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ORIGINAL ARTICLE

STUDY ON ISOLATION AND IDENTIFICATION OF CELLULOLYTIC BACTERIA FROM GUT (INTESTIONAL) OF DRY WOOD TERMITES

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ABSTRACT

The present study was conducted to isolate, identify aerobic and facultative anaerobic cellulolytic bacteria from gut region of the local drywood termites. The isolation is been carried out through gut sample serial dilution method. An isolated microorganism is cultivated in culture media. After growth of microorganisms at 34ºC identification of microorganisms was carried out by macroscopic examination. The bacterial species were tentatively identified by using the Bergey's manual. The isolates were using carboxy methl cellulose and cellobiose medium. The species were novel strains and identified as *Bacillus cereus, Klebsiella pnemoniae* and *Lysini bacillus***.** respectively based on biochemical analysis. Our results indicate that the dry wood termite gut is an attractive source for the study of novel cellulolytic microorganisms and enzymes useful for cellulose degradation.

Keywords: Cellulolytic Bacteria, Drywood Termites, Carboxy Methyl-Cellulose ,Cellobiose, biochemical.

1.INTRODUCTION

There are various species of bacteria in termite gut that are capable of degrading cellulose and hemicelluloses (wenzel et al., (2002). Termites are one of the most important of the soil insects that efficiently decompose lignocellulose with the aid of their associated microbial symbionts. Termites are said to dissimilate a significant proportion of the cellulose (74–99%) and hemicellulose (65–87%) components of lignocellulose they ingest Ohkuma (2003). It plays a key function in the tropics and sub tropics owing to their ability to decompose plant litter. They are especially known to thrive on lignocellulolytic materials such as barks, woods and plant materials (Leadbetter, 2007). Termites are among the most important lingocellulose-digesting insects and possess a variety of symbiotic microorganism in their hindguts, including bacteria (Brune, 2007). The diversity of termites gut communities is extra ordinarily high and the most abundant bacteria that have been identified from lower termites belong to the species of strict or facultative anaerobes (Nakashima et al., 2002).Therefore the main objectives of this study were to isolate and identify cellulolytic microbiota in the gut region on the termites.

2.MATERIALS AND METHODS

Collection of termites:

Termites were collected from decaying barks in the Annamalai University (11.3908ºN, 79.7148ºE). Termite specimens of all the populations collected (packed in scintillation vials in rectified alcohol) were identified from Zoological Survey of India, Kolkata and Forest Research Institute, Dehradun using the keys or descriptions of Chhotani [Chhotani OB .1997]. The details of collection site, date of collection, source, and name of the collector were mentioned on each vial. They were identified according to color, shap, size of their body and the size of their mandible dry wood termite. Termites were kept in container and fed with filter paper for one week.

Termites gut Bacteria of isolation:

The isolation of termites gut bacteria were performed according to Wenzel et al., (2002). After one week of feeding termites with filter paper, 10 termites were surface sterilized with 70% ethanol to remove contamination. Then they were rinsed in distilled water and allowed to air-dry for 1minute. Under aseptic condition, termites entire gut was picked out with a pair of sterilized medium and mixed with 15mL of medium containing 5g/L CMC, 0.1g/L CaCo3, pH 6.7.The culture was incubated for four weeks at 30°C.After incubation, 1mL of the culture was spread on cellobiose medium and incubated for 24hrs at 34C. Single colonies were obtained by quadrant streak and purified for subsequent studies.

Biochemical tests:

Each isolate was tested for its ability to grow in anaerobic condition by performing the OF test (Oxidative-fermentative) as described by Hugh and

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Leifson,(1953).Gram strain, indole, methyl, VP ,citrate utilization and starch hydrolysis isolates as described by Murinda et al., (2002).

Growth on CMC and cellulobiose:

Two different media were used to test the ability of bacteria to grow in a cellulolytic medium as described by wenzel et al (2002). The media were used to test the ability to grow in cellulolytic medium as described by Dickerman et al (1951).The medium contained 0.05g/100 mL cellobiose. The other components of the medium were 0.4g yeast extract, 1g malt extract and 1.2g agar per 100ml distilled water. The pH of the medium were adjusted to 7.0 and the medium was autoclaved at 120°C for 20min.The solidified medium was spread with the bacterial isolates and incubated at 39°C in aerobic and anaerobic conditions.

