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Application News

Statistical Analysis Software eMSTAT Solution™
Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer
MALDI-8020/MALDI-8030

Analysis of Compositional Protein Behavior by Bacterial Culture Time

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User Benefits

- Multivariate analysis tools in eMSTAT Solution can reveal differences that cannot be determined by visual appearance or bacterial counts.
- The annotation function can be used to estimate the composition proteins contained in a sample.
- MALDI-8020/30 is fast and can analyze large amounts of samples in a short time.

■ Introduction

Differences in culture conditions for microorganisms, such as medium, temperature, and time, can change what is detected when microorganisms are analyzed.

This is because environmental stress and the cultivation phase cause changes in the products in the organism. Therefore, when analyzing and identifying microorganisms, it is necessary to optimize the culture conditions and pretreatment methods under which the target substance is produced using standard microorganisms before culturing and analyzing unknown microorganisms. These conditions require consideration of multiple factors, and the number of samples to be analyzed is often large.

In this application news, as an example of the examination of the conditions for the bacterial body analysis, Escherichia coli strain DH5α, which is used as a calibration sample for the identification of microorganisms, was cultured, and changes in the composition proteins detected over time were analyzed using MALDI-8030 and eMSTAT Solution.

■ Bacterial culture and pretreatment conditions

Commercial liquid LB medium was used as the culture medium. 7 culture solutions were prepared by shaking at 37° C and incubating for 4, 8, 16, 24, 32, 52 and 56 hours. The culture was centrifuged at 3,200 *g* for 5 min, and the supernatant was decanted and resuspended with distilled water. The suspension was centrifuged at 3,200 *g* for 5 min and the supernatant was decanted and resuspended with distilled water. The OD600 value of the suspension was measured with a spectrophotometer to calculate the number of bacteria. (Calculated as 1×108 cfu/mL for OD600=1.) The suspension was then diluted with α-cyano-4 hydroxycinnamic acid (CHCA) solution so that the number of organisms when applied to the sample plate of MALDI-8030 (Fig. 1) was 10^5. 1 µL of each diluted solution was spotted on a sample plate, dried, and analyzed by MALDI-8030. The analysis conditions are shown in Table 1. The pretreatment and analysis time after the bacterial count calculation was about 1 hour.

Table 1 Analysis Conditions of MALDI-8030

Mass Range	: *m/z* 2,000-20,000
Polarity	: Positive
Matrix	: 10 mg/mL CHCA
Pulsed Extraction	: *m/z* 12,000

■ Growth of DH5α

The growth curve of cultured DH5α is shown in Fig. 2. At the time of 4 hours, the plant entered the logarithmic growth phase, and at the time of 8 hours, the plant entered the stationary phase, in which the balance between the number of bacteria increasing and the number of bacteria dying was maintained.

Fig. 1 MALDI-8030

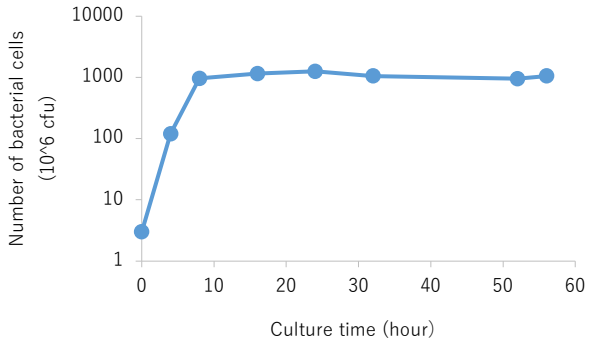


Fig. 2 The growth curve of cultured DH5α

■ Multivariate analysis with eMSTAT Solution

A total of 42 data were obtained, 6 data for each culture condition. Fig. 3 shows the results of multivariate analysis using eMSTAT Solution. The Score Plot compares each mass spectra and plots it based on feature similarity. The Loading Plot shows the *m/z* points that contribute to the separation of each piece of data. The growth curve shown in Fig. 2 was divided into two groups, the logarithmic growth phase and the stationary phase, but the multivariate analysis revealed three main groups. The Loading Plot shows that there are several characteristic peaks in the direction in which each group is plotted.

