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Extraction and Identification of Bacterial Cellulases from Termite Gut

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ABSTRACT

The termites have special ability to use different types of bacteria from their gut to break down plant components. The techniques for extraction and identification of bacteria that break down cellulose in termite stomachs, are discussed in this study. Certain bacteria, including *Pseudomonas* and *Klebsiella* are known to produce cellulases, which are essential for breaking down cellulosic materials into sugars in order to produce biofuel. The potential of termite gut bacteria in biotechnology and sustainable fuel sources is highlighted.

Keywords: Extraction, Identification, Termite, Bacteria, PCR

Introduction

Termites are abundant throughout the region, ranging from tropical to cooler regions and they may break down a variety of plant-based products. Termites seriously harm both organic and synthetic structures and even agricultural products. Annually causing significant financial losses. The variety of microbes found in termite guts makes them the most effective for the breakdown of lignocellulose-containing materials in the natural world [1]. The gut microorganisms are crucial for termites that consume wood. These bacteria facilitate effective wood digestion especially for the cellulose constituents [2].

Termite has two systems for the breakdown of cellulose. One of them is endogenous cellulose and cellulases from protozoa and symbiotic bacteria make up the other [3]. The majority of research on insect cellulolytic systems has focused on lower termites and wood-eating cockroaches which have a close evolutionary relationship. It is well known that, an expanded area of the hindgut of lower termites and wood-eating cockroaches have different types of bacteria [4].

Studies revealed that a single termite species is home to several hundred species of termite specific gut bacteria and that the microbiota is uniform within each host termite population [5]. Over the past ten years, rRNA gene-based molecular approaches have been used to study the communities of bacteria in termite stomachs. In China, termites *Odontotermes formosanus*, which grows underground fungi, is a major pest of plants and rivers. In *Odontotermes formasanus* the clones of *Clostridiales, Bacteroidetes, Proteobacteria* and *Actinobacteria* have been found [2]. *Trichoderma reesei, Penicillium janthinellum, Trichoderma reesei, Aspergillus kawachi, Aspergillus aculeatus, Penicillium janthinellum* and *Penicillium janthinellum* found in the gut of *O.formasanus* [6].

Many techniques are used for the isolation and identification of bacteria from the gut of bacteria which include dissection and preparation, Surface Sterilization and Homogenization, PCR-Based Genotypic Analysis and REP, BOX and ERIC-PCR based genotypic analysis. Many strains of bacteria are isolated from the gut of termite using various techniques and after isolation those strains are cultured in lab. Streak plate method is used for the purification of bacteria and after culturing it is shown that these bacteria are capable for the degradation of cellulose [6]. In this article, the techniques for the extraction of bacteria from the gut of termite are discussed.

Different methods for extraction of bacteria from termite gut

Dissection and Preparation:

Termites are collected from woody material and stored in a container. Termites were disinfected using 70% alcohol rinsing. Every termite was cut up into its head and body. The heads were removed using forceps and plastic rods were used to smash the bodies. Using a series of successive dilutions, the termites gut paste was utilized to isolate the bacteria. For the growth of cellulolytic bacteria the nutrient broth agar and CMC media were used [7]. After purification, the researchers separated cellulases from termite extracts using SDS-PAGE gels. The precise protein fractions were identified with the help of electrophoresis [6]. This technique offers easy access to the gut's contents, which are home of variety of cellulolytic bacteria. But it is time-consuming and needs to be handled carefully.

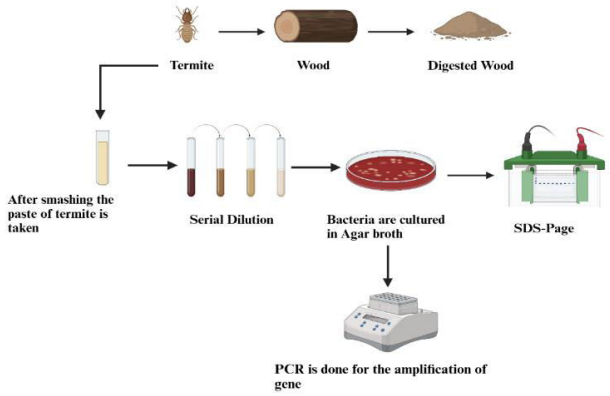

Fig. 1: Isolation and identification of cellulose bacteria from the gut of termite.

Surface Sterilization and Homogenization

The termites that had been gathered were immediately used to obtain DNA. Using sterile forceps, the entire guts of twenty randomly selected workers were removed from each colony. Using an Isoplant II kit (Nippon Gene) that chemically lyses bacterial cell walls and membranes with benzyl chloride, DNAs were recovered from the gut homogenates. A DNeasy tissue kit (QIAGEN) was used for the further purification of extract [8]. This technique minimizes microbial infection from outside sources. It is quite easy to understand and uncomplicated. But not all surface bacteria may be totally eradicated, there is possibility that sensitive bacteria will lose during homogenization.

