The Isolation of Bacterial Polysaccharides with Anti-inflammatory Activity

Yasuo KITA, Masao ISONO, Akira MISAKI,*
Koiti ENDO** and Hidemasa YAMASAKI**

Microbiological Research Laboratories,
Central Research Division, Takeda Chemical
Industries, Ltd., Osaka

*The Institute of Scientific and Industrial Research,
Osaka University, Osaka and **Department
of Pharmacology, Okayama University
Medical School, Okayama

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In the course of studies on biologically active polysaccharides, an acidic polysaccharide which was produced extracellularly by *Aerobacter* sp. IFO 12369 and which stimulated nonspecific host resistance,¹⁾ against pathogenic bacteria, was isolated and its chemical structure was elucidated.²⁾ More recently, it was found that an extracellular acidic polysaccharide from *Serratia piscatorum* IFO 12527 exhibits marked anti-inflammatory activity,^{3~5)} and its chemical and biological properties have been investigated.^{3~5)} The present communication deals with the isolation of other anti-inflammatory polysaccharides.

Approximately seventy strains of extracellular polysaccharide-producing bacteria were isolated from soils and sewage. The bacteriological properties of several microorganisms which produced polysaccharides with

TABLE I. ANTI-INFLAMMATORY ACTIVITIES OF CRUDE POLYSACCHARIDE PREPARATIONS

Bacterial strain which produced the polysaccharide ^{a)}	Anti-inflammatory activity ^{b)} (%)	
	1.0mg/kg(<i>i.m.</i>)	0.5mg/kg(<i>i.m.</i>)
<i>Serratia piscatorum</i>	94.0	85.4
<i>Aerobacter</i> sp. IFO 12369	71.0	n.d.
<i>Agrobacterium</i> sp. No. 34	101.5	64.6
<i>Pseudomonas</i> sp. No. 14	83.6	79.3
<i>Pseudomonas</i> sp. No. 16	81.8	68.3
<i>Bacillus</i> sp. No. 21	90.3	76.8
<i>Bacillus</i> sp. No. 37	87.9	62.2

^{a)} Crude polysaccharides isolated by ethanol precipitation from culture filtrates.

^{b)} Anti-inflammatory activity is expressed as percentage inhibition by the polysaccharide (dose: 0.5 or 1.0 mg/kg, *i.m.*) of the carrageenin edema of rat foot.

n.d.: not determined.

marked anti-inflammatory activity were examined according to "Bergey's Manual of Determinative Bacteriology."⁶⁾

Each microorganism was grown in a 200 ml flask containing 50 ml of a sucrose medium (sucrose 5%, (NH₄)₂SO₄ 0.1%, K₂HPO₄ 0.2%, KH₂PO₄ 0.15%, MgSO₄·7H₂O 0.02%, yeast extract 0.1% and CaCO₃ 0.3%, pH 7.0) at 28°C for 3 days on a rotary shaker. Polysaccharides were isolated by precipitation with 75% ethanol from the culture filtrates, and the precipitates were collected by centrifugation, washed with 75% ethanol, dissolved in water, dialyzed and lyophilized. The anti-inflammatory activities of the various polysaccharide preparations were examined by measurement of the reduction of rat carrageenin edema, as

TABLE II. COMPARISON OF ANTI-INFLAMMATORY ACTIVITIES, TOXICITIES AND CARBOHYDRATE COMPOSITIONS OF THE ACIDIC AND NEUTRAL POLYSACCHARIDES

Bacterial strain	Polysaccharide preparation ^{a)}	Anti-inflammatory activity (%)	Acute toxicity ^{b)}	Carbohydrate components			
		0.25 mg/kg (<i>i.m.</i>)					
<i>Serratia piscatorum</i>	Acidic fraction	70.0	M		Gal	Rha	GalUA
<i>Aerobacter</i> sp. IFO 12369	Acidic fraction	(71.0) ^{c)}	M	Man	Gal		GlcUA
<i>Agrobacterium</i> sp. No. 34	Acidic fraction	0	L				
	Neutral fraction	67.0	H	Man	Glc	Rha	Xyl
<i>Pseudomonas</i> sp. No. 14	Acidic fraction	0	L				
	Neutral fraction	93.0	H	Man	Glc	Rha	Fuc
<i>Pseudomonas</i> sp. No. 16	Acidic fraction	0	L				
	Neutral fraction	95.2	H	Man	Glc	Rha	
<i>Bacillus</i> sp. No. 21	Neutral fraction	76.6	H	Man	Glc		

^{a)} Polysaccharide preparation fractionated with Cetavlon.

^{b)} LD₅₀ (*i.m.* in mice), L: > 500 mg/kg, M: ca. 500 mg/kg, H: 500 mg/kg >

^{c)} Dose: 1.0 mg/kg (*i.m.*)

reported previously.⁴⁾

Among approximately 70 bacterial polysaccharides tested for biological activity, five preparations had marked anti-inflammatory activities. The results of primary screening and the species of the microorganisms, identified from their bacteriological properties, are listed in Table I. These active polysaccharide preparations were further fractionated by treatment with Cetavlon⁷⁾ to give acidic and neutral fractions. After precipitation with ethanol, they were dissolved in water, dialyzed, lyophilized and examined for anti-inflammatory activity and toxicity. As seen in Table II, anti-inflammatory activity was found in the neutral polysaccharide fractions of *Agrobacterium* sp. No. 34, *Pseudomonas* sp. No. 14 & 16 and *Bacillus* sp. No. 21, and in the acidic polysaccharides of *Serratia piscatorum* and *Aerobacter* sp. IFO 12369. The anti-inflammatory activities of these neutral polysaccharides appear to be accompanied by some toxic activity.

The carbohydrate compositions of these anti-inflammatory polysaccharides are shown in Table II. The polysaccharides (50 mg each) were hydrolyzed by heating with 1 N H₂SO₄ (2.5 ml each) at 100°C for 15 hr. After neutralization with BaCO₃, the hydrolysate was filtered, concentrated and the sugars were examined by paper chromatography. It is interesting that all the neutral polysaccharides with anti-inflammatory activity elaborated by *Agrobacterium* sp., *Pseudomonas* sp. and *Bacillus* sp. contain D-mannose and D-glucose as common sugar components. It was also noted that the three polysaccharides of Gram-negative bac-

teria contain L-rhamnose, in addition to D-mannose and D-glucose. The relationship between the sugar composition and anti-inflammatory activity of these biologically active polysaccharides is being further investigated.

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