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# Microbial Study and Functional Analysis of Hindgut Microbiota of *Odontotermes obesus*(Rambur):(Blattodea:Termitidae)

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## Abstract

The present study investigated microbial diversity in the hindgut microbiota of *Odontotermes obesus*(Rambur). Colonies were isolated both aerobically and anaerobically by using tryptic soy agar medium. Isolates were characterized and identified by means of biochemical tests. Based on tentative identification, all the isolates belonged to the Enterobacteriaceae family. All the colonies were screened for cellulase, amylase and invertase activity. Enzyme assays were performed for positive colonies. The enzyme assay for amylase activity was found to be highest for strain No.16 at 1.46 the highest invertase activity was found in strain No.17 at 1.332 the enzyme assay for cellulase activity was found to be highest for strain No.12 at 0. 09 The results showed that termite gut bacteria play a significant role in biofuel production in terms of plant decomposition and conversion to simple sugars.

***Keywords:*** *Odontotermes obesus*; Enterobacteriacae; cellulose; amylase; invertase

## Introduction

Termites are social insects belonging to the order Blattodea (Telmah et al., 2020). Termites are a large and important group of insects in terrestrial ecosystems that decompose lignocelluloses (Zeinab et al., 2021). Termites are economically the most important pests in the world and can cause mass destruction of cellulosic material without any alarm. Diet and gut microenvironment have been the primary determinants shaping microbial communities in termite guts (Mikaelyan et al., 2017). It is estimated that out of the damage caused by pests, approximately 70% is due to termites usually attacking wood, fallen logs, paper or any cellulose-containing material (Eggleton, 2000). Termite plays an important economic role in economic entomology, with the cost of damage to buildings, especially in developed countries in America and Asia, calculated to be millions of money. In recent years, there has been a large increase in the scientific literature concerning termites (Vargo and Husseneder, 2009), which reflects their economic importance and the availability of funding to support termite research. Various preventative and remedial strategies are currently used against pest species in the termite control industry (Su and Scheffrahn, 1998, 2000).

Most termite species are known for their economic importance and cause damage to agricultural crops (Ahmed et al., 2006). *Odontotermes obesus* is a widely distributed mound building termite. The mound density varied from 8-10 mounds/ha. The presence of microorganisms in the hindguts of termites allowed them to thrive on recalcitrant plant matter. Termite gut symbionts reside in the lumen or are attached to the wall of the hindgut region and can represent more than 40% of the termite's weight (Bignell D. E. 2000). Diverse microorganisms inhabit the intestinal tracts of all termite feeding groups (Brune, 1998; Brauman et al., 2001) at a high density of 106-107 cells per µl of gut volume (Schultz and Breznak, 1978; Bignell et al., 1980; Brauman et al., 2001). Protozoa and bacteria play a key role in host physiology and gut ecology. Microbial cellulases, amylases and invertases have shown their prospective application

in various industries, including paper and pulp, laundry, textile, biofuel production, and agriculture. Biotechnological conversion of cellulosic biomass is a potentially supportable approach to develop novel products and bioprocesses. Microbial enzymes have become the central biocatalysts due to their complex nature and widespread applications. Cellulases, amylases and invertases are inducible enzymes produced by a large microbial diversity including both fungi and bacteria, during their growth on cellulosic materials. Enzymatic saccharification of lignocellulosic materials such as sugarcane bagasse, corncob, rice straw, *Prosopis juliflora*, *Lantana camara*, switch grass, saw dust, and forest residues by cellulases for biofuel production is perhaps the most popular application currently being investigated (Sukumaran et al., 2005; Kuhad et al 2010; Gupta et al., 2011). Bioconversion of lignocellulosic materials into useful and higher value products normally requires multistep processes (Kuhad et al., 2010; Ghosh and Singh 1993, Wyman et al., 2005).

