EXTRACELLULAR POLYSACCHARIDES OF Bullera alba VKM Y-2141

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The intracellular polysaccharides formed by Bullera alba VKM Y-2141 have been investigated. This culture synthesizes a xyloglucuronomannan and a xylogalactomannan in a ratio of 8:1. The structure of the xyloglucuronomannan has been established: in it, to the main chain of an α-1,3-bound mannan are attached glucuronic acid and xylose residues by β-1,2- and β-1,4-bonds, respectively. A structural similarity of this polymer to the exoglycans of yeasts of the genus Cryptococcus has been noted.

Yeasts of the genus Bullera have scarcely been studied as producing agents of polysaccharides. We have begun investigations of polysaccharide formation in this group of microorganisms. It has been shown that the capsular yeast B. tsugae synthesizes in considerable yield an extracellular mannan similar to the mannans of yeasts of the genus Rhodoturula [1]. In the present paper we give the results of an investigation of the chemical composition and structure of the exoglycans formed by another species of yeast from the genus Bullera – B. alba VKM Y-2141.

The yeast B. alba produced an extracellular polysaccharide on Golubev's medium with a yield of 2.8 ± 0.4 g/liter. After its de-ionization and fractionation with the aid of Cetavlon, acid and neutral fractions were obtained in a ratio of 8:1. The homogeneity of the polymers isolated was established in gel chromatography on a column of Sepharose 4B. As a result of complete acid hydrolysis and subsequent paper chromatography (PC) it was shown that the acid fraction consisted of mannose, glucuronic acid, and glycose residues and from the quantitative ratio of the components determined with the aid of the GLC of the corresponding polyol acetates, it was a xyloglucuronomannan (Table 1). As is known, exoglycans with the same monosaccharide composition are synthesized by yeasts of the genus Cryptococcus under analogous conditions of cultivation. While having a common type of chemical structure, the extracellular polysaccharides of different types and strains of cryptococci differ in the quantitative ratios of the monosaccharides and the structures of the side chains in the polymer molecules [2].

According to the results of periodate oxidation, the xyloglucuronomannan contained more than 60% of 1 → 3-glycosidic bonds. On subsequent tetrahydroborate reduction of the oxidized products, intense spots of glycerol and weak spots of erythritol were detected on the chromatograms, which indicated a predominating amount of 1 → 2 bonds in comparison with 1 → 4 glycosidic bonds (Table 1).

The neutral fraction of the polysaccharide, which was a xylogalactomannan, contained galactose residues that did not undergo the action of sodium periodate (Table 1).

The core of xyloglucuronomannan obtained on the Smith degradation of the polymer consisted predominantly of mannose units resistant to the action of sodium periodate (Table 1). Its structure was confirmed with the aid of ^{13}C NMR spectroscopy. The spectra clearly revealed six signals belonging to an α-D-mannopyranose residue (ppm): 103.37 (C1), 70.99 (C2), 79.55 (C3), 67.49 (C4), 74.82 (C5), 62.37 (C6). The chemical shift of the C1 atom of the glycosylating mannose at 103.37 ppm corresponded to the 1 → 3 type of bond [3]. These results are identical with those obtained for linear α-1→ 3 mannans forming the main chain of the exoglycans of cryptococci [2, 4, 5].

As the result of the partial hydrolysis of the xyloglucuronomannan, an oligosaccharide fragment was isolated. On its subsequent hydrolysis with 2 N trifluoroacetic acid (2 mg of

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TABLE 1. Characteristics of the Exoglycans of B. alba

Specimen	Monosaccharide composition, %				$[\alpha]_D^{20}$, deg (c 0.25; 0.1 N NaOH)	Ratio of glycosidic bonds, %			Products of periodate oxidation with subsequent tetrahydroborate reduction
	Man	GlcA	Xyl	Gal		1→6 for nonreducing terminal groups	1→2 and/or 1→4	1→3	
Xyloglucuronomannan	73,6	18,2	8,2	—	+32	17,0	22,0	61,0	glycerol, mannose, erythritol
Xylogalactomannan	54,7	—	20,8	24,5	+14	19,0	11,0	70,0	Mannose, galactose, glycerol, erythritol (traces)
Core of a xyloglucuronomannan	98,0	2,0	—	—	−68	7,0	—	93,0	Mannose

TABLE 2. Values of the Chemical Shifts in the ^{13}C NMR Spectra of the Aldobiuronic Acid from B. alba

