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CHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF THE LIPOPOLYSACCHARIDES OF Yersinia kristensenii SEROVARS 0:12,25; 0:12,26; and 0:25,35

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A comparative and immunochemical characterization of the lipopolysaccharides of three serovars of Y. kristensenii has been performed and the ratios of the monosaccharides have been established. The results of the immunochemical investigations confirmed the serotyping of the microorganisms.

In recent years, from Yersinia enterocolitica – a microorganism causing an infectious disease of the gastrointestinal tract with a complex chemical pattern – another three types of Yersinia have been isolated on the basis of biochemical characteristics and DNA-DNA-hybridization: Y. kristensenii, Y. frederiksenii, and Y. intermedia [1]. No classification has been developed for these species and there is no information whatever on the structural investigation of the antigens.

In the present paper we give the results of a comparative chemical and immunochemical study of the lipopolysaccharides isolated from Y. kristensenii, serovar 0:12,26 (strain 103), 0:12,25 (strain 490), and 0:25,35 (strain 1647) which have common O-antigens. The lipopolysaccharides were extracted from an acetone powder of the microbial mass with aqueous phenol by Westphal's method [2]. The nucleic acids were separated by precipitation with Cetavlon. Table 1 gives analytical results for the lipopolysaccharides obtained. The smallest yield of lipopolysaccharide was obtained from the serovar 0:25,35, and it was distinguished by a low KDO content.

To establish their monosaccharide compositions, the lipopolysaccharides were subjected to acid hydrolysis, and the hydrolysates, in the form of polyol acetates, were investigated by PC and GLC. An amino acid analyzer was used to determine aminosugars. The ratios of the monosaccharides are given in Table 2. All the lipopolysaccharides had the same qualitative monosaccharide composition, including residues of D-glucose, D-galactose, D-glycero-D-mannoheptose, L-glycero-D-mannoheptose, fucosamine, galactosamine, and glucosamine.

All the lipopolysaccharides possessed serological activity. The results of cross-precipitation in the test system are shown in Fig. 1. All the lipopolysaccharides gave one distinct precipitation band with the homogeneous antiserum, but for the serovar 0:12,25 system another, weaker, precipitation band was observed which formed a spur from the precipitation band for the serovor 0:12,26 system.

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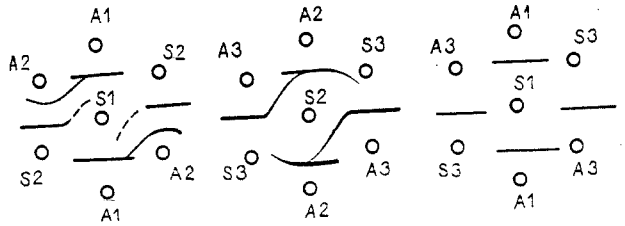

Fig. 1. Cross-precipitation of the lipopolysaccharides and antisera in a test system: S – antiserum; A – antigen. 1) For serovar 0:12,26; 2) for serovar 0:12,25; 3) for serovar 0:25,35.

TABLE 1. Analytical Results for the LPSs of Y. kristensenii

Serovar	Composition, %							
	yield of LPS	protein	nucleic acids	monosaccharides	heptoses	KDO	P	acetyl groups
0:12,26	3,1	4 6	2,2	57,1	5 0	3	1,4	1,4
0:12,25	5,8	3,9	1,2	43,6	3 3	1,5	1,9	2,5
0:25,35	0,9	5 4	1,6	48,8	4,0	0,8	1,2	2,6

The complete absence of cross-precipitation for the systems of serovars 0:12,26 and 0:25,35 confirmed the serotyping, with the absence of a common antigen. The partial cross-precipitation for the system of serovars 0:12,26 and 0:12,25 and for the system of serovars 0:12,25 and 0:25,35 confirmed the serotyping with the presence of one common determinant in each case.

The lipopolysaccharides were investigated in the indirect hemagglutination reaction (Table 3). For the homologous systems high titers of the antisera were characteristic, which fell for systems having a related antigen, while in the case of the complete absence of a common antigen no indirect hemagglutination reaction was observed.