The aim of the present research is to study the microbial diversity and functional analysis of the termite gut to utilize termite gut bacteria, which are economically important. The major outcome of the project is the identification of bacteria responsible for cellulose, starch and sucrose degradation to identify the enzymes that play a role in wood digestion, which in turn is a key factor for the biofuel industry. The other beneficiaries are agriculture and forestry departments.

## Materials and Methods

### Termite collection

Termite samples were collected from the botanical garden of Forman Christian College, Lahore.

***Figure 1:*** *Odontotermes obesus* from the Botanical Gardens of Forman Christian College and University.

### *Isolation of Hindgut*

Before dissection, termites were surface sterilized using 70% ethanol and rinsed with distilled water. The termite gut was dissected by using a fine dissecting needle, immediately transferred to phosphate-buffered saline and kept at -80°C until use.

### *Bacterial Culture and Identification*

The dissected gut was diluted in 100 ml autoclaved water. Ten microliters of diluted sample was spread on tryptone soy agar plates and incubated at 37°C for 24 hr. Single colonies were purified by the streak plate method and characterized using classical morphological i-e gram staining and biochemical methods by using Qts strips.

### *Anaerobic Culture*

Cultures were also grown anaerobically by using Gaspak jars containing an activated GasPak $H_2$ and $CO_2$ generator envelope. Single

colonies were purified by the streak plate method and characterized using classical morphological i-e gram staining and biochemical methods by using Qts strips.

### *Tentative Identification of Isolates*

The identification of bacteria is a crucial step in the study of microbial diversity from any environment. On the basis of colony morphology and biochemical tests, isolates will be identified.

Furthermore, their identification will be performed according to Bergey's Manual of Determinative Bacteriology (Bergey et al, 1974). It is considered one of the most important and authentic manuals used for identification purposes. It contains all important properties of identified bacteria on the basis of which they can be classified. It consists of information about phenotype, shape, size, cell arrangement, aerobic/anaerobic, gram staining, motility, spore formation, optimum growth temperature and biochemical assay results. This makes identification much easier (http://www.bergeys.org/pubinfo.html).

### *Enzyme assay*

### *Qualitative Screening for Amylase-Positive Strains*

The bacterial strains were grown on TSA plates containing starch for qualitative screening. After overnight incubation, iodine reagent was added to detect the presence of starch. Iodine reagent forms a complex with starch to form a blue–black color in the culture medium. Clear halos surrounding colonies indicate digestion of starch in the medium due to the presence of alpha-amylase.

### *Amylase activity quantification*

TSB containing starch was used for the estimation. Each culture tube contained 20 ml of medium and was inoculated with bacterial colonies positive for amylase and incubated at 27+1°C for 7 days at 140 rpm.

### *Procedure*

After seven days of incubation, the tubes were removed from the incubator, and the fermentation broth was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used for the amylase assay.

The amylase assay was conducted by mixing 500 µl of enzyme solution with 500 µl of soluble starch in 0.1 M sodium phosphate buffer (pH 7). The mixture was incubated for 15 minutes at 45°C. The reaction was terminated by adding 11ml of DNS by boiling it in a water bath for 10 minutes. Finally, the absorbance was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µg of starch under the assay conditions.

### *Qualitative Screening for Invertase Positive Strains*

TSB containing sucrose was used for qualitative screening. Each culture tube containing 5 ml medium was inoculated with bacterial culture, and phenol red was added as an indicator. The samples were incubated at 37°C for24 hours. Note the colour change from red to yellow. Yellow indicates positive colonies for invertase.

### *Invertase activity quantification*

Sucrose broth was used for the estimation. Each flask contained 50 ml of medium and was inoculated with bacterial colonies positive for amylase and incubated at 27+1°C for 5 days at 140 rpm.

### *Procedure*

The enzyme activity assay was carried out in a solution containing 100 µl of enzyme solution mixed with 100 µl of 0.5% sucrose in 10 mM acetate buffer. The mixture was maintained at 37°C for 30 min, and the rate of appearance of inverted sugar was determined by

the addition of 200 µl of DNS. The mixture was boiled for 10 minutes. The absorbance was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µg of sucrose under the assay conditions.

