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Detoxification Related Genes in Gut of *Coptotermes curvignathus*

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Abstract

Coptotermes curvignathus (*C. curvignathus*) are subterranean termites that feed on living-tree as their sole diet, which consist mainly of cellulose, hemicelluloses, lignin, plant allele chemical and other environmental residues such as insecticide. The xenobiotic compounds, plant allele chemical and insecticide are hazardous to termites health and need to be transported out of their body via xenobiotic and detoxification metabolism. This paper highlighted the potential enzymes that play vital role in the xenobiotic and detoxification metabolism. Transcriptomic data were generated from 200 termite's digestive system using Illumina HiSeq 2000. Raw data was trimmed and assembled by SOLEXAQA and Bowtie before loaded into Gene Ontology based data mining software, Blast2GO (B2G). The result showed that, *C. curvignathus* contain enzymes that involved in all three biotransformation phases of xenobiotic and detoxification metabolism, which included cytochrome P450s monooxygenases, glutathione S-transferase, carboxylesterase, UDP-glucuronyltransferases and N-acetyltransferase. The result of this study is the first insight into Cc xenobiotic pathway.

Keywords: *Coptotermes curvignathus*; Xenobiotic; Detoxification; Cytochrome P450; Glutathione S-transferase

Introduction

The most intensively studied wood feeder termites; *Reticuliformis sp.* And *Coptotermesformosanus* are known to feed on dry dead wood or partially degraded wood residue [1,2]. However, their close sister species *Coptotermes curvignathus* (*C. curvignathus*) is said to evolve and adapt to feed on living plant instead of decomposed wood residue [3]. This indicates that *C. curvignathus* able to overcome plant defense mechanism and achieve effective lignocelluloses biomass conversion. Lignocellulosic of living plant is mostly consisting of cellulose, hemicelluloses, lignin and other compound, such as plant allelochemical that act in plant defense mechanism.

Recent studies of termite transcriptomes have focused mainly on degradation of cellulose and hemicelluloses [1,2,4]. The study regards termite metabolizing on xenobiotic substances such as lignin, plant allele chemical and insecticide is hardly to be found. As being the oldest living fossil [5], termite is believed to have the ability to protect itself from xenobiotic residue through its metabolic pathway. Xenobiotic compound may undergo biotransformation phases to transform harmful toxin to unharmed residue and transported out of the host's body [6]. Thus, this paper is to highlight *C. curvignathus*'s metabolic resistance agent in combating xenobiotic compounds.

Experimental Procedure

Sample collection

Rubber wood were used for baiting and put next to *C. curvignatus* infected tree for about one or two months. Harvesting was done after bait was about 50-60% eaten, and kept in the dark container for several nights before proceeding with lab experiment.

RNA extraction and DNase treatment

About 200 worker termites were collected and gut dissected. Dissected gut was stored in liquid nitrogen and RNA extraction was carried out according to Sepasol Super G (Nacalai, Inc. http://www.nacalai.co.jp) protocol. The gut was homogenized in Sepasol Super G and sequently added with choloroform. After centrifugation, supernatant was collected and mixed in isopropanol. DNase treatment was carried out according to Sepasol Super G DNase treatment protocol.

Library preparation

The library was constructed using the TrueSeq RNA sample Preparation Kit (Illumina, Inc. http://www.illumina.com) according to manufacturer's Low Throughput (LT) protocol with some modification. 200 ng of total RNA was used as starting material. The mRNA enrichment was performed first using mRNA-ONLY™ Prokaryotic mRNA isolation Kit (Epicentre, an Illumina company), this step is used to substitute the fragment and purify step in the LT protocol. Subsequently, first and second cDNA was synthesized, unique adapters were then ligated. Double strand DNA (dsDNA) fragment with ligated adapter were enriched using 15 cycles of PCR. Library was then assessed for fragment size distribution using BioAnalyzer 2100. The concentration of dsDNA adapter-ligated library was then determined by quantitative PCR (qPCR). Library was diluted to 10nM and then pooled with other sample in equal volume for cluster generation.

Pooled libraries were loaded on individual lanes of IlluminaHiSeq 2000 flow cell. Sample were then sequence on IlluminaHiSeq 2000 with 100 bp pair-end reads, with image analysis and base calling performed with HiSeq Control Software. Raw flow-cell data was processed and demultiplexed using SolexaQA (Illumina) for the sample.

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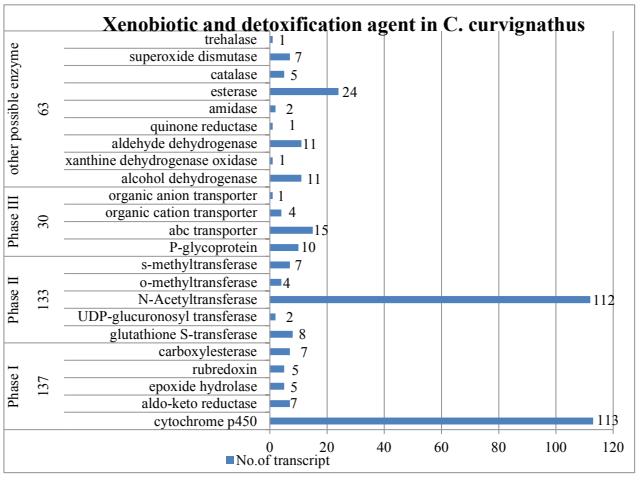

Figure 1: Bar chart of list xenobiotic and detoxification agent found from Coptotermes curvignathus EST library.

