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Antibacterial Activity of Cellulase Enzyme Isolated from Gut Microbiota of *Coptotermes ceylonicus* (Termite)

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Abstract: Gut microbiota of termites belonging to the species *Coptotermes ceylonicus* is known to contain plethora of enzymes of which cellulase is the most important among them. Cellulase was successfully extracted and isolated from the gut of these termites by using standard protocol. Cellulytic index was calculated and found to be 3.25. The extracted crude enzyme was partially purified by Dinitro Salicylic Acid (DNS) method. Extracted cellulase enzyme was screened for antibacterial activity against two bacterial species, *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) using well diffusion method. Partially purified cellulase enzyme showed effective antibacterial activity against *E. coli* (16 mm of zone of inhibition), however, there was no such effect observed in the *B. subtilis*. In this study we successfully isolated cellulase enzyme from the gut of *Coptotermes ceylonicus* and confirm its bactericidal effect against *E. coli*. Further studies are required to test the bactericidal effect of cellulase against different bacterial species.

Keywords: Termites, *Coptotermes ceylonicus*, Cellulase, Well diffusion method, *Escherichia coli*, *Bacillus subtilis*, Antibacterial activity

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Introduction

Termites (Order: Isoptera; Ranjith and Kalleshwaraswamy, 2021) play a great role in terrestrial ecosystem by recycling lingo cellulosic biomass, which refers to a mixture of cellulose, hemicellulose and lignin. Termite comprises of a complex assemblage of diverse species, roughly divided into so called higher and lower termites. Lower termites harbor a dense population of prokaryotes and Protists (single celled eukaryotes) in their gut. Higher termites comprise only one apical family (Termitidae) but more than

three quarters of all termite species. While they also harbor a dense and diverse array of prokaryotes, higher termites lack protists (Ohkuma, 2003). This association of gut cellulolytic protists with lower termites is a well-known example of mutual symbiosis. The protists endocytose wood or cellulose particles to produce acetate, which is then absorbed by termites as energy and carbon source (Ohkuma, 2003). Little is known of the fungus cultivating termite's digestive processes and the microbial associates

housed within the alimentary canal (Breznak, 2000). The termite gut, thus presents a novel habitat for searching new antibiotic derivative producing strains. The wide spread use of microorganisms in the chemical and pharmaceutical industries has come about as a result of the recognition that they are cheaper to use for synthesis of organic compounds such as antibiotics rather than to synthesize them chemically. Thousands of different antibiotics produced by fungi, *Actinomycetes* or unicellular bacteria have been isolated and characterized. Higher termites produce termicin and spinigerin antibiotics which are useful medically as well as for protection of plants from pathogenic fungi. Some antibiotic compounds isolated from termites have also been used for treating microbial infections such as sinusitis, bronchitis and skin infections (Lamberty, 2001). Overuse of antibiotics, poor quality and counterfeit drugs with the wrong level of active therapeutic ingredients have led to emergence of resistant microorganisms. Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness higher health care expenditures, and a greater risk of death (WHO, 2013). When infections become resistant to first line drugs, more expensive therapies must be used. The achievements of modern medicine are put at risk by multi - drug resistant microorganisms. Without effective antimicrobials for prevention and treatment of infections, the success of organ transplantation, cancer chemotherapy and major surgery would be compromised (Struelens, 2003). There is need to come up with cheaper and affordable sources of new antimicrobials and hence, the study of termites could be important in this respect. The present study is an attempt to isolate the cellulase enzyme from the gut of termite *Coptotermes ceylonicus* and to study its antibacterial activity.

Materials and Methods

Termite Collection:

Termites were collected from a wood shop in

Ayanavaram (Chennai, India). Termites were surface sterilized using 70% ethanol for 30 seconds and the whole body of termites was macerated using mortar and pestle. The paste obtained from the termite was used for isolation of the bacteria. The bacteria were inoculated on the media by swabbing technique.

Screening of Cellulase Producing Organisms:

Nutrient broth agar media was used for the inoculation of bacteria. Then for primary screening of cellulase producing bacteria, the bacteria from the colonies of nutrient broth agar plates were inoculated in the Carboxymethyl cellulose media as it preferably supports the growth of cellulolytic bacteria and pure culture was obtained by using the preparation of same media. The secondary screening of cellulase producing organisms was done by Congo red test. Agar plates prepared with 1% CMC strains were streaked and petri plates were incubated at 37 C for 48 h. Petri plates were flooded with 0.1% Congo red reagent and left for 20 min. Then the plates were washed with 1M NaCl. Clearance zones called halo zones are seen against the red color of Congo red for the positive test. Enzyme activity was indexed as:

$$\text{Cellulolytic Index} = \frac{\text{Diameter of zone} - \text{Diameter of bacterial colony}}{\text{Diameter of bacterial Colony}}$$

Only the isolates that produced a clear zone around the colony were chosen for further study. The index value was 3.25.

