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Application News

GCMS GCMS-TQ™8040 NX

Facilitation of Bio-manufacturing Research Through Metabolic Pathway Analysis Simulation

Yuki Nakagawa[1], Shuya Arakawa[2], Ryoichi Sasano[2], Shigetoshi Yoshikawa[3], Kazuki Tomita[4], Kenya Tanaka[4,5], Tomohisa Hasunuma[4, 5]
1 Shimadzu Corporation 2 AiSTI SCIENCE Co., Ltd. 3 Infocom Corporation
4 Graduate School of Science, Technology and Innovation, Kobe University
5 Engineering Biology Research Center, Kobe University

User Benefits

- By integrating the score space search function of SIMCA 18 with the metabolic pathway analysis function of the multi-omics analysis package, it is possible to simulate metabolic pathways and design experiments.
- By using multi-omics analysis packages, not only primary metabolite measurement data, but also cell and culture media data can be read to detect measurement items (digital biomarkers) with many features.

■ Introduction

Bio-manufacturing refers to the production of useful products using biological processes. This technology is at the heart of sustainable product development because it has less environmental impact than conventional chemical synthesis, such as reaction processes using fossil fuels at high temperatures and pressures. Metabolic pathway analysis is essential in bio-manufacturing because it allows us to understand how organisms take in nutrients and produce energy. For example, it is important to understand their metabolic processes when using microorganisms to produce ethanol from sugar. By understanding this process, you can optimize the production process of your products.

Conversely, metabolic pathway analysis requires extensive data processing and expertise, so expert support is essential.

To resolve these issues, we measured metabolites in bacterial cells and culture medium supernatants using a gas chromatograph mass spectrometer GCMS-TQ8040 NX equipped with an automated metabolomics pretreatment system SPL-M100 and then performed metabolic pathway analysis using a multi-omics analysis package. Using Multi-omics Analysis Package enables correlation analysis using metabolic pathways without requiring in-depth knowledge.

Fig. 1 GCMS-TQ™8040 NX with SPL-M100

To produce orsellinic acid, a precursor of small-molecule pharmaceutical raw materials, from acetyl-CoA, cells of 3 strains of E. coli BL21 (DE3) transfected with plasmids and culture supernatants of these strains were used for analysis (Fig. 2) [1].

Strain 1: Plasmid X transfected strain
Strain 2: Transfected with plasmids X and Y
Strain 3: Transfected with plasmids X, Y, and Z

Cultures of each strain were stopped at 12, 24, and 48 hours, and cells and supernatants were measured at n=3.

Strain 1: 12 hours (Cells n=3, supernatants n=3)
24 hours (Cells n=3, supernatants n=3)
48 hours (Cells n=3, supernatants n=3)
Strain 2: 12 hours (Cells n=3, supernatants n=3)
24 hours (Cells n=3, supernatants n=3)
48 hours (Cells n=3, supernatants n=3)
Strain 3: 12 hours (Cells n=3, supernatants n=3)
24 hours (Cells n=3, supernatants n=3)
48 hours (Cells n=3, supernatants n=3)

The sample preparation was performed according to the standard protocol of SPL-M100, and GC-MS used Multiple Reaction Monitoring (MRM) as the data collection mode to simultaneously measure 504 components such as amino acids, nucleic acids, fatty acids, and organic acids within 23 minutes of analysis time (Table 1).

Table 1 Analytical Conditions

GC-MS	
Oven Temp.	: 100 °C (4.5 min) → (15 °C /min) → 330 °C (1 min) Total 23.0 mins
Carrier Gas	: Helium
Injection Volume	: 30 µL

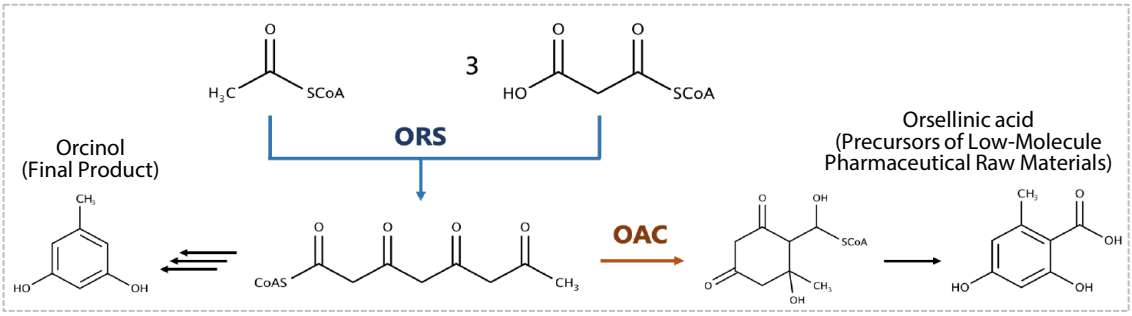

Fig. 2 Metabolic pathway of orcinol synthase and cyclization enzyme
(Targeting the formation of orsellinic acid, a precursor of small molecule drug materials, by introducing **OAC enzyme**)

