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Structural analysis of an extracellular polysaccharide produced by *Rhodococcus rhodochrous* strain S-2

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Abstract—A possibility has been suggested of applying the EPS produced by *Rhodococcus rhodochrous* strain S-2 (S-2 EPS) to the bioremediation of oil-contaminated environments, because its addition, together with minerals, to oil-contaminated seawater resulted in emulsification of the oil, increased the degradation of polyaromatic hydrocarbons (PAH) of the oil, and led to the dominance of PAH-degrading marine bacteria. To understand the underlying principles of these phenomena, we determined the chemical structure of the sugar chain of S-2 EPS. The EPS was found to be composed of D-galactose, D-mannose, D-glucose, and D-glucuronic acid, in a molar ratio of 1:1:1:1. In addition, 0.8% (w/w) of octadecanoic acid and 2.7% (w/w) of hexadecanoic acid were also contained in its structure. By ^{1}H and ^{13}C NMR spectroscopy, including 2D DQF-COSY, TOCSY, HMQC, HMBC, and NOESY experiments, as well as chemical and enzymatic analyses, the polysaccharide was shown to consist of tetrasaccharide repeating units with the following structure:

β-D-Glc*p*A
1
↓
2
→3)-α-D-Man*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→

Keywords: *Rhodococcus rhodochrous*; Extracellular polysaccharide; Bioremediation; Emulsification; Structure; NMR spectroscopy

1. Introduction

There have been a number of reports on the catabolic activities of *Rhodococcus* toward a wide variety of organic compounds, including such xenobiotics as polychlorinated biphenyls, aliphatic and aromatic hydrocarbons,

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and complex mixtures thereof, including crude oil.[1–4] It has been reported that mucoid strains of *Rhodococcus* showed good growth in the presence of hydrocarbons, whereas rough strains did not, but that the rough strains could grow in the presence of hydrocarbons by addition of the extracellular polysaccharides (EPS) produced by these mucoid strains.[5,6] Recently, we reported that the addition of EPS produced by *Rhodococcus rhodochrous* strain S-2 (S-2 EPS) together with minerals to oil-contaminated seawater resulted in emulsification of the oil, increased the degradation of polyaromatic hydrocarbons (PAH) of the oil, and led to the dominance of a species of PAH-degrading marine bacteria, *Cycloclusticus* sp., in the samples, suggesting the possibility of its application to the bioremediation of oil-contaminated environments.[7]

An understanding of the biochemical and biophysical properties of S-2 EPS and the isolation of genes and enzymes required for the synthesis and modification of it should lead to a better grasp of the principles underlying the protection from hydrocarbon toxicity and the promotion of oil-, especially PAH-, degradation by marine bacteria. Such knowledge will undoubtedly expand the possibilities of bioremediation for oil-contaminated marine environments. In this study, we present the composition of S-2 EPS and the chemical structure of its sugar chain.

2. Results and discussion

2.1. Purification of S-2 EPS

EPS produced by *R. rhodochrous* strain S-2 was extracted and purified by DEAE-Toyopearl column chromatography, being eluted with a linear gradient (0–1 M) of NaCl. This procedure gave a major single peak at approximately 0.3 M concentration of NaCl, and the fractions contained in this peak were combined as the S-2 EPS. Two minor peaks were also detected at 0 M (non-absorbed fraction) and approximately 0.1 M NaCl, and the former and the later peaks contained glucomannan and mannan, respectively (data to be presented elsewhere), which did not show the activities of S-2 EPS reported previously,[6,7] such as emulsification of the oil. Spectrophotometrically, no absorption was detected at 280 nm or at 255 nm, suggesting that the S-2 EPS did not contain proteins or nucleic acids. A single band was detected by cellulose acetate membrane electrophoresis of S-2 EPS (data not shown). The S-2 EPS was eluted as a broad single peak earlier than Dextran T2000 by Sephacryl S1000 gel-filtration chromatography (data not shown), suggesting its apparent molecular weight to be greater than 2,000,000. These data indicate that the S-2 EPS had been purified to homogeneity. S-2 EPS was a white fibrous material soluble in water and alkalis but not in acids, MeOH, EtOH, or acetone.

2.2. Compositional analysis

The monosaccharide content of S-2 EPS was determined by both H_2SO_4 hydrolysis followed by gas–liquid chromatography (GLC) analysis and trifluoroacetic acid (TFA) hydrolysis followed by HPLC analysis. Consequently, galactose, glucose, mannose, and glucuronic acid were detected in the molar ratios of 1:1:1:1. The absolute configurations of these three monosaccharides and carboxyl-reduced glucuronic acid were determined by GLC of acetylated (+)-2-octyl derivatives and all were shown to have the D configurations.

Methylation analysis was performed on both native and carboxyl-reduced S-2 EPS (Table 1). For both EPS, the data indicated that the D-galactose and D-glucose were substituted at the O-3 positions. The presence of 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl-D-mannitol in both methylated EPSs indicated that the S-2 EPS was branched at the D-mannose residues. In the methylation analysis of carboxyl-reduced S-2 EPS, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol was detected. In contrast, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol was detected in the methylated native S-2 EPS after carboxyl-reduction. These data indicate that D-glucuronic acid residues in the S-2 EPS were present at the non-reducing ends of side chains and that D-mannose residues were side-chain branching points.

Fatty acids were extracted from the alkali-hydrolyzate of S-2 EPS, but not from untreated S-2 EPS, suggesting that the fatty acids were bound to EPS by ester bonds. According to the GLC–MS analysis of methylated fatty

Table 1. Methylation analysis data of S-2 EPS

Derivatives	Molar ratio	
	Native EPS[a]	Carboxyl-reduced EPS
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol	0.75	N.D.
1,2,3,5-Tetra-*O*-acetyl-4,6-di-*O*-methyl-D-mannitol	0.87	0.69
1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol	0.69	0.82
1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol	1.00	1.00
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	N.D.	0.52

[a] The D-glucuronic acid residues contained in native EPS were reduced with $NaBH_4$ to the D-glucose residues after methylation.