PhD Departmental Seminar

Shortcut to microbial metabolome analysis starts with spatial profiling

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Throughput is one of the bottlenecks in microbial natural products research. Conventional workflow of culturing, fraction, and structural elucidation require either too much time or sample. Here, we are presenting a workflow combining rapid spatial profiling of ambient MS with powerful structural elucidation by tandem MS to facilitate the metabolome research of *Pseudoalteromonas*.

To achieve rapid spatial profiling for *Pseudoalteromonas*, liquid micro-junction surface sampling probe (LMJ-SSP) based MS is used combining with hyperspectral visualization for spectra interpretation. Agar plate cross-streaked with *P. rubra* and *P. tunicata* was imaged with a spatial resolution of 1mm without sample preparation. Hyperspectral visualization, which convert acquired spectral data into red-green-blue (RGB) color code through unsupervised multivariant analysis, makes sampled area with different metabolome profiles into image comprised of different colors. Colors in the hyperspectral image can be used to further generate a list of m/z list containing prodigine compounds corresponding to *P. rubra* and tambjamine compounds corresponding to *P. tunicata*.

As the biosynthesis pathway of macrocyclic derivative tambjamine MYP1 involves activation of terminal primary C-H bonds and Rieske oxygenase-catalyzed oxidative cyclization reaction, the metabolome of *P. tunicata* was further explored. Based on chromatographic separation and tandem MS fragmentation, a potential rotamer of tambjamine MYP1 was discovered in whole cells of *E. coli* expressing the relevant biosynthetic enzymes. Three innovative linear derivatives with hydroxyl and aldehyde groups are also discovered from *P. tunicata* extracts.

In summary, LMJ-SSP based auto-sampling platform was successfully proven capable of performing rapid spatial profiling on microbial agar plates. Unsupervised multivariant analysis-based hyperspectral visualization can determine potential connections between bacteria and potential natural products. In the metabolomic analysis of tamjamine analogues, innovative rotamer products were discovered in the oxidative cyclization reaction while additional derivatives were also found. A MS-based workflow that covers both initial stage screening and follow-up structural characterization of natural product is presented.