A general, high-throughput platform for molecular prototyping of microbial cell factories via optically guided mass spectrometry

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Project Goal:

To develop a general platform for high-throughput screening of microbial cell factories at the molecular level using advanced mass spectrometry approaches.

Abstract:

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (MS) imaging has been widely used for molecular analysis of biological samples, but manual procedures of sample preparation are rate-limiting. Recently, we developed automatic, optically guided MALDI-ToF MS analysis as a label-free method to enable high-throughput engineering of microbial cell factories[1]. Coupled with machine vision, automatic MALDI-ToF MS acquisition from randomly distributed colonies simplifies procedures for chemical phenotyping without liquid handling. This method was successfully applied to engineer and screen mutant strains that produce new antibiotic analogs or mixtures of glycolipid congeners with desirable composition. For both cases, large populations of colonies were rapidly surveyed at the molecular level, providing information-rich insights not easily obtained with traditional screening assays. Computational algorithms were also developed to process and visualize the resulting mass spectral data sets, whereby colorimetric readouts were overlaid with optical images to facilitate mutant recovery. After MALDI-ToF MS screening, follow-up analyses using high-resolution MS and tandem MS were readily performed on the same sample target. In our CABBI project, this platform will be applied to screen large libraries of engineered yeasts to synthesize lipid-derived products of custom chemical profiles with high efficiency. For preliminary results, when mutations were introduced into fatty acid synthetase genes, altered membrane lipid compositions in resulting yeast strains were robustly detected using optically guided MALDI MS. Utilizing standard microbiological techniques with routine microscopy and MALDI-ToF MS instruments, this simple yet effective workflow is applicable for a wide range of screening campaigns improving microbial cell factories.

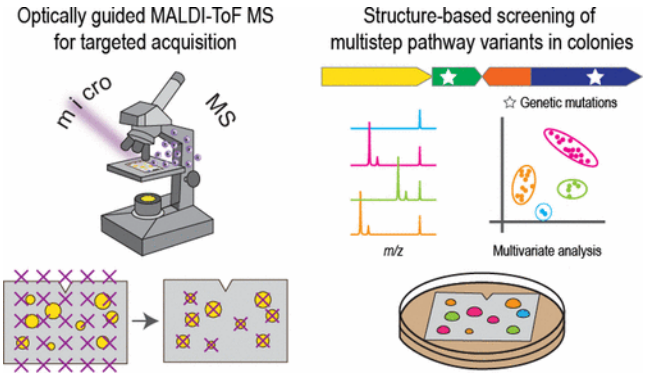

Figure 1. Schematic illustration of optically guided MALDI-ToF MS for automatic chemical profiling of microbial colonies and its application in biosynthetic pathway engineering.

References

1. T. Si, et al. "Profiling of Microbial Colonies for High-Throughput Engineering of Multistep Enzymatic Reactions via Optically Guided Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry." *Journal of the American Chemical Society*, 2017, 139(36), 12466-12473

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