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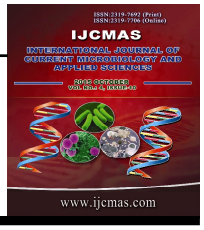

Original Research Article

Studies on Cellulolytic Activity and Structure of Symbiotic Bacterial Community in *Odontotermes parvidens* Guts

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Keywords

Isoptera, *Odontotermes parvidens*, CMC, Zd/Cd, Gram's iodine

A B S T R A C T

Termite (Isoptera) are the most lignocellulose digesting insects which harbour a great diversity of bacteria in their gut. These bacteria live in a symbiotic relationship. These bacteria were isolated from the termite gut and cultured to study the cellulolytic activity of these bacteria. The cellulolytic activity of these bacteria was observed by culturing them on Carboxymethyl-cellulose (CMC) media (Modified Czepak media). Then the cellulose digesting bacterial isolates were identified with the help of Garm's Iodine. It showed a clear zone or halo zone around the colony. It was observed that termite species *Odontotermes parvidens* harbours a total of 19 culturable bacterial isolates, out of which 15 showed cellulolytic activity. The maximum Zd/Cd ratio was shown by NSB215 and minimum by NSB26. These results showed the efficiency of bacteria to digest the cellulose content presen in their food.

Introduction

Termites (order Isoptera) can be divided into two groups, lower and higher termites. Lower termites are restricted to the diet of wood or grass. Higher termites, on the other hand, include soil-feeding (humivorous), wood and grass-feeding (xylophagous) and fungus-consuming species (Noirot,1992).

They are especially known to thrive on lignocellulolytic materials such as bark, woods and plant materials (Leadbetter, 2007). Termites are among the most important lignocellulose digesting insects and possess a variety of symbiotic microorganism in their hind gut including bacteria (Brune, 2007). These bacteria play a role in decomposing cellulose and hemicellulose (Paul *et al.*, 1993). The diversity of termite gut communities is extra ordinarily high. This study was culture dependent.Therefore, the main objectives of this study were to isolate and characterize cellulotytic bacteria in the gut of termite species *Odontotermes parvidens*. And to know the cellulose digesting efficiency.

The presence of steep gradients of oxygen and hydrogen in a particular proportion inside the gut creates a microhabitat for some microorganisms (Ohkuma, 2003).

The association of gut cellulolytic microbiota with termites is a well-known example of mutual symbiosis. The presence of gut microbiota help termite to survive on a diet of wood or cellulose. Higher termites harbor a dense and diverse population of gut prokaryotes (Ohkuma *et al.,* 2001) which endocytose wood or cellulose particles into their food vacuoles and degrade cellulose to produce acetate, which is in turn absorbed by termites as their energy and carbon source (Inoue *et al.,* 1997). These termites have endogenous cellulases and symbiotic microorganisms in their hindguts including flagellated protozoa, bacteria, and yeasts which are able to convert decaying wood into organic matter thereby enriching the soil (Li *et al.,* 2005).

Material and Methods

Collection of termites-Termite workers were collected from the tree bark in Kurukshetra University, Kurukshetra. Kurukshetra (India) is situated at $29^0$58 N latitude and $76^0$51 E longitude at about 250 m a.s.l. Approximately 100 termites were brought to the laboratoay and identified with the help of identification keys (Chhotani, 1997).

Preparation of termite homogenate- Termites were washed with sterile distilled water and surface sterilized with 70% (v/v) ethanol. Approximately 50 termite guts were extracted and kept in 1 ml of Phosphate Saline Buffer(PBS) and were preserved at -20^oC. Further the samples were vortexed for uniform distribution and this homogenate was used for isolation of bacterial strains.

Isolation of bacteria from termite gut- About 1 ml of termite homogenate was serially diluted (upto 10^{-5}) dilution using PBS. The nutrient agar medium (Peptone 5.0 g/L, Beef extract 3 g/L, yeast extract 3 g/L, Agar 2 g/L, pH 7$\pm$ 0.2) was prepared and sterilized at 15 lbs at 121.5^oC for 15 minutes. Sterilized melted and cooled medium was poured into petriplates. After solidification, samples from 10^{-4} and 10^{-5} were pipetted and spread gently on agar. These plates were incubated at 37^o for 24–48 hrs. After incubation the colonies of bacteria were picked and purified by repeated streaking on nutrient agar plates.

