



UNIVERSITI PUTRA MALAYSIA

***DIGESTOME OF THE TERMITE, COPTOTERMES CURVIGNATHUS
HOLMGREN AS A SOURCE OF NOVEL LIGNOCELLULASES***

SULIANA CHARLES NYUCONG

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By

SULIANA ANAK CHARLES NYUCONG

**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia in Fulfillment of the requirements for the Degree of Master of
Science**

August 2016

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Abstract of thesis presented to the senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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August 2016

Chair: Patricia King Jie Hung, PhD
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Researchers have made extensive efforts to determine the lignocellulose degrading potential genes in herbivorous insect, which might assist in reducing the cost of biofuel production via treatment of lignocelluloses. This project was carried out using *Coptotermes curvignathus*, a subterranean termite that has an important impact economically on both agricultural lands and plantations by causing deaths of crop plants, whether in sapling or mature form. Hence, the ability of *C. curvignathus* to feed on living trees and overcome the barriers and defenses of plants is intriguing. The objectives of this study are to discover the Carbohydrate-Active enzymes (CAZyme) related genes in *C. curvignathus*, illustrate a potential pathway for lignocelluloses degradation in the digestive system, and discover the genes involved in the defensive system. To achieve these objectives, a transcriptomic gene library was constructed using digestive tracts from 200 insects, from which mRNA was isolated to generate a cDNA library. The cDNA library was sequenced in Next Generation Sequencing via Illumina HiSeq 2000. Raw data were trimmed and assembled by SolexaQA and Bowtie before loading into Gene Ontology based data mining software, Blast2GO (B2G). Assembled transcriptome were loaded into CAZyme analysis toolkits to identify CAZyme genes present in the data. Data were also analysed based on insect innate immunity database (IIID). The result from the study showed the presence of expressed gene encoding lignocellulose of CAZyme residing in *C. curvignathus* that fed in different habitat, with about 68% of the common CAZyme families encoded transcripts from mineral habitat which were expressed to be higher than peat habitat. As compared to *Reticuliformis flavipes* and *Coptotermes formosanus*, ten CAZyme families (GH16, GH22, GH23, GH32, GH73, GT90, CE15, CBM3, CBM5 and CBM50) were found only in *C.*

curvignathus. In addition, in this study, expression profile for Auxiliary Activity (AA) of a new family of CAZyme is presented to show that lignin degradation is significant in *C. curvignathus* lignocellulosic digestion. The lignocellulose degrading pathway was illustrated in this study to show the complex mechanism on how *C. curvignathus* digest lignocellulose material. Lastly, this study has discovered several important expressed genes, such as GH22 and GH23 that are related to host immune system and detoxification. In conclusion, this project is hoped to adapt the usage of *C. curvignathus* CAZyme ability in industrial lignocellulose degradation and also improve the agricultural sector in the control of *C. curvignathus* through the finding of defence related transcriptome such as GH22 and GH23. It is also recommended to study each of the ten unique gene found in this study in detail via protein expression, which will determine the actual function in *C. curvignathus*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**DIGESTOM ANAI-ANAI *COPTOTERMES CURVIGNATHUS* HOLMGREN
SEBAGAI SUMBER BAHARU ENZIM LIGNOSELULOSA**

