Isolation and characterization of endoglucanases produced by microbes residing in the gut of *Coptotermes curvignathus* termite

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Abstract
Bacteria and enzymes in the gut of termites play an important role to digest lignocellulosic material. Coptotermes curvignathus is one of the very few destructive species that can infest living plants. In this study, five bacteria isolated from C. curvignathus gut; four aerobic Bacillus spp. and an anaerobic uncultured bacterium were identified to produce endoglucanase with molecular size of 11 kDa which is significantly smaller than the endoglucanase produced by Reticulitermes speratus. Biolog reader identification showed that TG117 and N45/1 were Bacillus cereus/thuringiensis, TG111 was Bacillus pseudomycoides and TG005 was Bacillus mycoides.

Endoglucanase produced by aerobic isolate NA45/1 showed promising potential as an industrial enzyme with significantly higher enzymtic activity than the commercial cellulase from Aspergillus niger (C1184 Sigma). Endoglucanase NA45/1 displayed enzymatic activity 0.3961 U at pH 9 and 45°C. The endoglucanase TG111 acted optimally at alkaline condition with 0.2294 U whereas endoglucanase TG117 functioned best at slightly acidic condition. This study showed that the termite gut has a wide range of endoglucanase enzymes with various optimum temperatures and pH.

Keywords: *Bacillus*, cellulose, enzyme activity, gut microbes, lignocellulosic.

Introduction

Termite and its gut microbes are believed to share at least 20 million years old relationship as indicated by fossil evidence[1]. Survivals of termites are depending on their gut symbiont present in extraordinarily high diversity. Worker caste termites are usually the target subject to study the gut symbiont as they are the diet provider of the colony. Workers forage and predigest plant biomass which is termite's sole diet before feeding to their colony members via trophallaxis[2,3].

The major constituent of plant cell walls is cellulose which is considered the most abundant carbon source and industrial feed stock for renewable energy. Cellulose is a linear polymer of glucose linked by β-1, 4 glycosidic bonds[4]. The beta acetyl linkages give rigidity property to the cellulose and it requires three types of enzymes to be fully digested to glucose. Endoglucanase hydrolyses the β-1, 4 bonds of the cellulose chains. Exoglucanase acts on non-reducing ends of the cellulose chains to cleave cellobiosyl units. β-glucosidase enzyme acts on non-reducing ends of cello-oligosaccharides where it cleaves glucosyl units[5].

Tokuda et al[6] reported that endo-β-1, 4 glucanase was detected mainly in the midgut of *N. takasgoensis* with molecular weight of 47 kDa. Several *Bacillus* have been isolated from termite gut aerobically and also other anaerobic cellulolytic bacteria[7].The high naturally diversity of bacteria such as *Bacillus strains* isolated from the decomposed rice brain and the gut of termite *Odontotermes formosanus* able to produce endoglucanase can be potential source as stable enzyme in compliment to industrial usage[8,9].

Termite gut is often regarded as a powerful plant biomass conversion reactor. They are the key organism in the tropic to degrade plant material. *C. curvignathus* is among the very few known termite species that has the ability to extract energy from living tree without pre-decomposed by other microbes[10]. This unique ability was exploited in this study to screen for potential endoglucanase for better biomass conversion.

Material and Methods

Termite Sampling and Gut Microbial Isolation: Sampling was carried out at Woodman Oil Palm Plantation Block 16 (N 03° 01' 00.6", E 112° 52' 51.7") at Semanok, Tatau, Sarawak. Baiting method was used to bait the termite. A total of five pieces of the rubber wood blocks (10 × 10 × 30 cm) were buried into in the soil near to the infected oil palm for two months. The rubber wood blocks were extracted after two months.

Gut contents of 100 worker *C. curvignathus* were inoculated into 100 mL of nutrient broth and incubated aerobically at room temperature for 24 hours while anaerobic culture were incubated in an anaerobic jar (Oxoid) at room temperature for two weeks .The aerobic and anaerobic culture in nutrient broth were then spread plated onto nutrient agar (Merck), incubated at room temperature for 24 hours. All colonies on the agar were

picked, subcultured three times and coded. All the isolates were subjected to endoglucanase activity screening

Endoglucanase Activity Screening: All aerobic and anaerobic isolates were screened for endoglucanase activity. Cultures of each isolate were tested on 1% CMC agar for endoglucanase activity. A total of 10 µL of the culture was pipetted and dropped onto the surface of CMC agar. The agar plate was then incubated at 37°C for two hours.