Detection of cellulase producing activity of isolated bacteria- The purified colonies were then transferred to the Carboxymethyl-cellulose (CMC) containing media (Modified Czepak media). These colonies were grown in circular batches on CMC agar. Then the bacterial colonies with cellulase activity were identified with the help of Gram Iodine. The cellulose hydrolysing activity was indicated by the appearance of clear zone around the bacterial growth. Morphological characterization of strains was done with the help of Gram's Stain. This study was aimed to isolate and characterize the cellulose digesting bacteria from the termite (*Odontotermes parvidens*) gut.

Results and Discussion

Screening, isolation and purification of cellulolytic bacterial species- A total of 19 bacterial isolates were cultured on nutrient agar media and CMC media. Screening and isolation procedure resulted in the isolation of 15 cellulolytic bacteria out of 19 from a single termite species (*Odontotermes parvidens*). These bacteria were showing differential cellulose digesting activity on CMC media.

Plate assay for cellulase production- The size of halos and colonies were used to compare the enzyme activity (Tomova *et al.,* 2014).

Table.1 List of efficient cellulolytic bacterial isolates

Sr. No.	Bacterial Isolate	Mean Zone Diametre (mm)	Mean Colony Diametre (mm)	Clear Zone (mm)	Ratio Zd/Cd
1.	NSB21	19	07	12	2.71
2.	NSB22	17	06	11	2.83
3.	NSB23	18	08	10	2.25
4.	NSB26	07	04	03	1.75
5.	NSB27	24	09	15	2.67
6.	NSB29	21	11	10	1.90
7.	NSB210	18	06	12	3.00
8.	NSB211	19	06	13	3.17
9.	NSB212	20	07	13	2.86
10.	NSB214	18	07	11	2.57
11.	NSB215	21	06	15	3.50
12.	NSB216	18	08	10	2.25
13.	NSB217	11	05	06	2.20
14.	NSB218	13	05	08	2.60
15.	NSB219	17	06	07	2.83

Table.2 Morphological characterization of bacterial isolates

Sr. No.	Bacterial Isolate	Colony morphology					Shape of Bacteria	Gram Staining
		Shape	**Surface**	**Margin**	**Colour**	**Pigmentation**		
1.	NSB21	Spherical	Raised	Entire	Light pink	Pink	Rod/Bacilli	+
2.	NSB22	Irregular	Flate	Irregular	Creamish white	None	Vibrio	+
3.	NSB23	Spherical	Raised	Entire	Cream	None	Spirillum	+
4.	NSB26	Spherical	Raised	Entire	Light pink	Pink	Small rods	_
5.	NSB27	Spherical	Raised	Entire	Red	Red	Thin rods	_
6.	NSB29	Spherical	Raised	Entire	Dark Brown	Brown	Thin filamentous rod	_
7.	NSB210	Spherical	Raised	Entire	Light pink	Pink	Small, thin rods	+
8.	NSB211	Spherical	Raised	Entire	Light yellow	Yellow	Thick rods in chain	+
9.	NSB212	Spherical	Raised	Entire	yellow	Yellow	Rods	+
10.	NSB214	Spherical	Raised	Entire	Cream	None	Thick rods	+
11.	NSB215	Irregular	Smooth	Irregular	Cream	None	Rods	+
12.	NSB216	Irregular	Raised	Irregular	Creamish yellow	None	Rods	+
13.	NSB217	Spherical	Smooth	Entire	Creamish white	None	Rods	+
14.	NSB218	Irregular	Flate	Irregular	Brown	Brown	Thin spirillum	+
15.	NSB219	Spherical	Smooth	Entire	Reddish Brown	Reddish Brown	Spirillum	+

Figure.1 Screening of cellulolytic bacteria by flooding the petri dishes with Gram's Iodine. A clearance zone surrounding a colony is an indicative of hydrolytic activity of cellulose

