68. Multidimensional Chemical Analysis of Microbial Communities by Integrating Fluorescence, Vibrational and Mass Spectrometric Microspectroscopic Imaging

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Project Goal: Our goal is to develop a multi-modal analysis program by integrating fluorescence, Raman, and matrix-assisted laser desorption ionization MS imaging (MALDI MSI) microspectroscopies and apply them to a system that closely mimics the rhizosphere. Specifically, we are developing a three-component system comprised of two microorganisms and a plant root. We seek to understand the biological interactions, processes, and communication events that occur at the intra- and inter-species level between the microorganisms as well as the interactions occurring between the plant root and the individual microorganisms by following the spatial and temporal characteristics of the various biomolecules within the system.

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Bacteria are the most abundant organisms on the earth and play significant roles in processes such as nitrogen and carbon cycling, mass transfer of transition metals, and degradation of organic contaminants in the environment. Many bacteria naturally aggregate and adhere to surfaces forming biofilms, which are multicellular communities held together by a self-produced extracellular matrix. Bacteria within biofilms live in a complex microbial community that exhibits primitive homeostasis, a circulatory system, and metabolic cooperativity.[1] Corresponding to differences in phenotype, bacteria growing in a biofilm show different gene expression profiles than planktonic cells.[2] As a result, bacterial cells within biofilms respond differently to environmental perturbations than their planktonic counterparts. Biofilms can convey either detrimental or beneficial effects, depending on the setting. Characterizing and spatially mapping the chemical and molecular composition of bacterial biofilms would provide valuable insight into the behaviour of biofilm-forming bacteria including the function of molecular chemical constituents, how the bacteria respond to external cues contained in the chemical or physical environment, the mechanisms of biofilm formation and maturation, and how they may be managed.

Despite the great importance of answering these questions, there are a relative dearth of methods that can provide spatiotemporal chemical information that would inform them. Thus, in this work, fluorescence, Raman and matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI) microspectroscopies have been applied for the analysis of three different bacterial species; *Pseudomonas* GM41, *Pseudomonas* GM17 and *Pantoea* YR343. Both biofilms and planktonic bacteria have been characterized for the three species allowing us to (1) visualize and identify different molecular species in their composition, (2) differentiate biofilms and planktonic bacteria based on their molecular composition, and (3) differentiate the different species based on their molecular and chemical composition.[4] These powerful characterizations are made possible by the use of multivariate statistical analysis.

Further work has focused on *Pantoea* YR343, which was isolated from the rhizosphere of poplar and displays a robust root colonization phenotype. Colonies of *Pantoea* YR343 have a yellowish color due to the production of carotenoids. Consistent with this, the Ramam spectrum of *Pantoea* YR343 displays prominent peaks at 1150 cm^{-1} and 1525 cm^{-1}, suggesting that the color may be due to the presence of a carotenoid. To test this, we deleted the *crtB* gene, which encodes a phytoene synthase that is responsible for converting geranylgenanyl pyrophosphate to phytoene, an early step in the biosynthesis of β-carotene. A *Pantoea* mutant strain lacking *crtB* grows with similar kinetics as a wildtype strain, but displays a whitish color, consistent with the loss of pigment. The Raman spectrum from the *crtB* mutant lacks the prominent peaks at 1150 cm^{-1} and 1525 cm^{-1}, consistent with our hypothesis that these peaks arise from a carotenoid species, as predicted. Further analysis to confirm the exact identity of the carotenoid molecule is underway using LDI. Future studies are also aimed at using multimodal analyses to examine microbial colonization of plant roots.

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