### *Qualitative Screening for Cellulase Positive Strains*

The bacterial strains were grown on tryptone soy agar containing CMC for qualitative screening. After overnight incubation, shaking solution was added to detect the presence of CMC. The procedure is as follows:

- Add 10 ml of congested solution to the top of the plate. The cells were incubated for 15 minutes to stain.
- Pour off congored.
- Add 10 ml of 1 M NaCl. Incubate for 15 minutes to destain the unound regions.
- Observe the clearing zone around positive colonies.

### *Cellulase activity quantification*

Tryptone soy broth containing CMC was used for the estimation. Each culture tube containing 20 ml of medium was inoculated with bacterial colonies positive for cellulase and incubated at 27+1°C for 7 days at 140 rpm.

### *Procedure*

After seven days of incubation, the tubes were removed from the incubator, and the fermentation broth was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used for the cellulose assay.

The cellulase assay was conducted by mixing 50 µL of enzyme solution with 50 µL of 0.5% CMC in 10 mM sodium acetate buffer. The mixture was incubated at 40°C for 30 minutes. The reaction was terminated by adding 100 µL of DNS and placing it in a boiling water bath for 5 minutes. Then, the mixture was diluted to1 ml by adding distilled water. Finally, the absorbance was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µg of cellulose under the assay conditions.

## Results

Ma Maximum number of bacterial colonies was grown on TSA medium, out of which 20 were randomly picked and characterized.

### *Characterization of bacteria isolated from the termite gut*

### *Morphological Characteristics of Aerobically Isolated Bacteria from the Termite Gut*

The colony color of the isolates was off-white. The colony shape in most of the cases was regular or circular. Margins of bacterial colonies were entire. All of the colonies had flat elevation. Most bacterial isolates were small/medium, ranging from 2-5 mm. Some of them have larger colony sizes of 6-7 mm. All of the cells were nonmotile except isolate no.4. Most of the strains were gram-negative bacteria, while some were gram-positive. All bacterial isolates were rod-shaped, as shown in Table 1.

| *Isolate* | *Colony Characteristics* | | | | | *Cell Characteristics* | | |
|---|---|---|---|---|---|---|---|---|
| | *Shape* | *Margin* | *Elevation* | *Color* | *Size (mm)* | *Motility* | *Shape* | *Gram Staining* |
| 1 | Irreg. | Ent. | Flat | OW | 5 mm | NM | R | -ve |
| 2 | Irreg. | Ent. | Flat | OW | 5 mm | NM | R | -ve |
| 3 | Cir. | Ent. | Flat | OW | 7 mm | NM | R | -ve |
| 4 | Irreg. | Ent. | Flat | OW | 4 mm | M | R | -ve |
| 5 | Cir. | Ent. | Flat | OW | 3 mm | NM | R | +ve |
| 6 | Cir. | Ent. | Flat | OW | 5 mm | NM | R | -ve |
| 7 | Cir. | Ent. | Flat | OW | 3 mm | NM | R | +ve |
| 8 | Cir. | Ent. | Flat | OW | 6 mm | NM | R | -ve |
| 9 | Irreg. | Ent. | Flat | OW | 5 mm | NM | R | +ve |
| 10 | Irreg. | Ent. | Flat | OW | 5 mm | NM | R | -ve |
| 11 | Cir. | Ent. | Flat | OW | 3 mm | NM | R | -ve |
| 12 | Cir. | Ent. | Flat | OW | 3 mm | NM | R | -ve |
| 13 | Cir. | Ent. | Flat | OW | 3 mm | NM | R | -ve |
| 14 | Cir. | Ent. | Flat | OW | 2 mm | NM | R | -ve |
| 15 | Irreg. | Ent. | Flat | OW | 2 mm | NM | R | -ve |
| 16 | Irreg. | Ent. | Flat | OW | 6 mm | NM | R | +ve |
| 17 | Cir. | Ent. | Flat | OW | 2 mm | NM | R | -ve |
| 18 | Irreg. | Ent. | Flat | OW | 3 mm | NM | R | -ve |
| 19 | Irreg. | Ent. | Flat | OW | 2 mm | NM | R | -ve |
| 20 | Cir. | Ent. | Flat | OW | 2 mm | NM | R | -ve |