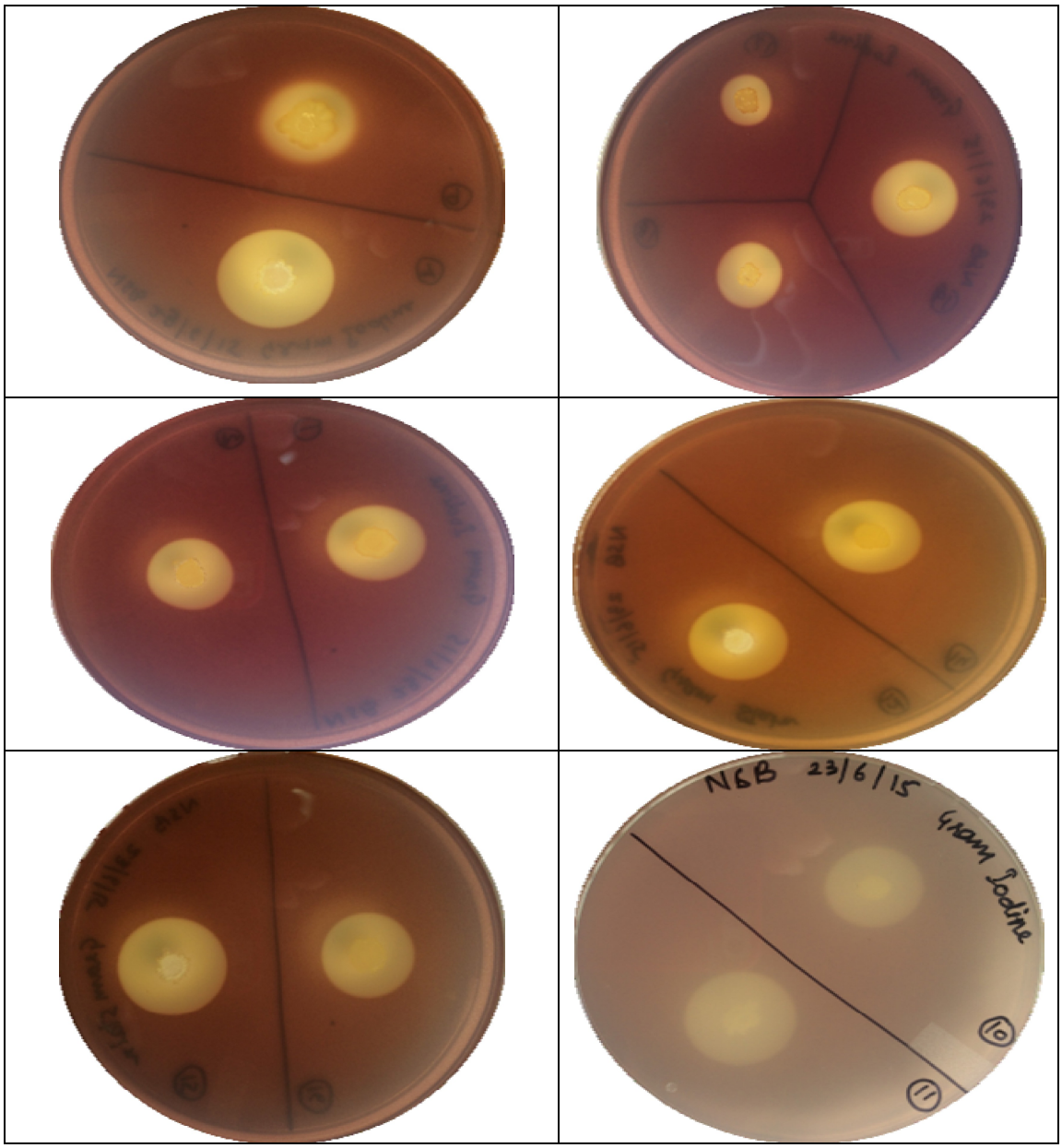
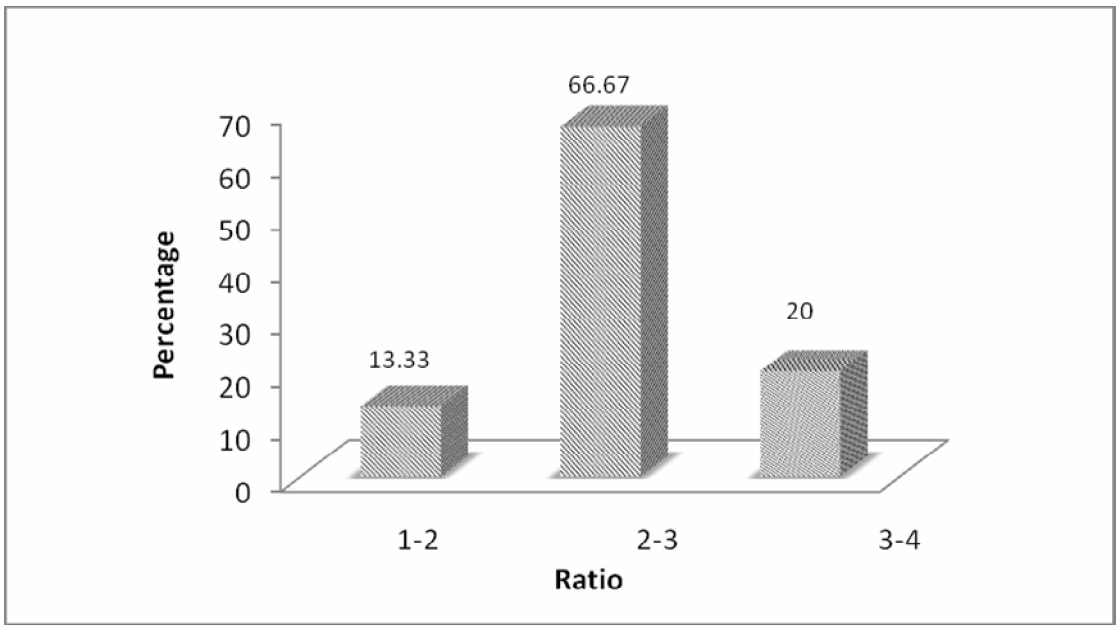


