Three-dimensional Metabolic Imaging of Live Bacterial Colonies by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation

Hang Li[1], Pranav Balan[2], and Akos Vertes[1]

[1]Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, The George Washington University, Washington, DC 20052

[2]Thomas Jefferson High School for Science and Technology, Alexandria, VA 22312

(Introduction) With the rising tide of antibiotic resistant bacterial strains, understanding microbial metabolism is of great importance. Conventional bioanalytical tools often have limited capabilities to provide molecular information on spatiotemporal metabolite distributions in microbial colonies.[1] Rapid characterization of metabolites and lipids in a microbial colony requires new bioanalytical tools. Here, we introduce laser ablation electrospray ionization (LAESI), in combination with ion mobility separation (IMS) and mass spectrometry (MS), to investigate microbial metabolism. Three-dimensional spatial distributions of metabolites in *Escherichia coli* (K-12) colonies were mapped using LAESI-IMS-MS.

(Methods) Microsampling of the bacterial colonies and the agar gel by LAESI utilized mid-IR laser pulses at 2940 nm to induce ablation by sudden energy deposition into the water content of the samples. The ejected ablation plume was intercepted by an electrospray for ionization. To generate the electrospray, 50% methanol solution with 0.1% acetic acid (*v/v*) was delivered at 500 nL/min flow rate through an emitter held at high voltage. The LAESI-generated ions entered a high performance quadrupole time-of-flight mass spectrometer (Synapt G2S, Waters Co., Milford, MA) for detection.[2] A traveling wave (T-wave) ion mobility separation (IMS) system was incorporated in the instrument. The gas phase ions became separated through collisions with the buffer gas molecules as they moved along the IMS cell.

A starter culture of *Escherichia coli* (K-12 strain) was grown in a Luria Broth (LB) medium in an orbital shaker (MaxQ 4000 Benchtop, Thermo Scientific Inc., Waltham, MA) at 37 ºC. After 24 hours of incubation, the liquid culture was inoculated on an LB agar plate to initiate a single

colony for MS study. Profiling and imaging of the *E. coli* culture was performed using a commercial LAESI source (DP-1000, Protea Biosciences, Morgantown, WV) integrated with the IMS-MS system.

(Preliminary data) Preliminary studies showed that over one hundred metabolites and lipids were detected by LAESI-IMS-MS from a single *E. coli* colony in positive and negative ion modes. The detected molecules belonged to several biosynthesis pathways, e.g., leucine/isoleucine and valine biosynthesis. In negative ion mass spectra, major membrane lipid peaks resulting from abundant phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) ions were found. The ablation mark over the colony indicated a spot size with a diameter of ~150 µm. The side view of a single *E. coli* colony showed its thickness between 80 µm and 100 µm. With a single laser pulse penetrating ~30 µm, depth profiling was achieved by acquiring individual spectra from consecutive laser pulses, and in three to four pulses the bottom layer was reached. Three-dimensional images were built from lateral rastering and depth profiling. For example, the *m/z* 188.2 ion-identified as acetylspermidine, and the *m/z* 704.5 ion-identified as PE(16:1/17:0) exhibited decreasing intensities in deeper layers (see Figure 1) with the former retaining stronger signal on the perimeter.

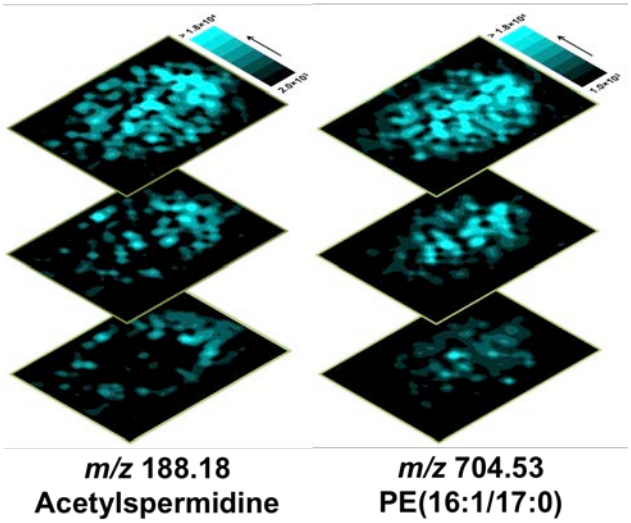

Figure 1. LAESI-MSI of a single *E. coli* colony at three layers of increasing depths. Ion maps correspond to acetylspermidine and PE(16:1/17:0).

(Novel aspects) Over one hundred metabolites and lipids were detected by LAESI-IMS-MS from a single bacterial colony within seconds. Our results show that high throughput metabolomic analysis and three-dimensional imaging can be performed by this method to probe a complex microbial community.

(1) Watrous, J. D.; Dorrestein, P. C. *Nature Reviews: Microbiology* **2011**, *9*, 683-94.
(2) Shrestha, B.; Vertes, A. *Analytical Chemistry* **2014**, *86*, 4308-4315.