EXPERIMENTAL

Descending chromatography was performed on FN-12 paper in the pyridine–butan-1-ol–water (4:6:3) system. Reducing sugars were detected with an alkaline solution of $AgNO_3$, aminosugars with a 0.2% solution of ninhydrin in acetone, and KDO with 2-thiobarbituric acid. GLC was performed on a Pye-Unicam 104 chromatograph with a flame-ionization detector in a glass column (0.4 × 150 cm) with the following phases: column A – the phase QF-1 (3% of Gas-Chrom Q, 100-120 mesh), and column B – the phase OV-225 (3% on Gas-Chrom Q, 100-120 mesh). Analysis was carried out in a program from 175 to 225°C (5°C/min).

The total amount of monosaccharides was determined by the method of Dubois et al. [3], protein by Lowry's method [4], heptoses by a modification of Sinilova's procedure [5], KDO by the method of [6], phosphorus by the method of [7], acetyl groups by the method of [8], and nucleic acids by Spirin's method [9].

Isolation of the LPSs. A dry acetone powder of the microbes was extracted with 45% aqueous phenol. The aqueous layer was freed from nucleic acids by precipitation with 2% Cetavlon, and was dialyzed and lyophilized.

Hydrolysis of the Lipopolysaccharides. An LPS (10 mg) was hydrolyzed in a sealed tube with 2 N HCl, 0.5 ml of TFA, or 1 N H_2SO_4 at 100°C for 3 h. The hydrolysates, in the form of polyol acetates, were investigated by PC and GLC. For the quantitative determination of amino sugars, an LPS (1 mg) was hydrolyzed with 4 N HCl at 100°C for 4 h and was evaporated several times with ethanol, and the hydrolysate was analyzed on a Biotronic LC-200 automatic amino acid analyzer in columns (0.22 × 6 cm) packed with DC-6A resin.

Preparation of Antisera. Antisera were obtained by immunizing rabbits with a suspension of microbial cells (1 million according to an optical turbidity standard). Cells that had been heated in the boiling water bath for 2 h were used to inject the animals intravenously at 3-day intervals with 0.25, 0.5, 1, and 2 ml of the antigen, respectively. Blood

TABLE 2. Monosaccharide Compositions of the LPSs

Serovar	Glc	Gal	Hep		FucN	GalN	GlcN
			D-D	L-D			
0:12,26	15,0	4,0	1,7	5,3	0,8	4,2	4,9
0:12,25	8,6	4,0	1,0	3,0	1,5	1,4	3,2
0:25,35	36,0	7,0	1,0	3,0	1,7	0,5	3,3

TABLE 3. Titers of the Antisera in the Hemagglutation Reaction

Titer of the antiserum	LPS		
	0:12,26	0:12,25	0:25,35
0:12.26	1:160000	1:64000	None
0:12,25	1:16000	1:320000	1:640
0:25,35	None	1:128000	1:160000

was taken a week after the end of the complete cycle of immunization from an auricular vein of the rabbit. The serum was separated at 3000 rpm and was investigated in immunochemical reactions.

Immunodiffusion in agar was carried out by Ouchterlony's method [10].

Indirect Hemagglutination Reaction. A. Activation of the Antigen. The lipopolysaccharides were kept in 0.1 N NaOH solution (0.2 ml) at 37°C for 1 h or in 0.5% triethylamine solution at 37°C for 16 h.

B. Preparation of Sensitized Erythrocytes. Ram erythrocytes (0.2 ml) that had been washed three times with physiological solution were mixed in phosphate buffer (10 ml, pH 7.2) with the activated lipopolysaccharide (0.2 ml) and the mixture was incubated at 37°C for 3 h. The sensitized erythrocytes were washed three time with phosphate buffer, and a 0.5% suspension was used in the hemagglutination reaction.

C. The antisera were titrated in polystyrene planchets in amounts of 100 µl with the addition to each of 100 µl of a 0.5% suspension of sensitized erythrocytes and the keeping of the resulting mixtures at room temperature for 3-5 h. The results were read in accordance with a four-cross system.

SUMMARY

The identity of the qualitative monosaccharide compositions of the lipopolysaccharides of Yersinia kristensenii serovars 0:12,26, 0:12,25, and 0:25,35 has been established.

The results of immunochemical investigations have confirmed the serotyping of these microorganisms.

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