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Wed. Jul 1, 2026 12:10 PM - 1:00 PM JST | Wed. Jul 1, 2026 3:10 AM - 4:00 AM UTC | Poster 1(4FFoyer+401+402)
[P005] Poster

12:10 PM - 1:00 PM JST | 3:10 AM - 4:00 AM UTC

[P03-363] Single-cell Raman analysis for *in situ* evaluation of lycopene productivity in recombinant *Escherichia coli*

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Keywords：Raman spectroscopy、metabolite、E. coli、Biomanufacturing

[Purpose]
Microbial biomanufacturing has gained attention as a sustainable alternative to productionprocesses that rely on fossil fuels. For the societal implementation of biomanufacturing, screening of microorganisms with the target metabolic function is essential. However, conventional metabolite detection by instrumental analyses such as LC–MS requires laborious, time-consuming sample preparation, limiting the throughput of screening. In this study, we focused on Raman spectroscopy, which enables non-destructive, label-free acquisition of molecular information, and investigated *in situ* metabolite analysis of recombinant *Escherichia coli*.
[Method]
coli expressing heterologous lycopene biosynthetic enzymes were used for metabolite detection. The low-producing and high-producing strains were GX-01 and GX-02. For single-cell Raman measurements, spectra were acquired with an exposure time of 5 s per measurement point. The obtained cellular spectra were decomposed by MCR-ALS, and metabolite analysis was performed by assigning the resolved spectra. To validate the feasibility of Raman-based evaluation of lycopene productivity, we compared Raman measurements with absorbance-based quantification. In the absorbance assay, lycopene extracted from cells was quantified by measuring absorbance at 474 nm. For Raman-based evaluation, 100 individual cells were measured, and the relative abundance of lycopene was calculated from the intensity of lycopene-associated peaks in the cellular Raman spectra.
[Results]
Spectral decomposition using MCR-ALS identified both the target metabolite, lycopene, and biomolecules involved in primary metabolism, including proteins and cytochromes. Evaluation of lycopene productivity using absorbance-based quantification showed that GX-02 produced 2.7 times more lycopene than GX-01. Raman-based analysis indicated a 2.8-fold increase in lycopene in GX-02, supporting the utility of Raman spectroscopy for *in situ* productivity assessment, with performance comparable to conventional methods. Additionally, non-destructive single-cell measurements identified the emergence of highly lycopene-producing cells within the population.
[Consideration]
For practical screening applications, further improving the speed and accuracy of Raman-based colony measurements will be essential. Although this study focused on lycopene, expanding case studies to a wider range of target products will be necessary to broaden the applicability of this approach. Such efforts will provide the foundation for developing this method into a platform for screening useful microorganisms, ultimately including candidates from natural samples.
[Conclusion]
This study demonstrated that Raman spectroscopy enables *in situ* metabolite detection

andevaluation of lycopene productivity in recombinant *Escherichia coli*. Nondestructive and label-free metabolite detection is expected to serve as a foundational technology for rapid screening and selection of candidate strains in microbial biomanufacturing.