

NOTES

Purification of Polysaccharide Antigen from *Leptospira biflexa* Strain Urawa

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Serologically active polysaccharide was isolated from the cells of *Leptospira biflexa* strain Urawa and purified. The constituents of this polysaccharide were characterized, and its serological specificity was partially examined.

It has been reported (3, 4, 8, 10, 11) that treponemal polysaccharide is composed of simple sugars, amino sugars, and a small amount of amino acid residues. Schneider (12), in a report on the genus *Leptospira*, discussed the isolation and chemical properties of the complement-fixing antigens from *L. icterohaemorrhagiae* and *L. canicola*. However, the polysaccharide antigens from *Leptospira* have not yet been purified, nor have the immunological properties of this polysaccharide been defined. This note deals with the isolation, purification, and chemical properties of polysaccharide antigen from *L. biflexa* strain Urawa.

The organisms were grown aerobically in serum-free Cox medium (2) for 7 days at 30 C, harvested by centrifugation, washed with physiological saline, and lyophilized. The dried cells were washed with acetone and further delipidated by repeated extraction with ether-ethanol (1:1) and then with chloroform-methanol (2:1).

Polysaccharides were removed by extracting a 5.22-g quantity of the delipidated cells with normal ammonium hydroxide for 42 hr at room temperature with continuous stirring. After being neutralized with acetic acid, the extract was centrifuged, and the supernatant fluid was dialyzed against running tap water for 48 hr. After dialysis, the solution was concentrated by evaporation under reduced pressure and deproteinized by the trichloroacetic acid procedure (1) and by the method of Sevag (13). A 246-mg quantity of the crude polysaccharide was recovered by precipitation with 10 volumes of ethyl alcohol.

As shown in Table 1, the crude polysaccharide

was composed of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose as determined by gas chromatographic analysis of the alditol acetates by using a column of 3% ECNSS-M on Chromosorb Q (120 to 200 mesh, 4 × 200 mm glass column); 2-deoxyribose, ribose, and three unidentified sugars were present as minor components. Galactosamine and glucosamine were also detected upon analysis for amino acids, and a protein content of 7.5% was present, as determined by the method of Lowry (7) by using bovine serum albumin as a standard.

To further purify the polysaccharide fraction, it was dissolved in a small amount of distilled water and subjected to ion exchange resin (Dowex 50W, H⁺ form) chromatography by using distilled water as the mobile phase. The eluate was concentrated in a rotary evaporator under reduced pressure, and Fehling reagent was added with stirring. The mixture was allowed to stand at 7 C for 12 hr.

The polysaccharide-copper complex thus formed was sedimented by centrifugation. The supernatant fluid containing the polysaccharides was neutralized with hydrochloric acid, immediately dialyzed against running tap water for 24 hr, and then chromatographed on columns of Amberlite IRA4B (CO₃²⁻ form) and Dowex 50W resin (H⁺ form), as described previously, to collect the purified product. This polysaccharide, designated LS-2, showed $[\alpha]_D = +48.4$ in water and a single peak in gel filtration on Sephadex G-100; however, it also contained a trace of protein as determined by the method of Lowry (7) and hexosamine as determined by the method of Elson and Morgan (6).

These findings suggest that this LS-2 fraction

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TABLE 1. Sugar compositions of polysaccharides of *Leptospira biflexa* strain Urawa

Sugars	Arabinose (R _t) ^a	Sugar compositions (molar ratios) ^b	
		Crude poly-saccharide	Purified-poly-saccharide (LS-2)
Unidentified sugar 1	0.30	0.26	0.13
Unidentified sugar 2	0.39	0.27	ND ^c
Rhamnose	0.59	0.64	0.76
Fucose	0.66	0.43	0.29
Ribose	0.88	0.40	ND
Arabinose	1.00	1.00	1.38
Xylose	1.61	0.47	0.40
Unidentified sugar 3	2.52	0.32	0.11
Mannose	2.63	1.45	0.74
Galactose	3.25	1.00	1.00
Glucose	3.48	0.57	0.45

^a The relative retention times are based on arabinose as unity.

^b Relative molar ratios are based on galactose as unity.

^c ND, Not detected.

was a highly purified polysaccharide of *L. biflexa* and, as shown in Table 1, rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose were the major constituents as determined by gas-liquid chromatographic analysis. Chemical investigation of this product is now in progress.

In the double diffusion test described by Ouchterlony (9), the LS-2 fraction gave a single band with anti-*L. biflexa* strain Urawa rabbit serum.

It has been previously reported by Elian and Nicoara (5) that two *L. biflexa* strains, Patoc and Sao Paulo, reacted with heterologous antisera. Therefore, we examined the serological cross reactivity of the LS-2 fraction with several antileptospiral and antitreponemal rabbit sera. It showed weak cross reactivity with anti-*L. hebdomadis* serum, but did not react with anti-*L. canicola*, *L. icterohaemorrhagiae*, or *Treponema pallidum*

Reiter sera in the ring test. These findings suggest that the LS-2 fraction is not a family-specific antigen. The genus specificity of LS-2 is being studied.

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