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PNNL-31973

Deciphering exopolysaccharide functional specificity via visualization of chemical motifs that mediate microbial-microenvironment interactions in soil micromodels

September 2021

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

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Report

Project summary: Exopolysaccharides (EPS) are carbohydrate-based matrices of microbial biofilms that microbes use to adapt to their surroundings. Microbial EPS also promotes soil aggregate stability and contributes toward soil health by reducing erosion caused by continued land use [1]. The chemical structure of EPS, even within single species biofilms, can vary greatly: from repeating units and branching patterns to the non-carbohydrate decorations [2, 3]. Despite the wealth of knowledge on EPS chemistry, many unresolved questions regarding the biological implications of EPS chemical variations remain. Here, we develop a mass spectrometry imaging toolbox that reveal how EPS chemistry enables microbial adaptation to different microenvironments such as specific moisture, soil-biofilm interfaces, and food source proximity. First, we developed emulated soil micromodels (ESMs), microfluidic channels that simulate the physical properties of soil, for biofilm formation in different microenvironments. Then, in order to map chemical features of EPS in these micromodels, we develop strategies for in-situ releasing of oligosaccharides using specific enzymes. Lastly, we optimized matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) to spatially analyze the released compounds in ESMs.

Introduction and Project background: Continued land use for crop production alters soil organic matter content, thereby influencing soil aggregate stability and health. Microbial EPS are binding agents that significantly contributes to the maintenance of soil aggregates and pore networks by diminishing the effects of erosion and promoting nutrient transport for stability of soils and terrestrial ecosystems [1,4]. The EPS material holds moisture within its polymeric matrix, swelling and shrinking despite wide fluctuations in environmental moisture content. In this way, soil microbial EPS maintains microbial communities during long periods of drought by providing hydration and nutrition [5]. The structural and compositional diversity of the EPS produced by single as well as multispecies microbial communities in soils influences the ability of EPS to maintain the health of soils and the ecosystems thriving within. Chemical modifications alter the physicochemical properties of the EPS polymer, which in turn affect bacterial environmental adaptability [2]. Moreover, the molecular motifs of EPS are spatially distinct based on microbial community structure at a given location in the biofilm [3]. The physical and chemical microstructure of soils also influences the spatial distribution of microbially derived EPS chemistry. Therefore, bulk approaches that average EPS chemistry cannot reveal the critical chemical features of EPS specific for microenvironments, and this project aimed to find a method that could link spatial with chemical information. We developed enzyme assisted MALDI MSI for EPS spatial chemical analysis of biofilms grown in microfluidic devices that simulated soil environments. Our approach utilizes specific enzymes to in-situ release oligosaccharides and then employs a UV laser to ablate and ionize released oligosaccharides from a sample spatially, linking chemical structure of oligosaccharides with their location in biofilm.

Data and Approach: We used sodium alginate, alginic acid and *Pseudomonas fluorescens* biofilms grown on the agar, liquid medium, and microfluidic devices to develop enzyme assisted MALDI MSI workflow for in situ microbial alginate biofilm analyses. Different enzyme preparation protocols (source of alginate lyase, specific activity, organic solvent stability, salt content, enzyme quantity applied, incubation time, buffers pH) were tested for optimal alginate degradation. Different biofilm cultivation strategies and various washing protocols (including buffet and organic solvent wash) were tested to allow maximal accessibility of the enzyme to alginate biofilm. We optimized MALDI matrix application and 15 Tesla MALDI FTICR parameters to achieve the highest sensitivity of oligosaccharide detection. To demonstrate applicability of the optimized

workflow in emulated soil micromodels, we fabricated and tested biocompatible ESMs from UV curable polymers that are compatible with direct MS analysis.

