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JBEI Microbial Communities Deconstruction Research Activities

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There exist several unexplored microbial communities that are promising sources of biocatalysts and metabolic pathways for hydrolysis of polysaccharides in plant cell walls (lignocellulose), such as those found on the tropical rain forest floor and within compost. The compost environment is nutrient rich, whereas the rain forest floor is nutrient deficient. Our approach is to examine both communities to increase the likelihood of discovering a variety of novel pathways for the biological deconstruction of lignocellulose. The tropical rain forest has developed metabolic pathways that quickly convert the available biomass into useful metabolites as evidenced by the scarcity of litter on the forest floor despite high net primary productivity. These communities therefore have great potential to provide enzymes with very high specific activities and rapid kinetics of biomass degradation. In contrast, composting is an industrial process managed under thermophilic conditions. Microbial communities in compost are dynamic and vary based on the extent of decomposition of the compost, moisture, temperature, and oxygen concentration. Studying compost communities, in particular composts enriched in lignocellulosic biomass (greenwaste composts), will facilitate the discovery of novel enzymes and pathways amenable to certain high-temperature, high ionic strength, and non-neutral pH biomass pretreatment conditions, as well as insights into how such pathways are regulated for optimal lignocellulose degradation in their respective environments. Forests, and in particular tropical rain forests, have evolved to obtain the maximum nutrients from leaf litter. Thus, over many millions of years, the soil microbial communities in these environments have evolved to recycle all biomass that results from the episodic and changing forest environment. For example, the microbial communities present in the wet tropical forest soils of Puerto Rico have optimized the conversion of lignin and cellulose to methane and methanol through 40-60 million years of evolutionary selection for rapid and complete decomposition of plant material. Wet tropical forests typical of those in Puerto Rico have some of the highest known rates of plant productivity on Earth and concomitantly high microbial rates of biomass breakdown. Due to abundant rainfall and high carbon availability, these soils are frequently anaerobic leading to methane production during decomposition. Studies have confirmed that because of this frequent anaerobiosis, prokaryotes assume a primary role in macromolecular decomposition. Wet tropical soils will provide fertile ground for the discovery of novel lignin and cellulose degrading enzymes, and for new and unique consortia capable of effectively functioning under a wide range of environmental conditions. The aerobic-anaerobic cycling that naturally occurs over time and space in these wet forest soils has made selected bacterial and archaeal communities extremely effective in converting lignocellulose to methane. In composting prokaryotes are dominant in the early stages of decomposition, while both prokaryotes and eukaryotes play a role later in the process. Selecting incubation conditions that enrich both groups is an important part of our experimental approach. The Microbial Communities Department will identify and isolate key lignocellulolytic enzymes and metabolic pathways that will be delivered to the Deconstruction Division enzyme engineering group and the Fuels Synthesis Division, respectively of JBEI. During the past year this group has constructed and tested a new MycoChip microarray for rapid identification of more than 11,620 fungal taxa. Early in the year we placed bags of switch grass and lignin baited "bug traps" in the soil of the tropical rain forest to enrich for lignocellulose degraders. In only 1 week we observed increases in phenol oxidase and peroxidase. We have also observed a very high microbial diversity in these samples. Respirometer studies of switch grass with tropical rain forest soil revealed very high rates of production of CO₂, CH₄ and H₂S from the inoculated switchgrass and more than 156 different taxa from the soil alone. We also established optimal wetting conditions for decomposition of corn stover and

switchgrass inoculated with green waste compost and incubated under aerobic and thermophilic conditions. We determined that there were more than 2,602 bacterial taxa associated with switchgrass inoculated with compost. Preliminary results on Titanium 454 pyrosequencing of one tropical rain forest soil sample revealed more than 2,700 cellulases, hemicellulases, and ligninases. Similar data on a compost sample are currently being analyzed. We intend to characterize the enzyme repertoire present in the metagenomic samples, and correlate that with differences in activity and community composition between compost and tropical rain forest soil.

