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Application Note

Life Sciences

O-Antigen Typing of *Escherichia coli* by MALDI-TOF MS Analysis of O-Antigen Polysaccharides

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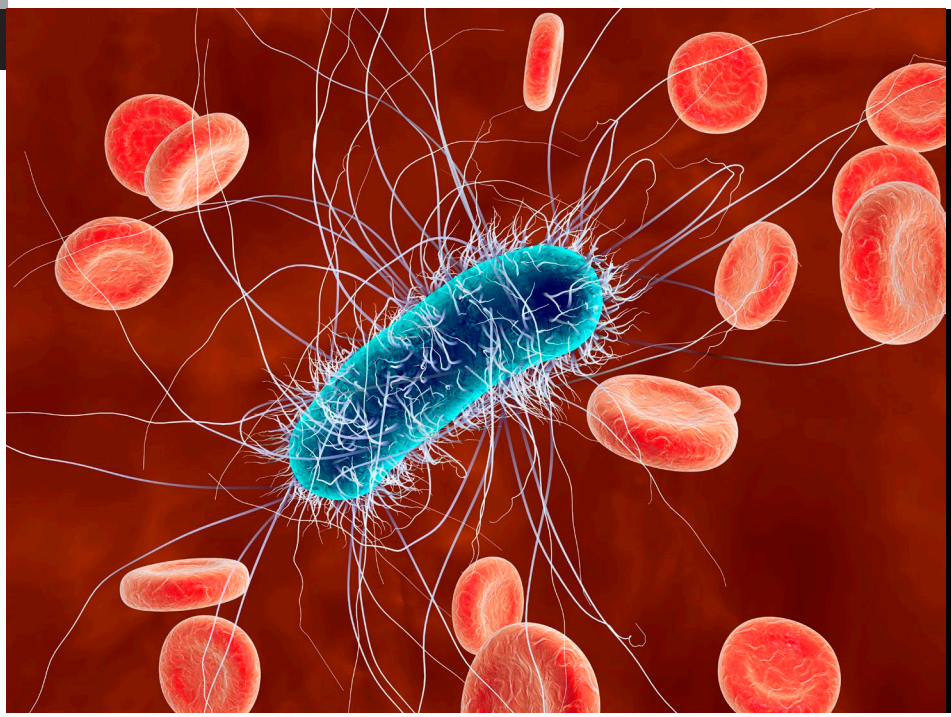

Life Sciences

■ Abstract

Microorganism typing is one of the most fundamental techniques for the diagnosis of infectious diseases. For *Escherichia coli*, the most common method is typing based on the structure of the O-antigen, which is one part of lipopolysaccharides (LPS) located on the surface of cells. That is achieved by an antigen-antibody reaction using an antiserum for the O-antigen (O-serotyping). However, due to the structural diversity in O-antigens of *E. coli*, O-serotyping requires more than 180 types of antiserum and significant labor. This article describes an example of O-antigen typing of *E. coli* strains using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which enables typing with a single reagent and a single assay.

1. Introduction

Serotyping is routinely employed in testing for foodborne illnesses and infectious diseases caused by microorganisms. It targets molecular determinants expressed on the bacterial surface, such as glycans and proteins.

For *E. coli* and *Salmonella*, O-serotyping targets the O-antigen, one structural constituent of cell-surface lipopolysaccharides (LPS). LPSs comprise three domains: lipid A, core oligosaccharide, and O-antigen (Fig. 1).

The O-antigen is a structure in which basic units (repeating units) composed of several monosaccharides are linked in a linear chain (O-antigen polysaccharide). O-serotyping is an immunological method based on using antisera to determine structural differences in O-antigen polysaccharides.

Enterohemorrhagic *E. coli* (EHEC) are pathogenic *E. coli* strains characterized by production of Shiga-toxin. EHEC cause severe abdominal pain and bloody diarrhea with a small number of bacteria. Among the O-serogroups of EHEC, O157 is the most frequently detected, followed by O26 and O103 (Table 1). However, typing the more than 180 O-serogroups of *E. coli* requires costly antiserum reagents and a significant amount of labor.

