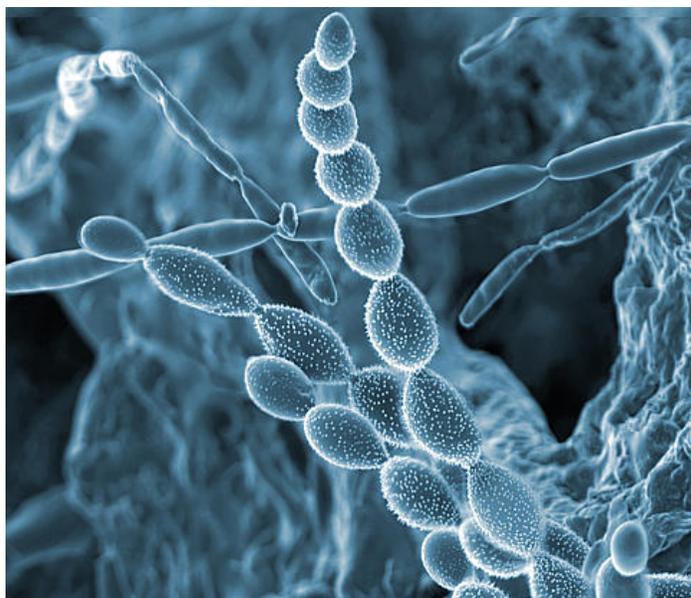


MICROBIOME RESEARCH OPPORTUNITIES

EMSL scientists develop unique capabilities to provide the user community with novel insights into microbial community functions and dynamics that lie at the foundation of systems biology. Using ultra-high resolution mass spectrometry (MS), spatiotemporally resolved measurements of dynamic systems, microscopy and spectroscopy, and modeling and simulation, EMSL provides unmatched multimodal analytical capabilities that are coupled with simulations for understanding the microbiome in natural systems.



Omics

<https://www.emsl.pnl.gov/emslweb/capabilities/mass-spectrometry>

https://www.emsl.pnl.gov/emslweb/capabilities/nmr_epr

<https://www.emsl.pnl.gov/emslweb/capabilities/cisa>

EMSL scientists and users are advancing the identification and interpretation of microbial signals within and between cells as well as environmental interactions of microbes and microbial communities with plants, soil and the atmosphere. EMSL houses a suite of measurement capabilities for metametabolomics, metaproteomics and meta-transcriptomics in support of systems biology and multi-omics integration efforts. These include high-end MS instrumentation (i.e. Orbitraps) for

quantitative meta-proteomics, and next-generation sequencing systems such as the Ion Proton™ system for massively parallel unbiased sequencing and metatranscriptome analysis of complex microbial communities, as well as transcriptome analysis of small, spatially defined bacterial clusters, isolated by laser capture microdissection and other cell isolation approaches. EMSL also offers a unique capability, an ultra-high performance 21 Tesla Fourier transform ion cyclotron resonance mass spectrometer -- one of only two available world-wide. It has unmatched ability to identify and quantify diverse proteins in fungal secretomes (top-down proteomics), allowing access to the functional capacity of the system, and analytes in complex environmental mixtures (i.e. dissolved or soil organic matter).

Top-down Proteomics

Top-down proteomics is aimed at functional characterization of intact proteins from natural or engineered plant or microbial systems to understand the effects of post-translational modifications and proteolytic processing events on their activity and stability. In contrast, the traditional bottom-up strategy, which involves the analysis of protein fragments, often destroys critical information about chemical modifications that alter protein structure and function. Similarly, activity-based proteomics enable highly specific tagging of microbial populations that participate in different metabolic/functional guilds of interest (e.g. lignocellulose degradation). These populations can subsequently be sorted using for example fluorescence activated cells sorting and interrogated using multi-omics to determine their identities and functional potential and their specific roles within the ecosystem. Advanced microfluidic capabilities offer users the unique ability to perform omics measurements with limited amounts of material (down to very small populations such as hundreds of microbial cells or a single eukaryotic cell). EMSL has been at the forefront of proteomics measurements at this level of sensitivity, opening up new possibilities such as proteomics of a single soil aggregate coupled with transcriptome analysis using approaches developed specifically for small amounts of starting RNA. Together, this suite of capabilities is enabling EMSL scientists and users to test hypotheses about the functional response of microorganisms, microbial communities and microbe-by-environment interactions.

