43. Opportunities for Cheating? Transcriptomic Analysis of Model Organisms Exposed to Shifting Communities and Substrates

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Project Goals: Plant cell wall polymers, such as cellulose, xylan, pectin (PGA), and lignin, must be degraded into monomers before being taken up and metabolized by microorganisms. Polymer degradation is catalyzed by extracellular enzymes produced by certain microorganisms (investors). However, production of extracellular enzymes is an energy-intensive process. Thus, microorganisms shift expression of extracellular enzyme genes depending on the availability of monomers that can be taken up. During plant decomposition, cheaters are microbes that do not contribute to extracellular enzyme pools but take up polymer degradation products released by the activity of other organisms. In this experiment our goal was to examine the transcriptional and physiological responses of model fungi growing on polymers to amendment with monomers. Then, we tested predictions about enzyme expression and monomer uptake (and therefore cheating) when grown together in a mixed culture.

The model fungi *Talaromyces stipitatus* and *Schizophyllum commune* were grown in microcosms containing cellulose as a carbon substrate, requiring investment in extracellular cellulolytic enzymes. After respiration stabilized, monomers of differing types and amounts were added to replicate microcosms to determine effects on organism transcriptomes and physiology.

Respiration and enzyme activity of *S. commune* shifted slowly but for a sustained period in response to monomer amendment. There was a stronger response to glucose and galacturonic acid amendment than xylose amendment. In contrast, *T. stipitatus* responded rapidly and strongly to monomer amendment, and responded more strongly to xylose than glucose or galacturonic acid.

We also observed dramatic shifts in *T. stipitatus* gene expression 7 and 53 hr after monomer amendment. RNA was isolated from microcosms using the MoBio PowerSoil RNA Isolation kit, treated with DNase, and then mRNA transcripts were sequenced using an Illumina mRNA- seq protocol at the Yale Center for Genome Analysis. Analysis of 40 samples yielded ~190 million paired end raw sequencing reads (total 2.6 billion bases) which were analyzed using TopHat and Cuffdiff. Over half of the glycosyl hydrolases (GHs) for which transcription was detected were significantly up- or down-regulated in response to increasing xylose amendment (33 of 61 genes; $P<0.05$). Up-regulated genes include both of the beta-xylosidases for which transcription was detected, whereas down-regulated genes include several glycosyl hydrolases involved in cellulose degradation (cellobiohydrolases, beta-glucosidases, and endoglucanases). Similarly, expression of over half of the major facilitator superfamily (MFS) transporter proteins were significantly up- or down-regulated (31 of 55 transcribed genes; $P<0.05$), most of them having been up-regulated. Interestingly, genes that were up-regulated (beta-xylosidases and most MFS transporters) tended to respond to the lowest concentration xylose amendment (0.77 mM); whereas genes that were down-regulated required a higher concentration of xylose before the significant down-regulation was observed (most of them required a concentration of 4.6 mM or higher).

The data described above indicate that *T. stipitatus* is adapted to rapidly responding to small changes in available monomer concentrations by increasing uptake and altering gene expression. *T. stipitatus* appears to respond more rapidly and at a lower monomer concentration than S. commune. Furthermore, *T. stipitatus* rapidly takes up and utilizes xylose at all concentrations, but xylan-degrading enzyme activity is only induced at the higher xylose concentrations. These traits should provide *T. stipitatus* the ability to opportunistically cheat when grown with S. commune by taking advantage of transient monomers. Next, we describe results from 13C- labeling to determine which of these organisms consumes monomers when growing in mixed culture.

We then tested the prediction that *T. stipitatus* should be a cheater when grown in co-culture with *S. commune*. We conducted mixed culture experiments and amended with 13C-labeled glucose and xylose to determine patterns in C consumption and investment in extracellular enzymes. DNA stable isotope probing (SIP) was performed to detect the 13C labeled genomes. Preliminary SIP data show that 7 hr after 13C-monomer amendment, the unlabeled *T. stipitatus* DNA decreased, while DNA corresponding with labeled *T. stipitatus* and unlabeled *S. commune* DNA slightly increased. As monomer concentration or incubation time increased, the microbial DNA became more strongly labeled by 13C. However, *S. commune* DNA never became fully labeled, indicating that growth was maintained on cellulose degradation products. Labeling of *T. stipitatus* DNA may be stronger, and will be confirmed by quantitative PCR of DNA fractions. Rapid uptake of *T. stipitatus* monomers resulting in 13C labeled genomes, coupled with our previously documented stronger reductions in expression of extracellular enzyme genes in response to monomer amendments, indicate cheating of *T. stipitatus* under the growth conditions we simulated.

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