After incubation, the whole CMC agar plate was flooded with 0.1% Congo red stain (Systerm) for 30 minutes. The Congo red solution was then discarded and the CMC agar plate was destained with 1 M NaCl (Merck) for 15 minutes. Clear zones observed indicate presence of endoglucanase activity.

Four aerobic isolates were found to have endoglucanase activity. These four isolates were then coded as TG005, TG117, TG111 and NA45/1. Only one anaerobic isolate was found to have endoglucanase activity. This anaerobic isolate was coded as ST1. All these five isolates were cultured in fermenters to mass produce the endoglucanases.

Mass Production of Endoglucanase: Aerobic isolates TG117, TG111, TG005 and NA45/1 were aseptically inoculated into 25 µL nutrient broths and incubated at room temperature for 24 hours. An anaerobic isolate ST1 was aseptically inoculated into 25 µL nutrient broth and incubated in an anaerobic jar at room temperature for 24 hours. All culture solutions were spread plated on nutrient agar to detect any contamination. A total amount of 10 µL of the pure isolates TG117, TG111, TG005 and NA45/1 solutions were transferred into new 1% carboxymethyl cellulose (CMC) broth (Calbiochem) and incubated at room temperature for two weeks. For anaerobic isolate ST1, 10 µL of the pure culture was transferred into a new 1% CMC broth and incubated in an anaerobic jar at room temperature for two weeks.

Purification of Crude Endoglucanase: Column chromatography was prepared by packing silica gel 60, 230-400 mesh ASTM (Merck) into the Pasteur pipette (Favorit 150 mm). 0.5M Ammonium acetate was used as mobile phase. A total amount of 800 µL crude protein extraction was pipetted into the column chromatography. A total of 100 µL of elution per tube for each fraction was collected. The fragments were tested on CMC agar to screen for endoglucanase activity.

Zymogram Analysis: Zymogram analysis for endoglucanase was performed with 10% Non-denaturing Polyacrylamide Gel Electrophoresis (Native-PAGE). Native-PAGE was performed using 5 mL of 10% separating gel and 2 mL of 5% stacking gel. A total of 20 µL of protein sample was mixed with 5× sample buffer and vortexed before loaded. The gel was run under 100 volts for 60 minutes. After Native-PAGE was done, the gel was incubated with 0.5% CMC agar for one hour. The agar plate was stained with 0.5% Congo red for 10 minutes and eventually washed with 1 M NaCl. Protein with endoglucanase activity would display clear bands against a red background.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE): The SDS-PAGE was performed to identify the molecular weight of endoglucanase produced by preparing 15% separating gel (Acros) and 5% stacking gel. A total of 20 µL of protein sample was mixed with 5× sample buffer and heated at 100 °C for 10 minutes before loading into gel. The gel was run under 100 volts for 60 minutes. The gel was stained with Coomassie brilliant blue R-250 for 15 minutes on the shaker and then destained with Coomassie gel destains.

Identification of Microbes: Each isolate was inoculated in nutrient broth at 37 °C for 24 hours. The cultures were proceeded for DNA extraction using the MasterPure™ DNA Purification Kit (EPICENTRE® Biotechnologies) according to manufacture recommended methods. DNA with good quality was amplified using Polymerase Chain Reaction (PCR) using *16S rRNA* universal primers which are 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR mix was prepared by adding 1 µL of each *16s rRNA* universal primer, 2 unit of Taq polymerase, 5 µL 10× Buffer A, 1.5 µL 50 mM $MgCl_2$, 2 µL of 2 mM $dNTP_s$ and 0.5 µL of template DNA at 2 ng. The total volume was made up to 50 µL. PCR was performed for 18 cycles at 95 °C denaturing for 30 seconds, 50 °C of annealing temperature for one minute and 72 °C extension for four minutes. After the 18 cycles, PCR products were stored at 4 °C.