The O-antigen polysaccharide can be regarded as a polymer because it is composed of repeating units. Polymers are one of the substances that are easily observed using MALDI-TOF MS. When the LPSs extracted from *E. coli* were analyzed, a mass spectrum was obtained in which the peak intervals corresponded to the size of the repeating units. Based on those findings, it was considered that the O-antigen type could be identified by observing the structural differences in the O-antigen polysaccharides based on mass spectral patterns (PAT. JP7365007). This study attempted, we try to identify the O-antigen types from mass spectra of O-antigen polysaccharide using bacterial isolates, including human-derived EHEC bacteria.

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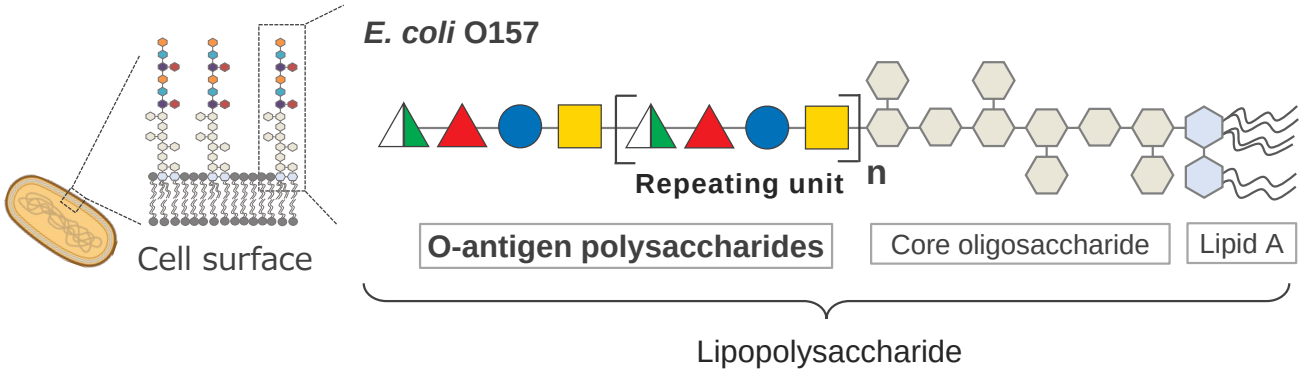

Fig. 1 Lipopolysaccharide and O-Antigen Polysaccharide
The O-antigen polysaccharide consists of several or up to dozens of repeating units composed of several monosaccharides.

2. Experiments

2 – 1 Materials

Strains of the major EHEC O-serogroups, namely O157, O26, and O103, were analyzed, as shown in Table 1. The analysis included type strains distributed by the Research Institute for Microbial Diseases (RIMD), Osaka University, as well as human-derived EHEC isolates maintained by the Osaka Institute of Public Health (OIPH) (Table 2).

2 – 2 Preparation

The preparation of O-antigen polysaccharide samples from bacterial cells was performed as previously described[2]. Colonies grown on Luria-Bertani agar media were suspended in purified water. That sample was heated at 90 °C for 10 minutes. After centrifugation, the supernatant was removed, and hydrochloric acid (100 mM final concentration) and sodium chloride (25 mM final concentration) were added to the pellet. After heating at 90 °C for 10 minutes, the supernatant obtained by centrifugation was used as the O-antigen polysaccharide sample solution. The matrix solution used was 2,5-dihydroxybenzoic acid (1.25 mg/mL DHB) dissolved in 50 % acetonitrile. 1.0 µL of the O-antigen polysaccharide sample solution was dropped onto a MALDI sample plate to dry, and 1.0 µL of the DHB matrix solution was spotted onto the plate to dry.

2 – 3 MALDI-TOF MS

O-antigen polysaccharides were analyzed using a benchtop MALDI-TOF MS (MALDI-8020, Fig. 2) system under the parameters listed in Table 3.

Table 1 O-Serogroup Prevalence of EHEC Strains in Japan

O-Serogroup	Number of Strains	Prevalence (%)
O157	147	83.5
O26	6	3.4
O103	4	2.3
O71	4	2.3
Other (9 types)	13	7.4
Untypable	2	1.1
Total	176	100.0

The O-serogroup prevalence of EHEC strains isolated in Osaka, Japan, between January 2023 to December 2023 [1].