Fluorescent In Situ Hybridization (FISH)

Termite guts were obtained, preserved and subjected to fluorescence probes that were tailored to certain bacterial strains. The ribosomal RNA of bacteria was bound by these probes. The samples were examined under a fluorescent microscope to observation and identification of bacterial communities, enabling the investigation of their composition and spatial distribution within the gut, once extra probes had been cleaned out [9]. It allows the direct observation of bacteria in their natural habitat while maintaining the interactions and geographical context. FISH may overlook some bacterial species since it is limited to identifying bacteria that can hybridize with available probes.

Methods used for identification of cellulases

Molecular identification of potential cellulose-degrading isolates

To identify different bacterial isolates, 16S rRNA gene was amplified, resulting in 1300bp amplicons. Strains including *Pseudomonas*, *Salmonella*, *Klebsiella*, *Serratia* and *Enterobacter spp*. were found in the analysis. In addition to that, cellulolytic bacteria from termite gut were identified by analyzing the 16S rRNA gene using universal primers (27F and 1492R). The PCR procedure were conducted in a BIORAD T100™ thermocycler and included an initial denaturation at 95°C for five minutes followed by thirty cycles of 95°C for thirty seconds, 55°C for one minute, 72°C for one minute and final elongation at 72°C for seven minutes [10]. It is highly specific for identification of bacterial species, but it requires sophisticated laboratory equipment and expertise.

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Table 1: For identification of bacterial strain used universal primers [11].

Sr. No.	Primers	Sequences of primers
1	REP F REP R	5'TCGICTTATCTGGCCTAC3' 5'TTTTCGTCGTCATCTGGC3'
2	ERIC F ERIC R	5'AAGTAAGTGACTGGGGTGAGCG3' 5'TGTAAGCTCCTGGGGATTCAC3'

PCR-Based Genotypic Analysis

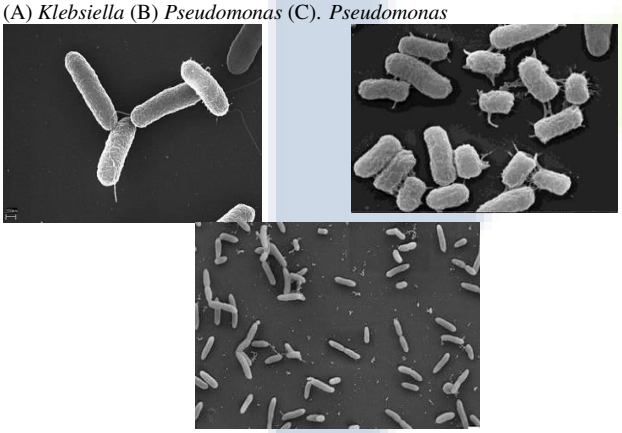
Using primers 27F and 1492R in a 50 µl reaction mixture, the 16S rRNA gene was amplified. The PCR protocol consisted of an initial denaturation at 94ºC for five minutes, followed by annealing at 55ºC for thirty seconds, extension at 72ºC for 1.5 minutes, and a final extension at 72ºC for ten minutes, with a subsequent hold at 4ºC [11]. PCR can be used for diagnosis of many diseases caused by diversity of bacteria and variety of experiments and analysis. False negative and false positive results may decreses the specificity & sensitivity [12].

REP and ERIC-PCR based genotypic analysis

Using REP and ERIC primers, REP-PCR fingerprinting identified substantial genetic variation across 16 isolates. Variability ranged from 0.59 to 1.00 for REP primers and 50-1300bp amplicons were produced by ERIC primers, indicating significant polymorphism [11]. REP-PCR and ERIC-PCR are an efficient method for identifying genetic variation and differentiating closely related microbial strains. Primer specificity may place restrictions on the results and amplification conditions and DNA quality may also affect them.

Results

The bacteria had been extracted and identified as shown in Fig. 2 *Salmonella* (A) *Klebsiella* (B) *Pseudomonas* (C). *Pseudomonas*



Applications:

The heroes of the biofuel industry are cellulases. Their role is critical in the breakdown of difficult cellulosic materials into simple sugars that can be utilized in the production of biofuels. Together, these enzymes which include β-glucosidases, cellobiohydrolases and endo-glucanases cut particular cellulose structural linkages [16]. An important industrial enzyme, cellulase contributes to about 20% of the global enzyme market [17]. Furthermore, it is anticipated that the commercial biofuel manufacturing sectors will in the near future drive a significant portion of the demand for this enzyme [18].

Conclusion

It is concluded that termites are amazing insects that may be found around the world and can harm both agricultural items and buildings. Because of the presence of unique bacteria in the gut of termites and they are able to break down difficult plant materials which makes them unique. In this study the use of cutting-edge methods like PCR and DNA sequencing to examine these microorganisms. Termites are gathered and cleaned their digestive tracts and different bacteria are identified. Many other techniques such as gel electrophoresis and SDS-PAGE also aid in the identification of certain bacterial strains. Certain bacteria that have been isolated from termites have the ability to efficiently break down cellulose. That's information about bacteria from termites gut not only fascinating but it also has useful applications. The enzymes derived from these microorganisms called cellulases are essential to the biofuel sector. They contribute to sustainable fuel sources by converting plant resources into sugars that are used to produce biofuel.

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