The PCR purification was performed according to QIAquick PCR Purification Kit (QIAquick®spin Handbook). Purified DNA was then packed and sent to First Base for sequencing. The sequenced results were then analyzed to nucleotide blast to the sequences deposited in the GenBank from National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) public database using the BLASTn software.

Characterization of the Microbes by Biolog Reader: Biochemical profiles of the four aerobic isolates TG117, TG111, TG005 and NA45/1 were analyzed using Biolog GEN III MicroPlate.™ Isolation of a pure culture was obtained from BUG+B agar media and then suspended in a special gelling inoculation fluid (IF-B). The cell suspension was inoculated into the GEN III MicroPlate™ with total volume of 100 µL at 33°C for 22 hours. After incubation, OmniLog® software was used to identify the microbes.

Determination of Optimum Enzymatic pH: The endoglucanase from isolates TG117, TG111, TG005,

NA45/1 and ST1 as well as *Aspergillus niger* endoglucanase (Sigma) were tested for optimum pH for endoglucanase activity. A total of 2 µg/mL endoglucanase were tested on different pH ranging from 3, 4, 5, 6, 7, 8 and 9 at 40°C for 30 minutes. Endoglucanase activity was measured based on glucose released from CMC broth after enzymatic hydrolyses. Enzyme activity was stopped by incubation in 100°C water bath for 10 minutes. An amount of 25 µL was used to evaluate glucose level according to D-Glucose assay (Megazyme) using GOPOD reagent.

Determination of Optimum Enzymatic Temperature: The endoglucanase from isolates TG117, TG111, TG005, NA45/1 and ST1 together with *A. niger* was tested for optimum temperature for endoglucanase activity. A total of 2 µg/mL endoglucanase were tested on different temperature ranging from 30 to 70°C with 5°C interval. Endoglucanase activity was measured based on glucose released from CMC broth via enzymatic hydrolyses. Enzyme activity was stopped by incubation in 100°C water bath for 10 minutes. An amount of 25 µL was used to evaluate glucose level according to D-Glucose assay using GOPOD reagent.

Determination of Enzyme Activity: Enzyme activity of the endoglucanase isolated from the TG117, TG111, TG005, NA45/1 and ST1 isolates was measured using D-Glucose assay. A total of 2 µg/mL of each endoglucanase were tested in 1% CMC broth, incubated at optimum condition respective to each endoglucanase. Endoglucanase TG117 was tested using 40°C at pH 5, TG111 was tested using 70°C at pH 9, NA45/1 was tested using 45°C at pH 9 and TG005 and ST1 were tested using 55°C at pH 6. Endoglucanase activity was measured based on glucose released from CMC broth via enzymatic hydrolyses. Enzyme activity was stopped by incubation in 100°C water bath for 10 minutes. An amount of 25 µL was tested as glucose level by using GOPOD reagent from D-Glucose assay. One unit (U) will liberate 1 µmole of glucose per minute.

D-Glucose Assay (Megazyme) Measurement: The amount of D-glucose (µg/mL) released from CMC was calculated based on the following formula:

D-glucose = (Absorbance of sample /Absorbance of D-glucose standard) × 1000

Enzyme Activity Formula: One unit of enzyme activity was defined as amount of enzyme that release 1µmol of glucose equivalent per minutes (U/mL):

[Amount of D-glucose (ug/mL) / 180.16 glucose molar mass (g/mol)] / 30 minutes

Statistically Analysis: All the triplicate data for the optimum pH, temperature and enzymatic activity data were statistically analyzed by one-way ANOVA to detect significant difference. Tukey's Studentized Range Test was used to separate the means. Statistical Analysis Software (SAS) version 9.1 was used to analyse the data.

Results and Discussion

Qualitative Evaluation of Endoglucanase Activity: Endoglucanase activity is shown in figure 1. Five isolates were identified to have endoglucanase activity by observing clear zone area.

