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Application News

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Liquid Chromatograph Mass Spectrometry

A Multiomics Approach Using Metabolomics and Lipidomics

Microorganisms including *Escherichia coli* and yeast are used to mass produce useful substances in a variety of industrial sectors such as food, biotechnology, and energy. In the food sector, for example, fermentation processes that utilize microorganisms are widely used for alcoholic beverages and fermented foods, and microbial breeding is performed for the purpose of more efficient fermentative production and the production of high value-added metabolites. In order to improve the production efficiency of useful substances and increase the production capacity of such high value-added compounds, it is necessary to monitor metabolic changes using metabolomics. Since monitoring of metabolic changes not only requires an understanding of the target substance but also an understanding of the metabolic changes of the precursor and intermediates, the metabolomics approach is expected to be very effective because of the ability to simultaneously analyze a large number of compounds. This research attempts to understand metabolic changes from a multiomics approach by evaluating metabolic changes using metabolomics and combining the lipidomics results of phospholipids. The sample used was *E. coli*, which efficiently produces the sulfur-containing metabolite ergothioneine. Thiosulfate or sulfate was added as a sulfur source for the synthesis of cysteine which acts as the substrate for ergothioneine. By applying the approaches of metabolomics and lipidomics, this article introduces an example of evaluating how related sulfur-containing metabolites change depending on cultivation progression.

LC/MS Analysis of *E. coli* Extracts

Minimal culture media with either 50 mM of thiosulfate or 100 mM of sulfate added as the sulfur source were used to cultivate *E. coli* in a jar fermenter. In order to evaluate metabolic changes depending on the progression of cultivation, some bacteria was collected from each culture suspension after 0, 24, 48, 72, 96, 120, 168, and 216 hours. After measuring the OD value of the collected *E. coli*, adjustment was performed to obtain OD = 2 at 1 mL, the culture medium was removed, and then the bacteria was rinsed with ultrapure water. Next, the Bligh-Dyer method was used to extract hydrophilic metabolites and phospholipids from the bacteria. After collecting the aqueous layer and chloroform layer and exsiccating using a centrifugal concentrator, the result was dissolved as required with ultrapure water and methanol for undergoing simultaneous analysis using the LCMS-8060. For metabolite analysis, simultaneous analysis was performed using the PFPP column method in version 2 of the LC/MS/MS method package for primary metabolites. For phospholipid analysis, simultaneous analysis was performed using the analysis conditions of the LC/MS/MS MRM library for phospholipid profiling. Table 1 lists the analysis conditions for each analysis. Fig. 1 shows example MRM chromatograms of primary metabolites and of phospholipids obtained by analyzing the *E. coli* extract cultivated using the culture medium with thiosulfate added (each chromatogram depicts the 0 hour mark and 72 hour mark).

Table 1 Analysis Conditions of Metabolomics and Lipidomics

Primary Metabolite Analysis (LC Analysis Conditions)	
Column	: PFPP column (2.1 × 150 mm, 3 µm)
Mobile phase A	: 0.1 % formic acid - Water
Mobile phase B	: 0.1 % formic acid - Acetonitrile
Flow rate	: 0.25 mL/min
Time program	: Linear gradient
Column oven temperature	: 40 °C
Injection volume	: 3 µL

Phospholipid Analysis (LC Analysis Conditions)	
Column	: C8 column (2.1 × 150 mm, 2.6 µm)
Mobile phase A	: 20 mM ammoniumformate - Water
Mobile phase B	: 50 % Acetonitrile/50 % 2-Propanol
Flow rate	: 0.3 mL/min
Time program (B conc.)	: Curved gradient
Column oven temperature	: 45 °C
Injection volume	: 3 µL

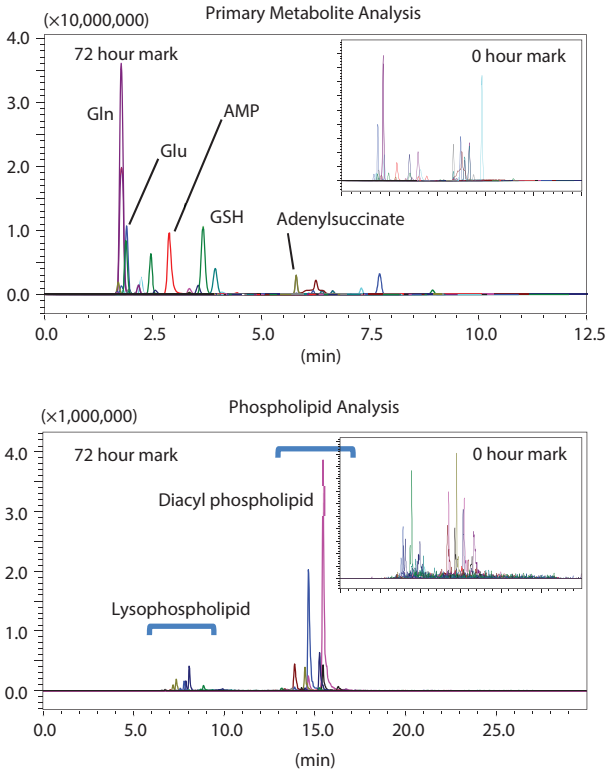

Fig. 1 MRM Chromatograms of *E. coli* Extracts Cultivated in Culture Medium with Thiosulfate Added (Primary Metabolites and Phospholipids)

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Evaluation of Metabolic Changes Using Multiomics

