Fructan from Erwinia herbicola

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Levan production by strains of Erwinia herbicola is common, and this property has some taxonomic significance for species differentiation within the “herbicola” group. The extracellular polysaccharide elaborated by strain 403 was characterized by nuclear magnetic resonance spectroscopy and methylation analysis. Results showed it to be a typical bacterial levan.

Extracellular polysaccharides of microorganisms are of interest in sugar technology because of their effects on the processing of raw material, sugar recovery, and quality. As a consequence, the recognition and classification of microorganisms found on sugar cane and during raw sugar production are important to the industry.

In a study of sugar cane and sugar mill isolates of the “herbicola” group of the genus Erwinia, 3 of 32 strains produced a viscous growth on nutrient agar containing 5% sucrose. This note describes the structural analysis of the extracellular polysaccharide elaborated by one of these strains, SR1403.

E. herbicola SR1403 was isolated, from a crushing mill at a sugar factory, on a medium (pH 5.5) containing (per liter): sucrose, 100 g; glucose, 10 g; tryptone (Oxoid) 10 g; yeast extract, 5 g; agar, 20 g (R. H. Tilbury, Ph.D. thesis, University of Aston, Birmingham, Ala., 1982). The organism is gram negative, yellow pigmented, nonmotile, and rod-shaped. Biochemical tests were performed as described by Edwards and Ewing (3). Positive results were recorded for: fermentation of glucose and lactose without gas production; catalase, growth on eosin-methylene blue and MacConkey agars; growth in Moeller KCN broth, gelatin liquefaction; the indole test; citrate utilization; and the Voges-Proskauer test. Negative results were noted for: the oxidase test; hydrogen sulfide production; decarboxylation of lysine, ornithine, and arginine; starch hydrolysis; the urease test; nitrate reduction; phenylalanine deaminase; the methyl red test; malonate utilization.

The characteristics of strain 403 appeared very similar to those of E. herbicola subsp. ananas (7), differing primarily by lack of motility. Levan production by E. herbicola subsp. ananas is common (2, 10), and this property has taxonomic significance for species differentiation within the herbicola group.

For polysaccharide synthesis, E. herbicola SR1403 was inoculated into synthetic cane juice medium (500 ml; Tilbury, Ph.D. thesis) in an Erlenmeyer flask (1 liter) and incubated as a batch culture with shaking for 2 days at 30°C. The medium (pH 5.4) contained: sucrose, 156 g; glucose, 14 g; KH2PO4, 1.5 g; MgSO4·7H2O, 0.5 g; CaSO4, 0.5 g; soluble starch, 1.0 g; tryptone (Oxoid), 5.0 g; yeast extract (Oxoid), 5.0 g; distilled water, 1 liter. Polysaccharides were recovered by the cold aqueous extraction method of Sutherland and Wilkinson (13). The culture medium was centrifuged at 10,400 × g for 45 min at 20°C, and the supernatant fluid was treated with portions of ethanol to fractionally precipitate its constituents (Fig. 1). The fractionation curve shows that the bulk material precipitated over the range 42 to 60% aqueous ethanol. The fraction precipitated over the range 45 to 55% was washed twice with 60% aqueous ethanol before dialysis under toluene to complete the removal of any low-molecular-weight impurities. The single inflection in the precipitation curve indicates some degree of homogeneity in the preparation. The weight of material recovered by lyophilization was 13.2 g.

The hydrolysate obtained by heating the preparation in 1 M sulfuric acid at 100°C for 4 h contained only fructose when examined by paper chromatography using the solvent system ethyl acetate-pyridine-water (8:2:1, vol/vol/vol). This indicated that the polysaccharide was a fructan. The mode of biosynthesis of these polysaccharides is such that each molecule should contain a terminal sucrose residue, with D-glucose on the nonreducing end (12), but examination of the hydrolysate by heavily overloaded paper chromatogram failed to reveal any D-glucose. This was probably a consequence of the molecular size of the polymer.

When examined by gel permeation chromatography on cross-linked Sepharose gels equilibrated to 7 M urea (Fig. 2), the fructan exhibited
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relative residues. Tomašić et al. (15) noted weak but detectable signals associated with succrose terminal endings in rye grass levan; this is probably a consequence of molecular size, since the grass levan averages 10 D-fructofuranose residues per terminal sucrose and the bacterial levans are very much greater in molecular size.

Although 13C nuclear magnetic resonance spectroscopy can distinguish between (2→6)-linked levans and (2→1)-linked inulins by the chemical shifts of the hydroxymethyl carbons, the positions of the linkages involved were established unequivocally by methylation analysis. The polysaccharide was methylated by the Hakomori method, and the methylation products were identified by combined gas chromatography-mass spectrometry as outlined by Jansson et al. (6). Component O-acetyl-O-methyl alditols were identified by retention times on two phases, 3% OV-225 isothermally at 170°C and 3% ECNSS-M (Allied Science Laboratories) isothermally at 160°C. Gas-liquid chromatography-mass spectroscopy was performed as follows: chromatography on OV-225 phase into a Varian MAT 311-SS 100 mass spectrome-

FIG. 1. Fractional precipitation by ethanol of polymeric material from clarified culture medium produced by E. herbicola SRI403. The relationship is the cumulative percentage of the total precipitate as a function of increasing ethanol concentration.

a high degree of polydispersity. Molecular size fractionation occurred in each of the gels, but an excluded portion was still evident in the most porous gel, Sepharose CL-2B. These gels have exclusion limits for dextran polysaccharides that range in molecular weight from $1 \times 10^6$ to $20 \times 10^6$.

The fructan had a specific optical rotation of $\alpha_{589} = 41.8$ (c, 1.7% in water). This negative rotation indicated the presence of a $\beta$-configuration in the linkages of the constituent fructose residues.

13C nuclear magnetic resonance spectroscopy confirmed this observation and demonstrated that the polysaccharide was a typical bacterial levan. The spectrum was obtained on a deuterated sample in deuterium oxide in a 10-mm probe at a concentration of 50 mg/ml. It was prepared by overnight acquisition on a JEOL FX-100 spectrometer at 70°C under deuterium lock and referenced externally to tetramethylsilane. The spectrum (Fig. 3) compared favorably with spectra of levans from rye grass (15) and Streptococcus salivarius (11). The principal feature of the spectrum was the six intense signals, which are consistent with an essentially linear chain of $\beta$-linked fructofuranosyl residues. The polysaccharide was methylated by the Hakomori method, and the methylation products were identified by combined gas chromatography-mass spectrometry as outlined by Jansson et al. (6). Component O-acetyl-O-methyl alditols were identified by retention times on two phases, 3% OV-225 isothermally at 170°C and 3% ECNSS-M (Allied Science Laboratories) isothermally at 160°C. Gas-liquid chromatography-mass spectroscopy was performed as follows: chromatography on OV-225 phase into a Varian MAT 311-SS 100 mass spectrome-

FIG. 2. Gel permeation chromatography of the 45 to 55% fraction. Portions were examined on columns (95 by 2.5 cm) of Sepharose CL-6B and CL-4B in 7 M urea and a column (80 by 5.0 cm) of Sepharose CL-2B in 7 M urea. Fraction size was 10 ml.