ANALYSIS OF GUT FLORA FROM DAMP WOOD TERMITES (*TRINERVITERMES* SPP.) AND EXTRACTION, CHARACTERIZATION OF CELLULASE FROM THE ISOLATE

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INTRODUCTION

Termites are the most copious terrestrial insects in many damp and dry areas of tropical and subtropical ecosystems [1-3]. They are called as “white ants;” however, they differ from ants in morphological and phylogenetical characters [4]. Termites are polymorphic insects, living in large groups of some hundred to several million individuals, comprised reproductive (Winged) forms together with numerous sterile soldiers and workers [5,6]. All termites live in colonies within the confines of excavations within wood above-ground, or in subterranean and epigeal nest systems [7]. Termites portray an immense role in terrestrial ecosystem by recycling biomass containing the mixture of cellulose, lignin, and hemicellulose [8-10]. Termites are soil insects that efficiently decays lignocellulose with the aid of the gut-associated microbes to simplify carbohydrate that later fermented to ethanol using fungi.

They are a key functional group of animals in the tropics and can achieve high dense population. Their numerous colonies have great influence in ecosystems.

METHODS

Collection of termites

Damp wood termites were collected and identified as *Trinervitermes* spp. based on their shape and parts. The termites were surface sterilized with 70% ethanol. The head was trimmed off, and the entire gut was removed with sterile forceps and mixed in a mortar and pestle [11].

Isolation of microbes from termite gut

The guts were homogenized in 1 ml of sterile distilled water, centrifuged at 10,000 rpm for 5 minutes to remove large gut debris. The supernatant was serially diluted and spread plate method was performed on nutrient agar, agar and plate count agar. Colonies were picked up from the agar plates after incubating at 37°C overnight and inoculated into respective broths [12].

Isolation and generic level identification

From the resultant Petri plate of enumeration study of crushed mixture of termites, nine types of bacterial colonies were obtained. These colonies were isolated and cultured in individual Petri plates [13].

For proper characterization, the colonies were labeled as NSP1, NSP2,……. NSP8. Standard procedures of bacterial identification such as gram staining, spore staining, capsule staining, motility, IMViC, and other essential tests were carried out to identify bacteria at least up to the generic level [14].

Screening of bacteria for cellulase production

Enzyme production

The isolated organisms were inoculated in 150 ml Erlenmeyer flask which contains 50 g of cellulose initially the moisture content of the substrate is determined. The minimal salt medium was prepared with the following content (g/L), and the pH of the minimal salt medium (MSM) is adjusted before sterilization [15].

Ammonium sulfate - 10 g, potassium phosphate - 3 g, magnesium sulfate - 0.5 g, calcium chloride - 0.5 g, yeast extract - 7 g, glucose (dextrose) - 15 g. The MSM was prepared and sterilized [16].
Congo red method
The different bacterial species were inoculated in the minimal medium and were kept in the shaker for 5-7 days. The medium was then filtered and centrifuged at 10,000 rpm for 15 minutes. The agar was prepared with the following composition and autoclaved at 121°C for 30 minutes. The medium was poured in the sterile Petri plates and left for 15 minutes. The agar was prepared with the following composition and autoclaved at 121°C for 30 minutes. The medium was poured in the sterile Petri plates and left for 15 minutes. 20µl of the supernatant of centrifuged bacterial medium was added to the wells made in plates using micro pipette. These were incubated at 25-30°C for 72 hrs. The Petri plates were logged with 1% of Congo red and left for 15 minutes. The detaining of the plates was done with 1 M NaCl solution for 15 minutes. The zone of clearance was observed for the cellulose hydrolysis surrounding the colonies [17].

Assay of cellulase
Endoglucanase assay was performed by incubating 1 ml of crude enzyme with 1 ml of 1% carboxy methyl cellulose (CMC) in 50 mM sodium citrate buffer (pH 4.8) at 50°C for 30 minutes. At different growth period, the matrix was washed with phosphate-buffered saline, and the cell-free extract was used for analysis. The bacterial crude extract was prepared by centrifuging 10 mL of cell-free extract at 5000 rpm for 15 minutes. The activity of cellulase was studied, using dinitrosalicylic (DNS) assay method. The assay was carried out as follows. Culture filtrate 0.2 mL was mixed with 1% CMC in a test tube and incubated at 40°C for 30 minutes. The reaction was terminated by adding 3 mL of DNS reagent. The tube was then incubated at 100°C for 15 minutes followed by the addition of 1 mL of salt solution. The optical density was taken at 575 nm against blank. One unit of cellulase activity is defined as the amount of enzyme required to liberate 1 µmol of reducing sugars per minute under the assay conditions [18].