Zymogram Analysis: Zymogram analysis in figure 2 showing endoglucanase activity from Native-PAGE was located on CMC agar. All the five isolates TG117, TG111, TG005, NA45/1 and ST1 showed one single clear zone band. Zymogram analysis in figure 2 revealed endoglucanase activity from all the isolates TG117, TG111, TG005, NA45/1 and ST1. All these endoglucanase showed clear band at the similar position. These endoglucanases were further detailed analysis by SDS-PAGE.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE): The SDS-PAGE in figure 3 showed the sizes of the endoglucanase produced by five bacteria isolates. All five endoglucanase from termite gut showed molecular weight of 11 kDa.

Endoglucanase produced by TG117, TG111, TG005, NA45/1 and ST1 isolates produced endoglucanase with molecular weight around 11kDa (Figure 3). This size is relatively smaller than other reported termite endoglucanases. Sister termite *Reticulitermes speratus* produced two endoglucanases, endo β-1, 4-glucanase with molecular weight of 41 kDa and 42 kDa[11]. Higher termites *Nasutitermes takasagoensis* produced endoglucanase at 47 kDa[6]. The small molecular size can be a plus point as increased surface area has a higher tendency to interface more efficiently[12]. In addition, the endoglucanase produced by *C. curvignathus* gut microbes was produced extracellularly which facilitated purification for industrial usage.

Identification of the Microbes: Almost full 16S RNA gene of isolate TG117, TG111, TG005, NA45/1 and ST1 were sequenced and sequencing was blast against NCBI database. Table 1 showed the BlastN result based on almost full sequence of 16S RNA. TG117, TG111, TG005 and NA45/1 were identified as *Bacillus* spp. in Firmicutes phylum and ST1 was unknown bacterium (Table 1).

Wenzel et al[13] reported several endoglucanase producing *Bacillus* isolated from the gut of termite *Zootermopsis agusticollis*. Aerobic *Bacillus* sp. was reported to assist wood feeding termites to digest cellulose and hemicelluloses in both hydrolytic and oxidative stages by producing endoglucanase and xylanase[14]. Isolate ST1 showed 95% similarity to the partial sequence 16S rRNA of an uncultured bacterium isolated from leaf of soybean[15]. Based on this blast result, isolate ST1 will be the first

reported anaerobic endoglucanase bacteria from *C. curvignathus*.

Characterization of the Microbes by Biolog Reader: Four aerobic isolates were tested on different type of carbon source utilization and chemical sensitivity (Table 2). All the isolates were able to utilize dextrin, D-maltose, D-trehalose, D-cellobiose, D-glucose-6-phosphate and D-fructose-6-phosphate as carbon sources. All the isolates were also able to utilize amino acid such as gelatine, L-serine, methyl-pyruvate, L-malic acid and Acetoacetic acid. Isolates TG117, TG111 and NA45/1 showed negative result on gentiobiose and sucrose usage. Isolate TG117 and TG005 showed negative result for D-fucose and L-fucose. All isolates were able to survive on below 8% sodium chloride and also 1% sodium lactate. However, all the isolates were sensitive towards the antibiotics.

Bacillus species has ability to produce acid from D-glucose, starch hydrolysis, casein hydrolysis, citrate utilization, tyrosine degradation, growth at pH 5.7 and growth in 7% NaCl[16]. *Bacillus* species utilize different types of carbon source and have ability to tolerance variable chemicals. *Bacillus* species were previously reported as able to degrade complex starch substrate into simple sugars like maltose and glucose[17].

Determination of Optimum Enzymatic pH: Five isolates showed different optimum pH for their endoglucanase activity (Table 3). TG111 and NA45/1 had the highest endoglucanase activity in alkaline condition (pH 9). TG005 and ST1 showed highest endoglucanase activity at pH 6. TG117 showed highest endoglucanase activity at acidic condition (pH 5).

Result of this study showed that *C. curvignathus* contains several endoglucanase microbes that function optimally at a wide range of pH. The optimum pH of endoglucanase produced by four aerobic cultures (TG117, TG111, TG005, NA45/1) and an anaerobic culture (ST1) from *C. curvignathus* gut microbes ranged from 5 to 9. Wood feeding termites were found to have steep pH gradient in their gut[18]. The pH of midgut was reported at neutral (pH 7) and when travelled across to hindgut, the pH rose to pH 10[18]. This means these endoglucanase were able to work at their optimum pH at the physiological pH provided by different niches of termite gut.

