SHIMADZU

Application News

No.B49

MALDI-TOF Mass Spectrometry

Application of MALDI-TOF MS for Discrimination of Species Possessing Highly Conserved Ribosomal RNA Gene Sequences

A microorganism identification system which uses a matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF MS), unlike any of the conventional methods, is attracting attention recently as it offers a fast, simple bacterial identification method. The conventional 16S rRNA gene sequence analysis method is widely used for quick identification of bacteria, but due to the high homology of 16S rRNA gene sequences depending on the bacterial strain, there are cases where identification may be difficult.
Here, using the AXIMA Microorganism Identification System, consisting of the AXIMA MALDI-TOF MS developed by Shimadzu in conjunction with our database search software for microorganism identification, we introduce the results of our investigation of the identification capability of the MALDI-TOF MS in discriminating among strains of *Escherichia*, in which the homology rate of the 16S rRNA gene sequence is greater than 95 %[1].

Note: For Research Use Only. Not for use in diagnostic procedures.

Fig. 1 shows the microorganism identification working flow using the AXIMA Microorganism Identification System.

1) The microorganism sample is harvested. Analysis can be conducted on a very small amount of sample, consisting of as few as 10^5 cells/sample well.
2) The harvested bacteria cells are applied to a MALDI-TOF MS sample plate well. The applied bacteria cells are mixed with a MALDI ionization assist agent referred to as a matrix, and the sample preparation is completed when the sample has dried.
3) Identification and classification of the types of microorganisms in the sample are conducted by analyzing the prepared bacterial sample using the AXIMA microorganism identification system which incorporates a MALDI-TOF MS. The identification is accomplished through comparison of the mass spectral data for each type of microorganism in the system database with the MALDI-TOF MS measurement data obtained from the microorganism sample.

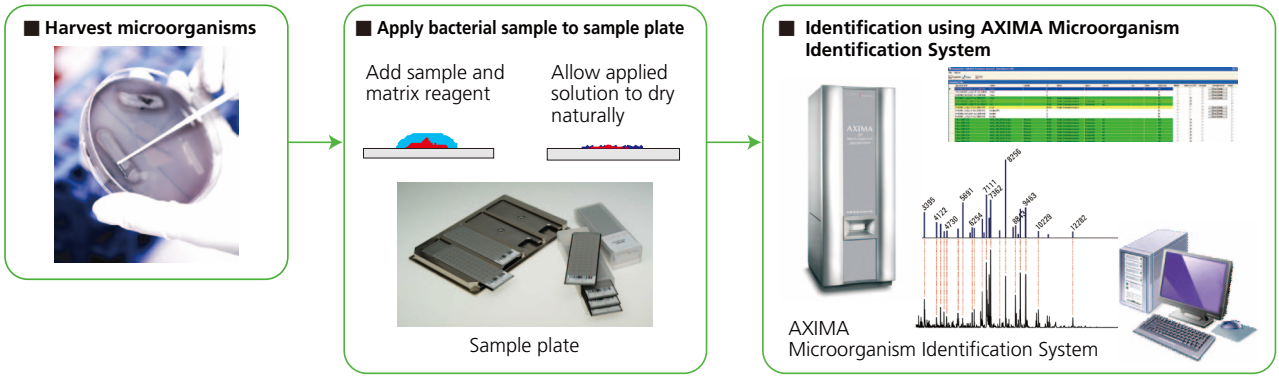

Fig. 1 Schematic Overview of Analysis of MALDI-TOF Mass Spectra of Intact Cells of Microorganisms Using AXIMA Microorganism Identification System

Table 1 Agreement Rate of 16S rRNA Gene Sequences (From Muroi et al. (2011) with permission from Pharmaceutical Society of Japan)

Species	Strains	No.	1	2	3	4	5	6	7	8	9	10	11
E. coli	NBRC 12734	1											
	NBRC 3972	2	99.1										
	NBRC 12713	3	99.7	99.3									
	NBRC 13168	4	99.4	99.5	99.3								
	NBRC 13891	5	99.9	99.3	99.8	99.5							
	NBRC 13893	6	99.7	99.3	100.0	99.3	99.8						
	NBRC 3301	7	99.7	99.0	99.7	99.2	99.5	99.7					
	NBRC 102203	8	99.5	99.0	99.5	99.2	99.7	99.5	99.3				
	NBRC 14237	9	99.7	99.2	99.7	99.3	99.8	99.7	99.4	99.6			
E. fergusonii	NBRC 102419	10	99.6	98.9	99.5	99.3	99.6	99.5	99.7	99.3	99.5		
E. hermanii	NBRC 105704	11	97.4	97.4	97.3	97.5	97.5	97.3	97.1	97.2	97.4	97.1	
E. blattae	NBRC 105725	12	95.9	96.0	96.0	96.0	96.0	96.0	95.8	95.8	95.9	96.0	95.8

