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Identification of Novel Cellulolytic Bacterial Strains from Termite Guts for Efficient Biomass Conversion

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ABSTRACT

Termites as eusocial insects, play a vital role in the terrestrial ecosystem by recycling lignocellulosic biomass comprising cellulose, hemicellulose, and lignin. Their ability to efficiently degrade plant material is facilitated by symbiotic microorganisms in their gut, particularly cellulolytic bacteria, which convert cellulose into metabolizable sugars such as glucose. This study focuses on isolating and identifying cellulose-degrading bacteria from the gut of termites belonging to the genus *Odontotermes*, which are major contributors to litter decomposition. The research involved isolating bacterial strains from termite gut samples and their characterization through standard morphological, physiological, and biochemical tests. The cellulolytic potential of the isolates was evaluated using carboxymethyl cellulose (CMC) agar plates, and the presence of cellulolytic enzyme activity was further confirmed using molecular techniques. The findings from this study enhance our understanding of termite gut microbiota and their role in lignocellulose degradation, with potential applications in biofuel production, waste management, and industrial enzyme development.

Keywords: *Biomass, Termites, Cellulolytic bacteria, Odontotermes, lignocellulose*

1. INTRODUCTION

Termites are eusocial insects that play a crucial role in terrestrial ecosystems by recycling lignocellulosic biomass, which primarily consists of cellulose, hemicellulose, and lignin (Breznak & Brune, 1994). As major decomposers of dead plant material, termites contribute significantly to nutrient cycling and soil enrichment. Their efficiency in lignocellulose degradation is attributed to their symbiotic gut microbiota, which hydrolyzes complex plant polymers into simpler sugars that can be metabolized (Hongoh, 2010; Brune, 2014). Studies indicate that termites can decompose 74–99% of cellulose and 65–87% of hemicellulose from their diet, highlighting their remarkable lignocellulolytic capability (Ohkuma, 2003; Wenzel et al., 2002).

The order Isoptera is broadly classified into lower and higher termites. Lower termites primarily rely on a dense population of gut symbionts, including prokaryotic bacteria and eukaryotic protists, to digest cellulose (Brune & Ohkuma, 2011). In contrast, higher termites (Family: Termitidae) lack protists but compensate with an even more diverse bacterial community in their hindgut (Mathew et al., 2002). This microbial ecosystem is structured around syntrophic interactions, where cellulolytic bacteria ferment plant polymers, generating short-chain fatty acids (SCFAs) such as acetate, which serves as a primary energy source for the host (Pester & Brune, 2007). Additionally, termite gut microbiota contributes to global biogeochemical processes by participating in carbon and nitrogen cycling (Tayasu et al., 1994; Brune, 2014).

2. ROLE OF GUT BACTERIA IN CELLULOSE DIGESTION

The digestion of lignocellulose in termites is facilitated by a combination of endogenous enzymes and microbial cellulases. Termite-derived enzymes, such as endo-β-1,4-glucanase and β-glucosidase, belong to glycosyl hydrolase family 9 (GHF9) and are secreted from salivary glands and the midgut (Tokuda et al., 1997; Ni & Tokuda, 2013). However, in lower termites, a significant portion of cellulose digestion occurs in the hindgut, where unhydrolyzed fibers undergo further microbial processing (Ohkuma, 2003).

Gut bacteria play a central role in lignocellulose breakdown by producing a diverse array of cellulolytic enzymes, including endoglucanases, exoglucanases (cellobiohydrolases), and β-glucosidases, which work synergistically to degrade cellulose into fermentable sugars (Tsegaye et al., 2019; Upadhyaya et al., 2012). Cellulolytic bacteria isolated from termite guts predominantly belong to Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Watanabe & Tokuda, 2010; Mikaelyan et al., 2015). Among them, *Clostridium* spp. and *Fibrobacter* spp. are recognized as highly efficient cellulose degraders due to their ability to produce extensive cellulases and hemicellulases (Liu et al., 2019; Warnecke et al., 2007).

Furthermore, *Treponema* spp. and *Bacteroides* spp. contribute to the fermentation of cellulose-derived sugars into SCFAs (Pester & Brune, 2007; Hongoh et al., 2008).

Higher termites, in particular, have developed a highly specialized bacterial community for lignocellulose digestion. For instance, *Fibrobacteres-–Bacteroidetes* groups are abundant in their hindgut and function as key cellulose degraders in the absence of protists (Mikaelyan et al., 2015). Some species of higher termites also rely on CO_2-reducing acetogenesis rather than methanogenesis to optimize the efficiency of cellulose fermentation (Pester & Brune, 2007).

Given their critical role in lignocellulose degradation, termite gut microbiota represents a promising resource for the discovery of novel cellulolytic bacteria with potential applications in biotechnology, including biofuel production, waste management, and industrial enzyme development (Liu et al., 2019; Warnecke et al., 2007). The present study aims to isolate and identify cellulolytic bacteria from the gut of termites belonging to the genus *Odontotermes*, a major contributor to litter decomposition in forest ecosystems (Mikaelyan et al., 2015). Bacterial isolates will be characterized using standard morphological, biochemical, and molecular techniques, and their cellulolytic activity will be evaluated on carboxymethyl cellulose (CMC) agar plates. This study seeks to enhance our understanding of termite gut microbiota and explore their potential for sustainable biotechnological applications.