Determination of Optimum Enzymatic Temperature: Most of the isolates showed higher endoglucanase activity at temperature higher than 40°C (Table 4). TG111 showed the highest optimum endoglucanase activity at 70°C whereas TG005 and ST1 had optimum endoglucanase activity at 55°C. NA45/1 showed optimum endoglucanase activity at 45°C and TG111 at 40°C.

Isolate TG117 had optimum endoglucanase activity at pH 5 and 40°C, both isolates TG005 and ST1 had optimum endoglucanase activity at pH 6 and 55°C, isolate TG111 had optimum endoglucanase activity at pH 9 and 70°C and isolate NA45/1 had optimum endoglucanase activity at pH 9 and 45°C (Table 2 and 3). The wide optimum temperature of endoglucanase activity by these termite gut microbes explained the high thermal tolerant of Rhinotermitidae. *Coptotermes formosanus* is reported tolerable at temperature within range from 7.2 to 45.9°C and *Reticulitermes flavipes* is within range from 1.5 to 44.9°C[19]. Endoglucanase isolated from this study can act on higher temperature condition up to 70°C. *C. curvignathus* can confine in a living tree inner gallery which may suffer from high temperature in its habitat whereas its sister termites can confirm themselves in soils habitat up to 15 cm depth[19]. Enzymes that work optimally at high temperature allow optimum metabolism of termite host.

Isolate NA45/1, *Bacillus* sp. showed similarity optimum condition toward *Bacillus circulans* which was isolated from decomposed rice bran that produced endoglucanase with optimum activity at pH 9, temperature 45°C[7]. Tokuda et al[6] reported that endoglucanase produced from higher termite had optimum activity at pH 5.8 and temperature 65°C.

Among five isolates, endoglucanase from isolates TG111 had the highest optimum pH (pH 9) and temperature of 70°C. Therefore endoglucanase from TG111 may be good alternative to thermostable crude endoglucanase produced by *Apergillus fumigatus* which can withstand temperature from 60 to 70°C without losing functional 3D structure and activity[20].

Determination of Enzyme Activity: Table 5 shows that isolate NA45/1 had significantly higher enzyme activity (0.3691 U) than the other five isolates including the commercial enzyme from *Aspergillis niger* when given the same substrate. The enzymatic activity was higher than the commercial enzyme cellulase *A. niger* (Sigma). High enzyme activity may enhance utilization of biomass for fuel production employing cellulase in obtaining better yields and novel activities[21].

Conclusion

In conclusion, *C. curvignathus* contains gut microbes that produced small endoglucanase molecular size, 11 kDa. The optimum pH and temperature of these enzymes were wide, ranging from pH 5 to 9 and at 40 to 70°C. Endoglucanase from isolate TG111 was able to function under high temperature of 70°C and at an alkaline pH of 9 while NA45/1 showed the highest enzyme activity of 0.3961 U as compared to all endoglucanases tested. Thus, the endoglucanase produced from *C. curvignathus* was potentially to be commercialized for future biomass industrial application and biofuel production.

Table 1
BLASTN result of isolates based on almost full sequence of 16S RNA

Samples	Closest neighbor	E value	Identities (%)	Accession number
TG117	*Bacillus thuringiensis* strain GTG-6S	4e-158	100	JX 283457.1
TG111	*Bacillus cereus* strain KNUC52	0.0	99	AY 279196.1
TG005	*Bacillus* sp. A1144	0.0	100	JX 266312.1
NA45/1	*Bacillus* sp. 2410I	0.0	100	DQ 067208.1
ST1	Uncultured Bacterium	0.0	95	FN 421645.1

Table 2
Characteristics of the aerobic isolates on different type of carbon source utilization assays and chemical sensitivity assays

