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CHARACTERIZING LIGNIN DEGRADATION IN A FUNGUS-CULTIVATING TERMITE

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MAIN CONCLUSION

Young termite workers rapidly depolymerize and degrade even the most recalcitrant wood lignin structures facilitating polysaccharide cleavage by symbiotic fungi [1].

INTRODUCTION

Lignin is a heterogenous plant cell wall polyphenol derived primarily from hydroxycinnamyl alcohols via combinatorial radical coupling reactions containing ether- and C-C linkages [2]. Its depolymerization is challenging, making lignin a major barrier to gaining access to stored energy within lignocellulosic materials [3]. Termites from the subfamily Macrotermitinae form a monophyletic group of species that have cultivated fungi (*Termitomyces* spp.) using wood materials and are able to decompose lignocellulose almost completely [4]. Fungus-cultivating termites have evolved in mutualistic association with multiple microbial symbionts, not only with lignocellulose-digesting basidiomycetes fungi, but also with gut microbiota associated with their fungus comb [5]. The way Macrotermitinae termites and symbiotic microbiota circumvent the lignin barrier and cleave polysaccharides into easily digestible sugars remains a mystery [5]. In this study, we examined the degradation mechanisms of wood lignin and polysaccharides through each stage of the plant substrate flow using a laboratory rearing colony of *Odontotermes formosanus* [6].

MATERIALS AND METHODS

We characterized the fungus comb and sound poplar wood using SEM and chemical compositional analyses. We then determined the transit time of wood particles through the guts of young worker termites using food-stain marker feeding experiments. Then, to gain molecular-level understanding, we performed solution-state ^{1}H–^{13}C heteronuclear single quantum coherence nuclear magnetic resonance (HSQC NMR) spectroscopy on whole-cell-wall material from the fungus comb. Pyrolysis gas-chromatography-mass-spectroscopy (Py-GC-MS) was used to identify releasable lignin-derived phenols from the fungus comb. High performance anion exchange chromatography (HPAEC) analysis was used to separate and identify oligosaccharides found the non-hydrolyzed fractions of the fungus comb materials.

RESULTS AND DISCUSSION

Wood fibers collected from the fungus comb at the fresh, middle-aged, and mature stages revealed a progressively smaller average particle length of 44.6, 29.0, and 14.4 μm,

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respectively. Chemical composition analysis showed a loss of 13%, 45%, and 60% of their lignin in the fresh, middle-aged, and mature comb samples, respectively. From the food-stain marker feeding experiments, the passage of stained wood through the young worker gut was 3.30 ± 0.04 hours. HSQC NMR spectroscopy of whole-cell-wall material from the fungus comb was used to elucidate the relative distributions of the various lignin subunits, characterized by their specific interunit linkages, as well as the polysaccharide profile. From the NMR spectra, the lignin aromatic regions gave syringyl-to-guaiacyl (S/G) ratios of fresh (B), middle-aged (C), and mature (D) fungus combs of 5.23, 6.61, and 16.39, respectively, compared with 1.68 in sound poplar wood (A) (**Figure 1**). Therefore, the G units were preferentially degraded in all fungus combs relative to the S units. In the lignin sidechain regions, the β-ether units decreased by 28% going from the sound wood to the fresh fungus comb, followed by a further 57% from fresh to the middle-aged comb, but no further depletion in the mature fungus comb. The resinol units also showed a substantial decrease with 56% depletion in the fresh fungus comb and complete removal by the middle-aged comb. Phenylcoumarans, spirodienones, and cinnamyl alcohol end-units had also essentially disappeared before the material made it to the fresh fungus comb. Ring methoxyl groups are appreciably removed, and lignin sidechains are extensively cleaved leaving a residue (fresh fungus comb) almost completely devoid of various condensed units that are identifiable by their characteristic C-C linkages. Py-GC-MS found vanillic acid in high abundance in the fresh fungus comb (8.3%), suggesting that the majority of β-ether cleavage takes place in the young worker gut, but is followed by further cleavage by the fresh comb microbiome. HPAEC analysis demonstrated that the fungus-comb microbiome contributes significantly to polysaccharide cleavage, enriching the mature comb in cellulosic-based oligomers for the old-worker termite to consume.

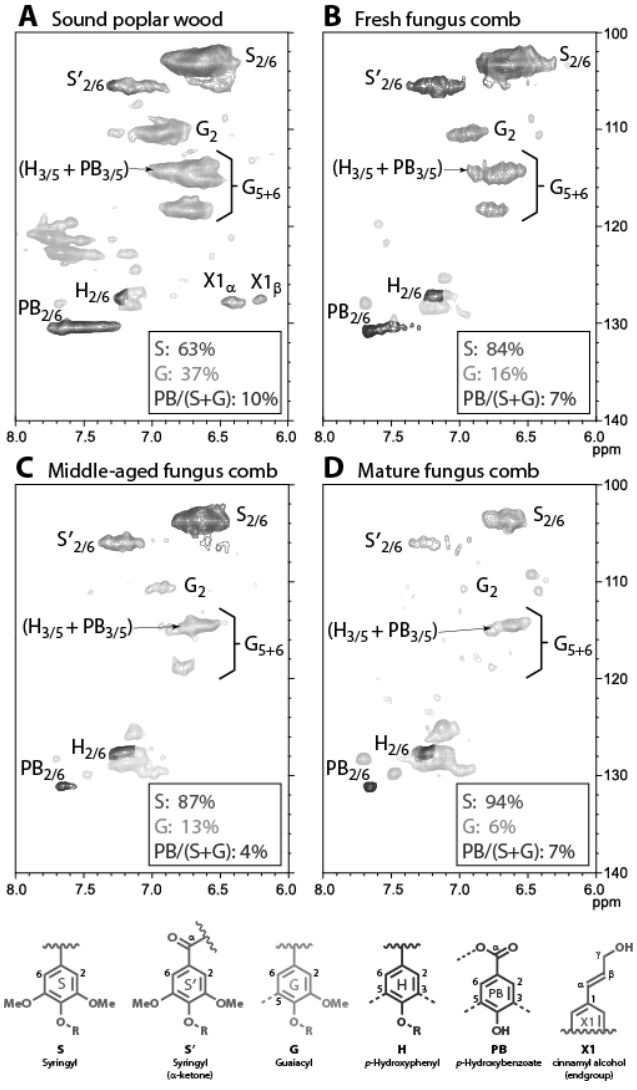

Figure 1. ^{1}H–^{13}C HSQC NMR spectra of cell walls from fungus comb samples showing degradation of the lignin aromatic units as the fungus comb matured.

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