Structure of the Core Oligosaccharide from Lipopolysaccharide of *Erwinia carotovora*

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The lipopolysaccharide of *Erwinia carotovora* was analyzed by quantitative sugar analysis, methylation analysis, and chromic oxide oxidation. This led to the following structure of the core oligosaccharide:

\[
\beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\alpha_\text{Hep} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\alpha_\text{Hep} \quad \beta_\text{Glc} \\
\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\alpha_\text{Hep} \quad \beta_\text{Hep} \quad \alpha_\text{Glc} \quad \beta_\alpha_\text{Hep} \quad \beta_\text{Glc}
\]

*Erwinia* are gram-negative bacteria known to cause tissue-maccerating and necrotic diseases of plants. According to numerous investigations (2), the bacterial phytopathogenicity is influenced by the cell wall lipopolysaccharide (LPS). Recent results have shown that some *Erwinia* strains are sensitive to the phage Mu (3). LPS from *Erwinia carotovora* has been found to carry the receptor structure for this phage (7). For a detailed investigation of Mu receptor and a better understanding of bacterial pathogenic mechanisms, we initiated a structural study of the LPS from *Erwinia carotovora*.

*Erwinia carotovora* B374 was grown at 32°C in dYT medium (10) overnight, and LPS was extracted by the phenol-chloroform-ether procedure (4). Two percent of the bacterial dry weight was extracted as LPS. It contained 9.15% glucose and 8.78% heptose. The LPS was characterized by polyacrylamide gel electrophoresis, which could distinguish between rough and smooth strains (Fig. 1). LPS was visualized by the silver stain method (13). The migration pattern of *Erwinia carotovora* LPS was similar to those from other "rough" bacteria such as *Escherichia coli* K-12, suggesting that the polysaccharide contains a core oligosaccharide only. Particularly, the *Erwinia* LPS contained some background staining which could be caused by glycans coextracted with LPS (9).

The LPS structure was analyzed by the following strategy. The core oligosaccharide was cleaved from the lipid A moiety by hydrolysis with 1% acetic acid at 100°C for 2 h. The insoluble lipid A was removed by centrifugation, and the supernatant was purified by gel filtration on a Sephadex G-50 column (1 by 80 cm) with 0.1 M pyridine acetate (pH 5.5) as eluant. Carbohydrate-containing fractions were identified by the phenol-sulfuric acid procedure (1). The core oligosaccharide eluted in a molecular weight range of ca. 1.200. Quantitative analysis by gas-liquid chromatography showed that it consisted of 9.6% glucose and 10.3% heptose.

The anomic configuration of the glycosidic linkages was determined by the CrO$_3$ oxidation procedure (5). Quantitative analysis of the acetylated oligosaccharide after CrO$_3$ oxidation showed that 50% of the glucose was destroyed and the heptose remained intact. This indicates that one glucose residue has $\alpha$ configuration and one has $\beta$ configuration, whereas all heptoses are in $\alpha$ configuration.

For methylation analysis, the oligosaccharide was lyophilized and dephosphorylated by 60% HF at 0°C for 30 min (8). The HF reagent was removed by lyophilization, and the oligosaccharide was again purified by gel filtration on Sephadex G-50. The dephosphorylated oligosaccharide was subjected to methylation analysis by the method of Stellner et al. (12). The partially methylated alditol acetates and their ratios from the analysis by gas-liquid chromatography–mass spectrometry are listed in Table 1.

The carbohydrate components were not present in equal molar amounts, indicating that the methylated oligosaccharide was microheterogeneous. There was a reduced ratio of 3-linked glucose and an elevated amount of 3-linked heptose compared with the other constituents. This led to the conclusion that terminal glucose and heptose moieties were partially lacking. From these data it could be calculated that the methylated carbohydrate consisted of a mixture of an oligosaccharide with two glucosees and three heptoses (32%), of an oligosaccharide with one glucose and three heptoses (33%), and of an oligosaccharide with two glucosees and two heptoses (35%). Based on this calculation, the ratio of glucose to heptose in the mixture would be 2.5 to 3.0, which is in agreement with the quantitative analysis of the oligosaccharide.

For the elucidation of the arrangement of the monosaccharides in the LPS, core-defective mutants of *Erwinia carotovora* were isolated by selection of phage Mu-resistant colonies. Quantitative analysis of LPS from one mutant gave 9.06% glucose and 21.6% heptose. This mutant apparently lacked one glucose unit. It was subjected to CrO$_3$ oxidation. Subsequent quantitative analysis revealed that the remaining glucose unit was resistant to the oxidation, indicating that it

<table>
<thead>
<tr>
<th>Partially methylated alditol acetate</th>
<th>Ratio</th>
</tr>
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<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-1,6-di-O-acetyl-glucose</td>
<td>1.00</td>
</tr>
<tr>
<td>2,3,4,6-tri-O-methyl-1,5,6-di-O-acetyl-glucose</td>
<td>0.67</td>
</tr>
<tr>
<td>2,3,4,5,6-penta-O-methyl-1,5,6-di-O-acetyl-heptose</td>
<td>1.00</td>
</tr>
<tr>
<td>2,3,4,6,7-tetra-O-methyl-1,3,5,7-tetra-O-acetyl-heptose</td>
<td>1.52</td>
</tr>
<tr>
<td>2,3,4,6,7-tetra-O-methyl-1,3,5,7-tetra-O-acetyl-heptose</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Partially methylated alditol acetates were subjected to gas chromatography–mass spectrometry and monitored by total ion scan with a Carlo Erba 2101, Varian CH7A/SS188 system. Then 1 $\mu$L of a solution in chloroform was injected onto a capillary column (25 m by 0.32 mm) coated with methyl phenyl silicone (Chrompack CP Sil 8) at a column temperature of 100°C and a temperature program of 3°C/min to 240°C. Carrier gas was helium at a flow rate of 1.8 mL/min.

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was bound to heptose in α-glycosidic linkage. These results led to the following structure for the complete oligosaccharide from *Erwinia carotovora* LPS:

$$\text{Hep} \beta \text{Glc}_1\alpha\text{Glc}_1\\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha\text{Glc}_1\alpha$$

The core structure of the LPS is similar to those of other rough strains, especially *Escherichia coli* B (6, 11) with a difference at the terminal end. *Escherichia coli* B contains a Glcα1-3Glcα1 unit, whereas *Erwinia carotovora* has a Glcβ1-6Glcα1 terminus.

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**LITERATURE CITED**