Sugar residue	Chemical shift, ppm.					
	C1	C2	C3	C4	C5	C6
D-Manp α	93,14	79,66	70,51	68,20	73,28	61,70
β	94,76	81,83	74,26	68,20	77,00	61,92
D-GlcpA β(β)	104,77	73,28	76,25	72,49	76,25	174,8
β(α)	(102,78)	(73,58)				

TABLE 3. Results of an Analysis of Methylated Derivatives of the Monosaccharides in the Xyloglucuronomannan of B. alba*

Methyl derivatives of monosaccharides	Xyloglucuronomannan	
	native	reduced
2,3,4-Me_3Xyl	1,0	1,0
2,3,4,6-Me_4Glc	—	2,0
2,4,6-Me_3Man	4,1	4,3
4,6-Me_2Man	2,2	2,3
2,6-Me_2Man	1,0	1,2

*The ratios of the methyl derivatives calculated relative to the component present in smallest amount are given.

sample, 0.1 ml of TFA, 100°C, 10 h), mannose and glucuronic acid were identified with the aid of PC. Table 2 gives the values of the chemical shifts in the ^{13}C NMR spectra of the aldobiuronic acid isolated. The presence of twenty signals corresponds to a disaccharide fragment. Twelve signals related to α,β-D-manno-pyranose and the others to β-D-glucuronic acid. The splitting of the C1 signal of glucuronic acid as a function of the configuration of the glycosidic center of the mannose is evidence in favor of the presence of a 1 → 2 bond in the disaccharide. The spectra were interpreted with the aid of the spectra of model compounds [3]. Analysis showed that the fragment isolated had the structure

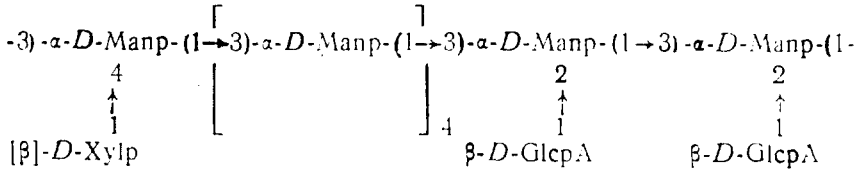
β-*D*-GlcpA-(1→2)-α-*D*-Manp.

The results of the methylation of the xyloglucuronomannan confirmed those obtained with the aid of the methods described above. Both a native and a reduced sample were subjected to methylation. The completeness of the reduction of the uronic acid residues in the polymer was checked with the aid of NMR spectroscopy. The reduced sample gave no absorption bands

at 1640 and 1725 cm^{-1}, which indicated the absence of carboxy and ester groups from it. Likewise, on complete acid hydrolysis followed by PC, clear spots corresponding to glucose were detected on the chromatogram.

2,3,4,6-Tetra-O-methyl-glucose was detected in the products of the methylation of the reduced polymer, which indicated the terminal position of the glucuronic acid in the polysaccharide. From the ratio of 2,4,6-tri-O-methylmannose, 2,4-di-O-methylmannose, and 4,6-di-O-methylmannose, approximately 4:2:1, it follows that to four unsubstituted mannose units in the core there were three substituted units, two of them being substituted in position 2 (Table 3). As already stated, the substituents in positions 2 of the mannose chain are glucuronic acid residues. The substituent in position 4 of the mannose units is probably xylose, since the amount of 2,6-di-O-methylmannose corresponds to the amount of 2,3,4-tri-O-methylxylose (Table 3).

On the basis of the facts given above, we can give a possible formula of the structural unit of the xyloglucuronomannan of B. alba:



Thus, the main structural elements of the molecule of the xyloglucuronomannan of B. alba are analogous to those of the heteroglycans of yeasts of the genus Cryptococcus investigated previously, for which an α-1,3-bound core consisting of mannose with attached glucuronic acid and xylose residues is also characteristic [2]. With the same type of structure, these polymers differ by the number of side chains and the degree of branching. The B. alba polysaccharide is the least branched of all the polymers of similar structure investigated.

EXPERIMENTAL

The chromatographic analysis of the monosaccharides and sugar alcohols was carried out in the solvent system butan-1-ol–water–ethanol–ammonia (40:49:10:1), and that of the oligosaccharides in the ethyl acetate–acetic acid–water (9:2:1) system on Filtrak FN-11 paper. Preparative PC was conducted on FN-18 paper. The chromatograms were visualized with aniline hydrogen phthalate and an ammoniacal solution of silver nitrate.

