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PNNL-32115

PhenoProfiling

Mapping phenotypic outcomes to molecular determinants of biochemical activity

September 2021

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Background and Significance

The aggregate genomes of the trillions of microorganisms within soil, animal hosts, and aquatic systems encode for an extensive functional capacity for myriad biochemical activities. C, N, P, and S metabolism, synthesis of signaling molecules and vitamins, mineralization, and other activities are essential to microbe, community, and plant physiology, and more broadly to climate, water, animal, flora, and human health. The current understanding of the molecular basis for the function of microbial communities stems primarily from comparative metagenomic and metatranscriptomic studies. These same tools are employed to ascertain the impacts to the community resulting from perturbations, such as climate change, emerging pollutants, fires, and seawater infiltration for environmental communities, and dietary changes, xenobiotic exposure, and various disease states for the human gut microbiome. Such studies can identify the *potential* for a specific function, but they cannot determine that a particular cell is functionally active, nor can they determine the molecular architecture required for function. In short, genes and transcripts alone fail to reveal the complex subcellular arrangement of proteins and molecules that elicit a given phenotype in a microbial cell.

In order to map phenotypic outcomes to molecular determinants, we proposed an integrated functional profiling platform, PhenoProfiling, that has at its core activity-based protein profiling (ABPP), which is uniquely suited to selectively isolate functional microbes in complex systems and sets the stage for high-throughput characterization studies. ABPP utilizes small molecule probes (activity-based probes, or ABPs) to covalently label enzymes based on catalytic activity. ABPs may also mimic naturally occurring molecules such as vitamins, lipids, natural products, or carbohydrates to enable an understanding of the microbes and proteins that act upon those molecules. ABPs are multimodal, such that the reporting of protein binding events can be readily varied, resulting in a versatile functional measurement platform that can be used for imaging, fluorescence-activated cell sorting (FACS) followed by DNA sequencing, and multi-omics. The bulk of ABPP-driven data focuses on individual proteins, rather than whole cells displaying a phenotype of interest. Herein, the approach enables the capture of living, functional cells, based upon phenotype for subsequent analyses by an array of predictive phenomics capabilities, followed by innovative computational modeling strategies to map functions back to ecosystems.

Research Design and Methodology

The power of PhenoProfiling lies in selective single-cell isolation of living microbes from complex communities based upon a desired phenotype using ABPs paired with FACS. The isolated microbes can be further characterized by imaging and multi-omics analyses, cultured, and/or used for additional studies for deep molecular profiling of the determinants of one or more target phenotypes. Isolation of whole cells enables identification of all the enriched proteins in phenotypically-interesting microbes, rather than being limited to the catalytically active enzyme, providing a more robust molecular landscape for a particular function. Additionally, the isolated living cells can be used to create new microbial communities that perform a desired biochemical activity with high efficiency. Our proposed PhenoProfiling is still somewhat manual, but by future coupling with highly sensitive microfluidic, omic, and imaging technologies it will become a robust, high-throughput capability.

Summary of Research Results

We proposed to quantify the phenome of microbial communities through the enrichment of selected activities using PhenoProfiling. Using ABPs, we isolated highly active consortia from a native soil microbiome, targeting cellulose and lignocellulose degradation. We utilized two different activity-based probes (ABPs, Figure 1) to enrich soil microbial communities for specific activities. To profile cellulose degradation, we enriched for glucosidase and cellobiosidase activities using a reported probe with established capabilities, GH4a. To enable flow cytometric analysis, we appended a fluorophore (BODIPY, or BDP) to the existing click chemistry handle. This minor modification does not impact the portion of the probe that enables target selectivity, but allows for fluorescence-enabled visualization. For profiling of lignocellulose degradation, xylosidase and xylobiosidase activities were targeted using SY-F161, obtained from the Overkleeft lab. The selectivity of this probe is established, and the existing attachment of Cy-5 enabled flow cytometric analysis without further modification.

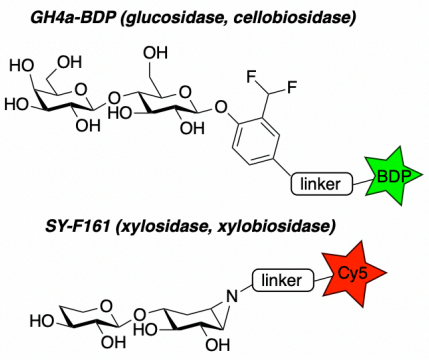

Figure 1: Structures of ABPs used for activity enrichment.

Microbes were extracted from triplicate Warden soil by vortexing the soil samples with a tris-buffered saline (TBS) plus 0.02% tween 20 mixture. Extracted cells were purified from soil debris by use of a nycodenz gradient. Microbial extracts were combined and washed and resuspended in TBS without tween. Cells were then recovered overnight in MOPS media supplemented with either xylose or cellobiose. Microbes were then resuspended in MOPS buffer containing the appropriate ABP, then pelleted and resuspended in a nucleic acid stain prior to analysis by flow cytometry.

