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Application News

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Liquid Chromatography Mass Spectrometry

Application of Metabolomics to Microbial Breeding

Microorganisms have been used for many years to produce useful materials in a wide range of industrial fields, including food, biotechnology, and energy. In the food sector, microorganisms are used to produce foods by the fermentation process, such as alcoholic beverages and fermented foods, and in the biotechnology sector microorganisms are used for the large scale production of amino acids and antibiotics. In the energy sector, it is anticipated that microorganism will be used for biofuel production, though lowering the cost presents an outstanding problem. Although microorganisms are already used in the production of a variety of useful materials, genetic modification and breeding is still performed with the aim of improving production efficiency. Metabolomics presents useful tools for the evaluation of metabolic changes during microorganism breeding, and for understanding metabolic changes related to a target material and its precursors and intermediates. By deepening our understanding of the metabolic pathways involved in material production, metabolomics is expected to result in more efficient materials production. In this article, we discuss an example of LC/MS analysis of how the sulfur-containing metabolites vary during culture of a cysteine-producing *Escherichia coli* (*E. coli*) when either thiosulfuric acid or sulfuric acid is added as the sulphur source during cysteine synthesis.

LC/MS Analysis of *Escherichia coli* Extract

We cultured *E. coli* in minimal media to which thiosulfuric acid or sulfuric acid was added as a sulfur source. To evaluate metabolic changes during culture, some *E. coli* were collected from the culture suspension were collected at 3, 4, 5, 6, 7, 8, and 9 hours of culture. The optical density (OD) of the collected *E. coli* was measured before the media components and *E. coli* were quickly separated by filtration. *E. coli* extract was then prepared by breaking down the isolated *E. coli* in methanol. After removing methanol by centrifugal concentration, the extract was adjusted to an appropriate dilution with ultrapure water and used for LC/MS analysis. Metabolites were analyzed simultaneously using the analytical conditions of an ion pairing method (LCMS-8040) and non-ion pairing method (LCMS-8050) obtained from an LCMS method package [primary metabolites]. Table 1 shows the analytical conditions of each method. Fig. 1 also shows an MRM chromatogram (both at 6 hours into culture) for each analytical method obtained by analysis of *E. coli* extract after culture in thiosulfate-containing medium. The main peaks detected when the ion pairing method was used were amino acids, coenzymes, and nucleic acid-related compounds, and the main peaks detected when the non-ion pairing method was used were amino acids, organic acids, and nucleic acid-related compounds.

Table 1 Analytical Conditions for Ion Pairing Method and Non-Ion Pairing Method

Ion Pairing Method (LC Analytical Conditions)	
Column	: RP column
Mobile Phase A	: 15 mmol/L Acetate, 10 mmol/L Tributylamine - Water
Mobile Phase B	: Methanol
Flowrate	: 0.3 mL/min
Mode	: Gradient elution

Non-Ion Pairing Method (LC Analytical Conditions)	
Column	: RP column
Mobile Phase A	: 0.1 % Formic acid - Water
Mobile Phase B	: 0.1 % Formic acid - Acetonitrile
Flowrate	: 0.25 mL/min
Mode	: Gradient elution