Gel chromatography was carried out by the method described previously [5]. GLC was conducted on a Tsvet-163 instrument using a glass column (0.4 × 150 cm) containing 3% of OV-225 on Chromaton (0.315-0.400 mm). Polyol acetates were investigated at a temperature of 190°C with a rate of flow of helium of 40 liters/h; acetates of partially methylated methyl glycosides were investigated with programming of the temperature from 120 to 220°C/2°C at a rate of flow of helium of 30 liters/h.

The ^{13}C NMR spectra of the oligosaccharide and of the modified polysaccharides were obtained on a Bruker AM-300 instrument (FRG) in D_2O at 40°C using methanol as internal standard (50.15 pm). The chemical shifts are given relative to tetramethylsilane. IR spectra were taken on a UR-20 instrument. Specific optical rotations of solutions of the polymers were measured on a Perkin-Elmer 241 automatic polarimeter.

Isolation of the Polysaccharide. A culture of B. alba VKM-Y-2141 was used. Fermentation was performed in a glucose-peptone medium with phosphate buffer (Golubev's medium) by a procedure described previously [4]. The extracellular polysaccharides were isolated from the native solution by precipitation with ethanol (1:3 by volume). The polysaccharide raw material obtained was dissolved, and the solution was treated with KU-2 cation-exchange resin (H^+) and fractionated by means of a 5% solution of Cetavlon with the addition of a 0.05 M solution of $Na_2B_4O_7$ (1:1 by volume). The precipitate was separated off by centrifugation (5000 rpm) and was washed twice with water, and the acid fraction of the polysaccharide was precipitated with ethanol (1:3 by volume). The neutral fraction was obtained from the supernatant by precipitation with ethanol. Both fractions were treated with KU-2 cation-exchange resin (H^+) and were reprecipitated with ethanol. The precipitates were washed with ethanol and with acetone and were dried in vacuum at 40°C.

Complete Hydrolysis. A polysaccharide (20 mg) was hydrolyzed in 0.5 ml of 2 N H_2SO_4 at 100°C for 4 h. The hydrolysates were neutralized with $BaCO_3$. The monosaccharides were

identified with the aid of PC. To determine the quantitative ratio of monosaccharides, 50 mg of a polysaccharide was treated so as to obtain polyol acetates [4] and these were studied with the aid of TLC.

Periodate Oxidation. A polysaccharide (50 mg) was kept in a 0.015 M sodium metaperiodate solution (50 ml, 4°C, 120 h). The glycosidic bonds were calculated by a generally known method [6]. The polyols were identified with the aid of PC. For this purpose, the oxidized polymer (20 mg) was treated with $NaBH_4$ (50 mg) for 16 h, and the pH of the reduced product was brought to 5-6 by the addition of 2 N CH_3COOH. The solution was de-ionized with KU-2 (H^+) and EDE-2P (OH^-) ion-exchange resins and was evaporated to dryness, and the residue was evaporated three times with methanol. The polyalcohol obtained was hydrolyzed (1 ml of 0.5 N HCl, 100°C, 6 h).

Smith Degradation. To obtain the core, 200 mg of the xyloglucuronomannan was dissolved in 50 ml of water and was treated with 50 ml of a 0.1 N solution of sodium metaperiodate. The mixture was kept at 20°C for 48 h. The excess of periodate was decomposed with ethylene glycol (2 ml) and the reaction mixture was dialyzed against distilled water for two days. The solution was evaporated to 50 ml, 400 mg of $NaBH_4$ was added, and the mixture was kept for 12 h and was then dialyzed (for two days) and evaporated to 40 ml. Hydrolysis was carried out with the addition of 3.6 ml of 6 N HCl to the solution (20°C, 7 h). The resulting solution was neutralized, subjected to dialysis, and lyophilized.

Partial Hydrolysis. The xyloglucuronomannan (500 mg) was hydrolyzed with 1 N H_2SO_4 (33 ml, 100°C, 4 h) and the resulting mixture was neutralized with $BaCO_3$. The precipitate was separated off by centrifugation. The supernatant was treated with KU-2 anion-exchange resin (H^+) and was then passed through a column (1 × 10 cm) containing AV-17 anion-exchange resin (OH^-). Elution was performed first with water to eliminate the neutral fraction and then with 1 N HCOOH to isolate the acid fraction. The latter was concentrated by evaporation. An aldobiuronic acid was isolated with the aid of preparative PC.

Reduction of the xyloglucuronomannan was carried out by Fasio's method in a modified variant [5].

Analysis by the Methylation Method. Native and reduced samples of the xyloglucuronomannan (50 mg) were methylated twice by Hakomori's method [7]. The completeness of methylation was determined from their IR spectra. The completely methylated products were analyzed in the form of acetates of partially methylated methyl glycosides.

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