Substrate	TG117	TG111	TG005	NA45/1
Dextrin	+	+	+	+
D-Maltose	+	+	+	+
D-Trehalose	+	+	+	+
D-Cellobiose	±	±	±	±
Gentiobiose	-	-	±	-
Sucrose	-	-	±	-
D-Turanose	-	-	±	±
β-Methyl-D-Glucoside	±	-	±	-
D-Salicin	< -	+	±	-
N-Acetyl-D-Glucosamine	+	+	+	+
N-Acetyl-β-D-Mannosamine	-	±	-	-
α-D-Glucose	+	+	+	+
D-Mannose	-	-	-	±
D-Fructose	+	+	+	+
D-Galactose	±	±	-	-
Inosine	+	+	+	+
Stachyose	-	-	-	-
D-Raffinose	-	-	-	-
α-D-Lactose	-	-	-	-
D-Melibiose	-	-	-	-
N-Acetyl-D-Galactosamine	-	-	-	-
N-Acetyl Neuraminic Acid	-	-	-	-
3-Methyl-Glucose	-	-	-	-
D-Fucose	-	±	-	±
L-Fucose	-	±	-	±
L-Rhamnose	-	-	-	-
D-Sorbitol	-	-	-	-
D-Mannitol	-	-	-	-
D-Arabitol	-	-	-	-
Glycerol	+	+	+	±
Myo-Inositol	-	-	-	-
D-Aspartic acid	-	-	-	-
D-Serine	±	±	+	±
L-Pyroglutamic Acid	-	-	-	-
Pectin	-	-	±	-
L-Galactonic Acid Lactone	-	-	-	-
Mucic Acid	-	-	-	-
Quinic Acid	-	-	-	-
D-Saccharic Acid	-	-	-	-
P-Hydroxy-phenylacetic Acid	-	-	-	-
D-Malic Acid	-	-	-	-
Y-Amino-Butyric Acid	-	-	-	-
α-Hydroxy-Butyric Acid	-	-	-	-

D-Glucose-6-phosphate	+	+	+	+
D-Fructose-6-phosphate	+	+	+	+
Gelatin	+	+	+	+
Glycyl-L-Proline	±	±	±	±
L-Alanine	±	±	±	±
L-Arginine	±	±	±	±
L-Aspartic Acid	±	±	±	±
L-Glutamic Acid	±	±	±	±
L-Histidine	±	±	±	±
L-serine	+	+	+	+
D-Galacturonic Acid	±	±	±	+
D-Gluconic Acid	+	< -	±	±
D-Glucuronic Acid	±	±	±	< -
Glucuronamide	±	±	±	< -
Methyl-Pyruvate	+	+	+	+
D-Lactic Acid Methyl Ester	±	±	±	±
L-Lactic Acid	±	±	±	±
Citric Acid	±	±	±	±
Bromo-Succinic Acid	±	±	±	±
L-Malic Acid	+	+	+	+
Tween 40	±	±	±	±
α-keto-Glutaric Acid	-	-	±	±
β-Hydroxy-D, L-Butyric Acid	±	-	-	-
α-Keto-Butyric Acid	-	±	-	-
Acetic Acid	±	±	±	±
Acetoacetic Acid	+	+	±	+
Propionic Acid	-	±	-	-
Formic Acid	±	+	±	±
pH6	+	+	+	+
PH5	-	-	-	-
1% Sodium Chloride	+	+	+	+
4% Sodium Chloride	+	+	+	+
8% Sodium Chloride	±	-	<-	+
1% Sodium Lactate	+	+	+	+
Fusidic Acid	-	-	-	-
D-Serine	+	+	+	+
Troleandomycin	-	-	-	-
Rifamycin SV	-	< -	±	< -
Minocycline	-	-	-	-
Lincomycin	-	-	-	-
Guanidine Hydrogen Chloride	+	+	±	+
Niaproof 4	-	-	-	-
Vancomycin	-	-	-	-
Tetrezolium Blue	-	-	-	-
Tetrazolium Violet	-	-	-	±
Nalidixic Acid	< -	-	-	-
Potassium Tellurite	+	+	+	+
Aztreonam	+	+	+	+
Sodium Butyrate	+	+	+	+
Lithium Chloride	±	±	+	+
Sodium Bromate	±	±	+	±
Identification	*Bacillus cereus/ thuringiensis*	*Bacillus pseudomycoides*	*Bacillus mycoides*	*Bacillus cereus/ thuringiensis*

