.9	–	–	35.4	26.2	36.3	2.1
		HMCB	0.65	–	–	–	47.6	52.4	–

waste from the oils and fats factories of Uzbekistan) and cottonplant stems ground in a ball mill to a particle size of 0.1-0.3 mm. Cultivation was performed by the deep method at 28-30°C in 500-ml Erlenmeyer flasks containing 200 ml of nutrient medium with pH 6.5 for 18 days on circular shaking machines at the rate of 250 rpm. Samples were taken every three days for analyzing their carbohydrate contents. Total carbohydrates were determined by Dubois's method [5].

Treatment of the Cultures. After the end of growth the fungal mycelium was separated from the CL by vacuum filtration on a Büchner funnel and was carefully washed with distilled water. The biomass was triturated with quartz sand until the mycelial hyphae had been completely broken down. The homogenate was centrifuged at 8000 rpm for 15 min. The SNL was separated from the homogenate and its contents of carbohydrates, difficultly and readily hydrolyzable polysaccharides, and hemicellulose were determined.

The acid hydrolysis of the polysaccharides was conducted with 2 N H_2SO_4 in the boiling water bath for 7-12 h. The solutions were neutralized with $BaCO_3$, filtered, and evaporated in vacuum. The hydrolysis products were investigated by PC on FN-3 and 11 paper (Germany) in the solvent system butan-1-ol–pyridine–water (6:4:3), the sugars being revealed with acid aniline phthalate and urea.

Isolation of the Glucan. CL II was evaporated to half volume and was treated with a fourfold volume of ethanol. The resulting precipitate was separated off and was washed with acetone to dewater it.

Isolation of the WSPSs. SNLs I and II were evaporated to half volume and precipitated with ethanol (1:4 v/v). The resulting precipitates were washed with acetone.

Isolation of the PcSs. The homogenate was extracted twice with 0.5% solutions of oxalic acid and ammonium oxalate (1:1) at 70°C for 3 h. The extracts were separated off, evaporated to half volume, and precipitated with ethanol. The precipitate was separated off and dried in vacuum.

Isolation of the HMCs. After the isolation of the PcSs, the residue was treated with 10% KOH solution at room temperature for 24 h. The extract was filtered and was neutralized with 80% CH_3COOH. The resulting precipitate was separated off and dewatered with acetone. This gave HMCA. The mother solution was dialyzed against distilled water. The dialysate was evaporated and precipitated with ethanol, and the precipitate was washed with acetone, giving HMCB.

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