Table 2 Experimental Strain

O-Serogroup	Source	Collection Num.
O157	Type strain	RIMD 0509516
	Human-derived	2023H092
	Human-derived	2023H093
O26	Type strain	RIMD 0509624
	Human-derived	2023H059
	Human-derived	2023H132
O103	Type strain	RIMD 0509463
	Human-derived	2023H041
	Human-derived	2023H178

Fig. 2 MALDI-8020 Benchtop Linear MALDI-TOF MS

Table 3 Analytical Parameters of MALDI-8020

MS Analysis Conditions	
Ion type	Linear positive
m/z measurement range	500 - 10000
Matrix	DHB
Pulsed extraction	PE 2,300 Da
Laser Irradiation Conditions	
Irradiation count	5 shots × 256 profiles
Repetition frequency	200 Hz
Laser intensity	85
Fixed operation	Raster selected spots Width 1000 µm / Height 1000 µm Spacing between points 50 µm

3. Results and Discussion

3 – 1 Structure and size of O-antigen polysaccharides

O-antigen polysaccharides consist of a few or up to dozens of linear repeating units composed of several monosaccharides[3]. Fig. 3 illustrates the structures of repeating units in *E. coli* O157, O26, and O103. For instance, the repeating unit of the O157 is composed of four monosaccharides: *N*-acetylrhamnosamine (RhaNAc), fucose (Fuc), glucose (Glc), and *N*-acetylgalactosamine (GalNAc).

The molecular weight of a repeating unit is determined by subtracting the molecular weight of the H_2O lost during glycosidic bonding from the total molecular weight of its constituent monosaccharides. Accordingly, the molecular weight of the repeating unit in O157 is approximately 699 Da. Similarly, the molecular weights of the repeating units in O26 and O103 were calculated to be approximately 537 Da and 1003 Da, respectively.

3 – 2 Mass spectra of O-antigen polysaccharides

Fig. 4 shows the mass spectrum of the O-antigen polysaccharide sample prepared from the type strain of *E. coli* O157.

In the mass spectrum, periodic peaks (*m/z* 1438.9, *m/z* 2137.5, *m/z* 2836.0, and *m/z* 3534.6) at intervals of approximately 699, corresponding to the molecular weight of the repeating unit, were observed. As shown in Fig. 5 (A), similar periodic peaks with intervals of approximately 699 were also observed in the mass spectrum of human-derived EHEC O157 strains, as consistent with the results of the type strain.

E. coli O26 and O103 were also examined to verify whether this method can be applied to O- serogroups other than *E. coli* O157. Figs. 5 (B) and (C) represent the mass spectra of O-antigen polysaccharide samples prepared from the type strain and human-derived EHEC strains of O26 and O103, respectively. Like *E. coli* O157, periodic peaks with intervals of approximately 537 were observed for O26, and periodic peaks at intervals of approximately 1003 were observed for O103 in both the type strains and human-derived EHEC strains.

For *E. coli* O157, O26, and O103, the O-antigen typing was achieved by comparing the calculated molecular weight of the repeating unit in the O-antigen polysaccharide with the periodic peak intervals observed in the mass spectrum.