Note: +, positive; -, negative; ±, borderline; < -, mismatched positive, Biolog reader

Table 3
Amount of D-glucose released from CMC substrate at 40°C incubation for 30 minutes at different substrate pH

pH	D-glucose (µg/0.1mL)					
	TG117	**TG111**	**TG005**	**NA45/1**	**ST1**	**C**
3	675.6^{b}	653.3^{b}	708.4^{bc}	659.1^{b}	709.9^{b}	141.1^{d}
4	534.1^{bc}	363.4^{c}	695.8^{bc}	474.2^{cd}	398.7^{cd}	384.7^{c}
5	905.1^{a}	675.5^{b}	$8311.^{ab}$	394.9^{d}	221.4^{e}	661.0^{b}
6	395.7^{cd}	307.7^{c}	939.0^{a}	550.0^{bc}	1054.6^{a}	879.7^{a}
7	626.5^{b}	274.2^{c}	634.5^{cd}	574.8^{bc}	300.6^{de}	696.5^{b}
8	592.4^{b}	290.6^{c}	280.2^{e}	254.8^{e}	471.3^{c}	377.9^{c}
9	290.4^{d}	881.2^{a}	498.9^{d}	1051.9^{a}	699.9^{b}	320.2^{c}

Note: Mean comparison with same alphabetical number indicated no significant different across the column at $p<0.05$ by Tukey's Studentized Range Test. Positive control, C was using a commercial enzyme cellulase *Aspergillis niger*.

Table 4
Amount of D-glucose released from CMC substrate at pH 5 incubated for 30 minutes at different temperature

Temperatur e(°C)	D-glucose (µg/0.1mL)					
	TG117	**TG111**	**TG005**	**NA45/1**	**ST1**	**C**
30	599.4^{b}	358.1^{c}	403.8^{cd}	416.1^{d}	501.1^{bcd}	363.8^{cd}
35	584.6^{bc}	419.0^{c}	419.0^{cd}	595.2^{b}	583.2^{bc}	404.1^{cd}
40	929.2^{a}	100.5^{d}	500.8^{bc}	471.3^{d}	588.1^{b}	411.9^{cd}
45	511.7^{bcd}	462.9^{bc}	535.4^{ab}	768.6^{a}	426.8^{d}	566.2^{b}
50	540.7^{bc}	494.0^{bc}	566.2^{ab}	429.6^{d}	427.5^{d}	340.4^{d}
55	252.7^{e}	571.9^{b}	630.6^{a}	442.3^{cd}	925.7^{a}	940.6^{a}
60	389.7^{de}	493.7^{bc}	392.7^{d}	430.2^{d}	566.7^{bcd}	484.9^{bc}
65	454.0^{cd}	413.5^{c}	575.0^{ab}	515.9^{bcd}	634.9^{b}	571.4^{b}
70	460.3^{bcd}	905.6^{a}	538.1^{ab}	557.1^{bc}	600.8^{b}	448.7^{bcd}

Note: Mean comparison with same alphabetical number indicated no significant different across the column at $p<0.05$ by Tukey's Studentized Range Test. Positive control, C was using a commercial enzyme cellulase *Aspergillis niger*.

Table 5
Enzyme activity of endoglucanase isolates incubated at optimum pH and temperature

Samples	pH	Temperature (°C)	Enzyme activity (U/mL)
TG117	5	40	0.2057^{cd}
TG111	9	70	0.2294^{c}
TG005	6	55	0.1911^{d}
NA45/1	9	45	0.3961^{a}
ST1	6	55	0.1959^{cd}
Positive Control cellulase *Aspergillis niger*	6	55	0.3414^{b}

Note: Mean comparison with same alphabetical number indicated no significant different across the column at $p<0.05$ by Tukey's Studentized Range Test

