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Aromatic compound degradation by the wood-feeding termite *Coptotermes formosanus* (Shiraki)

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ABSTRACT

Wood-feeding termites (WFT) have proven to be highly efficient for wood digestion. There is evidence to support the hypothesis that there are ligninolytic enzymes existing in the gut of WFT responsible for wood pretreatment toward cellulose utilization. Elucidating the mechanism of biomass pretreatment through lignin modification in termites will help to develop more efficient lignocellulosic biofuel production processes. The in-vivo degradation of aromatic compounds with different substructures, including dyes, lignin model monomers and dimers, and lignin sulfonate, by *Coptotermes formosanus* (Shiraki) was investigated. The degradation of aromatic compounds was determined using pyrolysis-gas chromatography/mass spectrometry. The results revealed that WFT were able to metabolize the conjugated aromatic structures and that the degradation efficiency is higher in the foregut and midgut regions than in the hindgut. This is the first time that evidence has been provided to show different aromatic compound degradation in the separate gut segments of a termite. This study provides information on the *C. formosanus* (Shiraki) lignin modification phenomenon, and it demonstrates that phenomenon's potential in the breakdown of the plant cell wall. Understanding this lignin modification could contribute to technology that will supplant current harsh pretreatment protocols for plant cell walls and thereby better facilitate the conversion of cellulose and hemicellulose.

1. Introduction

Improving the efficiency of lignocellulosic biomass pretreatment is essential for economic cellulose hydrolysis and lignocellulosics-based biofuel production (Mosier et al., 2005). Wood-feeding termites (WFT) are created by nature to utilize wood as their sole food source and are capable of efficient wood-cellulose degradation in a matter of hours (utilization of 65%–99% cellulose and hemicellulose in wood within 24 h) (Esenther and Kirk, 1974; Wood, 1978), instead of weeks, as in fungal and bacterial systems (Chen et al., 2010). This makes WFT a promising model organism for biological pretreatment of lignocellulose under natural conditions. Currently, how termites accomplish the rapid wood-cellulose conversion process is at the center of scientific curiosity. Since cellulose and hemicellulose are protected by lignin from being damaged (Chabannes et al., 2001; Böerjan et al., 2003), lignin removal, or at least modification, should be required for wood-cellulose hydrolysis (Lorenz et al., 2009). Brune et al. (1995a), Brune et al. (1995b) found the existence of an oxygen gradient in termite guts and demonstrated it to be a critical co-substrate for degradation of aromatic compounds. By collecting the respired $^{14}CO_2$ after feeding termites on natural and synthetic ^{14}C labeled lignins and related compounds, Butler and Buckerfield (1979) and Cookson (1987) demonstrated the disruption of synthetic lignin and wood lignin by the WFT. Furthermore, characterization of natural polymeric lignin after WFT digestion, and identification of low-molecular-weight products from wood-lignin disruption (Geib et al., 2008; Ke et al., 2010), have provided considerable insights into the chemistry of lignin modification by WFT. However, the relationship of lignin degradation/modification in different gut compartments of WFT is less understood, as previous studies relied on the overall degradation of synthetic lignin by WFT. We propose the hypothesis that WFT have evolved an efficient lignin modification process that is dependent on the particular gut compartments for further efficient wood-cellulose hydrolysis.

Lignin itself is a heterogeneous natural polymer with phenylpropane chains linked by carbon–carbon and ether bonds (Feldman et al., 1986). The exact chemical structure and specific reactions involved in its biodegradation are not yet clear. The complexity of lignin structure has continually challenged researchers to characterize it prior to and following biological digestion in order to understand the biomass digestion

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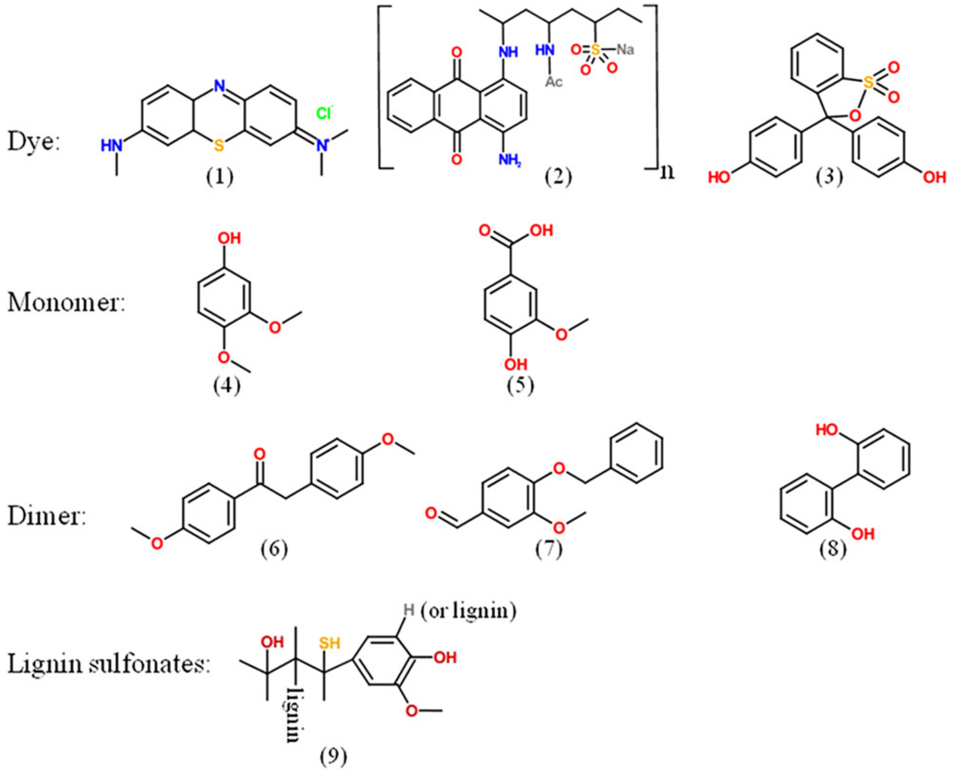

