学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on MALDI Glycotyping: Development of a Structure-Based Typing Method for Bacterial O-Antigens
(MALDI Glycotyping による 0 抗原構造に基づく型分類法の開発に関する研究)

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) is a powerful analytical technique that enables high-sensitivity, high-resolution analysis of biopolymers. In recent years, MALDI-MS has gained attention as a promising tool for microbial identification based on species-specific protein profiles. However, conventional protein-based approaches are insufficient for distinguishing serotypes within a species, which is an essential task in epidemiological surveillance and infection control. Among these serotypes, O-antigens, which are glycan components located at the outermost layer of lipopolysaccharides (LPS) in Gram-negative bacteria, play a pivotal role. For example, *Escherichia coli* possesses approximately 187 known O-antigen types, each differing in monosaccharide composition and glycosidic linkages. Traditional methods for O-antigen typing, such as serological agglutination and PCR, rely on serotype-specific reagents. Therefore, untested strains need to undergo stepwise screening, which is both time-consuming and labor-intensive. This procedural bottleneck can significantly delay epidemiological investigations, particularly when rapid identification of diverse or emerging O-antigen types is required.

To address these limitations, this study focused not on proteins but on the glycan structures of O-antigens as direct targets for identification. Prior to this work, I established a novel MALDI matrix, DAN/DHB/Na, which selectively ionizes glycans and enables direct detection of glycan moieties from both purified and unpurified glycoproteins without complex pretreatment such as desalting or column separation. In this context, the use of MALDI-MS to distinguish glycan structures was termed MALDI glycotyping. Building upon these findings, this thesis aims to establish a novel analytical approach for the direct detection of O-antigen glycan structures. Using glycan structural information, MALDI glycotyping enables rapid, one-step identification of both known and previously uncharacterized O-antigen types without the need for serotype-specific reagents.

In Chapter 2, the development and foundational validation of MALDI glycotyping are presented. A simplified workflow comprising washing, mild acid hydrolysis, and centrifugation was constructed to enable rapid glycan detection. By employing the DAN/DHB/Na matrix, glycan-specific signals were obtained within one hour from bacterial inoculation to analysis. Application of this method to six strains of Gram-negative bacteria, including *E. coli* O157, yielded distinct *m/z* signals corresponding to their known O-antigen structures. The method enabled both subtype differentiation and the structural characterization of previously unreported O-antigens. Furthermore, the technique demonstrated the capacity to simultaneously identify both bacterial species and O-antigens from a single colony and proved effective in mixed culture environments, demonstrating its potential for practical identification applications.

Chapter 3 focuses on improving the accuracy of O-antigen identification by optimizing the matrix composition, using *Yersinia pseudotuberculosis* as a model organism. It was observed that the original DAN/DHB/Na matrix produced heterogeneous adduct ions, which potentially complicated structural

interpretation. To address this issue, a new matrix formulation (DAN/DHB/K) was employed to unify adduct ion formation. This modification led to improved spectral clarity and enhanced structural interpretation accuracy. Application to four *Y. pseudotuberculosis* strains confirmed the presence of O-antigen signals consistent with previously reported structures. Notably, even O-antigens with identical molecular weights were distinguishable based on subtle differences in their detected signal patterns. These findings demonstrated that MALDI glycotyping can distinguish fine structural variations between serotypes and that the method's discriminative capability can be further enhanced by accumulating reference spectra.

Chapter 4 evaluates the broad applicability and clinical potential of MALDI glycotyping. A total of 71 strains of *E. coli* and *Shigella* were analyzed using both positive and negative ion modes. Over 80% of strains yielded identifiable O-antigen profiles. The use of negative ion mode significantly enhanced the detection of acidic O-antigens, which had previously been difficult to analyze. MALDI glycotyping successfully distinguished structurally similar O-antigens with identical molecular weights. Furthermore, in 10 of the 71 strains, results from MALDI glycotyping were inconsistent with the reported structure of the registered O-antigen types. To clarify these discrepancies, conventional serotyping was conducted for all 10 strains. As a result, two of the strains were identified as having new O-antigen subtypes that differed from the reported structures. In four other strains, serotyping revealed different O-antigen types from those originally registered, and the redefined O-antigen structures were consistent with those identified by MALDI glycotyping. However, for the remaining four strains, the O-antigen types could not be determined by serotyping. For these untypeable strains, all previously reported O-antigen structures of *E. coli* and *Shigella* were compiled, and a comparative analysis was performed to examine whether the detected glycan components matched any known structures. Based on this analysis, candidate O-antigen types were successfully narrowed down. Overall, these findings underscore the potential of MALDI glycotyping as a robust platform for high-throughput and structure-based serotyping, with the capability to complement and refine traditional methods.

In conclusion, this dissertation demonstrates that MALDI glycotyping enables rapid, straightforward, and highly accurate identification of bacterial O-antigens based on their glycan structures, with the entire process completed within one hour. The method integrates compositional features and spectral patterns to enable the differentiation of known O-antigen types and the identification of previously uncharacterized structures. Future expansion of glycan spectral databases will further enhance the precision and breadth of this technique. Ultimately, MALDI glycotyping provides a practical, structure-based alternative to conventional serotyping and offers a promising platform for clinical identification, food safety monitoring, and microbial epidemiology.