FPase assay was performed using 30 mg strip of Whatman filter paper No. 1 under the same conditions with incubation time of 1 hr [19].

The amount of protein in culture supernatant was determined using the method reported by Lowry et al. [20].

Optimization of nutritional parameters for cellulase production
Bacillus strain that had the ability to utilize various additional carbon sources. It was observed that CMC gave maximum cellulase activity at 1.8% concentration for enzyme production when compared to other carbon sources [21].

Effect of pH on enzyme production
Optimization was carried out using minimal medium of 2, 4, 6, 8 and 10. The pH was adjusted by using 1 N HCL or 1 N NaOH. Then, minimal salt medium was enriched with CMC powder. Autoclaved and then different bacterial (NSP1, NSP3, NSP4, and NSP5) batch cultures were maintained [22].

Effect of temperature on enzyme production
Optimization was carried out by placing the substrates containing minimal salt medium and the bacterial (NSP1, NSP3, NSP4, and NSP5) at different temperatures of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C and 55°C [23].

RESULTS

Identification study:
The organisms isolated were tabulated in Table 1.

Screening of cellulase from isolated organism
Congo red method was used to identify the potential strain from collection of bacteria isolates identified from the gut of termites (Fig. 1).

Enzyme activity
The enzyme activity was estimated using CMC as a substrate by DNS method. Table -2 lists the FPase and Endoglucanase activity of different microorganism isolated.
Table 1: Characterization of the different bacterial sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gram</th>
<th>Spore</th>
<th>Capsule</th>
<th>Shape</th>
<th>Motility</th>
<th>Biochemical test</th>
<th>Organisms identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>-</td>
<td>-</td>
<td>Bacillus sp.</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>-</td>
<td>-</td>
<td>Bacillus sp.</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Bacillus sp.</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Serratia sp.</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Salmonella typhi</td>
</tr>
<tr>
<td>NSP,</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacilli</td>
<td>+</td>
<td>-</td>
<td>Proteus vulgaris</td>
</tr>
</tbody>
</table>

I: Indole; MR: Methyl red; VP: Voges prokauer; Ci: Citrate; Co: Coagulase; U: Urease; N: Nitrate; H₂S: Hydrogen sulfide

Table 2: FPase and endoglucanase assay results for the isolated strains of bacteria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organisms identified</th>
<th>Cellulase activity</th>
<th>FPase</th>
<th>U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP,</td>
<td>Bacillus sp.</td>
<td>36.7±0.023</td>
<td>3.75±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Bacillus sp.</td>
<td>49.5±0.023</td>
<td>4.75±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Escherichia coli</td>
<td>17.9±0.023</td>
<td>1.75±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Staphylococcus aureus</td>
<td>36.6±0.023</td>
<td>3.65±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Bacillus sp.</td>
<td>27.1±0.023</td>
<td>2.75±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Serratia sp.</td>
<td>11.7±0.023</td>
<td>1.15±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Bacillus sp.</td>
<td>8.5±0.023</td>
<td>0.85±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Salmonella typhi</td>
<td>11.2±0.023</td>
<td>1.15±0.023</td>
<td></td>
</tr>
<tr>
<td>NSP,</td>
<td>Proteus vulgaris</td>
<td>9.2±0.023</td>
<td>0.92±0.023</td>
<td></td>
</tr>
</tbody>
</table>

*Mean square error

Effect of pH on cellulase activity (filter paper and endoglucanase assay)

The optimal pH for endoglucanase activity is found to be at pH 6 for bacterial cellulase. Increasing or decreasing the pH beyond this resulted in decline in enzyme activity. Cellulase yield by NSP appeared to depend on pH value. Results illustrated in Fig. 3 clearly show that the optimum pH for maximum production of cellulase. It was observed that the activity decreased with more increase in pH which indicates reduction in the activity [26-27].

CONCLUSION

The strong relationship between the microbes and the termites was well established. The organisms present in the midgut of termites are responsible for the degradation of lignocellulose material (Wood). By exploiting this phenomenon, the organisms were isolated from the termites and identified for the production of cellulase enzyme. From these isolated, the potential producers were identified and characterized at different temperature and pH. These organisms could be considered as effective candidates for the production of cellulase in large scale.

REFERENCES