The general gating strategy for cell sorting is shown in Figure 2. Cells were discriminated from soil debris using the nucleic acid stain fluorescence, then analyzed for probe fluorescence. Unstained, unlabeled controls were used to determine gates. “All cells”, “ABP negative”, and “ABP positive” gates were sorted into tubes for inoculation into fresh media and 16S analysis, as well as directly into a plate (500 cells/well) containing MOPS media and commercially-available methylumbelliferone (MUB) substrates for kinetic analysis of relevant activities. MUB substrates are commonly used to determine the activity of specific enzymes through the release of a fluorescent MUB molecule concomitant with enzyme-mediated cleavage, so that fluorescence directly correlates to the concentration of MUB as well as enzyme activity.

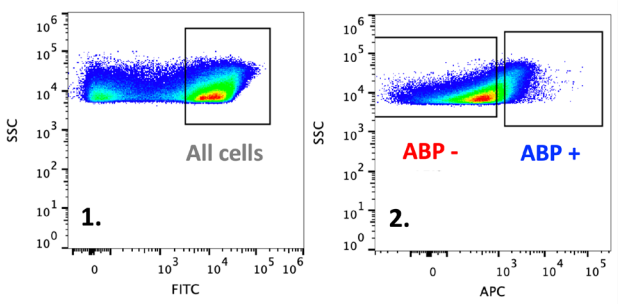

Figure 2: Representative data for general gating strategy for FACS. Cells are discriminated from debris using Syto staining in the first step, then ABP positive and ABP negative populations are gated in the second step, based on unlabeled controls.

Immediately following sorting, kinetic assays were used to track both growth (OD600) and activity (MUB fluorescence) over the course of 48 hours. Activity was then compared for each sorted population at a specific time point. OD600 values were compared at the same time to ensure that

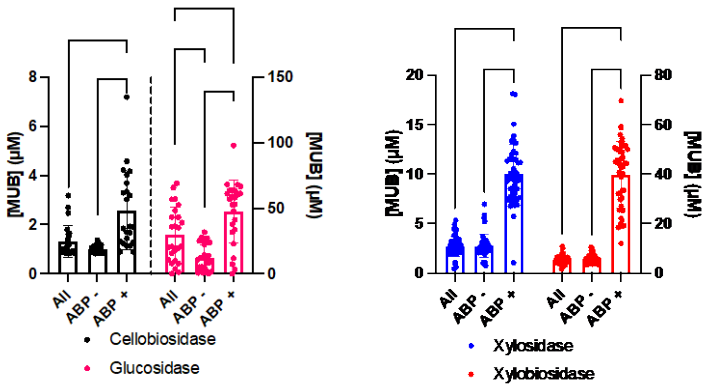

Figure 3: MUB-based activity assays show significant differences in cellobiosidase and glucosidase activity following enrichment with GH4a-BDP (left, 24 hours) and xylosidase and xylobiosidase following enrichment with SY-F161 (right, 18 hours).

differences in activity were not the result of variances in microbial growth. Using both ABPs, we observed significant differences in activity between the ABP negative and ABP positive populations (Figure 3). Data is a summary of 28 (for GH4a-BDP) and 48 (for SY-F161) experimental replicates of each sorted population.

Due to our interest in the development of stable, highly active microbial consortia, we additionally inoculated fresh media with each of the sorted populations, as well as direct microbial extract, to determine if activity enrichment is maintained following longer periods of growth (Figure 4). Cultures were incubated for 48 hours prior to adjusting for cell density and resuspension in MOPS buffer containing MUB substrates to assay activity. ABP positive communities maintained elevated activity over the ABP negative unenriched populations, although differences were less pronounced than in initial assays.

With enriched consortia in hand, we will identify not only the microbes using 16S amplicon sequencing, but also the metabolite and protein architecture associated with high biochemical activity using proteomics and metabolomics. Genomic analysis for the data presented here is underway.

In conclusion, we have successfully enriched a native soil microbiome for activities necessary for cellulose and lignocellulose degradation without relying on tedious and time consuming culturing techniques typically used to select for certain activities. These enriched communities were found to maintain differences in activity for up to 48 hours, as determined by direct kinetic assays of the given activities. This rapid process is applicable to a variety of activities and microbiomes.

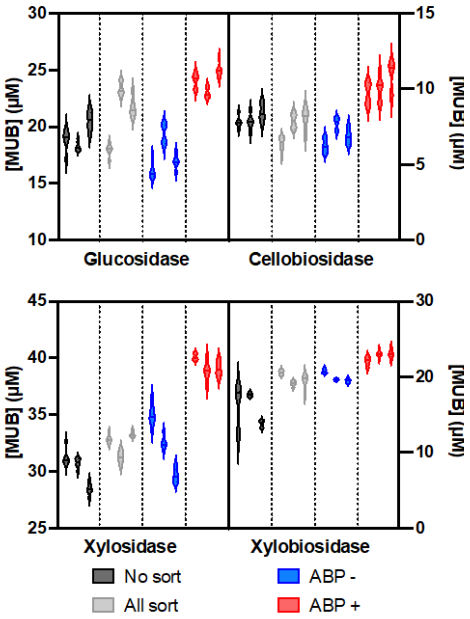

Figure 4: Activity assays confirm that activity differences are maintained for up to 48 hours following growth of enriched communities.

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