High throughput approaches for investigation of microbial interactions within synthetic microbial communities

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Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses 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 and interactions to their *in situ* activities. Recently, we reported the implementation of BONCAT (Biorthogonal Non-Canonical Amino Acid Tagging) to capture the translationally active cells in soils[1] from Oak Ridge, TN. When comparing microbial populations from two soil depths incubated under the same conditions for seven days, we found that active populations ranged from 25 – 75% of total cells, and accounted for 3-4 million active cells per gram of soil. The BONCAT positive cell fraction for each depth was recovered by fluorescence activated cell sorting (FACS) and identified by16S amplicon sequencing. On average, 86% of sequence reads recovered from the active community shared >97% sequence similarity with cultured isolates from the same location suggesting that we can use our existing isolate collection to construct synthetic communities (SynComs) designed to mimic active populations and investigate relevant microbial interactions, especially resource partitioning.

Currently, tracking growth rates and resource use in mixed communities is technically challenging. Significant progress has been made using sequencing-based approaches. However, there are limitations in sensitivity and throughput. Use of mass spectrometry for analysis of strain specific protein biomarkers represents an alternative and promising complementary approach. This approach is routinely used for rapid, highly sensitive identification of bacteria in clinical settings. We are adapted this protein-profiling approach for use with stable isotope probing to both determine community structure and track resource partitioning in our BONCAT positive SynCom experiments. This is accomplished by using strain-specific biomarker profiling with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Initial results show that this approach accurately quantified individual strain abundance in a mixed community of six different bacterial strains. Using exometabolomics profiling, we have predicted co-culture resource partitioning and are now feeding the SynComs with stable isotope labeled forms of these metabolites. With this approach, we will track label incorporation into newly synthesized protein biomarkers to compare metabolite use in pure cultures vs. SynComs. These integrated technologies will provide important new insights into resource competition and cross-feeding within sediment communities to help address our science goals.

References

[1] E. Couradeau, J. Sasse, D. Goudeau, N. Nath, T.C. Hazen, B.P. Bowen, R. Chakraborty, R.R. Malmstrom, T.R. Northen, Probing the active fraction of soil microbiomes using BONCAT-FACS, Nat. Commun. 10 (2019). doi:10.1038/s41467-019-10542-0.

ENIGMA is a Scientific Focus Area Program at Lawrence Berkeley National Laboratory and is supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.