Enzyme Assay:

Cellulase activity was measured following the method of Miller (1959). Briefly, a reaction mixture composed of 0.2 ml of crude enzyme solution and 1.8 ml of 0.5% carboxy methylcellulose (CMC) in 50 mM sodium phosphate buffer (pH 7) was incubated at 37 C in a shaking water bath for 30 min. The reaction was terminated by adding 3 ml of DNS reagent. The color was then developed by boiling the mixture for 5 min. OD of samples was measured at 575 nm against a blank containing all the reagents minus

the crude enzyme (Table 1).

Table 1: Enzyme Assay

S.NO	Cellulase Sample	Enzyme Activity IU/ml
1.	Blank	0
2.	Crude Enzyme	0.1498

Purification of Cellulase by Ammonium Sulfate Precipitation:

The prepared crude enzyme was saturated with solid ammonium sulphate at different concentration of 40, 60, and 80 g. The mixture was kept overnight at 4 C in a magnetic stirrer. Then the mixture was centrifuged and the pellet was dissolved in 50 ml of 1M Sodium Phosphate buffer at pH 7.0 for further purification (Table 2). The partially purified sample was assayed for enzyme activity by using DNS method.

Table 2: Partial Purification of Cellulase

S.No	Cellulase Samples	Enzyme Activity IU/ml
1.	Crude Enzyme	0.1498
2.	Partial Purification	0.1383

Antibacterial Assay by Well Diffusion Method:

Preparation of Mueller Hinton agar was allowed to autoclave by sterilization process. The media was poured into petri plates and allowed to solidify without any contamination. Bacterial strains *Escherichia coli* and *Bacillus subtilis* were used to swab the plates (gram-positive and gram-negative bacterial strains are used). Wells were made in the plate. The well at the center was added with 5 µl of Tetracycline and other wells were filled with crude enzyme at differed concentration of 20, 40, 60, 80 µl. It was incubated at 37 C for 24 h. The diameters of any inhibition zones formed around the wells were then measured.

Results and Discussion

In the present study, after termite maceration,

nutrient broth agar media was prepared, and used for the inoculation of bacteria. Screening of cellulase producing bacteria, was done by inoculating the nutrient broth agar plates in the Carboxymethyl cellulose and pure culture was obtained by using the preparation of same media (Fig. 1). The enzyme was successfully extracted from the gut of the termites and the crude enzyme was measured by spectrophotometric method which was later screened for antibacterial activity against two bacterial species, *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) using well diffusion method (Fig. 2). Partially purified cellulase enzyme showed effective antibacterial activity against *E. coli* (16 mm of zone of inhibition) (Fig. 1), however, there was no such effect observed in *B. subtilis* (Fig. 2), suggesting that termite extracted cellulase was most effective against *E. coli* (Table 3).

Fig. 1: Agar plates

Table 3: Antibacterial Activity

S.NO	Bacteria	Antibacterial Activity	
		Results	Diameter of Zone (mm)
1.	<i>E. coli</i>	+	16
2.	<i>B. subtilis</i>	-	-

In this study the isolate showed antibacterial activity against gram-negative *E. coli* of a diameter of 16 mm and showed no bacterial activity against gram-positive *Bacillus subtilis*. This variation is due to inability of the antibacterial compound to inhibit both gram-positive and gram-negative

Fig. 2: Antibacterial activity in *Escherichia coli* and *Bacillus subtilis*. (A)- *Escherichia coli* showed inhibition zone around the colony (diameter 16 mm); (B)- In *Bacillus subtilis* there is no inhibition zone around the colony.

bacteria growth in the respective media set up and could be due to variation in the cell wall permeability of bacteria to different drugs (Struelens, 2003). In past, Aswani *et al.* (2015) have reported that the cellulase produced by the isolate from the gut of *Macrotermes michaelseni* showed similar antibacterial activity against *E. coli*. Thus, the present study reveals that the social worker termites are one of the important insect for isolating potential microorganisms producing novel cellulase enzyme by the confirmation of screening method and determination of enzyme activity which has many applications in different industries.

Conclusion

It is concluded from this study that cellulase enzyme secreted by termites belonging to species, *Coptotermes ceylonicus* has antibacterial effect upon *Escherichia coli* but has no effect on *Bacillus subtilis*. Further studies are needed to evaluate about antibacterial effect of this cellulase enzyme upon other bacterial species.

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