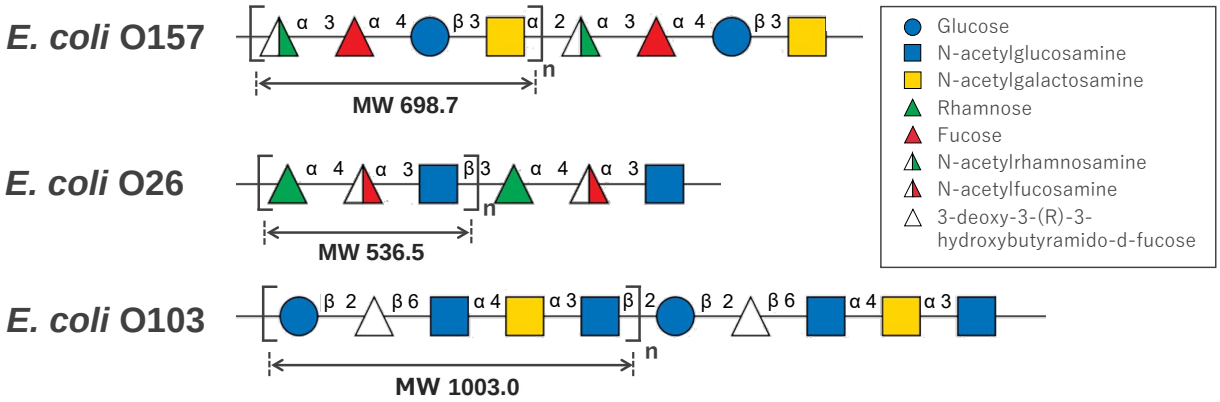

Fig. 3 Structure of O-Antigen Polysaccharides and Molecular Weights (MW) of Repeating Units

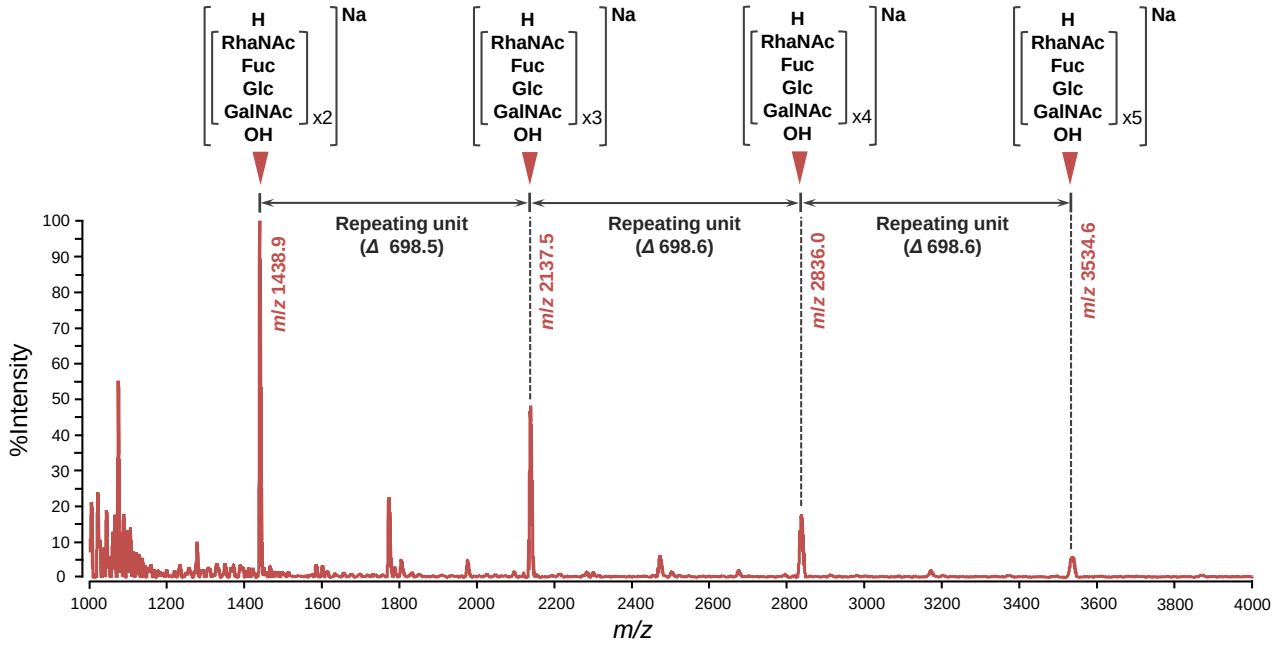

Fig. 4 Mass Spectrum of O-Antigen Polysaccharide Sample Prepared from *E. coli* O157

