

Mass Spectrometry-Based High-Throughput Quantification of Bioproducts in Liquid Culture

Background/Objective

To screen metabolites efficiently for high-throughput metabolic engineering, fast and information-rich methods are needed. Mass spectrometry (MS) can characterize a broad range of analytes but often requires multiple sample clean-up and extraction steps. We previously showed that liquid extraction surface analysis (LESA), an image-guided surface analysis approach, can be coupled with MS to directly sample metabolites from a surface, circumventing the time-consuming sample preprocessing steps. Here we examined a workflow to combine the advantages of LESA–MS and an acoustic liquid handler with stable isotope-labeled internal standards for fast, absolute quantitation of microbial metabolites from high-throughput liquid cultures.

Approach

We implemented an acoustic liquid handler for sample preparation to enable an entire 96-well plate of samples to be transferred in a single step. We then applied a self-produced, isotope-labeled culture to each sample to correct for the matrix effect and achieve absolute quantitation. Finally, automated sample analysis was performed using LESA–MS. We tested the workflow by quantitatively screening for itaconic acid, triacetic acid lactone (TAL), and free fatty acids and compared the results to established methods.

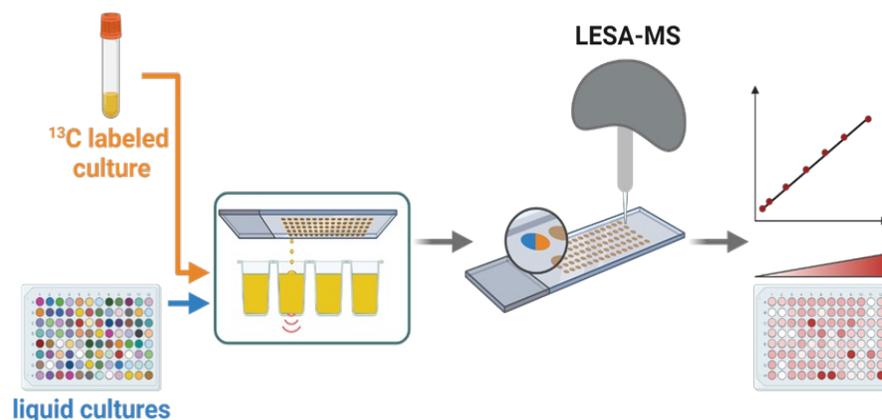
Results

With this workflow, we achieved absolute quantitation at a throughput of **60s per sample** in total. The workflow is also widely applicable to other strain cultures and products. We successfully screened itaconic acid, TAL, and free fatty acids, with quantitative results comparable to liquid and gas chromatography-based methods.

Significance/Impacts

A fast and accurate quantitative screening approach for multiple microbial metabolites across different yeast strains and mediums further enables high-throughput metabolic engineering of yeasts.

Zhou, S., Fatma, Z., Xue, P., Mishra, S., Cao, M., Zhao, H., Sweedler, J.V. Feb. 15, 2023. “Mass Spectrometry-Based High-Throughput Quantification of Bioproducts in Liquid Culture.” *Analytical Chemistry* 95(8):4067-4076. DOI: 10.1021/acs.analchem.2c04845.



Scheme of LESA-MS-based metabolite quantitation workflow.