Note

Anti-tumor Polysaccharide from
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Received June 10, 1982

Beverages such as beer, cider and milk turn ropy when contaminated by some microorganisms.^{1~3)} Some strains of lactobacilli^{1,2,4)} and streptococci⁵⁾ are responsible for the ropiness because they produce mucous polysaccharides. We have found that a polysaccharide produced by one of such strains (*Lactobacillus helveticus* var. *jugurti*) shows anti-tumor activity. Here we describe purification procedures for and physicochemical properties of this polysaccharide.

L. helveticus var. *jugurti* was cultured statically in a medium containing 10% whey powder (hydrolysed with a proteolytic enzyme, Amano A) and 0.5% beer yeast extract (Ebiosu) at 37°C for 24 hr. After removal of the cells by centrifugation (10,000 rpm, 10 min), polysac-

charide was precipitated by adding a 1/2 volume of ethanol and collected by centrifugation (3,000 rpm, 10 min). The precipitate was redissolved in water. After clarification at 10,000 rpm for 10 min, the above procedure of ethanol precipitation was repeated three times. The crude preparation of polysaccharide was then dissolved in 25 ml of 0.05 M phosphate buffer (pH 6.0) and applied to a DEAE-cellulose column (2.5 × 21 cm) equilibrated with the same buffer. Elution was performed with the same buffer and then the buffer containing 0.5 M sodium chloride, and the eluate was collected in 10 g fractions. The non-adsorbed polysaccharide fraction was obtained by washing with the same buffer with a 92.5% yield (Fig. 1). Protein fractions were recovered by washing the column with the high salt buffer solution. A small amount of protein contaminating the polysaccharide fraction was removed by second DEAE- and CM-cellulose column chromatographies (data not shown). The non-adsorbed polysaccharide fraction thus obtained was lyophilized after dialysis against water at 4°C for 4 days. The lyophilized polysaccharide was dissolved in 0.02% sodium azide solution and applied (1 ml) to a Sepharose 2B column (2.5 × 40.5 cm) which had been equilibrated with the same solution. The elution volume of the polysaccharide was almost the same as that of Blue Dextran 2000 and a single peak was observed for the polysaccharide (Fig. 2). The sedimentation pattern of the polysaccharide in 0.2 M phosphate buffer (pH 7.3) is shown in Fig. 3 (the photograph was taken by Dr. S. Kaminogawa, the University of Tokyo, for which the authors are very grateful). A solution of the polysaccharide (0.1%) was centrifuged at a speed of

FIG. 1. DEAE-Cellulose Column Chromatography of Polysaccharide from *Lactobacillus helveticus* var. *jugurti*.

See text for procedures. Sugar content was determined by the phenol-H₂SO₄ method¹⁰⁾ and protein content by the Cu-Folin method.¹¹⁾

●—●, polysaccharide; ○—○, protein.

FIG. 2. Gel Filtration of Polysaccharide from *Lactobacillus helveticus* var. *jugurti*.

See text for procedures. Sugar content was determined by the phenol- H_2SO_4 method¹⁰⁾ and content of Blue Dextran from the color density at 600 nm.

●—●, polysaccharide; ○—○, Blue Dextran.

FIG. 3. Sedimentation Pattern of Polysaccharide from *Lactobacillus helveticus* var. *jugurti*.

The polysaccharide was dissolved in 0.2M phosphate buffer (pH 7.3) and centrifugation carried out at 25°C (concentration, 0.1%, 51,200 rpm).

51,200 rpm. The polysaccharide formed a sharp single Schlieren peak with a sedimentation coefficient of about 8S at 25°C. The specific optical rotatory power, $[\alpha]_D^{18}$, was +33.2° in water, the pH of a 0.1% solution was neutral. Elementary analyses revealed the composition: C, 42.2%; H, 6.9%; O, 50.4%; N, 0.0%. The purified polysaccharide was hydrolysed in 2N sulfuric acid at 100°C for 4 hr. The hydrolysate was neutralized with barium carbonate and the precipitate removed by filtration. The filtrate thus obtained was examined by various analytical means. For gas-liquid chromatography, the sample solution was com-

FIG. 4. Gas-Liquid Chromatogram of TMS-Sugars of Hydrolysed Polysaccharide from *Lactobacillus helveticus* var. *jugurti*.

Column, 0.3 × 300 cm, glass; packing, 5% SE-52/Chromosorb W, 60~80 mesh; detector, hydrogen FID; column temp., 150~230°C (3°C/min); injection and detector temp., 260°C; carrier gas flow rate; N_2 , 60 ml/min · atm.

a, galactose; b, glucose.

TABLE I. EFFECT OF POLYSACCHARIDE ON ASCITES SARCOMA-180

Sarcoma-180 cells (1×10^5 /mouse) were inoculated intraperitoneally into ICR mice.

Treatment schedule	Total dose (mg/kg)	Median survival day	ILS (%)	60-day survivors
—	—	18	0	0/8
Days 1~9	20	44	144	1/7
	40	>60	>233	4/7
	80	>60	>233	4/6

pletely evaporated and treated with reagents for trimethylsilylation (TMS). After heating at 100°C for 5 min, a portion of TMS-sugars was injected into a gas-liquid chromatograph apparatus (Shimadzu, GC-4BM) equipped with a glass column (0.3 × 300 cm, 5% SE-52/Chromosorb W, 60~80 mesh). The column temperature was gradually increased from 150°C to 230°C at a rate of 3°C/min and the temperature of the FID was 260°C. The flow rate of gas was 60 ml/min · atm. The gas-liquid chromatogram of TMS-sugars of the hydrolysate (Fig. 4) shows the component sugars of the polysaccharide to be

glucose and galactose. Enzymic analysis⁶⁾ of both sugars showed the glucose/galactose ratio to be 1.9~2.2. Anti-tumor activity of the polysaccharide was tested against ascites Sarcoma-180 by injecting the polysaccharide intraperitoneally. Daily injection of the polysaccharide for nine successive days increased the life span of the mice (Table I): Mice given a 20 mg/kg dose had an increased life span (ILS) value of 144%; the ILS value for a 40 or 80 mg/kg dose was greater than 233%; four mice survived with a 40 or 80 mg/kg dose.

We described the polysaccharide produced by *Lactobacillus helveticus* var. *jugurti* as being composed of only glucose and galactose in a ratio of 2:1. The polysaccharide differs in the ratio of component sugars from that of Kefiran (glucose/galactose=1) reported by Rivière *et al.*⁷⁾ It also differs in component sugars from a dextran-like polysaccharide reported by Williamson,¹⁾ a polysaccharide (glucose, galactose, galacturonic acid and 6-deoxytalose) reported by Wang *et al.*,⁴⁾ and that (mannose, rhamnose and mannuronic acid) reported by Ranganathan *et al.*⁵⁾ A large number of reports dealing with anti-tumor polysaccharides⁸⁾ attribute the activities to host-mediated ones. The polysaccharides had no cytotoxic effects on tumor cells *in vitro*. The polysaccharide characterized in this study also showed no effect on the growth of HeLa cells *in vitro*.⁹⁾ Therefore, the anti-tumor activity of the polysaccharide against ascites Sarcoma-180 may also be based on

its host-mediated actions.

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