Extracellular Products Mediate Bacterial Synergism in Cellulose Degradation

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Project Goals:

1) Investigate the links between microbial biodiversity and plant biomass decomposition rates

2) To understand the molecular bases of microbial interactions during plant biomass decomposition

It has been demonstrated that microbial species richness influences ecosystem level processes such as litter decomposition, CO_2 flux and nitrogen cycling, however it is unclear how species interactions and dynamics affect these ecosystem services [1]. Understanding how microbial species coexist and interact during plant biomass decomposition is essential for the establishment of links between biodiversity and carbon cycling. Natural microbial communities often harbor thousands of species, making it difficult to identify the functions and interactions of individual community members. To overcome these limitations, some researchers have utilized synthetic microbial consortia as simplified models of natural microbial communities. This approach allows manipulation of species diversity and taxonomic composition as well as control of environmental variables. Using this bottom-up approach, Tiunov and Scheu [2] manipulated the species diversity of communities of fungal decomposers (ranging from 1 to 5 species) and identified that facilitative interaction plays a major role in decomposition processes.

In order to simplify the richness and interactions in decomposer communities, we established soil-derived microbial consortia enriched on pretreated switchgrass. Five bacterial genera were consistently isolated from these consortia: (A) *Cellulomonas*, (B) *Cellulosimicrobium*, (C) *Ensifer*, (D) *Pseudomonas* and (E) *Ochrobactrum*. To investigate the social interactions among these isolates, we manipulated the species richness of 31 bacterial consortia representing all the possible combinations of isolates (from mono-cultures to five species in combination) and assessed their growth and decomposition rates on different carbon sources: glucose, polyglutamic acid, xylan and cellulose.

Interestingly, we found that facilitative interactions among members of the consortia emerged on complex substrates and, in general, multispecies consortia outperformed the decomposition and productivity rates obtained by mono-cultures (Figure 1). These results have

important implications on our understanding of microbial symbiotic interactions and microbial coexistence in nutrient-poor environments.

Genome sequencing of the five strains revealed that the increase in the CAZy domains (or lignocellulolytic enzymes) repertoire of the multispecies consortia do not explain the observed performance of the consortia.

We further analyzed the most cellulolytic pair of strains (*Cellulomonas*-*Pseudomonas*) to understand the molecular bases of the synergism during cellulose degradation Figure 1B). Enzymatic activity profiling of secreted proteins also revealed that there are no synergistic interactions between the enzymes secreted by different species. These results indicate that exometabolites are likely to mediate the coexistence of these species during plant biomass decomposition. We utilized untargeted GC-MS to analyze the extracellular metabolites of the mono- and co-cultures to identify the metabolites involved in facilitative interactions. Vitamins and growth factors were identified as potential mediators of microbial interactions during plant biomass decomposition. One example is the nicotinic acid which is detected in the mono-culture of *Pseudomonas*, but showed decreased peak area in the co-culture, indicating its consumption by *Cellulomonas*. Moreover, the co-culture of *Cellulomonas* and *Pseudomonas* was found to produce high amounts of some organic acids of industrial interest (included in the list created by the Biotechonology for Biofuels: [3]), such as malic acid and phenyllactic acid. Our findings represent an important step toward understanding the molecular bases of microbial interactions during plant biomass decomposition.

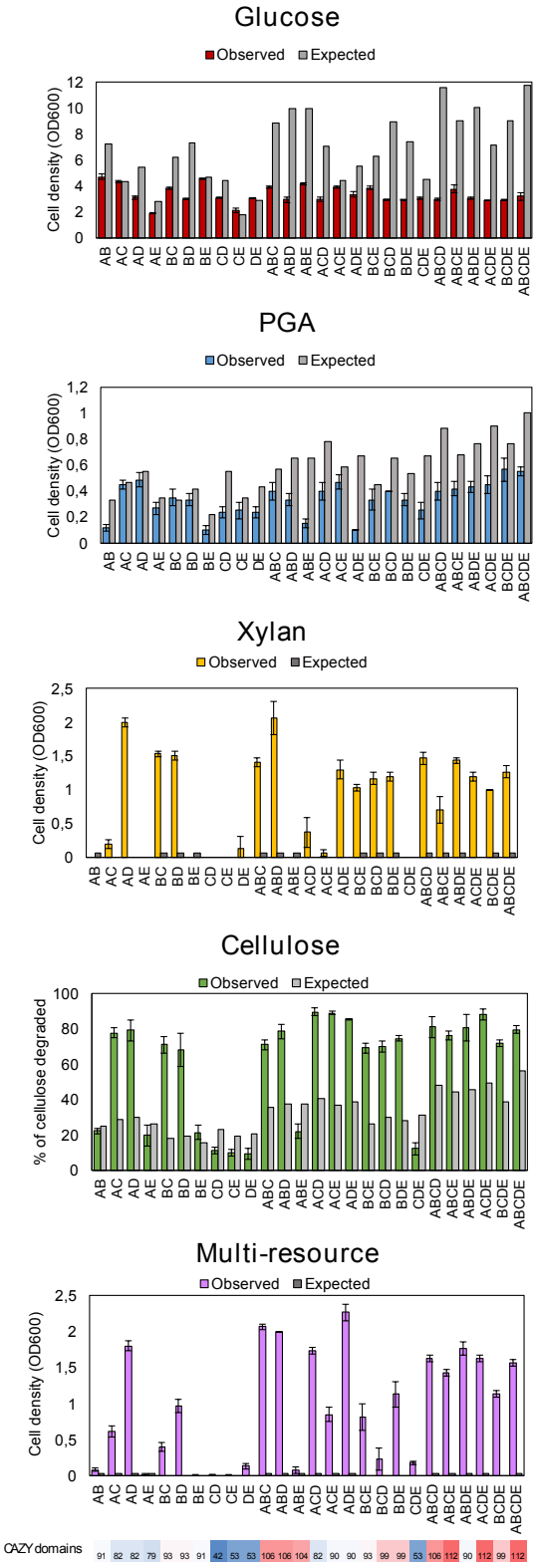

Figure 1. Effect of species richness and composition on total growth and degradation rate of various plant biomass polymers. Expected values were calculated by an additive model of the OD600 of monocultures growing at the same polymer.

References

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