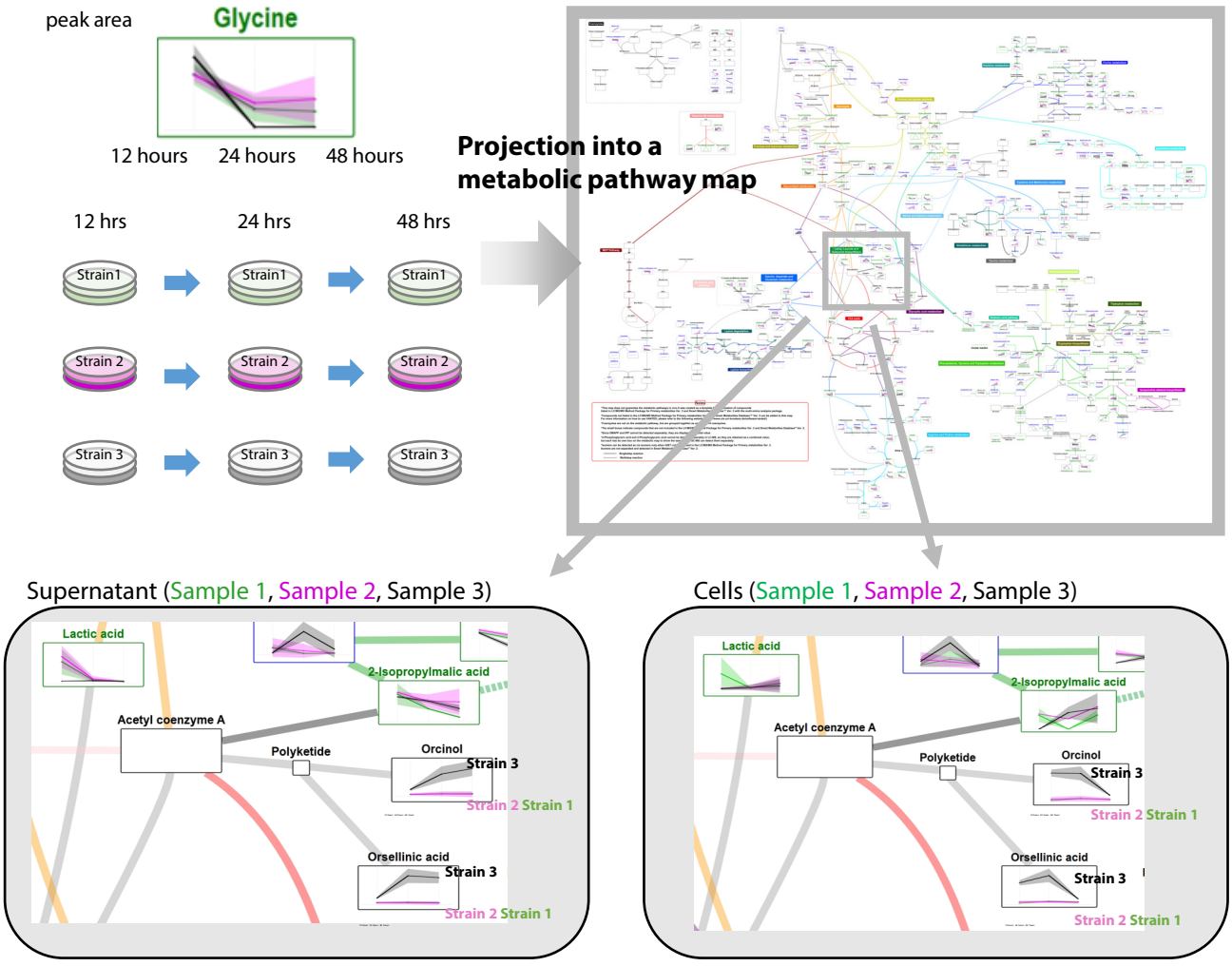

Fig. 3 Comparison of the time course of three samples on metabolic pathways

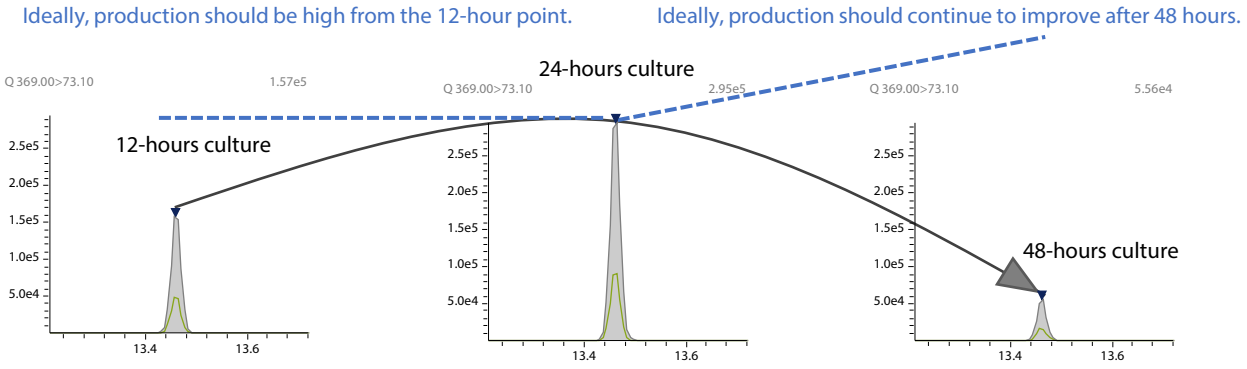

Fig. 4 Chromatogram of orsellinic acid in the cells of sample 3

■ Analysis of Changes Over Time on Metabolic Pathways

When n=3 was measured for each of the 3 samples incubated for 12, 24, and 48 hours, approximately 400 compounds were detected in each sample. The maximum production of orsellinic acid was found in Sample 3 (Fig. 3). This suggests that gene Z, which was introduced into sample 3 by plasmid, is important for orsellinic acid production. However, after 12 hours, the accumulation of orsellinic acid in the cells of Sample 3 decreased significantly. Since the supernatant contains more orsellinic acid than the cells and the supernatant does not decrease after 12 hours, it is possible that orsellinic acid was eluted from the cells into the supernatant. Alternatively, orsellinic acid may form a polymer after a certain concentration and time, or orsellinic acid may serve as a substrate and produce other compounds.

Based on these results, we found that the issues in this experiment were maintaining the accumulated amount of Sample 3 after 12 hours or improving the production amount within 12 hours (Fig. 4).

By observing the metabolic pathway of orsellinic acid formation, we will optimize the amount of orsellinic acid produced and promote ecological research on Lichens (bioindicators of environmental health).