Symbol: Cir, Circular; Irreg., Irregular; Ent., Entire; Off white; M, Motile; NM, Nonmotile;C, R, Rods; -ve, Gram-negative; +ve, Gram-positive

***Table 1:*** Morphological characteristics of bacteria isolated aerobically from the termite gut.

### *Biochemical Characterization of Isolated Bacterial Strains*

### *Biochemical Characterization of Bacterial Strains Isolated Aerobically from Termite Gut*

All of the bacterial strains isolated aerobically showed positive results for OPNG, CIT (except isolate 2), MALO, VP, GEL, GLU, MALT, SUC, CAT and MANN. All isolates showed negative results for INOS, ADO and RAF. Most of the strains showed negative results for LDC, ADH and ODC, except strain nos. 4, 16 and 18, which showed positive results. All of the strains showed negative results for the $H_2S$ and urease tests. Only strains 1, 2 and 20 showed positive lactase test results, as shown in Table 2.

| Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OPNG | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| CIT | + | - | + | + | + | + | + | + | + | + | + | - | + | + | + | - | + | + | - | + |
| MALO | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + |
| LDC | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | + | - | - |
| ADH | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| ODC | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | + | - | - |
| $H_2S$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| UREA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| TDA | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | + | - | + | - | _ |
| IND | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| VP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| GEL | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| GLU | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| $NO_3/N_2$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| MALT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SUC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MANN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ARAB | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | - | + | + |
| RHAM | + | + | + | + | + | + | + | + | + | + | - | - | + | - | + | - | + | + | - | - |
| SORB | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - | - | + | + | - | - |
| INOS | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - | - |
| ADO | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| MEL | - | + | + | + | + | + | + | + | + | + | - | - | - | - | + | - | - | - | - | - |
| RAF | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

+ positive, - negative

***Table 2:*** Biochemical characteristics of bacteria isolated aerobically from the termite gut.

### *Biochemical Characterization of Bacterial Strains Isolated Anaerobically from Termite Gut*

All of the bacterial strains isolated anaerobically showed positive results for OPNG, except isolate nos. 2, 3 and 15. All of the isolates showed positive results for VP, GEL, GLU, MALT, SUC, and MANN. All isolates showed negative results for INOS, ADO and RAF. All of the strains showed negative results for LDC, ADH and ODC. All of the strains showed negative results for the $H_2S$ and urease tests, as shown in Table 3.

| Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OPNG | + | - | - | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + |
| CIT | + | + | - | + | + | - | + | + | - | - | + | - | - | - | - | + | + | + | + | - |
| MALO | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | + | + | + |
| LDC | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ADH | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ODC | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $H_2S$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| UREA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| TDA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| IND | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| VP | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| GEL | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| GLU | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| $NO_3/N_2$ | - | - | -/+ | - | - | - | - | - | -/+ | - | - | - | - | - | - | - | - | - | - | -/+ |
| MALT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SUC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| MANN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ARAB | - | + | + | + | + | + | + | + | + | + | - | + | + | - | + | + | + | - | + | + |
| RHAM | - | + | + | + | + | - | - | - | + | + | - | - | + | - | - | - | - | + | + | - |
| SORB | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| INOS | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ADO | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| MEL | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| RAF | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

+ positive, - negative

***Table 3:*** Biochemical characteristics of bacteria isolated an aerobically from the termite gut.

### *Tentative identification of isolates*

The tentative identification of the isolates was performed with the help of Bergey's Manual of Bacteriology and through biochemical characterization. It is important to note that the identification is tentative and needs to be further confirmed by advanced molecular identification techniques. In this case, the identification was performed on the basis of the morphological features of the isolates and their biochemical tests. The strains identified are shown in Table 4.

| Colony no | Organism identified |
|---|---|
| 1,2,20 | *Enterobacter intermedius* |
| 3,6,8,10,1112,13,14,15,17,19 | *Yersinia pestis* |
| 4, | *Serratia liquefaciens* |
| 5,7,9,16 | *Mycobacterium smegmatis* |
| 18 | *Shigella sonei* |

***Table 4:*** Tentative identification of aerobically grown isolates.

### *Tentative identification of anaerobically grown isolates*

All the isolates found anaerobically were Yersinia pestis.