In this analysis we confirmed the existence of 49 primary metabolite components and 56 phospholipid components. We can also see from the MRM chromatograms in Fig. 1 that the metabolites and phospholipids in the bacteria change significantly depending on the culture time. Fig. 2 shows the results of comparing the area ratios of metabolites associated with sulfur-containing metabolites, such as cysteine which acts as the substrate for ergothioneine, for each culture with either thiosulfate or sulfate added and for each culture time (vertical axis represents area ratio and horizontal axis represents time). Fig. 2 also shows the changes over time of phospholipids including phosphatidylethanolamine and phosphatidylserine (vertical axis: area, horizontal axis: time). Changes in metabolites during cultivation due to differences in the sulfur source were verified on the metabolic pathway associated with cysteine synthesis in Fig. 2. In particular, during cultivation that occurs after the transition from the log phase to the stationary phase (after the 72 hour mark), we see an increase in compounds associated with cysteine synthesis in the culture medium with thiosulfate added. Likewise looking at changes in phospholipids, we see a large increase in phospholipids such as PE and PS in the log phase of the culture medium with sulfate added. Since PE in *E. coli* is biosynthesized with PS as the substrate, this suggests that PE, which is a major component of cell membranes, is biosynthesized preferentially (because the increase in PE occurs earlier than PS in the culture time). Moreover, PS that has the structure of serine, which is positioned upstream of the cysteine synthesis pathway, may affect the cysteine synthesis pathway in the case of insufficient serine, which serves as the substrate. The metabolomics approach therefore allows us to see that differences in the sulfur source added to the culture medium affect the production capacity of sulfur-containing metabolites, such as cysteine. Furthermore, overlaying lipidomics data facilitates a much deeper understanding of the associated metabolic changes. While this report discussed metabolic changes that occur over the course of *E. coli* cultivation and focused on the sulfur-containing metabolites related to cysteine production, more detailed evaluation of metabolic changes is possible by combining metabolomic analysis and lipidomic analysis using a triple quadrupole mass spectrometer.

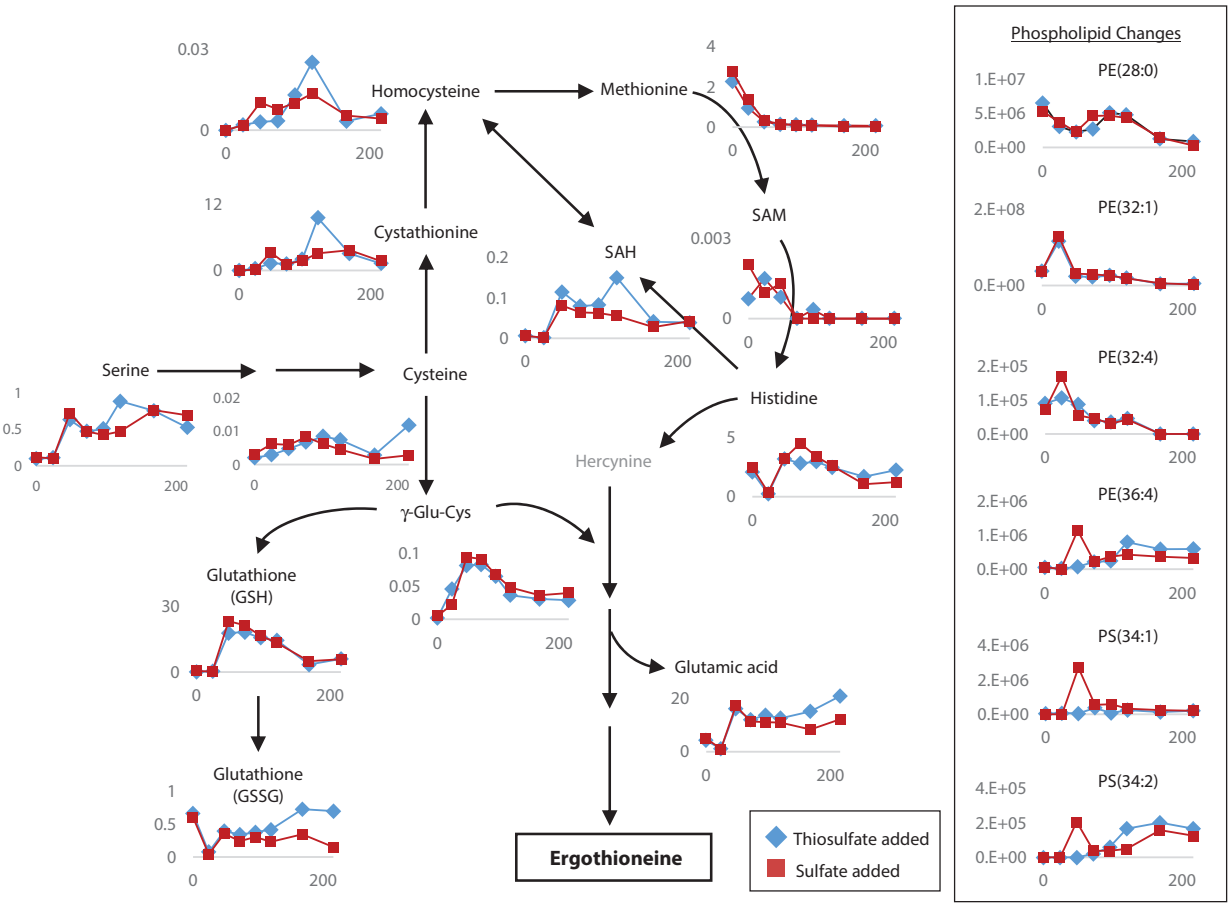

Fig. 2 Changes in Sulfur-Containing Metabolites and Phospholipids in *E. coli* Cultivated in Culture Media with Thiosulfate and Sulfate Added

* The *E. coli* sample was provided by Iwao Ohtsu and Yusuke Kawano from the Headquarters for International Industry-University Collaboration at the University of Tsukuba.
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