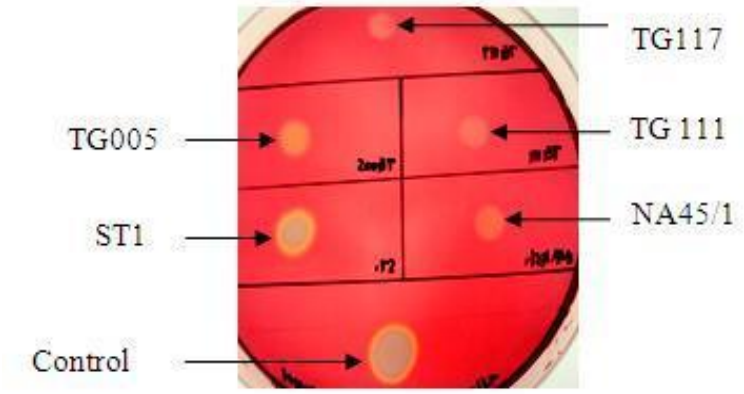

Figure 1: Expression of endoglucanase activity by gut microbes from *Coptotermes curvignathus*. Clear Zone on 1% CMC agar indicated endoglucanase activity. Control was *Aspergillus niger*.

TG117 TG111 TG005 NA45/1 ST1

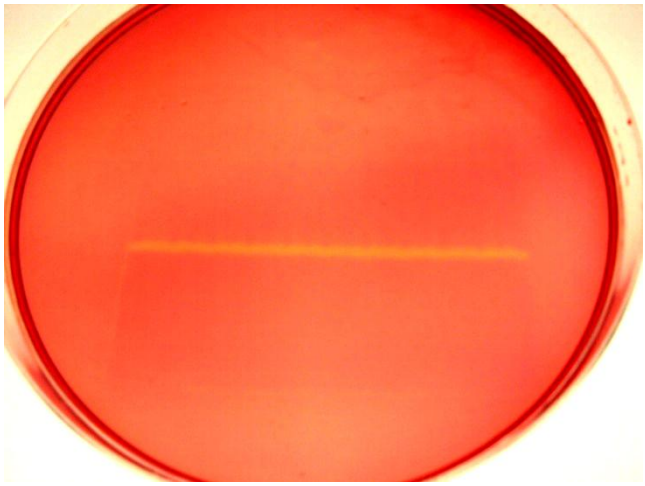

Figure 2: Zymogram analysis of endoglucanase activity on 10% Native-PAGE and activity was located on CMC agar.

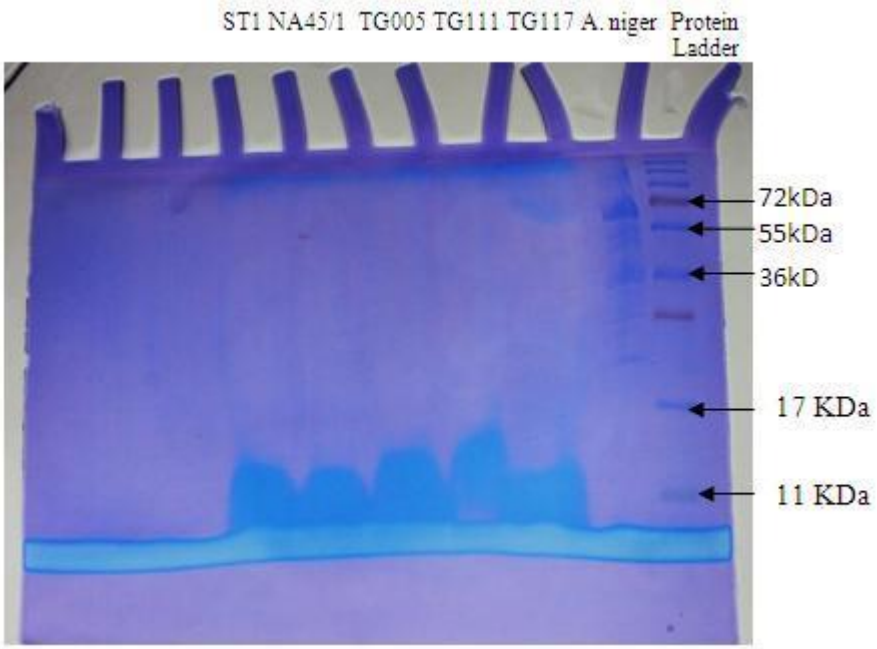

Figure 3: SDS-PAGE analysis of all the isolates. TG117, TG111, TG005, NA45/1 and ST1

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