Oleh

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Penyelidik telah melakukan pelbagai usaha dalam mendedahkan potensi degradasi lignoselulosa di sebalik serangga herbivora, yang berkemungkinan membantu dalam mengurangkan kos penghasilan bahan api bio melalui rawatan lignoselulosa. Projek ini dijalankan dengan menggunakan anai-anai bawah tanah, *Coptotermes curvignathus* yang telah memberi impak ekonomi ke atas pertanian dan perladangan dengan menyebabkan kematian pokok tanaman, tidak kira anak pokok ataupun pokok matang. Oleh itu, kebolehan *C. curvignathus* memakan pokok hidup dan selamat dari pertahanan pokok adalah sangat menarik. Objektif penyelidikan ini adalah menemui gen berkaitan dengan enzim karbohidrat aktif (CAZyme) di dalam *C. curvignathus*, mengilustrasi laluan berkemungkinan degradasi lignoselulosa di dalam sistem pencernaan, dan menemui gen yang terlibat dalam sistem pertahanan. Dalam mencapai objektif ini, perpustakaan gen transkriptomik dibina dengan menggunakan sistem pencernaan daripada 200 serangga, yang mana pengasingan mRNA dijalankan dalam penyediaan perpustakaan cDNA. Perpustakaan cDNA ini dianalisis jujukan dengan menggunakan instrumen Next Generation Sequencing Illumina HiSeq 2000. Data mentah dirapikan dan dipasangkan dengan menggunakan SolexaQA dan Bowtie sebelum dimuatnaikkan ke dalam perisian pangkalan data Gene Ontology, Blast2GO (B2G). Transkriptom terpasang juga dimuatnaikkan ke dalam kit analisis CAZyme, dan dibandingkan dengan pangkalan data imun semula jadi serangga (IID). Keputusan kajian mempamerkan persamaan dalam kehadiran ungkapan gen pengekodan lignoselulosa CAZyme melalui pemakanan *C. curvignathus* dalam habitat berbeza dengan sebanyak 68% daripada persamaan keluarga CAZyme dalam habitat mineral menunjukkan kod transcript yang

lebih tinggi berbanding habitat gambut. Setelah dibandingkan dengan *Reticuliformis flavipes* dan *Coptotermes formosanus*, kajian ini menemui sepuluh keluarga CAZyme (GH16, GH22, GH23, GH32, GH73, GT90, CE15, CBM3, CBM5 dan CBM50) yang hanya dijumpai di dalam *C. curvignathus*. Selain itu, dalam kajian ini profil Auxiliary activity (AA), keluarga CAZyme yang baharu juga dilaporkan untuk menunjukkan kepentingan degradasi lignin dalam pencernaan lignocellulose *C. curvignathus*. Laluan degradasi lignoselulosa telah diilustrasikan untuk menunjukkan mekanisme kompleks tindakan *C. curvignathus* dalam mencerna bahan lignoselulosa. Akhir sekali, penyelidikan ini telah menemukan beberapa gen, seperti GH22 dan GH23, yang berkaitan dengan perumah imun dan detoksifikasi. Secara ringkas, projek ini diharapkan dapat mengadaptasikan keupayaan CAZyme *C. curvignathus* dalam industri degradasi lignoselulosa dan juga menambahbaik sektor pertanian dalam mengawal *C. curvignathus* melalui penemuan gen pertahanan seperti GH22 dan GH23. Kajian lanjutan ke atas setiap satu daripada sepuluh gen unik ini melalui pengekspressan protein juga amatlah digalakkan untuk mengesahkan fungsi-fungsi yang sebenar di dalam *C. curvignathus*.

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“I can do all things through God who strengthen me” Philippians 4:13

With warm regards,

Suliana anak Charles Nyucong

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LIST OF ABBREVIATIONS

CAZyme	Carbohydrate Active Enzyme
mRNA	messenger Ribonucleic acid
cDNA	complementary Deoxyribonucleic acid
RNA	Ribonucleic acid
PO-MINERAL	prokaryote in mineral habitat
PO-PEAT	prokaryote in peat habitat
PO-ACCE	prokaryote in Accelerase treated
PO-CELL	prokaryote in Cellic treated
PE-MINERAL	prokaryote and eukaryote in mineral habitat
AA	auxiliary activities
GH	glycosyl hydrolase
GT	glycosyl transferase
CE	carbohydrate esterase
CBM	carbohydrate-binding Module
IMD	Immune deficiency
JNK	Jun amino-terminal Kinase
AMPs	Antimicrobial peptides
PGRRP	peptidoglycan recognition protein
SCR	scavenger receptor
GST	glutathione-S-transferases
UGTs	UPD-glucuronosyltransferases
ATP	adenosine triphosphate
ABC	ATP binding cassette
ROS	reactive oxygen species
LiCl	Lithium chloride
PCR	polymerase chain reaction
qPCR	quantitative PCR