Lichens and certain fungi produce orsellinic acid (a secondary metabolite with antimicrobial activity) to inhibit the growth of other microorganisms and maintain their habitats. Lichens are very sensitive to air quality, and the presence or diversity of a particular Lichens may reflect the level of air pollution in the area[2]. Analysis of the metabolic pathway of orsellinic acid, such as this one, could facilitate studies that scientists are using Lichens to monitor the environment and assess the accumulation of heavy metals and toxic substances in the atmosphere.

■ Metabolic Pathway Simulation

SIMCA18 (Infocom Co., Ltd.) provides a standard score space search function that allows you to select arbitrary positions on the principal component analysis score plot and back-calculate metabolite composition. Using this function, metabolic pathway analysis can be simulated by projecting the area value of the back-calculated metabolite group onto the metabolic pathway in a multi-omics analysis package.

In this study, to maximize intracellular orsellinic acid accumulation in Sample 3 within a short period of time, we selected a metabolite composition that maximizes accumulation at 12 hours. The results showed that the decrease in alanine and glycine caused a higher accumulation of orsellinic acid at 12 hours, while the decrease was more pronounced at 12~48 hours.

■ Summary

In this study, cells of 3 strains of Escherichia coli BL21 (DE3) were transfected with plasmids to produce orsellinic acid, a precursor of small-molecule pharmaceutical raw materials. Culture supernatants of these strains were used for analysis.

Using the standard score space search function of SIMCA18, we could simulate the metabolism of orsellinic acid formation and the metabolite composition at that time to maximize the accumulation of orsellinic acid quickly.

