

75. Multimodal chemical and architectural characterization of *Pseudomonas aeruginosa* microbial communities

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Project Goals: We are performing untargeted in situ chemical imaging of microbial communities. Here we combine complementary information collected from mass spectrometry imaging (MSI), confocal Raman microscopy (CRM), and electron microscopy (EM) to study the spatio-temporal dynamics underlying *Pseudomonas aeruginosa* bacterial biofilm formation and development. Each technique offers a unique advantage: MSI uses an ion's mass-to-charge (m/z) ratio to provide in-situ chemical identification and chemically specific ion imaging, CRM is a nondestructive light spectroscopy technique that differentiates compound classes by their inelastic scattering profile, and EM measures secondary electron emission profiles to provide an architectural map of the sample surface. Application of this combined approach highlights inter-film chemical heterogeneity and developmental differences both during biofilm development and resulting from different nutritional sources.

Many microorganisms, such as the gram-negative bacterium *Pseudomonas aeruginosa*, exist in highly cooperative communities that exhibit collective gene expression and coordinated behavior resembling that of a multicellular organism. The coordination of this ensemble is accomplished by way of a chemical communication system, termed quorum sensing (QS), in which the activation of genes and therefore downstream protein synthesis and phenotypical differentiation is controlled as a function of population density; each cell secretes a baseline level of a chemical messenger and when the population density reaches a threshold level this messenger activates or deactivates the transcription of certain genes, leading to phenotypical expression and behavior exhibition. For example, the transition of a *P. aeruginosa* cell from the planktonic phenotype into that of a sessile biofilm, a process that results in expression changes in over 10 % of the genome, is largely dictated by the local population density and the concentration of specific QS chemical messengers.

Here we present the combination of two untargeted chemical imaging capabilities, namely SIMS imaging and CRM, in conjunction with the architectural characterization provided by EM, for in situ identification and chemical mapping of the QS molecules and their downstream products across the surface of *P. aeruginosa* biofilms. We primarily focus on the class of biomolecules known as quinolones, which are pivotal for gene control and biofilm development. The prominent quinolones studied here are the two QS molecules 2-heptyl-3-hydroxy-4(1H)-quinolone, or *Pseudomonas* quinolone signal (PQS), and its precursor 2-heptyl-4(1H)-quinolone (HHQ), as well as a host of structurally similar molecules, a category that includes 2-nonyl-4(1H)-quinolone (NHQ), 2-Heptyl-4-hydroxyquinoline N-oxide (HQNO), 2-undecyl-4-hydroxyquinoline (C11:db-UHQ), C9:db-NHQ, C9-PQS, and C9:db-PQS.

In an early study, we developed a correlative methodology that utilizes an array of nanoparticle (NP) microdroplets as fiducial markers for accurate navigation and easy sample transfer between analytical

systems.¹ For this analysis, a precisely spaced microdroplet grid is first applied to the dried biofilm surface with a chemical inkjet printer. The sample is then independently subjected to both CRM and cluster secondary ion mass spectrometry (SIMS), while recording the precise grid coordinates of each chemical signature. Finally, the regions of interest identified from the correlated CRM/SIMS work are analyzed using EM, which facilitates the assignment of chemical information to specific spatial constructs. Using this methodology we discovered highly localized chemical pockets containing HHQ and PQS, in addition to several other quinolones, specifically distributed over exposed cells on the film surface. The results of this experiment, as well as those of numerous follow-up studies, show that architecturally similar areas of the film can have vastly different chemical compositions, possibly suggesting phenotypical differentiation in purportedly clonal cellular consortiums.

Our studies have also revealed dramatic changes in chemical composition both during film development and under different sources of nutrition. We prepared separate cultures of *P. aeruginosa* on either glucose or glutamate as the nutritional source and monitored their development at 2 different stages: in the planktonic state (overnight culture), and after 24 hours of film growth. A combination of SIMS and CRM reveals that planktonic organisms grown on both glucose and glutamate produce low concentrations of several quinolones including HHQ and 2-nonyl-4(1H)-quinolone (NHQ). By 24 hours of film growth dramatic differences between the nutritional conditions begin to emerge. Highly localized quinolone regions are found to be protruding from the glutamate film, while the glucose film shows a much more diffuse distribution. These results highlight the power of our multimodal analytical approach for biofilm study and are of great interest to the microbiology community.

SIMS imaging of 24 hour *P. aeruginosa* films grown on glucose and glutamate. PQS and HQNO are isomers.

References

1. Lanni, E.J. et al. Correlated imaging with C60-SIMS and confocal Raman microscopy: Visualization of cell-scale molecular distributions in bacterial biofilms. *Analytical Chemistry* 86, 10885-10891 (2014).

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