RESEARCH in Progress

Coalition for Advanced Wood Structures
a university, industry, government partnership

Effect of Chitosan on the Termite Digestome

The importance of wood as a construction material is unequivocal, and although market competition is increasing with development of new products, consumer preference for renewable and sustainable materials is playing a vital role. One propaganda target of competitive industry is chemically treated wood. Recent concerns about copper-based preservatives have motivated the forest products industry to find environmentally safe alternatives that meet the demands of green construction. One promising non-copper wood protection agent is chitosan. Industrial quantities of chitosan can be inexpensively synthesized from chitin, a waste polysaccharide generated during shrimp processing. Importantly, chitosan exhibits activity against both wood decay fungi and some species of insects. However, the activity of chitosan against termites is still unexplored.

Background

Termites are social insects that can be both appreciated and abhorred: *appreciated* because they play a vital role in maintaining resilient forests, decomposing nutrients locked up in the trees and returning them back to the soil and air; *abhorred* because they can become pests, causing an estimated $11 billion of damage annually to construction wood in the United States. Most of that damage is attributed to the eastern subterranean termite, *Reticulitermes flavipes*, a commonly encountered native insect found in the eastern half of the United States.

The termite diet of wood is highly recalcitrant and requires a joint digestive effort of the termite and its microbial symbionts. The symbionts are restricted to the hindgut portion of the alimentary canal (Fig. 1, top) where their densities can reach amazing proportions

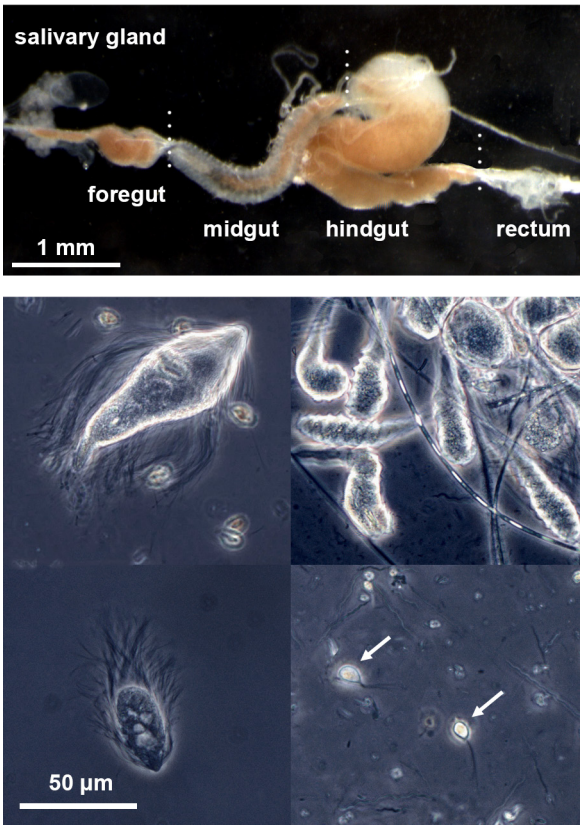

Figure 1—Sections of the alimentary canal dissected from *R. flavipes* (top panel); examples of flagellate protists found in the hindgut (bottom panel).

of 10^6 to 10^8 microbes per termite. Numbered among these microbes are about a dozen species of protists (Fig. 1, bottom), several hundred or more species of bacteria, and a few species of archaea. Recent studies have shown that the termite diet can influence the composition of the symbiont community, the expression of transcripts that encode wood-degrading enzymes, and the enzymes themselves.

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Objective

The objective of this study is to examine the potential for using chitosan as a wood preservative against termites. We aim to answer three questions: (1) does chitosan possess termiticidal properties? If yes, then (2) will termites be able to adapt to chitosan-treated wood by adopting symbionts that are not affected by chitosan antimicrobial properties? If not, then (3) what biological mechanism is responsible for resistance?

Approach

Question 1 will be answered using standard feeding tests to establish the toxicity threshold of chitosan-treated wood (Fig. 2). Three different populations of *R. flavipes* will be screened against different levels of chitosan-treated wood. Once the toxicity threshold is determined, wood samples will be treated with predetermined concentrations of chitosan, and then half the treated samples will be exposed to environmental conditions for 6 weeks to allow for establishment of chitosan-resistant microorganism population on wood. All wood samples will then be conditioned and exposed to termites. Assuming all three populations of termites tested can consume wood treated with chitosan, the termite hindguts will be pooled for DNA and RNA extractions.

Figure 2—Standard termite feeding test, showing undamaged, protected (samples 6 and 7) and attacked control wood (samples 31 and 32) with termites over the samples. (Courtesy of Linda Sites, Mississippi State University.)

Because of the complexity of the bacterial symbiont community, the only practical method that can capture changes in its composition and help answer question 2 is targeted amplicon sequencing, a metagenomics analysis that involves system-wide profiling of a specific DNA sequence. Because protists are far less complex than bacteria, changes in protist composition will also be assessed by direct counts taken under the microscope.

Resistance to chitosan will necessarily rely on a biological function that involves expression of protein(s) or protein product(s), wherein lies the answer to question 3. Because RNA, the direct precursor of protein expression, is much easier to work with and identify than protein, a metatranscriptomics analysis will be performed. Identification of total termite and symbiont RNA and their putative roles in the hindgut will be based on matching alignments of nucleotide sequence against accumulated knowledge in a public database such as the National Center for Biotechnology Information.

Expected Outcomes

The research will provide insight to the potential for using chitosan, an environmentally safe preservative, to protect wood, including its termiticidal properties and effectiveness against termites and their entire symbiont population. Studying the DNA and RNA profiles of termite gut systems will give insight to the mechanism of wood degradation by termites that can be used to improve current approaches to protection and to develop novel means of protection. This information can also lead to mining of enzymes to further facilitate wood conversion to energy and value-added products. The metagenomics component of this project will provide a wealth of data that can be mined for future studies.

Timeline

The project was initiated in April 2015; research on termiticidal properties (question 1) will be completed by March 2016. Research on adaptation of termites to chitosan and potentially resistant symbionts (question 2) will be completed by March 2018. Research on biological mechanisms of resistance (question 3) will be completed by March 2020.

Cooperators

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