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Title	Uncovering a variant 0111 0-antigen : a structural analysis by MALDI-glycotyping and NMR spectroscopy
Author(s)	Lee, June Chelyn
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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Uncovering a variant O111 O-antigen: a structural analysis by MALDI-glycotyping and NMR spectroscopy (MALDI グリコタイピングと NMR による大腸菌 O111 抗原変異構造の発見と同定に関する研究)

Gram-negative bacteria exhibit extensive glycan diversity, particularly in their surface polysaccharides, which play critical roles in immune evasion and host-pathogen interactions. The O-antigen polysaccharide (OPS) structures serve as the antigenic component, linked to Lipid A via the core polysaccharide to form LPS. These structures vary in glycan composition, sugar topology, linkages, and chain lengths, and are often modified with noncarbohydrate components such as phosphate, acetyl, pyruvate or alkyl. This diversity results from genetic variation, including horizontal gene transfer and nucleotide substitutions, contributing to bacterial adaptation, pathogenicity, and immune evasion. Importantly, understanding OPS structural variations is essential for developing effective serogrouping methods, supporting epidemiological studies, and guiding vaccine development.

Of particular interest in our study is the *Escherichia coli* O111 strain, a highly pathogenic strain associated with severe human infections. Its O-antigen consists of a pentasaccharide repeating unit, with two 3,6-dideoxyhexose (colitose) residues linked to a glucose residue within a trisaccharide backbone. Traditional serotyping methods often fail to distinguish closely related structures, leaving the exact structure of a variant O111 O-antigen unresolved prior to this study. Our preliminary work using the ultra-fast MALDI glycotyping method provided evidence of a possible variant OPS in O111. To advance this work, we optimized the MALDI glycotyping method to analyze *E. coli* O111 O-antigen containing acid-labile groups (i.e., colitose residues) in both commercial LPS and *E. coli* cell culture. We further elucidated the structure of the variant OPS using NMR techniques.

Chapter 1 provides a general background on glycan diversity in bacterial strains and an overview of the common methods used for OPS typing in serogrouping.

In Chapter 2, MALDI-TOF MS analysis revealed that strain JNBP02191 exhibited an OPS repeating unit (RU) mass of 787.32 Da, consistent with the established O111 structure. In contrast, strain JNBP02254 and its corresponding commercial LPS-B variant displayed an altered RU mass of 828.46 Da, attributed to the substitution of a hexose residue with an N-acetylhexosamine (HexNAc) residue. LIFT-TOF/TOF fragmentation analysis confirmed that this modification occurred at the non-reducing end and extended across consecutive RUs, highlighting structural heterogeneity within the O111 serogroup.

In Chapter 3, a detailed NMR analysis was conducted on the variant OPS, termed OPS-B, to further elucidate its structural variations at the atomic level. To achieve this, 1D and 2D NMR techniques, including HSQC, TOCSY, COSY, NOESY, and HMBC, were employed to fully assign the monosaccharide residues, their glycosylation positions, and anomeric configurations. The NMR analysis revealed a pentasaccharide O-antigen substructure that differed from the reported O111 O-antigen by the substitution of the terminal glucose residue with an N-acetylglucosamine residue on the repeating unit backbone. Although overlapping signals and partial colitose cleavage posed challenges, the integration of multiple NMR techniques enabled a comprehensive structural characterization of OPS-B.

In conclusion, this study demonstrates the effectiveness of MALDI-TOF MS glycotyping in rapidly distinguishing OPS variants and underscores the complementary role of NMR in resolving complex polysaccharide structures. The findings provide new insights into antigenic variability within the O111 serogroup and have significant implications for bacterial classification, pathogen detection, and vaccine design. Future research should focus on the immunological impact of these structural differences and further refine analytical methodologies for enhanced OPS characterization.