Discovery And Optimization Of Lignocellulolytic Bacteria From Puerto Rican Rainforest Soils

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Tropical soils in Puerto Rican rain forests have some of the highest decomposition rates recorded in the world, with almost total mass lost in decomposing plant material within one year. These soils are capable of deconstructing biofuel plant materials to basic components, like ethanol, methane or methanol. Rapidly fluctuating redox conditions are characteristic of the highly weathered soils of upland humid tropical forests, which are dominated by Fe-oxide mineralogy and have relatively low sulfate availability. The frequent episodes of anoxic conditions make it likely that these decomposing consortia are primarily bacteria, not fungi as are usually observed in temperate systems. Previous lab incubation under fluctuating redox conditions permitted simultaneous methanogenesis, N_2O production, and iron reduction, all accompanied by steady CO_2 production. The objective of this research is to define field conditions that result in characteristic high methane production in Puerto Rico forest soils from decomposing plant materials, and determine whether different microbial communities break down different plant materials. Towards this end, we designed a field experiment and accompanying laboratory incubations that would allow us to investigate the rates, controls and mechanisms of switchgrass decomposition in tropical rainforest soils. In June of 2008, we buried litterbags filled with switchgrass in four different forest types in the Luquillo LTER, located at the El Yunque National Forest in Puerto Rico, USA. The four forest types vary from more aerobic soils, warmer temperatures and annual precipitation on the order of 1,000 mm, to fluctuating redox soils, to mostly anaerobic soils, cooler soil temperatures and annual precipitation that can exceed 4,000 mm.

The experimental design included 4 field sites, 6 time points, and bags buried in pairs, one for. At each of 6 time point, litter bags and soil are collected from the field and assayed for microbial community analysis using 16S ribosomal DNA PhyloChip, potential enzyme activity (β-glucosidase, endoglucanase xylosidase, chitinase, phenol oxidase and peroxidase), and mass loss as indicators of decomposition. In the driest site, which we expect to also have the highest rates of decomposition, we also buried biosep beads baited with lignin (using unbaited beads as controls) as bug traps to identify and isolate microbes specifically able to decompose lignin. This site was also instrumented with oxygen sensors to measure oxygen levels in soil on an hourly basis over the course of this year-long incubation, and ultimately to correlate decomposition, enzyme activity and microbial community composition with oxygen availability at the end of the experiment. Concomitantly with the field experiment, we are using fresh soil to inoculate mini-reactors with dried ground switchgrass and incubate anaerobically to enrich for lignocellulose-degrading organisms. The initial inoculation of rain forest soil with switchgrass resulted in significant CO_2, CH_4 and H_2S production compared to uninoculated, anaerobic soil incubations, as well as a substantial change in microbial community composition. Switchgrass amendment resulted in significant change in 147 taxa compared to the 1847 detected in the soils. With switchgrass addition to soil, Archaea, methanogens, enteric bacteria, Bacilli and Clostridia were significantly increased, while Acidobacteria, Burkholderia and Verrucomicrobial were significantly reduced in the microbial community. Further passages of the soil microbial community with switchgrass as the sole carbon source has resulted in a low-richness, anaerobic microbial community capable of efficiently converting switchgrass to methane and carbon dioxide as well as depolymerizing cellulose, hemi-cellulose, and lignin in the process.