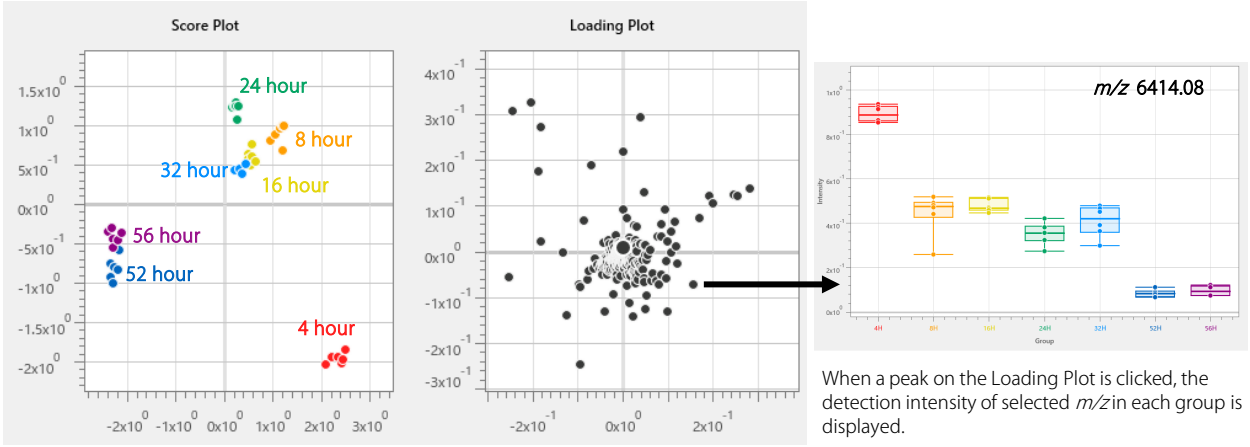
■ Protein estimation by annotation

These characteristic peaks were annotated using gene information to estimate proteins. The gene information of the DH5α strain was obtained from the National Center for Biotechnology Information (NCBI) database and translated into protein primary sequence information to calculate the theoretical molecular weight of the proteins that could be detected, and for some proteins, the mass values after the post-translational modification were used based on references. Fig. 4 shows the Peak Matrix that incorporates this information into the eMSTAT Solution. When checked against the Loading Plot, the protein was deduced for the seven peaks indicated by the arrows. HdeB is annotated in duplicate with S16, but the mass values of *m/z* 9068 for HdeB and *m/z* 9060.36 for S16 suggest that it is a close numerical HdeB. For these peaks, the frequency of detection in each group showed that other than HdeA/HdeB, five proteins were detected in all groups in ribosomal proteins.

Since these proteins are thought to be produced from the beginning of the culture, it is assumed that the differences due to these proteins are not caused by the presence or absence of peaks, but by differences in the detection intensity. On the other hand, HdeA/HdeB were detected 8 hours after the growth became constant. HdeA/HdeB are chaperones that are activated under stress conditions after saturation of culture.

■ Conclusion

The eMSTAT Solution was used to analyze the changes in the composition proteins of the bacterial cells over time. Even in a sample with many peaks, such as a fungus, we were able to take advantage of the gene information and annotation functions to consider the differences in features between groups based on the inferred protein information.



When a peak on the Loading Plot is clicked, the detection intensity of selected *m/z* in each group is displayed.

Fig. 3 Results of Multivariate Analysis with eMSTAT Solution

m/z	Annotation	ANOVA	4H(6)	8H(6)	16H(6)	24H(6)	32H(6)	52H(6)	56H(6)
9743.1615	HdeA	1.0728E-34	0	6	6	6	6	6	6
4366.4949	50S_L36	2.149E-22	6	6	6	6	6	6	6
5464.8250	50S_L36	1	0	1	0	0	0	0	0
7155.4187	50S_L35	1	1	0	3	1	2	4	4
5383.3315	50S_L34	1.7554E-33	6	6	6	6	6	6	6
6240.7242	50S_L33	0.52413	5	2	3	1	2	0	0
6318.5068	50S_L32	2.192E-33	6	6	6	6	6	5	4
7874.4455	50S_L31	3.3942E-17	6	6	6	6	6	5	4
6414.0821	50S_L30	1.044E-20	6	6	6	6	6	6	4
7276.6734	50S_L29	0.015685	6	6	6	6	6	6	6
8878.4124	50S_L28	8.8827E-22	6	6	6	6	6	1	3
8996.4393	50S_L27	3.9232E-23	6	6	6	6	6	1	0
10701.273	50S_L25	0.73419	3	0	0	0	0	0	0
11202.779	50S_L23	1	3	0	0	0	0	0	0
9556.3687	30S_S5_partial	1.1443E-14	6	6	0	1	0	0	0
8372.1134	30S_S21	6.4416E-20	6	6	6	6	6	0	0
9547.3077	30S_S20	1	0	0	1	1	0	0	0
10303.026	30S_S19	2.5899E-06	6	4	5	1	3	0	0
9067.5841	30S_S16,HdeB	1.6776E-28	0	6	6	6	6	6	6
10141.574	30S_S15	1	4	0	3	1	3	0	0

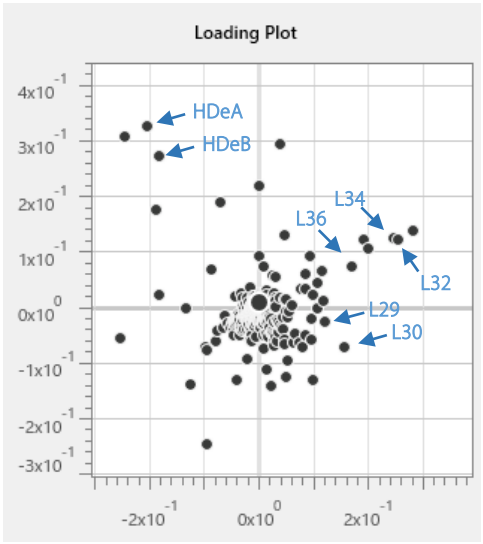


Fig. 4 Annotation Results Left: Peak Matrix, Right: Loading Plot

<References>

Michelle Q. Carter, Jacqueline W. Louie, Clifton K. Fagerquist, Omar Sultan, William G. Miller, Robert E. Mandrell Evolutionary Silence of the Acid Chaperone Protein HdeB in Enterohemorrhagic Escherichia coli O157:H7 Applied and Environmental Microbiology p. 1004–1014

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