Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 3 (4): 288-292, 2008
ISSN 1816-4935

Isolation and Identification of Three Species of Bacteria from the Termite *Coptotermes curvignathus* (Holmgren) Present in the Vicinity of University Putra Malaysia

[1]M. Ramin, [1]A.R. Alimon, [2]N. Abdullah, [1]J.M. Panandam and [3]K. Sijam
[1]Department of Animal Science, [2]Department of Microbiology,
[3]Department of Plant Protection, University Putra Malaysia,
43400, Serdang Selangor, Malaysia

Abstract: In this study the lower termite *Coptotermes curvignathus* (Holmgren) and higher termite *Macrotermes gilvus* (Hagen) were identified from different parts in the vicinity of University Putra Malaysia (UPM). We isolated three enteric bacteria from the hindgut of *Coptotermes curvignathus* (*Holmgren*). All isolates were facultative anaerobes. The isolates were identified as *Enterobacter aerogenes*, *Enterobacter cloacae* and *Clavibacter agropyri* (*Corynebacterium*) by using BIOLOG assay and Bergey's manual. The bacteria were able to assimilate carboxymethylcellulose (CMC) and cellobiose.

Key words: Higher and lower termite, gut bacteria, isolation, identification, biolog reader, lignocellulose

INTRODUCTION

Termites are insects from the order Isoptera which in Greek *isos* means equal and *pteron* means wing (Thorne and Carpenter, 1992). They are usually called white ants. They are small to medium in size with a dull white to light and dark brown body (Varma *et al*., 1994) and characterized by their colonial behavior. Termites are among the most important lignocellulose-digesting insects and possess a variety of symbiotic microorganisms in their hindguts, including bacteria, Archaea and Eukarya (Konig, 2006). Termites can be classified into six families and fifteen subfamilies (Lee and Wood, 1971) which the first five family belong to lower termites and the sixth family belongs to higher termites.

The main difference between higher and lower termites is that flagellates (protozoa) are present in the gut of only lower termites, whereas no protozoa were found in higher termites gut (Varma *et al*., 1994). It is also known that higher termites decompose cellulose by using their own enzymes (Ohkuma, 2003).

The diversity of termite gut communities is extraordinarily high and the function of each group of symbionts is poorly known. There are many kinds of bacteria with different functions in termites gut which have been isolated and identified. They were hemicellulose-degrading bacteria (Schafer *et al*., 1996), lignolytic bacteria (Borji *et al*., 2003), cellulolytic bacteria (Wenzel *et al*., 2002), aromatic-degrading bacteria (Harazono *et al*., 2003) and nitrogen-fixing bacteria (Frohlich *et al*., 2007). The most abundant bacteria which have been identified from both higher and lower termites belong to the species of strict or facultative anaerobes. *Staphylococcus* and *Bacillus* are the most abundant bacteria in termites gut (Breznak, 1982; Konig, 2006). Recently, *Serratia marcescenes* and *Enterobacter aerogenes* have been identified from Formosan termite *Coptotermes formosanus* (Adams and Boopathy, 2005). Oxygen can permeate into the gut through the body wall (Brune *et al*., 1995) and this could be lethal to the anaerobic endosymbionts if not removed. The role of facultative anaerobic

Corresponding Author: M. Ramin, Department of Animal Science, University Putra Malaysia, 43400, Serdang Selangor, Malaysia Tel: +60173979683 Fax: +60389432954

anaerobes may be to scavenge the oxygen that can permeate to the gut, effectively keeping the gut anaerobic. There is no report on isolation and identification of local termites gut bacteria. However, the identification of facultative anaerobic bacteria in *Coptotermes curvignathus* (Holmgren) has not been described in published work. Therefore, the main objective of this study was to identify termites from the vicinity of UPM and to isolate and identify enteric bacteria specially, facultative anaerobic bacteria with the capability of degrading cellulose in the gut of the lower termite identified in the vicinity of UPM. These facultative microbes may have a role to play in the microbial ecology of termites gut by removing oxygen and maintaining anaerobiosis for the strict anaerobic symbionts.

MATERIALS AND METHODS

Collecting and Identification of Termites

Termites were collected from the vicinity of UPM wood's near college twelve and mosque from their nest on the ground and traps which were made to catch them. The traps were boxes with a plastic cover which were placed in a pit near their nest and left for about 20-30 days. All collected termites were transferred to the Faculty of Forestry, for identification. The isolation and identification of bacteria was conducted in the microbiology lab located in the campus of UPM.

Isolation of the Bacteria

The termites collected were identified as *Coptotermes curvignathus* (Lower termite) and *Macrotermes gilvus* (Hagen) (Higher termite). The lower termites *Coptotermes curvignathus* were kept in a container with filter papers as their feed. The container was kept in a dark at room temperature with 60% humidity. New slightly wet filter papers were supplied daily for one week. Ten worker termites were surface sterilized with 70% ethanol and then washed in sterile distilled water. Under sterile conditions, the entire guts were removed from the abdomen by using a syringe and mixed with 10 mL 0.85% NaCl. 0.5 mL of the suspension was mixed with 4.5 mL medium 1 which contained 5 g L^{-1} *Carboxymethylcellulose* CMC, 0.2 g L^{-1} yeast extracts. The mixture was incubated at 30°C. After 3 days the culture was spread on nutrient agar plate and pure colonies were obtained by several subsequent culturing and plating.

