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Structural Characterization of an Exopolysaccharide from the Myxobacterium *Sorangium Cellulosum* NUST06

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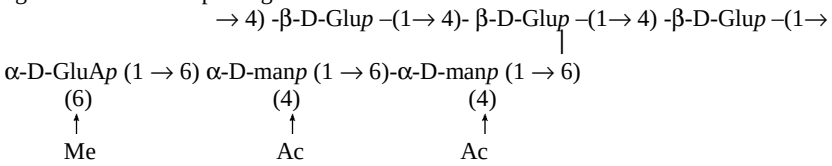
Abstract: The chemical structure of an exopolysaccharide (EPS) produced by *Sorangium cellulosum* NUST06 was investigated. The EPS was found to have molecular masses greater than 2×10^6 Da, and consisted of D-glucose, D-mannose and D-glucuronic acid in a ratio of 5:4:1. The analytical results of methylation and nuclear magnetic resonance spectra suggested the EPS consist of following tetrasaccharide repeating units:



Keywords: Exopolysaccharide, chemical structure, *Sorangium cellulosum.*

The large amounts of extracellulular matter produced by myxobacteria were composed of EPS and proteins that demonstrated as required factor for cell-cell cohesion[1]. There were few reports on the structure and bioactivities of the EPS in myxobacteria and all focus on myxobacterial extracellular matter were their role in myxobacteria social interactions[2]. Studies of the chemical structure of these molecules, constituent identification, and chemical and physical properties are essential for understanding their possible applications and eventually to improve them by using fermentation technology and genetically altered microbial strains[3]. In this paper, we first reported the purification and structural characterization of EPS produced by *Sorangium cellulosium* NUST06, a myxobacterium isolated from salt soils.

Crude EPS obtained from the ethanol precipitate of the cultured broth by *S.cellulosum* NUST06 was treated by trichloroacetic acid and dialyzed against distilled water with a yield of 51%. The polymeric fraction was desalted on Sephrose 4B with a yield of 90%. The purified EPS was contained at least 96% sugars and trace amounts of proteins (less than 0.2%). The average molecular weight of EPS was estimated from a calibration

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curve of standard dextrans obtained by gel filtration on Sepharose 4B to be about 2×10^6. Hydrolysis of the EPS with 2 mol/L trifluoro acetic acid yielded D-glucose, D-mannose and D-glucuronic acid as principal constituents in a ratio of 5:4:1.

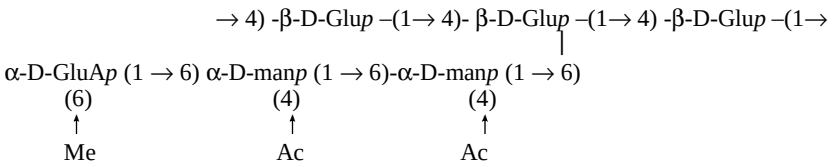
Analysis of the partially methylated alditol acetates, obtained from the permethylated EPS after acid hydrolysis, revealed for the EPS presence of a terminal glucose. As shown in **Table 1**, the major glucose residues were assigned as (1→4 and /or 6)-linked residues. The chain sugars were identified 1,4-linked glucose and branched mannose residues. All mannose residues were present as (1→ 6)-linked.

Table 1 GC-MS spectrometry analysis of the permethylated EPS from *S.cellulosum*

Methylated sugar (as alditol acetate)	Retention time (min)	Proportion of sugar (%)	Mode of linkage
2,3,4,6-Tetra-O-MeGlc	5.41	9.6	(Glcp)1→
2,3,6-Tri-O-Me-Glc	7.20	20.2	→4(Glcp)1→
2,3,4-Tri-O-MeMan	7.83	18.4	→6(Glcp)1→
2,3- Di-O-MeGlc	8.90	6.5	→4,6(Glcp)1→

The ^{13}C NMR spectrum of the EPS was conducted, low field signals at 103.3, 102.3, 102.4, 100.5 ppm were assign to the C-1 of β-D-glucose, D-glucuronic acid, and α-D-Mannose residues. The signals at 20.2 and 63.3 ppm exhibited that sugars of the EPS could be substituted with acetyl and methyl groups. The resanomeric configurations were assigned by comparing the ^{13}C NMR spectrum of the EPS with the data in literature[4,5], and the chemical shifts were shown in **Table 2**.

The results of methylation and ^{13}C NMR analysis suggested the EPS consist of the following possible repeating unit:



There is relatively little information about expolysaccharide production by *Sorangium cellulosum***,** and until now it is not clear whether these organisms are likely to prove to be a useful source of polymers. However, our results suggested that the EPS was a heteropolysaccharide whose repeating units consisted of β-D-glucose, D-glucuronic acid, and α-D-mannose. It seemed to be closely related to xanthan polymers[3]. Therefore, it will be interesting, after full structure elucidation, to obtain the information about the physical properities of the new exopolysaccharide.

Table 2 The chemical shifts of ^{13}C NMR of the EPS from *S. cellulosum*

Carbon	Chemical shifts of the EPS from S.cellulosum (ppm)			
	→4-β-D-Glcp	→4(6)-β-D-Glcp	→4(6)-α-D-Manp	→(6)-β-D-GlcAp
C-1	103.3	102.3	100.5	102.4
C-2	73.2	73.2	79.0	73.9
C-3	76.9	71.2	70.3	71.8
C-4	81.1	81.1	72.8	72.6
C-5	75.3	75.8	72.9	76.2
C-6	61.6	67.3	67.3	174.6
$-O^1CO^2CH_3$			^{1}164.2, ^{2}20.2	
$-OCH_3$				63.3

Experimental

Isolation and purification of EPS

The EPS was isolated and purified according to previously described methods[6]. Gel filtration chromatography was conducted with a Sepherose 4B (Pharmacia) column (1.5 by 60 cm), and the polysaccharides were eluted with 50 mmol/L phosphate buffer, pH 7.2, at the rate of 1 mL/min. Fractions containing polysaccharides were collected, and the total sugar content in each fraction was determined by the phenol-sulfuric acid method[7].

Chemical analysis of EPS

Monosaccharide compositions of EPS were determined by gas-liquid chromatography (GLC) of O-methyloxime acetate derivatives obtained after acid hydrolysis of polysaccharides (6 h, 100°C) in a 2 mol/L trifluoroacetic acid (TFA) solution[8]. EPS methylation was carried out by previously described methods[9]. The methylated EPS was hydrolyzed with 2 mol/L trifluoroacetic acid at 100°C for 6 h, reduced with sodium borohydride, and followed by acetylation with acetic anhydride-pyridine. The alditol acetated of partially methylated sugars were analyzed by GC and GC-MS, using a fused-silica capillary column (0.25 cm × 30 cm) of DB225 and a column (0.3 cm × 2 cm) of 3% ECNSS-M. NMR spectra were obtained on a Bruker AMX-500 instrument (125.75 MHz for ^{13}C NMR) at 50°C. Before the analysis, EPS samples were exchanged twice in D_2O with intermediate lyophilization and then dissolved in 0.5 mL of D_2O to a final concentration of 30 mg · mL^{-1}.

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