The bacterial strains that were investigated are listed in Table 1. The 16S rRNA gene sequence homology rate of the nine *E.coli* strains is 99 – 100 %. The homologies between *E. coli* and *E. fergusonii* is 98.9 – 99.7 %, and between *E. blattae* and *E. hermanii* and other bacterial strains 95.8 – 97.5 %, and even with differences at the species level, high homology rates are seen. It can thus be expected that discrimination using 16S rRNA gene sequence analysis would be difficult.

In this investigation, we conducted cluster analysis of the indices of the peak matching rate between MALDI-TOF MS measurement data obtained from each of the microorganism samples, and attempted to identify strains with high 16S rRNA gene sequence homology. The results of the cluster analysis conducted using the AXIMA Microorganism Identification System software functions are shown in Fig. 2. Aside from *E. blattae* and *E. hermanii*, even *E. fergusonii*, with its extremely high 16S rRNA gene sequence homology with *E. coli*, forms clusters different from *E. coli*, indicating that distinguishing between these bacterial species is possible using MALDI-TOF MS.

The above results show that discrimination among types of bacteria is possible by MALDI-TOF MS even when it is difficult by 16S rRNA gene sequence analysis. The technique of identifying and classifying microorganisms by MALDI-TOF MS can be expected to become a powerful supporting tool in future situations where conventional techniques are inadequate.

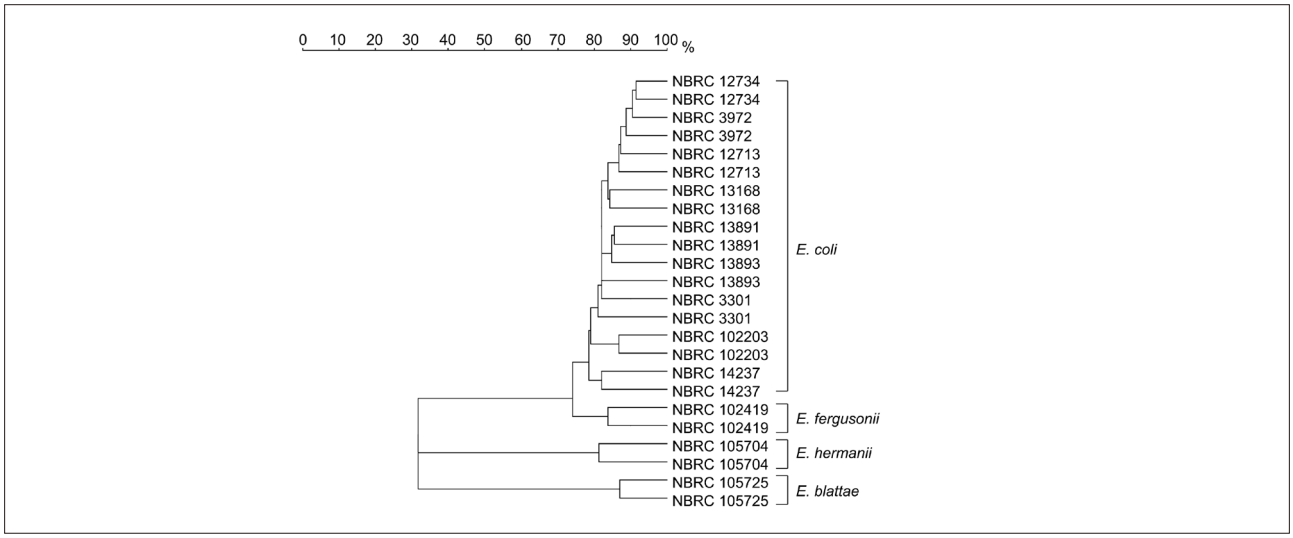

Fig. 2 A Dendrogram of MALDI Profiles Generated using a Single Link Agglomerative Clustering Algorithm. All strains were analyzed in duplicate. From Muroi et al. (2011) with permission from the Pharmaceutical Society of Japan.

[Reference]
1) Masashi Muroi, Keisuke Shima, Yasuyoshi Nakagawa, and Ken-ichi Tanamoto, Biol. Pharm. Bull. 2011, 34(3), 430-432
2) Acknowledgments
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