### *Amylytic Potential of Bacterial Isolates*

A total of 10 positive isolates were selected for enzyme production, and their respective electrolytic activity was estimated. The enzyme assay for amylase activity was found to be highest for strain no.16 with 1. 46 U/ml (figure 2).

### *Invertase Activity*

A total of 20 positive isolates were selected for amylase quantification. The highest amylase activity was found in strain no.17 at 1.332 U/ml (figure 3).

### *Cellulolytic Potential of Bacterial Isolates*

A total of 19 positive isolates were selected for enzyme production, and their respective electrolytic activity was estimated. The enzyme assay for cellulase activity was found to be highest for strain no.12 at 0.09 U/ml (figure 4).

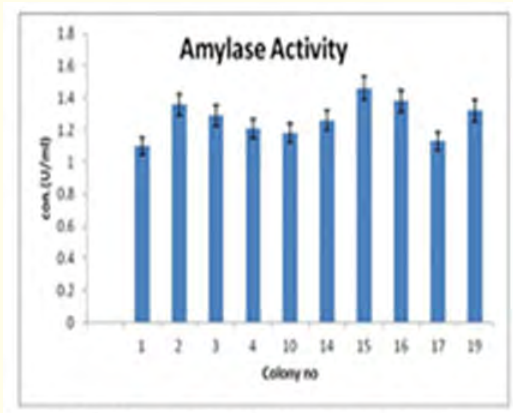


Figure2 Amylytic activity estimation of selected colonies. Values were shown as means of at least three measurements ± SD

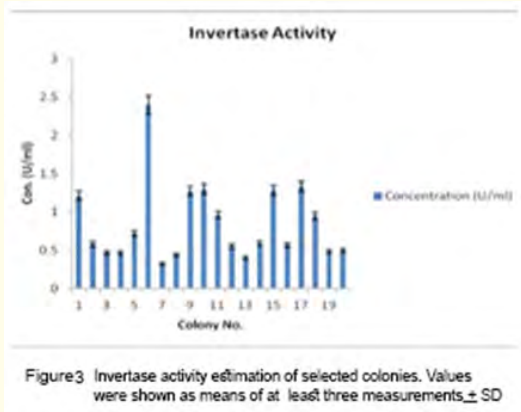


Figure3 Invertase activity estimation of selected colonies. Values were shown as means of at least three measurements ± SD

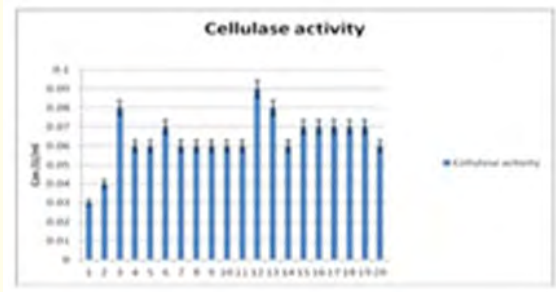


Figure4 cellulase activity estimation of selected colonies. Values were shown as means of at least three measurements ± SD

## Discussion

The aim of the present study was to examine the microbial diversity present in the hindgut of higher termites (*Odontoterme obesus*). The termite gut and the fungus comb harbor a wide variety of novel genera and species (Katoh et al., 2002; Shinzato et al., 2005; Long et al., 2010; Ohkuma & Brune, 2011). In this research microbes associated with the termite gut and their potential to degrade cellulose, starch and glucose were studied. The breakdown of cellulose and hemicelluloses was performed by gut microbes and host cellulase genes present in the termite (Kuhnigk & Ko¨nig, 1997; Yang et al., 2004; Huang et al., 2008). The study includes isolation and characterization of bacteria and then screening of cellulase-, amylase- and invertase-positive bacteria for enzymatic assays.