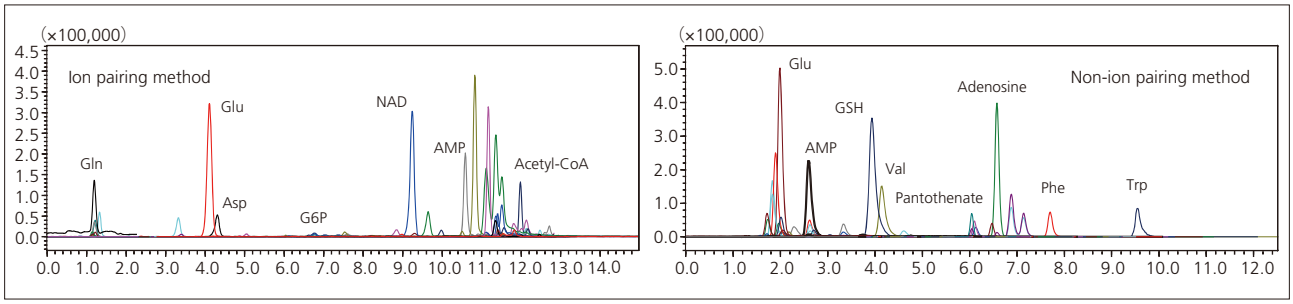
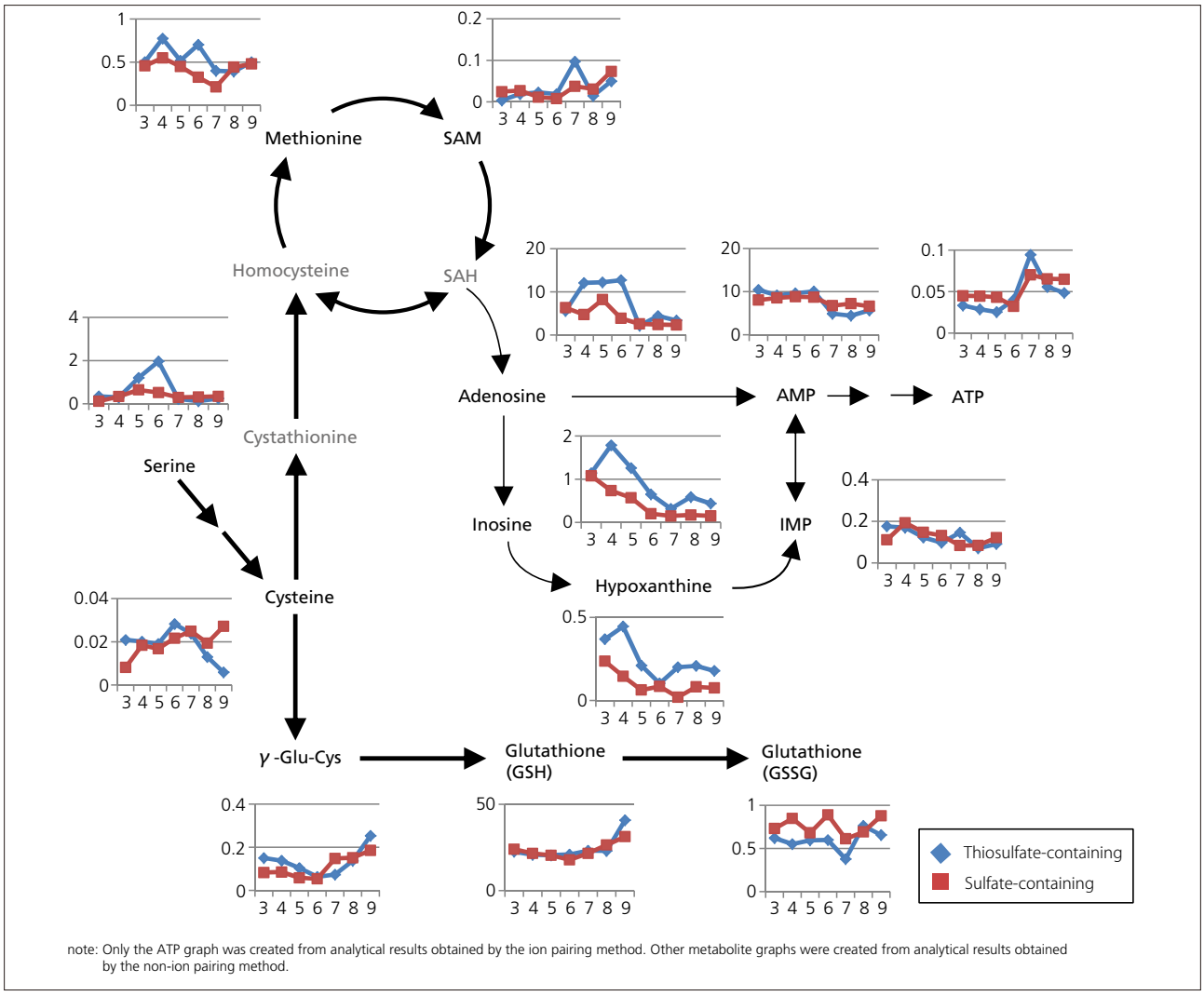


Fig. 1 MRM Chromatograms of *Escherichia coli* Extract After Culture in Thiosulfate-Containing Medium

Change in Sulfur-Containing Metabolites over Time

Based on the results we obtained, the area ratio of metabolites that are related to sulfur-containing metabolites, such as cysteine, over the course of culture in thiosulfate-containing or sulfate-containing medium are compared in Fig. 2 (vertical axis: area ratio, horizontal axis: time). Fig. 2 shows there were changes in metabolites over culture time caused by using different sulfur sources. At around six hours into culture, when glucose became exhausted, in the thiosulfate-containing medium we observed a fall in cysteine (seven hours onwards) and an increase in serine (at six hours), which is upstream in the metabolic pathway. In the thiosulfate-containing medium we also confirmed an increase in nucleosides (adenosine and inosine). This shows how metabolomics can be used to understand how adding different sulfur sources to media affects the productivity of sulfur-containing metabolites such as cysteine.

In this article we looked at how metabolites change during the course of *E. coli* culture, focusing on sulfur-containing metabolites that are linked to cysteine production. The results also show how the primary metabolites method package and a triple quadrupole mass spectrometer can be combined to evaluate changes in various other metabolites that are important to living organisms, such as amino acids, organic acids, and nucleic acid-related compounds.



note: Only the ATP graph was created from analytical results obtained by the ion pairing method. Other metabolite graphs were created from analytical results obtained by the non-ion pairing method.

Fig. 2 Changes in Sulfur-Containing Metabolites in *Escherichia coli* Cultured in a Thiosulfate- or Sulfate-Containing Medium

E. coli samples were provided by Iwao Ohtsu and Yusuke Kawano of the Integrated System Biology Course, Department of Biological Sciences, Graduate School of Biological Sciences, Nara Institute of Science and Technology.

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