Defining host-symbiont collaboration in termite lignocellulose digestion

“The view from the tip of the iceberg”

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Termites have the unique ability to exploit lignocellulose as a primary nutrition source. Traditionally, termite lignocellulose digestion has been considered as a gut-symbiont-mediated process; however, in recent years the importance of host digestive capabilities have become apparent. Despite this growing understanding, how digestive enzymes from different origins specifically collaborate (i.e., additively or synergistically) has remained largely unknown. In a recent study, we undertook translational-genomic studies to address these questions in the lower termite Reticulitermes flavipes (Isoptera: Rhinotermitidae) and its symbiotic gut fauna. We used a combination of native gut tissue preparations and recombinant enzymes derived from the host gut transcriptome to identify synergistic collaborations between host and symbiont, and also among enzymes produced exclusively by the host termite. These findings provided important new evidence of synergistic collaboration among enzymes in the release of fermentable monosaccharides from wood lignocellulose, and laid a foundation for future integrative studies into termite digestion, symbiosis and eusociality.

In the termite gut, lignocellulose is digested to release glucose and pentose sugars, which serve as inputs for essential metabolic pathways.1,2 For nearly a century the paradigm was that termite digestion of lignocellulose was mediated solely by microbial symbionts located in the hindgut paunch (Bacteria, Archaea and Protista).3,3-8 Although symbionts participate in lignocellulose digestion and other important metabolic processes,9-11 mounting evidence demonstrates that termite-derived activities are also responsible for this digestion. Despite early evidence suggesting host-based lignocellulose digestion capabilities in termites,4,12-14 absolute evidence of host digestive capabilities finally came with the identification of an endogenous termite cellulase gene having exclusive expression in symbiont-free salivary gland tissue.15 Since this determination, through the use of integrative molecular biology and biochemistry studies, considerable evidence of host digestion capabilities has accrued,2,16-23 now extending beyond cellulases to lignases and phenol-oxidases.18,24,25 However, despite a growing understanding of host and symbiont-mediated digestion capabilities, the relative contributions of these mutualistic partners to digestion (i.e., monosaccharide release) have remained essentially unknown.

To address some of these outstanding questions we recently published a study which sought to quantify the collaborative lignocellulose digestion capabilities of host and symbiont.26 We used the “lower” termite Reticulitermes flavipes, which has three distinct gut regions (Fig. 1A), hosts 12–13 cellulolytic protists,6 and thousands of bacterial and archaeal symbionts (Boucias et al. In preparation). These studies were enabled by three important experimental advances. First, techniques to isolate viable protein extracts from host and symbiont gut fractions were...
developed. The host gut fraction consisted of salivary gland, foregut and midgut tissues; whereas, the symbiont fraction included the hindgut and its microbial flora and fauna (Fig. 1A). Second, we have developed colorimetric monosaccharide detection assays that enabled rapid quantification of glucose and pentose released from pine wood lignocellulose and other cellulosic substrates in digestion assays.26 Third, we have produced three highly pure recombinant host enzymes and their relative degrees of synergy measured using lignocellulose and hemicellulose substrates. (C) Candidate host and symbiont enzymes that are the focus of ongoing research.

**Host-Symbiont Synergy**

Pine wood lignocellulose, the same material used to provision laboratory termite colonies, was used as a substrate in digestion assays with native gut tissue preparations. After 10–20 h incubations, released glucose and pentose were quantified using colorimetric methods noted above. Interestingly, the host fraction accounted for ~33% of glucose released and the symbiont fraction for about ~66%, indicating a clear enzymatic division of labor between host and symbiont. Levels of released pentose were significantly lower than levels of released glucose and showed a non-significant 40:60% split among host and symbiont fractions. In agreement with these findings, previous sequencing efforts revealed nearly twice as many cellulase-coding transcripts as hemicellulase-coding transcripts in the *R. flavipes* gut metatranscriptome. In a final native tissue experiment we compared monosaccharide release in individual host and symbiont incubations to pooled, “whole-gut” incubations. Interestingly, in this experiment pooled whole-gut incubations provided a synergistic increase in saccharification relative to the additive effects of each fraction alone. These were important results because they provided seminal evidence that host and symbiont collaborate synergistically, rather than additively, in lignocellulose digestion.

**Synergy among Host Enzymes, but with Tradeoffs**

Studies were conducted with recombinant host enzymes that used a number of substrates, most notably, pine lignocellulose and beechwood xylan. When combined, the Cell-1 and β-gluc cellulases showed >300-fold and >70-fold increases in amounts of glucose released from pine lignocellulose and beechwood xylan, respectively, relative to each enzyme alone (Fig. 1B). However, the three-enzyme combination of LacA + Cell-1 + β-gluc released a smaller amount of glucose from pine lignocellulose than the two-enzyme combination of Cell-1 + β-gluc, indicating some type of inhibition to be occurring (Fig. 1B). Conversely, the same three-enzyme combination released greater glucose from beechwood xylan than the Cell-1 + β-gluc combination (Fig. 1B), indicating that LacA significantly enhances glucose release from hemicellulose by host cellulases, presumably through lignin-hemicellulose disassociation. These findings were significant because they showed glucose release capabilities from various forms of cellulose and lignocellulose by two host cellulases and a phenol-oxidizing laccase.

In subsequent kinetic analyses, reductions in $K_m$ and $V_{max}$ for the recombinant β-gluc enzyme in the presence of glucose revealed that glucose un-competitively inhibits β-gluc. This finding explained the limited glucose output from pine lignocellulose by the three-enzyme combination. This phenomenon, termed **end product inhibition**, suggests the existence of a catalytic tradeoff among host enzymes. If such inhibition is truly physiologically significant, it supports the hypothesis that symbiont-assisted digestion evolved to its present-day status because it enhanced overall digestive efficiency. In addition to improving digestive efficiency, symbiosis also is thought to have facilitated group living in termites and eventually, evolution of eusociality.
Directions

In conclusion, our recently reported study revealed synergistic collaboration in lignocellulose digestion by a lower termite on two levels: (1) between host and symbiont and (2) among host enzymes. While compelling, we only consider these findings as a metaphorical “tip of the iceberg.” An important next step in this research will be to test other recombinant host phenol-oxidases/lignases, as well as symbiont cellulases and hemicellulases in combination with the three enzymes already tested (Figs. 1B and C).18,24,30

Moreover, complementary genomics studies have recently been completed that investigated: (1) prodakroyte diversity via 454 pyrosequencing of 16S PCR amplicons (Boucia et al. In preparation), (2) differential transcript expression in response to lignin feeding via quantitative 454 pyrosequencing (Sethi et al. In preparation) and (3) host and protist-symbiont metagenic expression in response to cellulose and lignocellulose feeding using host-symbiont “digestome” micro-arrays (Raychoudhury et al. In preparation). Such an integrative approach, using a combination of translational biochemistry and functional genomics/transcriptomics will provide informative, and novel results that deepen our understanding of termite digestion, symbiosis and sociality.

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