To explore the diversity of the microbial population, the termite gut was aseptically dissected, diluted and used for bacterial growth in tryptic soy agar medium.

Microbial strains isolated both aerobically and anaerobically showed some level of diversity. In terms of colony morphology, almost all of the colonies range from colors like off white. Similarly, only entire margins were observed for both types of isolates. The shape of the cells varies from circular to irregular for anaerobes and circular for aerobes. All isolates had flat elevation. Most of the cells of aerobes were nonmotile, gram-negative, rods, except for a few that were gram-positive. Only cells of isolate 4 showed motility. All anaerobes were nonmotile, gram-negative rods.

All of the isolates were catalase positive. Regarding cytochrome oxidase, all isolates showed negative results. In the majority of the isolates, urease activity was not detected. No isolates produced $H_2S$ gas. Few of the bacterial strains showed positive results for LDC, ODC and ADH. Both aerobes and anaerobes showed positive results for gelatin. A similar pattern was noticed for the VP test, which was positive for almost all isolates, indicating the presence of acetoin in those isolates. The starch hydrolysis test results varied, as half of the isolates were able to hydrolyze starch, and some could not. Most of the bacterial strains had the ability to utilize sugars such as maltose, sucrose, lactose and mannose.

Many important commercially valuable strains were isolated and identified in this study. The strains were screened for amylase, invertase and cellulase activity to use them for ethanol production. Insects have endogenous enzymes and symbiont enzymes that are more efficient if lignocellulosic materials are used as a source of glucose metabolism (Willis et al., 2010). All of the strains isolated aerobically belonged to the *Enterobacteriaceae* family. Important identified isolates were *Enterobacter intermedius*, *Yersinia pestis*, *Serratia liquefaciens*, *Mycobacterium smegmatis* and *Shigella sonei*. Strains isolated anaerobically were *Yersinia pestis*.

The enzyme assays for three enzymes, amylase, invertase and cellulase, were examined by methods recommended by the International Union of Pure and Applied Chemistry (IUPAC).The isolated strains were checked for their enzymatic activity. Significant results

were obtained in terms of substrate degradation for amylase, invertase and cellulase activity. The enzyme assay for amylase activity was found to be highest for strain no.16 at 1.46 The highest amylase activity was found in strain no.17 at 1.332.

The enzyme assay for cellulase activity was found to be highest for strain no.12 at 0.09 The extracellular cellulase activity of filter paper cellulose (FPC) only ranged from 0.012 to 0.196 U/mL, while for endoglucanase ranged between 0.162 and 0.400 IU/ml (Jantje et al., 2013).As isolates were identified tentatively on the basis of biochemical tests only, further study can be performed to confirm these isolates. Moreover, their role in the conversion of complex sugars to simple sugars is important to look over. It can open doors to new horizons. The mechanisms behind these termite gut microbe interactions are extremely important and an interesting topic to study.

## Conclusion

The key objective of this work was to isolate termite gut microbial diversity. Different microbial isolates were found in this study. The microbial enzyme study in this research shows significant results in terms of sugar conversion or substrate degradation. Microbial diversity in the hindgut microbiota of Odontotermes obesus (Rambur) and their enzymatic activity show that termite gut bacteria play a significant role in biofuel production in terms of plant decomposition and conversion to simple sugars. Further study can be performed to produce ethanol by using termite gut microbes.

## Novelty Statement

Microbial diversity in the hindgut microbiota of Odontotermes obesus (Rambur) and their enzymatic activity show that termite gut bacteria play a significant role in biofuel production, in terms of plant decomposition and conversion to simple sugars.

## References

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