Identification of the Bacteria

After several sub culturing, pure cultures of bacteria were obtained and the Gram stain and OF test (anaerobic test) were performed. The medium 1 for OF test contained 2.0 g L^{-1} peptone, 5 g L^{-1} NaCl, 0.3 g L^{-1} KH_2 PO_4, 3 g Agar and 3 mL 1% Bromthymol blue. All materials were dissolved together and the pH was adjusted to 7.1. Five mL of medium 1 was added to each 13 cm diameter test tube and sterilized at 121°C for 20 min. After autoclaving, 0.5 mL filter sterilized glucose was added to each test tube. Two test tubes were inoculated with each bacterial isolate and one test tube was covered with a layer of sterile melted Vaseline. The tubes were incubated at 30°C for 18-24 h.

The Biolog reader was used for identification of bacteria by using the right kit base on the Gram stain result. The pure cultures of bacteria were inoculated into the biolog broth and the turbidity of the inoculum was adjusted according to the Biolog protocol. One hundred and forty five microliter of the inoculum was pipetted into each well of the 96 well microplates and incubated for 4-6 or 16-24 h depending on the growth of bacteria and the ability of Biolog reader to analyze the results.

Growth on CMC and Cellobiose Agar

Pure cultures were grown on two different media, were one medium contained 0.05 g mL^{-1} cellobiose and another contained 0.05 g mL^{-1} carboxymethylcellulose. The other components of the medium were 0.4 g yeast extract, 1 g malt extract, 1.2 g agar per 100 mL distillated water. The pH was adjusted to 7.09 and autoclaved at 120°C for 20 min. The solidified medium was spread with the three bacterial isolates and incubated at 30°C for 24 h (Wenzel *et al*., 2002).

RESULTS AND DISCUSSION

The termites which were collected from the nest above the ground were identified as higher termites *Macrotermes gilvus* (Hagen) or fungus growing termites belonging to the family *Termitida* and subfamily *Macrotermitinae*. The higher termite *Macrotermes gilvus* (Hagen) is abundant in Malaysia and these termites don't have bacteria with the capability to digest cellulose and the digestion is done by their own enzymes which are secreted by their gut and salivary glands. The nest of the higher termite is shown in (Fig. 1). The other species of termite identified as *Coptotermes curvignathus* (Holmgren) belonged to lower termites in the family *Rhinotermitidae* and subfamily *Coptotermitinae*. Three pure isolates of the bacteria were obtained from *Coptotermes curvignathus* (Holmgren). Table 1 shows the characteristic of isolate 1.

Fig. 1: The nest of *Macrotermes gilvus* (Hagen), picture taken in UPM

Table 1: Characterization of isolate 1

Test	Isolate 1
Gram stain (24 h)	+
Catalase	-
Motility	-
Cell morphology	rod
OF	+
Sucrose	-
L- Fucose	+
D- Galactose	+
Mannan	+

+: Positive reaction, -: Negative reaction (John *et al*., 1994)

Table 2: Characterization of isolate 2 and 3

Test	Isolate 2	Isolate 3
Gram stain (24 h)	-	-
Oxidase (24 h)	-	-
Motility	+	+
OF	+	+
Cell morphology	rod	rod
Maltose	+	+
Sucrose	+	+
Glycogen	+	+
Citric acid	+	+
Uridine	+	+
Inosine	+	+
Acetic acid	+	+
L-Fucose	+	-
D- Cellobiose	+	+

+: Positive reaction, -: Negative reaction (John *et al*., 1994)

The bacterial cells were gram-positive, rod shaped, facultative anaerobe and non-motile. Based on the Bergey's manual and biolog reader, Isolate 1 was identified as *Clavibacter agropyri* (*Corynebacterium*) this kind of bacteria has not been identified elsewhere from the termite *Coptotermes curvignathus* (Holmgren). Zitzelsberger *et al*. (1987) has reported that a *Corynebacterium equi* was able to degrade a model compound of lignin bounds and also it has been demonstrated that a *Corynebacterium* was able to assimilate different phenolic compounds (Deschamps *et al*., 1980). Table 2 shows the characteristic of Isolate 2 and 3. The isolates were rod shaped, gram-negative, facultative anaerobe and non-motile. Isolate 2 was identified as *Enterobacter aerogenes* and Isolate 3 as *Enterobacter cloacae*. The lignocellulolytic activity of *Enterobacters* was investigated by Borji *et al*. (2003). Deschamps *et al*. (1980) has demonstrated that the *Enterobacter* is able to assimilate different phenolic compounds. The *Enterobacter aerogene* and *Enterobacter cloacae* had also been isolated from the termite *Coptotermes formosanus* by Adams and Boopathy (2005). The ability to degrade hemicellulose has also been demonstrated by a bacteria belonging to the group of *Enterobacteriaceae* which was isolated from the termite *Mastotermes darwiniensis* (Froggatt) and *Zootermopsis angusticollis* (Hagen). Kuhnigk *et al*. (1994) had isolated and identified bacteria from the termite *Mastotermes darwiniensis* and *Nasutitermes nigriceps* which were identified as *Enterobacter aerogenes* and *Enterobacter cloacae* and they were able to degrade lignin monomers.

In this study all three bacterial isolates were able to grow on CMC and cellobiose media indicating their cellulolytic capability. Wenzel *et al*. (2002) had detected some cellulolytic bacteria from the gut of termite *Zootermopsis angusticollis* and they have used CMC medium for their identification. *Enterobacter aerogenes* and *Enterobacter cloacae* has been investigated and the capability of these bacteria is clear to perform a series of anaerobic reactions such as O-demethylation, decarboxylation (Kuhnigk *et al*., 1994). The facultative bacteria with the capability of degrading lignocellulose compounds described in this study are found on the periphery of the termite gut while the strict anaerobes are in the center of the gut.

Further research is needed to better understand the ecology of these microbes and to study the role of these bacteria in industrial applications.

ACKNOWLEDGMENTS

We thank Mr. Mohammad Zawawi, Ali Nargeskhani for their technical supports in this study and Mr. Cheong Yew long for identifying the termites.

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