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Carbohydrate Research 340 (2005) 439–447
Carbohydrate RESEARCH

Structure of the exopolysaccharide produced by *Enterobacter amnigenus*

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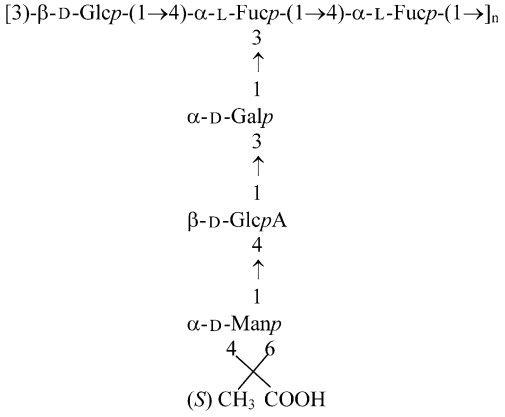
Received 8 November 2004; accepted 8 December 2004

Abstract—The bacterial species *Enterobacter amnigenus* was isolated from sugar beets harvested in Finland. It produced an exopolysaccharide rich in L-fucose, which gave viscous water solutions. Its primary structure was determined mainly by NMR spectroscopy and ESIMS of oligosaccharides and a polysaccharide with decreased molecular weight, obtained by Smith degradation of the O-deacetylated native polymer.



Keywords: *Enterobacter amnigenus*; L-fucose; Polysaccharide structure; NMR spectroscopy; Mass spectrometry

1. Introduction

Some microbial polysaccharides, in addition to their thickening, gelling or emulsifying properties, offer an alternative source to some rare sugars, thus offering a

Abbreviations: L-4dThr, 4-deoxy-L-threitol; ISV, ionspray voltage; OR, orifice potential.

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doi:10.1016/j.carres.2004.12.008

controllable way to manufacture required amount of unusual monosaccharides such as L-fucose (6-deoxy-L-galactose). L-Fucose and its oligosaccharides have potential applications in the medical field due to their anticancer and anti-inflammatory effects. Polysaccharides rich in L-fucose can be also used as skin moisturising agents in the cosmetic industry. The chemical synthesis of L-fucose is laborious and suffers from low yield; direct extraction from brown algae is costly and subject to seasonal variation.[1]

Fucose is present in a wide variety of organisms. In mammals, fucose-containing glycans have important roles in blood transfusion reactions, selectin-mediated leucocyte-endothelial adhesion, host–microbe interactions and numerous ontogenic events including signaling events by the Notch receptor family. Alterations in the expression of fucosylated oligosaccharides have also been observed in several pathological processes, including cancer and atherosclerosis.[2]

The major pathway for the biosynthesis of L-fucose in prokaryotic cells involves conversion of GDP-D-mannose to GDP-L-fucose, which in turn is the fucosyl donor for polysaccharides, glycoproteins and glycolipids.[3]

In a former survey, the slime production of 600 microorganisms from sugar beets collected from different parts of Finland was studied.[4] One hundred and seventy of them produced exopolysaccharides (EPS), of which 35% were heteropolysaccharides, although previously it was believed that 95% of polysaccharides produced by sugar beet spoilage organisms were homopolysaccharides, like levan or dextran. One of the isolates was identified as *Enterobacter amnigenus*, which produced, at the optimum temperature of 30 °C, a heteropolymer containing D-glucose, D-galactose, L-fucose and D-mannose.

In this report, the determination of the primary structure of *E. amnigenus* EPS is described.

2. Experimental

2.1. Bacterial culture and EPS purification

EPS production was carried out in a 10 dm^3 Biostat® E bioreactor (B. Braun Biotech International, Germany) in 6 dm^3 growth vol. Growth medium consisted of 10 g/L yeast extract (LAB M, MC1), 20 g/L bactopeptone (LAB M, MC24) and 80 g/L (inoculum 40 g/L) sucrose (BHD), 11.8 g/L $K_2HPO_4{\cdot}3H_2O$ (Merck), 3 g/L KH_2PO_4 (Riedel-de Haen) and 2 g/L $MgSO_4{\cdot}7H_2O$ (Merck). The inoculum was prepared in 250 mL shake flasks using 50 mL growth vol. Growth medium was inoculated with *E. amnigenus BPT 165* (laboratory collection in Helsinki University of Technology). Shake flask cultures were grown 6 h in a Certomat® HT (B. Braun Biotech International, Germany) shaker cabin at 32 °C and 150 rpm. The inoculum (300 mL) was added to the bioreactor. Aeration rate (1 vvm), growth temperature, foam level, dissolved oxygen tension (DOT) and pH were measured and/or controlled by the bioreactor control unit. Broth viscosity was measured using a Brookfield DVII+ viscometer with a small sample adapter. Cultivation was stopped after viscosity had reached its maximum. Proteins from the cultivation broth were hydrolysed using alkaline protease (Alcalase, Novo). Treatment with this protease resulted in removal of most of the acetyl groups. Therefore, the EPS was O-deacetylated completely with 0.01 M NaOH at room temperature for 5 h[5] and subsequent structural determinations were performed on this material. Diafiltration against RO water (Pellicon Mini with Biomax 1000, Millipore Inc.) was used to remove amino acids, cultivation metabolites and impurities to get the crude polysaccharide. The polymer was precipitated in 5 vol of cold acetone, and after solvent evaporation, it was recovered by lyophilisation.

2.2. Analytical procedures

Analytical GLC was performed on a Perkin Elmer Autosystem XL gas chromatograph equipped with a flame ionisation detector and an SP2330 capillary column (Supelco, 30 m), using He as the carrier gas. The following temperature programs were used: for alditol acetates, 200–245 °C at 4 °C/min; for methylated alditol acetates, 150–250 °C at 4 °C/min. GLC–MS analyses were carried out on a Hewlett–Packard 5890 gas chromatograph coupled to a Hewlett–Packard 5971 mass selective detector. Separation of trimethylsilylated methyl glycosides and of trimethylsilylated (+)-2-butyl glycosides was obtained on a HP1 column (Hewlett–Packard, 50 m).

2.3. Composition analysis

Hydrolysis of the EPS was carried out with 2 M trifluoroacetic acid at 125 °C for 1 h, while hydrolysis of oligosaccharides was performed at 100 °C for 6 h. Alditol acetates were prepared as previously described.[6] Methanolysis was performed with 1 M HCl in MeOH (Supelco) at 85 °C for 18 h according to Dudman et al.[7] Trimethylsilyl derivatives were obtained incubating the mixture of methyl glycosides with the Sylon HTP kit (3:1:9 HMDS + TMCS + pyridine, Supelco) at room temperature for 1 h. The products were dried under a stream of N_2, dissolved in *n*-hexane and centrifuged to remove insoluble materials. The clear supernatant was subjected to GLC analysis. The absolute configuration of the sugar residues was established via GLC analysis of the derived trimethylsilylated (+)-2-butyl glycosides.[8,9]