Platforms for natural product discovery: Monitoring spatiotemporal dynamics of metabolites from microbe co-culture using ambient mass spectrometry.

Soil harbors complex microbial communities where interactions are mediated by diverse secreted chemicals. Microbes are often cultured in close contact, or "co-cultured", to mimic these community interactions. Chemically mapping these dynamics provides insight into metabolite-based interactions and helps prioritize isolation of bioactive molecules. However, conventional chemical analysis methods, like bulk solvent extraction, are destructive and often lose rich spatiotemporal data, while common mass spectrometry imaging techniques have sample preparation or surface-feature requirements that make it challenging to map metabolites on the same sample over time. This work uses the liquid microjunction surface sampling probe (LMJ SSP) paired with mass spectrometry to spatiotemporally map metabolites of isolated and co-cultured soil microbes in custom chambers modeling micro-ecological interactions.

Strains of *Streptomyces* and *Penicillium* were grown together in 3D-printed tracks to create a controlled co-culture environment, then sampled with an automated direct micro-extraction technique using the LMJ SSP. Metabolite profiles were obtained over several days to track changes in activity along the surface of growing microbes and surrounding agar, revealing the molecular interplay between the co-cultured organisms, and with their environment. We observed the initial production and movement of known microbial metabolites, like griseofulvin analogues and prodiginines, and novel compound masses correlated with microbial interaction zones. Overall, this tool provides a robust, preparation-free method for mapping microbial metabolite dynamics, enabling mechanistic insight into microbial ecology and informing natural product isolation and discovery.