Figure.2 The ratio of the diameter of the zone of clearance to the diameter of the colony and the percentage of the bacterial isolates in each range of ratios



All the 15 bacterial isolates showed clear zone or halo zone around colonies with Gram's Iodine (Fig. 1). These isolates showed moderate to high cellulolytic activity (Ahmad Bashir *et al.,* 2013). Out of these 15 only 2 bacterial isolates showed zone diametre to colony diametre ratio (Zd/Cd) below 2.00. Out of 15 isolates, 11 isolates showed cellulolytic activity in the range of Zd/Cd ratio 2.0-3.0. and only 2 which were showing highest activity above 3.0 (Table 1). The diameter of halo- zone also a measure of cellulose degesting ability of bacterial strains (Upadhyaya *et al.,* 2012). The maximum cellulolytic activity, clearzone diameter was shown by NSB27 and NSB215 i.e. 15 mm but the Zd/Cd ratio was maximum in case of NSB215 i.e. 3.50, while the minimum cellulolytic activity, clear zone was shown by NSB26.

There are total 13.33% isolates which were showing Ratio Zd/Cd in the range of 1–2, 20% showing range between 3–4 and maximum isolates i.e. 66.67% showing range between 2–3 (Fig. 2).

Morphological characterization of bacterial isolates- Bacterial isolates were characterized with the help of colony morphology and Gram's staining method. These isolates showed variety of colonies on nutrient agar media. Out of 15 bacterial isolates, 12 isolates were Gram positive and only 3 isolates were Gram negative. Out of these 15, 11 isolates were rod shaped, 3 spiral and 1 vibrio shaped (Table 2).

There are many microbes present on the earth which are capable of degrading cellulose but some of these bacteria harbour insect gut like *O.parvidens*. These bacteria share highly symbiotic relationship with termite and colonozing all ecological niches (Gibson and Hunter, 2010; Harris *et al.,* 2010). This termite species harbour a large number of bacterial isolates out of which only 15 isolates could be cultured in the laboratory. These bacteria showed the remarkable cellulose digesting ability. The presence of clear zone showed the cellulose efficiency of the bacteria.

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