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CHAPTER 1

INTRODUCTION

Damage caused by termites is detrimental to economic gain. Homes, offices, and other structures made from wood tend to be infected and destroyed by termites (UNEP, 2000). Wood-based furniture is also attacked and damaged by this isopteran. Their ability to destroy furniture and buildings has been recognized by agricultural and horticultural practitioners. Termites are known to feed on mango, oil palm, coconut, acacia and other softwood trees, such as rubber trees (Chey & Intachat, 2000; Kirton *et al.*, 1999; Eggleton *et al.*, 1995; Waterhouse, 1993).

Hardwood and ornamental trees have also been reported to be infected and damaged by termites, including teak (Wylie *et al.*, 1998, Tho, 1992). Among those destructive termite species, subterranean termites have been singled out as responsible for most of the destruction. Subterranean termite colonies are usually found below soil level, making them hard to detect in a particular area, unless they have already infected wood (UNEP, 2000). *Coptotermes* spp. and *Reticuliformis* spp. are within the subterranean termite families that recently have been reported to cause destruction in buildings and agriculture (Kirton *et al.*, 2005).

As wood has high lignocellulose content, wood-feeding termites are possibly useful for the global fuel industry in producing cost saving biofuel (Dermibas, 2009; Himmel *et al.*, 2007). Lignocellulose pretreatments have been carried out in developing countries; however, most of them are not cost effective. This is due to the stages of treatment needed to fully degrade lignocellulosic biomass into lignin, cellulose and hemicelluloses, which are subsequently treated to produce biofuel (Cheng, 2009; Mousdale, 2008). Wood-feeder termites are able to chew and damage woods, furniture, buildings and trees. Thus, wood-feeder termites are a potential 'gold mine', harbouring genes to encode lignocellulosic degrading enzymes that may be able to reduce the cost of the multiple stages in lignocellulose pretreatment.

Coptotermes spp. and *Reticuliformis* spp. are two potential groups of subterranean termites that can be used as sources of a novel lignocellulose enzyme. Several studies carried out by Do *et al.* (2014), Zhang *et al.* (2012), Tartar *et al.* (2009) and Warnecke *et al.* (2007) have revealed lignocellulose degrading genes in termite guts. According to these reports, termite guts contain cellulose- and hemicellulose-degrading enzymes, with other possible degrading enzymes that

can act on polymers such as chitin and α -carbohydrate. Besides lignocellulosic degrading enzyme encoding genes, the abilities of termites in protecting the colony and themselves have been studied at the molecular level. Trees possess their own proteins and genes to protect from injury or attack by insects (Geisler-Lee *et al.*, 2006). Some of the proteins produced are recognized as secondary metabolites, or allochemicals. In addition, in agriculture or horticulture, a planted tree may be sprayed with an insecticide. Hence, it is important to understand the defence system residing in termites that provides the ability to feed on trees without harming their own body.

One *Coptotermes* spp. that has been recognized as a living-tree killer in plantations is *Coptotermes curvignathus* (*C. curvignathus*) (UNEP, 2010). *C. curvignathus* has been reported to destroy many living palm oil trees, whether saplings or mature trees, in Malaysia and Indonesia. Meanwhile in India, *C. curvignathus* has been reported to damage mango and coconut trees. For these reasons, this particular species is a potential source for discovering novel gene encoding enzymes and proteins involved in lignocellulosic degradation and the termite defence system.

Few reports have described the biology of *C. curvignathus* and none have described its characteristics at the molecular level. This project was carried out to contribute to the understanding of *C. curvignathus* at the molecular level.

The objectives of this research are:

1. To discover the genes encoding Carbohydrate-Active enZymes (CAZyme) in *C. curvignathus*;
2. To illustrate the lignocellulosic degrading gene pathway;
3. To discover the genes involved in the defence system of *C. curvignathus*, including immunity and detoxification genes.

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