

Linking Community Composition and Resource Utilization

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

There is an urgent need to improve our understanding of the connections between microbial community composition to their *in situ* activities. Exometabolomic methods provide direct measures of metabolite use and production and these data are increasingly available for diverse collections of isolates. However, the degree that isolate metabolic activities are conserved in communities is unclear due to the difficulty in measuring growth rates and resource use in mixed communities. Mass spectrometry based proteomics is routinely used for bacterial protein profiling and can be used for the identification of bacteria. Here, we developed a defined medium, the Northen Lab Defined Medium (NLDM), to characterize bacterial isolates and use protein profiling to track both community structure and resource use partitioning among community members.

NLDM not only supports the growth of diverse soil bacteria but also is defined and therefore suited for exometabolomic profiling. Metabolites included in NLDM were selected based on their presence in R2A medium and soil collected from the Oak Ridge Field Research Center (ORFRC), elemental stoichiometry requirements, as well as knowledge of metabolite usage by different bacteria [1]. NLDM supported the growth of 108 of the 110 phylogenetically diverse soil bacterial isolates (spanning 36 different families) tested and all of its metabolites were trackable through liquid chromatography mass spectrometry analysis.

Next, we constructed a five-member bacterial Synthetic Community (SynCom) based on our previous analysis of the translationally-active microbes present in soil samples from the ORFRC [2]. Resource utilization of the 5 individual SynCom members as well as the SynCom itself was examined using NLDM and exometabolomic profiling. Simultaneously, quantification of the community structure on NLDM was determined using proteomics.

The combination of exometabolomic profiling, using the newly developed NLDM medium, and proteomics enables simultaneous monitoring of population dynamics and substrate use partitioning in mixed communities. Next steps will include integrating stable isotope probing (SIP) into the current setup to track substrate usage by each individual member within the community.

References

[1] Jenkins, S. et al. Construction of Viable Soil Defined Media Using Quantitative Metabolomics Analysis of Soil Metabolites. *Front. Microbiol.* 8, 2618 (2017).

[2] Couradeau, E. et al. Probing the active fraction of soil microbiomes using BONCAT-FACS. *Nat. Commun.* 10, 1–10 (2019).

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