Data analysis

Raw data was trimmed based on (1.) base quality, Q_{phred} = 20, and (2.) sequence length > 50 bp. Bowtie was used to screen for phiX reads and other contaminant in sequencing process. The paired end and singleton were assembled and analysed using OASES.

Result and Discussion

A total of 25295 transcripts were generated from *C. curvignathus* library. About 373 of the transcripts were identified as possible genes in xenobiotic and detoxification metabolism. Of that amount, 113 transcripts were cytochrome P450 monooxygenase (CYP), seven transcripts were carboxylesterase and eight transcripts were glutathione S-transferase (Figure 1). These are the known enzymes that serve as important xenobiotic and detoxification agent. As summarize in Figure 1, there were five genes involved in phase I biotransformation, five genes in phase II biotransformation, four genes of phase III biotransformation and nine genes viewed as other possible enzyme or gene.

According by Xu et al. [7], Phase I biotransformation detoxification enzymes represent the most abundant class of xenobiotic-metabolizing enzymes. Phase I, are basically enzymes of hydrolysis, reduction or oxidation which introduce reactive groups into molecules, increasing water solubility. Most organisms exist with the presence of CYP were among the phase I biotransformation detoxification enzyme. CYP has been reported to have key roles of plant-insect interaction especially insecticide resistant [8,9]. CYP is also reported to in toxicants metabolized-nicotine, acetaminophen, procarcinogenic substances, benzene and polyaromatic hydrocarbons. Besides CYP, another known xenobiotic and detoxification metabolism agent are carboxylesterase.

Phase II biotransformation enzyme act on by products of Phase I transformation. Those listed in Figure 1 such as glutathione S-transferase (GST), UDP glucuronosyltransferase (UGT) and N-acetyltransferase are known to add bulky side group onto toxic compound to increase their hydrophilicity, then facilitating the excretion of the compound from the host body [10]. GST usually catalyse the conjugation of glutathione to electrophilic toxic molecules, increasing their solubility meanwhile UGT acts as catalysts for the transfer of glycosyl group from UDP-glucose to a variety of acceptor molecules [6]. GST in insects has been widely observed due to their involvement in defense against insecticide, which mainly organophosphate, organochlorides and cyclodienes. Unlike GST, there is not much evidence or information on the role of UGT in detoxification of xenobiotic in insects.

Based on the transcriptome of *C. curvignathus*, termites contain enzymes that enable them to defend against xenobiotic compounds through an elaborated three-phase xenobiotic-detoxification system.

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References

1. Zhang D, Lax AR, Henrissat B, Coutinho P, Katiya N, et al. (2012) Carbohydrate-active enzymes revealed in Coptotermesformosanus (isoptera: rhinotermitidae) transcriptome. Insect Mol Biol 21: 235-245.
2. Tartar A, Wheeler MM, Zhou X, Coy MR, Boucias DG, et al. (2009) Parallel metatranscriptome analyses of hosts and symbiont gene expression in the gut of the termite Reticulitermesflavipes. Biotechnology for Biofuels 2: 25.
3. King JH, Mahadi NM, Bong CF, Ong KH, Hassan O (2013) Bacterial microbiome of Coptotermes curvignathus Holmgren (Isoptera: Rhinotermitidae) replects the co-evolution of species and dietary pattern. Insect Science.
4. Raychoudhury R, Sen R, Cai Y, Sun Y, Lietze VU, et al. (2013) Comparative metatranscriptomic signatures of wood and paper feeding in the gut of the termite Reticulitermesflavipes (Isoptera: Rhinotermitidae). Insect Mol Biol 22: 155-171.
5. Takuya Abe, David Edward Bignell, Masahiko Higashi (2000) Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic Publisher.
6. Andrew Parkinson, 1996. Chapter 6: Biotransformation of Xenobiotics. McGraw-Hill, Inc. 5th edition.
7. Xu C, Li CY, Kong AN (2005) Induction of Phase I, II, and III drug metabolism/transport by xenobiotics. Arch Pharm Res 28: 249-268.
8. Scott J.G, Liu N, Wen Z (1998) Insect Cytochrome P450: Diversity, insecticide resistance and tolerance to plant toxins. Comparative Biochemistry and physiology 121: 147-155.
9. Jeffrey GS (1999) Cytochrome P450 and insecticide resistance. Insect Biochemistry and Molecular Biology 29: 757-777.
10. Pemberton KD, Barrett J (1989) The detoxification of xenobiotic compounds by Onchocercagutturosa (nematode:filarioidea). Int J Parasitol 19: 875-878.