UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF ENDOGLUCANASE PRODUCED BY MICROBES RESIDING IN THE GUT OF COPTOTERMES CURVIGNATHUS HOLMGREN TERMITE

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By

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ISOLATION AND CHARACTERIZATION OF ENDOGLUCANASE PRODUCED BY MICROBES RESIDING IN THE GUT OF *COPTOTERMES CURVIGNATHUS* HOLMGREN TERMITE

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Chair: Patricia King Jie Hung, PhD
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This research was carried out to isolate and identify endoglucanase producing microbes from the digestive system of wood termite *Coptotermes curvignathus* Holmgren as well as to characterize endoglucanase produced by these microbes based on their optimum pH, temperature, and enzymatic activity. Five endoglucanases producing bacteria were isolated, four were molecularly identified as aerobic *Bacillus spp.* and the other one was an unknown anaerobic bacterium. Based on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis, endoglucanases produced by these isolates were similar in molecular size, at 11 kDa. These endoglucanases were relatively smaller than the endoglucanases that is produced in *Reticulitermes speratus* salivary glands, which were reported at 41 kDa and 42 kDa. *Reticulitermes speratus* is phylogenetically close to *Coptotermes curvignathus* Holmgren, and both feed on wood. Identities of the endoglucanase producing bacteria were further confirmed using BIOLOG phenotypic analysis. Isolates TG117 was identified as *Bacillus thuringiensis*, NA45/1 as *B. cereus*, TG111 as *B. pseudomycoides*, and TG005 as *B. mycoides*. Among the five endoglucanases tested, endoglucanase produced by *B. cereus* NA45/1 showed the highest enzymatic activity, 0.40 UmL^{-1} at pH9 and 45°C. Endoglucanase *B. cereus* NA45/1 also had significantly higher enzymatic activity when compared to the commercial cellulase from *Aspergillus niger* (C1184 Sigma). Endoglucanase *B. pseudomycoides* TG111 performed optimally at alkaline condition pH9 and 70°C with enzymatic activity at 0.23 UmL^{-1}. Endoglucanase *B. thuringiensis* TG117 had the highest enzymatic activity at 0.21 UmL^{-1} when acted in an acidic condition, pH5 and at temperature, 40°C. Both isolates *B. mycoides* TG005 and unknown anaerobic ST1 has their maximum enzymatic activity at pH6 and temperature at 55°C. This study showed that *C. curvignathus* Holmgren had a wide range of endoglucanases, where optimum temperature and pH for maximum enzymatic activities varies widely. With this array of endoglucanases, *C. curvignathus* Holmgren that feed mainly on living plant-based diet would be able break down cellulose into oligomers and reducing sugars that will subsequently be broken down to fermentable glucoses to sustain life.

Abstrak tesis yang dikemukakan kepada Senat University Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PEMENCILAN DAN PENCIRIAN ENDOGLUKANASE YANG DIHASILKAN OLEH MIKROB YANG TINGGAL DI DALAM USUS ANAI *COPTOTERMES CURVIGNATHUS* HOLMGREN

Oleh

MONICA HII HUNG LING

Januari 2016

Pengerusi: Patricia King Jie Hung, PhD
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Kajian ini dijalankan untuk memencilkan dan mengenalpasti endoglukanase yang dihasilkan oleh mikrob dalam sistem pencernaan anai-anai *Coptotermes curvignathus* Holmgren, serta pencirian endoglukanase yang dihasilkan oleh mikrob tersebut berdasarkan pH, suhu dan aktiviti enzim optima masing-masing. Antara lima pencilan bakteria yang menghasilkan endoglukanase, empat pencilan bakteria dikenalpasti secara molekul sebagai aerobik *Bacillus* spp. dan satu lagi pencilan adalah bakteria anaerobik yang belum dikenalpasti. Berdasarkan analisis *Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis* (SDS-PAGE), endoglukanase yang dihasilkan oleh pencilan ini mempunyai saiz molekul yang sama iaitu 11 kDa. Saiz endoglukanase ini adalah lebih kecil daripada endoglukanase yang dihasilkan dari kelenjar air liur anai-anai spesis *Reticulitermes speratus*, di mana ia dilaporkan bersaiz 41 kDa dan 42 kDa masing-masing. *R. Speratus* adalah spesis terdekat dengan *C. curvignathus* Holmgren secara filogenetik dan kedua-duanya makan kayu. Identiti endoglukanase yang dihasilkan oleh bakteria ditentukan dengan menggunakan BIOLOG fenotip analisis. Pencilan TG117 dikenalpasti sebagai *Bacillus thuringiensis*, NA45/1 sebagai *B. cereus*, TG111 sebagai *B. pseudomycoides*, dan TG005 sebagai *B. mycoides*. Di antara lima endoglukanase yang telah dikaji, endoglukanase yang dihasilkan oleh *B. cereus* NA45/1 menunjukkan aktiviti enzim yang paling tinggi, iaitu 0.40 UmL^{-1} pada pH9 dan suhu 45°C. Endoglukanase *B. cereus* NA45/1 menunjukkan aktiviti enzim yang lebih tinggi daripada selulase komersil daripada *Aspergillus niger* (C1184 Sigma). Manakala, endoglukanase *B. pseudomycoides* TG111 mempunyai aktiviti enzim paling tinggi dalam keadaan beralkali, iaitu 0.23 UmL^{-1} pada pH9 dan suhu 70°C. Endoglukanase *B. thuringiensis* TG117 pula mempunyai aktiviti enzim paling tinggi dalam keadaan berasid, iaitu 0.21 UmL^{-1} pada pH5 dan suhu 40°C. Kedua-dua pencilan *B. mycoides* TG005 dan anaerobik yang belum dikenalpasti ST1 mempunyai aktiviti enzim yang maksimum pada pH6 dan pada suhu 55°C. Kajian ini menunjukkan bahawa *C. curvignathus* Holmgren mempunyai pelbagai jenis endogukanase yang berfungsi pada pH dan suhu yang berbeza. Keadaan ini boleh membolehkan *C. curvignathus* Holmgren untuk mencernakan selulosa kepada oligomer dan gula penurun diikuti dengan seterusnya glukosa untuk kemandirian anai-anai yang hanya bergantung kepada tumbuhan sebagai makanan tunggalnya.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the supervisory committee were as follows:

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LIST OF ABBREVIATIONS

Α	Alpha
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
Β	Beta
BSA	Bovine Serum Albumin
°C	Degree Celsius
CAZy	CArbohydrate-Active EnZymes
Cm	Centimeter
CMC	Carboxymethyl Cellulose
dNTP	Deoxy Nucleotide Triphospohate
DNA	Deoxy Ribonucleic Acid
GOPOD	Glucose oxidase/ peroxidase
M	Molar
Mg	Milligram
$MgCl_2$	Magnesium Chloride
Min	Minute
mL	Milliliter
Mm	Millimeter
µL	Microliter
µg	Microgram
µmol	Micromole
Nm	Nanometer
NA	Nutrient Agar
NaCl	Sodium Chloride
NCBI	National Center for Biotechnology Information
Native-PAGE	Non-denaturing Polyacrylamide Gel Electrophoresis
pH	Potential hydrogen
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
ST	Special Topic
TG	Termite Gut
%	Percent

CHAPTER 1

INTRODUCTION

Termites and its gut microbes are believed to share at least 20 million years of relationship as indicated by fossil evidence (Wier *et al.*, 2002). Hongoh *et al*. (2005) reported that diverse gut bacteria have co-evolved with their host termites and became a stable symbiotic complex. Some members of this symbiotic microbiota are found to be host specific at genus level (Hongoh *et al*., 2005). Although the gut of termite is very small in volume (1 to 2 µL), it contains a rich community of microbes (10^5 to $3x10^7$ cells per mL of termite gut) (Breznak & Leadbetter, 2006; Eutick *et al*., 1978). These gut microbes have a great impact on termites survival and their specialized diet (Radek, 1999). Cooperation of both termite and its gut symbionts enable a high efficiency in biomass conversion into energy and nutrient needed by both host and microbes (Scharf, 2015; Radek, 1999).

Termite feeds solely on lignocellulosic materials (Ohkuma, 2008). They have high lignocellulose degradation efficiency. This ability is very intriguing since lignocellulose is known to be a very robust material. Thus far, several studies have concluded those microbes that residing in the wood feeding termite can degrade lignocellulose and produce large amounts of hydrogen as intermediate (Pester and Brune, 2007). The ability of lower termites in degrading cellulose is well established partly attributed to its symbiotic protists. Other than their symbiont counterparts, termites are also known to produce their own endoglucanases in their salivary gland (Lo *et al*., 2011; Khademi *et al*., 2004). Endoglucanase is a cellulase that hydrolyses 1, 4-β bonds of the cellulose chains (Li *et al*., 2006). The beta acetyl linkage (β-1, 4 glycosidic bonds) gives rigidity property to the cellulose. In order to fully digest cellulose to glucose, it requires three types of enzymes: (1) Endoglucanase to hydrolyse β-1, 4 bonds of cellulose chains, (2) exoglucanase that cleaves cellobiosyl units on their non-reducing ends, and (3) β-glucosidase cleaves on cellobiosyl and other cello-oligosaccharides to glucose (Li *et al*., 2006).

Termites are known to be economically important insect pests especially in peat soil oil palm plantations in Malaysia covering 5.01 million hectare where 13.3% constitutes peat soil (Kon *et al*., 2012). One of the common termite species in oil palm plantations is *Coptotermes curvignathus* Holmgren where it can infest as early as 12 months after field planting and may kill more than 5.3% of the palms in a year (Masijan *et al*, 2006). This termite is among the very few known termite species that has the ability to feed on plant living tissues without the need of their diets to be predigested by other microbes (Chan *et al*., 2011). This indicates *C. curvignathus* Holmgren have the ability to achieve effective lignocellulose biomass conversion. This fascinating characteristic was investigated in this study with a focus to isolate and characterize different endoglucanases that can be found in the termite digestive system. The knowledge and findings of these endoglucanases can be used to improve present biomass conversion enzyme technology. Thus, the objectives of this study were to: (1) isolate and identify microbes residing in the gut of *C. curvignathus* Holmgren termites that can produce endoglucanases, (2) characterization of these endoglucanases based on their optimum pH and temperature to give the highest enzymatic activity.

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