Fig. 1. Structures of the aromatic model compounds used in this study. 1 – azure B; 2 – poly B-411; 3 – phenol red; 4 – veratryl alcohol; 5 – vanillic acid; 6 – desoxyanisoin; 7 – benzylvanillin; 8 – 2,2′-biphenyldiol; 9 – lignin sulfonate.

mechanisms. This is especially true in the case of WFT, because of the difficulty of gut sample collection. Meanwhile, one of the unique properties of the lignin-degrading enzymes is the low substrate specificity (Kirk and Tien, 1984; Muñoz et al., 1997). Substrates vary from polymeric lignin to dimeric and monomeric lignin model compounds, along with other low-molecular-mass aromatic compounds. Hence, specific reactions and their biochemistry could probably be elucidated through investigations of the metabolism of lignin substructure models, especially the metabolism of specific structures or linkages in different segments of the termite gut. These model compounds are produced by plants and transformed from lignin during its depolymerization, and the simple structures make them easier to study in the presence of the ligninolytic enzymes in the termite gut and the absence of lignin. Previous studies showed that the ligninolytic activities in biological systems could be studied by observing the modification/degradation of dyes, lignin monomers, lignin dimers, and lignin sulfonate (Archibald, 1992; Cui et al., 1993; Kuhnigk and Konig, 1997; Kato et al., 1998; Harazono et al., 2003). However, there has been no report on direct observation of structural modification on aromatic compounds in termite guts.

In this study, termite degradation experiments with nine aromatic model compounds are described. We employed Py-GC/MS to demonstrate the biodegradation of various compounds.

1.1. Background

Compound 1 was employed in an assay for lignin-type peroxidases. It became discolored when converted to veratraldehyde by lignin-type peroxidases (Archibald, 1992). Likewise, compound 2 is a blue, anthraquinone-based polymeric dye (Glenn and Gold, 1983) that has been shown to be useful as a lignin model in studies on lignin biodegradation (Cui and Dolphin, 1991; Nerud et al., 2004). Only fungi with known lignin-degrading ability were able to decolorize compound 2 (Platt et al., 1985). Compound 3 exists as a red crystal with an aromatic structure, and its decolorization has been used by researchers to indicate radical-generating reactions catalyzed by laccase and other ligninolytic enzymes (Nerud et al., 2004; Sathiya et al., 2007). Monomeric lignins, such as compounds 4 and 5 (Fig. 1), could be oxidized by lignin peroxidases (LiP) or manganese peroxidases (MnP) (Leisola et al., 1987; Hattori et al., 1988; Cui and Dolphin, 1992; Harazono et al., 2003). In softwood lignin, the minor lignin substructures of arylglycerol-β-aryl (β-*O*-4) and 5-5 lignin model compounds represent 50% and 5% of

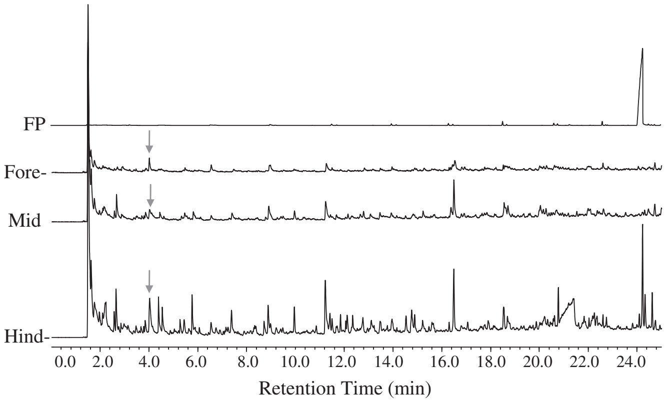

Fig. 2. Pyrogram. Filter-paper-fed-termite-gut content as the negative control. FP: filter paper. The guts from filter-paper-fed termites were used as a negative control and compared to the target-compounds-fed termite gut for aromatic compound degradation. The guts isolated from filter-paper-fed termites were considered to be lignin-free except the toluene peak at 4.079 min (pointed out by the arrows). All the other peaks are carbohydrate-related and fatty acids.