<References>

1) Filamentous Fungi-Derived Orsellinic Acid-Sesquiterpene Meroterpenoids: Fungal Sources, Chemical Structures, Bioactivities, and Biosynthesis, Hua Gao et al., accessed on June 6, 2024
2) Lichens as Bioindicators, National Park Service, accessed on June 6, 2024

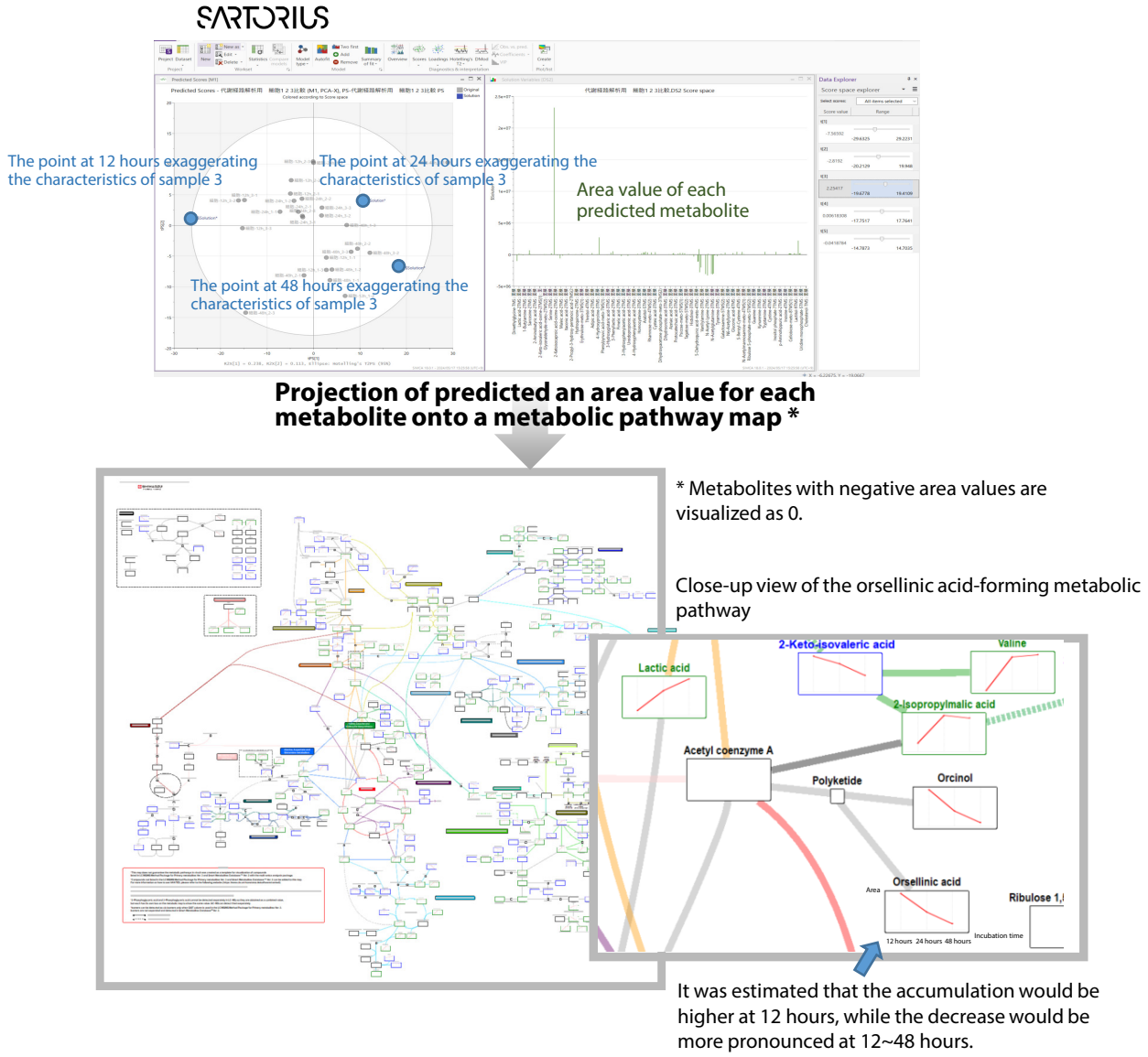

Fig. 5 Projection of the results from the score space search function of SIMCA18 onto metabolic pathways in a multi-omics analysis package

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