Integrated Metabolomics

Integrated, state-of-the-art nuclear magnetic resonance (NMR) spectroscopy and MS of various types – ion mobility spectrometry, gas chromatography, Fourier transform ion cyclotron resonance MS – enable EMSL to provide increased identification coverage of the metabolomes of microbial communities, their hosts and their environments, and to identify the mechanisms by which metabolites involved in communication and interaction are generated, transported and sequestered within microbial communities. Scientists at EMSL and Pacific Northwest National Laboratory are coupling these measurements with molecular dynamics simulations using the NWChem code (http://www.nwchem-sw.org/index.php/Main_Page) to achieve accurate identification of metabolites without the use of chemical standards.

Dynamic Multimodal Imaging

<https://www.emsl.pnl.gov/emslweb/capabilities/microscopy>

EMSL offers unique capabilities for dynamic multimodal imaging of metabolites, lipids and proteins in microbial communities with (sub)cellular resolution, based on fluorescence and MS-based imaging. Ambient ionization mass spectrometry imaging approaches facilitate *in-situ* molecular imaging of microbes (e.g. the rhizosphere) to directly map chemical distributions of intact molecular species (metabolites, proteins) employing liquid extraction surface analysis laser ablation electrospray ionization; and nano-desorption electrospray ionization with 1-200 micron spatial resolution. Super resolution fluorescence imaging and nanoscale secondary ion mass spectrometry (nanoSIMS) (coupled with combinatorial fluorescence *in-situ* hybridization or FISH) can be used to quantify the expression of multiple genes of interest in individual cells to reveal mechanistic details about elemental flux within the microbiome with submicron resolution. Combined MS, FISH and nanoSIMS experiments will enable linking biota with processes at the submicron scale.

Extending to the nanoscale for both labeled and unlabeled components, serial section transmission electron microscopy tomography and slice-and-view focused ion beam/scanning electron microscopy allow 1-10 nm spatial resolution reconstructions of whole cell architecture and internal ultrastructure, while helium ion microscopy provides nanoscale topographical and surface imaging ideal for studying plant/microbe interactions of intact specimens. Finally,

structures of microbial soluble and membrane protein complexes are performed using EMSL's cryo-electron microscopes with direct electron detectors or the onsite MS (i.e. native MS) and NMR capabilities. Altogether, the array of imaging instrumentation housed within EMSL provides unique opportunities for correlative, multimodal and integrative bioimaging across scales perfectly suited for microbiome research.

Stable Isotope Probing

Stable isotope probing (SIP) measurements can be used to measure key elemental fluxes (e.g. C, N, O) and provide critical insights into microbial processes, including nutrient flow through environmental microbiomes, eukaryotic/microbe interactions (e.g. plant/rhizome), and the ultimate fate of plant and microbial residues as stable soil carbon. EMSL offers a suite of SIP measurement technologies for omics (RNA, proteins, metabolites via RNA-Seq, MS and NMR) and multimodal imaging, as well as software for steady state metabolic flux analysis. Because SIP experiments are inherently temporal in nature, this approach addresses both the spatial and temporal aspects of microbial community dynamics.

Modeling and Simulation

<https://www.emsl.pnl.gov/emslweb/capabilities/computing>

The spatial organization of microbiomes in their environment, and the interactions between the individual microbes can be simulated using individual-based modeling. In such simulations, users can combine the reaction processes of microbes (inferred from genome data) with transport processes (such as diffusion of nutrients) to query the cell-by-cell and cell-by-environment interactions. Depending on the level of detail needed and the computing resources available, each microbe can be represented by reactions that summarize cellular metabolism, or with complete metabolic models. Software such as Biocellion enables these type of discrete, individual-based modeling simulations run on high-performance computing systems (using the Cascade system at EMSL).

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For more information about EMSL: www.emsl.pnnl.gov

EMSL, the Environmental Molecular Sciences Laboratory, is a national scientific User Facility sponsored by the Department of Energy's Office of Biological and Environmental Research in the Office of Science. Located at Pacific Northwest National Laboratory in Richland, Wash., EMSL offers an open, collaborative environment for scientific discovery to researchers around the world.

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