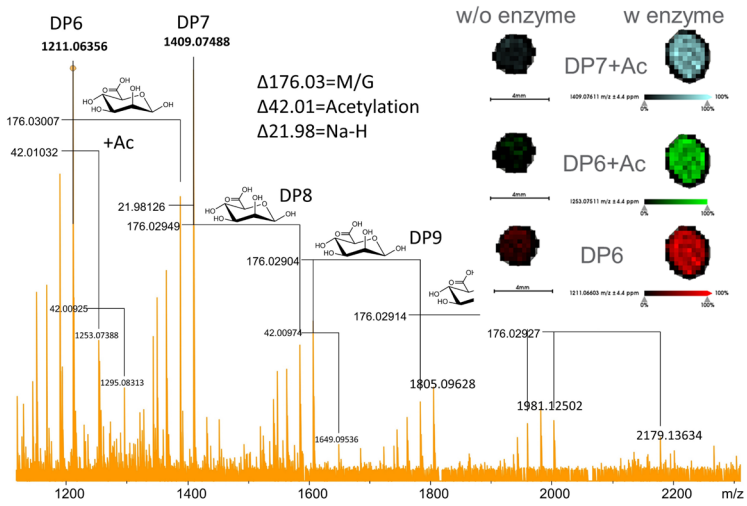

Figure 1. MALDI mass spectrum of alginate standard after treatment with enzyme (alginate lyase)

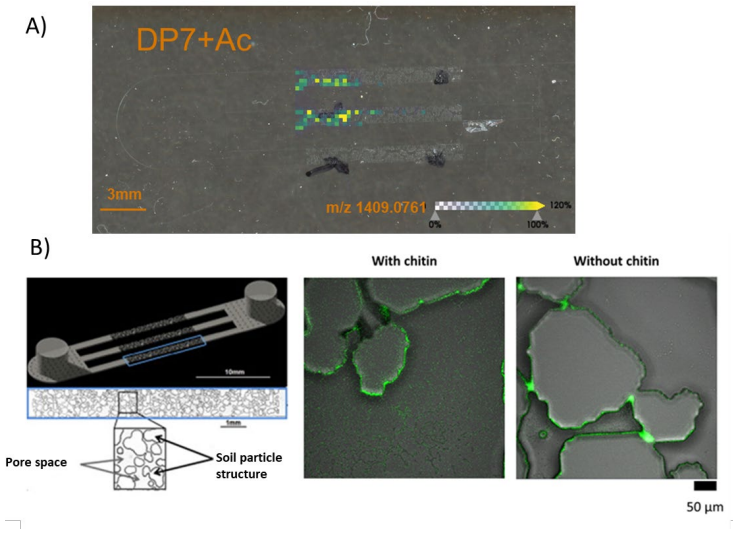

Figure 2. Emulated soil micromodels for EPS chemical analysis. A) Detection of acetylated alginate in microfluidic channel B) *P. fluorescens* biofilm formation (green) around soil aggregate like structures visualized by confocal microscopy

Results and Accomplishments: Figures 1 and 2 illustrate our main results and accomplishments in this project. After optimization of enzyme preparation, we were able to visualize alginate chemical features in the MALDI mass spectrum: different degrees of polymerization (identified as 176.03 repeating units) and chemical modification such as acetylation (identified by 42.01 mass shifts). As mentioned in the Background section, terminal sugar sequences, unusual structures, or non-carbohydrate modifications are more likely to be involved in specific EPS roles. In our workflow, by employing a "soft" ionization imaging approach, these decorations are preserved and visualized in the biofilms. This preservation of glycan modification is a great advantage compared to traditional LC-MS analyses that not only lack of providing spatial information but require alkaline conditions and hence lead to the losing of these ester bounded decorations. We also demonstrated how enzyme in-situ application allows revealing alginate chemical features at their original localization (insert). Figure 2 illustrate the application of emulated soil micromodels in EPS spatial chemical analysis. We tested different materials for biocompatible UV-curable polymer based microfluidic device fabrication and found that epoxy 603 polymer provides superb compatibility with MALDI MS analysis: low laser fluence needed for threshold signals, constant total ion current during imaging run, low background signals, and high signal to noise ratio for carbohydrates as analytes. Figure 2b illustrate P. fluorescence biofilm formation around the soil like particles in presence or absence of chtin. As we write this report, we are running our enzyme-assisted approach to this device and we are expecting to see different chemical features along the biofilm and in different nutrient conditions.

Impacts/Benefits: Our workflow and methodology developed here shows it has potential in analysis of spatial chemical features of EPS in bacterial biofilms. We used *Pseudomonas fluorescens* biofilms as an alginate producer but our methodology with slight modifications (enzyme used) can be applied to other soil microbial biofilms and even microbial cocultures cultures. Spatial characterization of EPS chemistry in soil – like environments will facilitate better understanding of influence of EPS chemistry in maintaining soil health during crop development and continued land use. A manuscript describing this approach is in preparation for submission in mSystems.

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