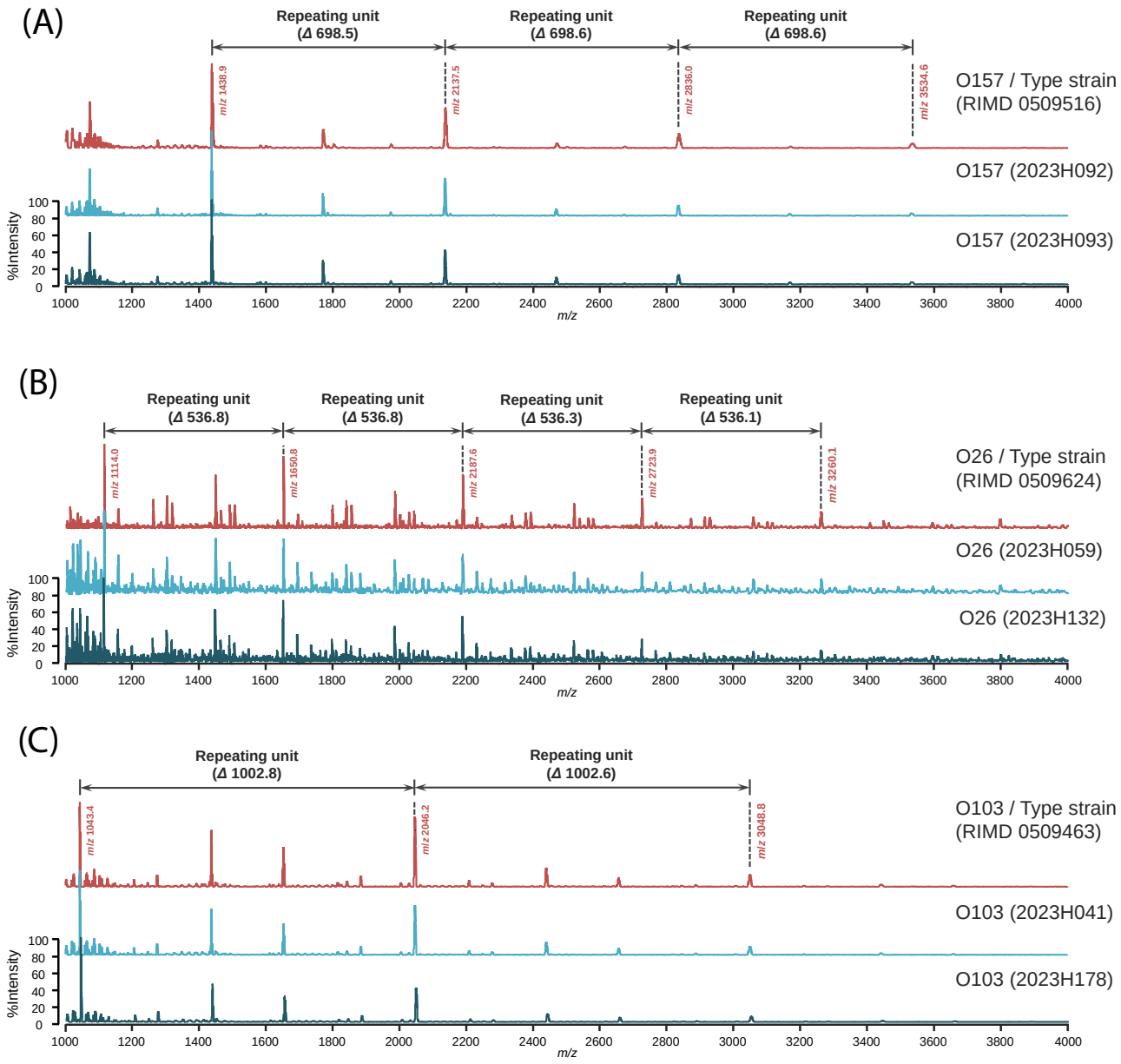

Fig. 5 Mass Spectra of O-Antigen Polysaccharide Samples Prepared from *E. coli* (Type Strains/EHEC Human Strains)

(A) *E. coli* O157, (B) *E. coli* O26, (C) *E. coli* O103

4. Conclusion

This Application Note article demonstrated that the structural differences of O-antigen polysaccharides could be observed as mass spectral patterns from *E. coli* strains in a single assay using a single reagent. This O-antigen typing method using MALDI-TOF MS was applicable not only for type strains but also for EHEC strains (O157, O26, and O103) isolated from humans. This method is an effective tool for determining O-antigen types. In the future, the possibility of typing other O-serogroups will also be investigated.

<References>

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