3.RESULTS AND DISCUSSION

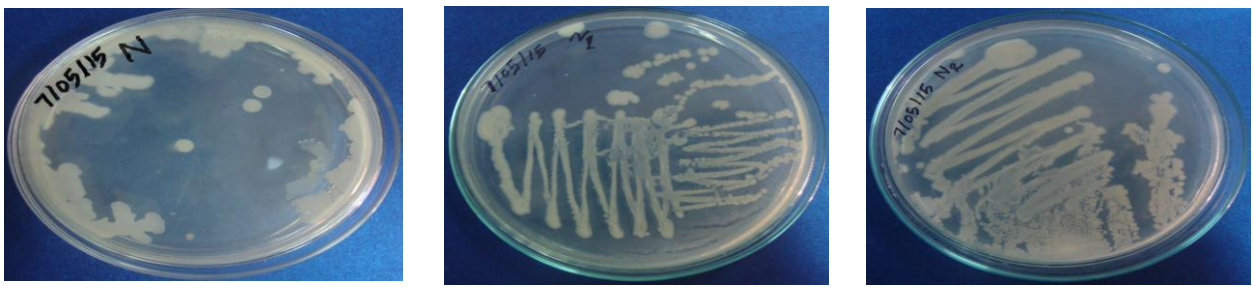
The termite was identified as dry wood termite that was from the lower termite family kalotermitidae. The characteristic (size, color, shap, of the pronotum, marginal teeth, mandible), Messenger et al. (2003). In this study, three bacterial species were isolated from the gut of dry wood termites. Three isolates were found to be cellulolytic bacteria. Three Bacterial species grow well on CMC and Cellulobioes. The OF test (Oxidative-fermentative) for these species were positive, which indicates their ability to grow in both aerobic and anaerobic condition. All bacterial cells gram, shaped, facultative anaerobe and motile based on the Bergey's manual Brenner et al. (2005). (Table.1)**.**The *bacillus.Sp* bacterial cells were gram-positive, rod shaped, facultative anaerobe and motile and several bacillus species identified, have been isolates from the termite was species were *bacillus.Sp* termite gut (Kutnigh et al., 1994). *Bacillus.Sp* has been isolated from the Niger Deta mangrove swamp and soils are capable of growing, have potential for used in biodegradation of PE. Seon- Yong chung et al., (1994) have isolated, characterized and identified bacteria *bacillus.Sp* from the gut of the soil feeding with possible antibiotics and enzymes for industrialization.

They were identified by the fact that they were aerobic, rod-shaped and gram-positive cells. Lysinibacillus are Gram-positive, rod-shaped, and round-spore-forming bacterial genus of the family Bacillaceae. Many bacterial species isolated from the digestive tracts of the woodborers belong to the genus Lysinibacillus (Bosire Carren.M et al 2013). It is expected that bacteria found a mutual symbiosis within the digestive tract of the woodborers. Bacteria inhabit the soil or wood and develop significantly when there are easily degradable organic nutrients. The bacterial community inside the digestive tract of woodborers may pertain to at least four physiological groups: plant growth promoters, free-living nitrogen fixers, biocides and phosphate solubilizers. Lysinibacillus is commonly found in the isolated from t insect gut (Maji et al., 2012).

Klebsiella pnemoniae bacterial were gram-positive, rod shaped, facultative anaerobe and non-motile. The role of the *Klebsiella pnemoniae* in the primitive termite gut had isolated and identified bacteria from the *Bombyx mori* which are able to cellulose degradation as reported by Anand et al. (2010). Studying the culture dependent and culture independent methods is important to evaluate the variations obtainable in different ecological and environmental condition of the termite gut microbial diversity Husseneder et al. (2003). Termites are common insects in tropical and subtropical regions and thrive on wood and catalytic materials. In this background study has led to the isolation and identification of the bacterial isolates with cellulolytic activity from the gut of dry wood termites under environmental conditions. Therefore, cellulolytic bacteria isolated from the present study may be used in the degradation of cellulolytic materials to increase their digestibility and possibly. From this study three bacterial species of cellulytic bacteria were isolated from the gut of the dry wood termite. They were identified *Bacillus cereus, Klebsiella pnemoniae and Lysinbacillus*. Further investigatation is very much required to characterization of cellulolytic bacteria which are crucial for bioful production and industrial application.

Fig 1. Showing the Microscope field view of the selected bacteria

Fig 2. Showing the Microscope field view of the selected bacteria



Bacillus. cereus ***Klebsiella pnemoniae*** ***L. bacillus***

Table 1: Isolation and Morphological characters of organisms

Cheracteristic	*Bacillus.cereus*	*Klebsiella pnemoniae*	*Lysini bacillus*
Grams	+	-	+
Morphology	Rod	Rod	Rod
Motility	Motile	Non-Motile	Motile
Indole	-	-	-
Methyl red test	+	-	-
V-P test	+	+	-
OF(Oxidative fermentative)	+	+	+
Oxidase test	+	-	-
Citrate Utilization	+	+	+
TSI	Acid/Alk	Acid/Alk	Acid/Alk

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