In recent years, 16S rRNA gene sequencing has become an indispensable tool for analyzing bacterial communities within termite guts. This culture-independent method allows for a comprehensive assessment of microbial diversity, including the identification of non-culturable species (Warnecke et al., 2007). For example, a study on *Reticulitermes flavipes* employed near full-length 16S rRNA gene sequencing to reveal a complex gut microbiota, highlighting the presence of diverse bacterial taxa involved in lignocellulose degradation (Shinzato et al., 2005). The application of 16S rRNA gene sequencing facilitates the calculation of alpha and beta diversities, providing insights into the richness and evenness of bacterial species within individual termite guts and across different termite species or colonies (Bourguignon et al., 2017). Moreover, differential abundance analyses can identify specific bacterial taxa that are overrepresented or underrepresented under various dietary or environmental conditions, shedding light on the functional roles of these microorganisms in lignocellulose digestion (Warnecke et al., 2007).

Complementing 16S rRNA gene sequencing, DNA barcoding serves as a powerful tool for species identification and specimen authentication. By utilizing standardized short DNA regions, such as the mitochondrial cytochrome c oxidase subunit I (COI) gene, DNA barcoding enables accurate identification of bacterial species, even those that are morphologically indistinguishable (Hebert et al., 2003). The integration of DNA barcoding with 16S rRNA gene sequencing enhances the resolution of microbial community analyses, allowing for precise taxonomic classification and discovery of novel cellulolytic bacteria within termite guts (Purty & Chatterjee 2016).

3. MATERIALS AND METHODS

Sample Collection

Termites (*Odontotermes* sp.) were collected from a naturally occurring colony near Kizhuparamba, Areekode, Malappuram district, Kerala (Figure 1). The colony was located in an area characterized by decaying wood, a known habitat for this species. Healthy adult termites were carefully harvested using sterile plastic bottles to prevent contamination during transport. This collection methodology follows that of previous studies on termite-associated microbiota (Korsa, et al., 2023).

Surface Sterilization

A total of 10 adult termites were selected for the study based on their size and activity. To remove potential surface contaminants, termites were subjected to surface sterilization using a 5-minute immersion in 70% ethanol, followed by two rinses with sterile distilled water. The sterilized termites were then dissected under sterile conditions: the heads were separated using forceps, and the bodies were crushed with a sterile glass rod to release gut contents. This method was adapted from studies focusing on bacterial isolation from the termite gut.

Bacterial Isolation

The gut homogenates were cultured on Carboxymethylcellulose (CMC) agar medium to isolate cellulolytic bacteria. CMC is commonly used for isolating microorganisms that can degrade cellulose, a primary component of the termite gut microbiota (Yadav et al. 2024). Plates were incubated at 30°C for 48 hours, and bacterial colonies were sub-cultured into fresh medium for further characterization. The selective medium and incubation conditions used here are based on methods widely employed in similar microbial isolation studies (Yadav et al. 2024).

Morphological and Motility Characterization

Isolates were characterized morphologically by Gram staining (Bergey 1994) to determine their gram reaction, shape, and arrangement. The motility of bacterial isolates was assessed using the hanging drop method (Bergey 1994). This technique involves placing a drop of bacterial suspension on a glass slide and observing for movement under a microscope, a critical test for identifying motile bacteria, particularly those with flagella.

Biochemical Characterization

A suite of standard biochemical tests was performed to classify the bacterial isolates further:

- **Indole Test:** Detects the ability of bacteria to produce indole from tryptophan.
- **Methyl Red Test:** Identifies mixed acid fermentation.
- **Voges-Proskauer Test:** Used to detect acetoin production.
- **Catalase Test:** Identifies the presence of catalase enzyme, which decomposes hydrogen peroxide into water and oxygen.
- **Starch Hydrolysis Test:** Determines the ability of bacteria to hydrolyze starch using amylase enzymes (Chauhan et al. 2020).
- These tests are widely used for bacterial identification and are referenced in studies (Devaraj & Kesti 2019).

Molecular characterization

DNA Isolation

Bacterial genomic DNA was extracted using a commercial kit (Qiagen, Germany) according to the manufacturer's instructions. The DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and its quality was assessed by agarose gel electrophoresis. This method follows established protocols for DNA extraction in microbiological studies (Makonde et al., 2013)

Analysis of Isolated DNA by Agarose Gel Electrophoresis

The quality and integrity of the isolated DNA were confirmed using agarose gel electrophoresis. A 2% agarose gel was prepared with TAE buffer, and DNA samples were loaded alongside a molecular weight marker (Thermo Fisher Scientific). The gel was stained with ethidium bromide and visualized under a UV transilluminator, as described by Sambrook and Russell (2001). This step is essential for ensuring that DNA is intact and suitable for downstream applications such as PCR amplification (Fig. 01).

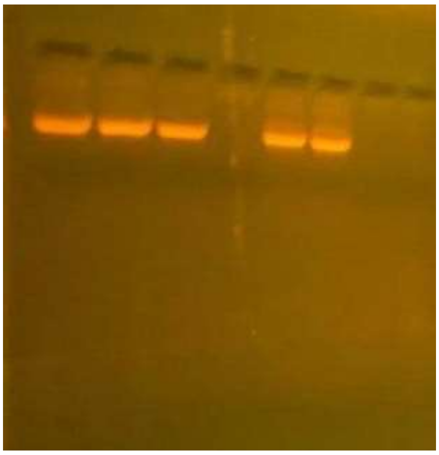


Fig.01: Showing band isolation of genomic DNA from the isolated bacteria

PCR Amplification of 16S rRNA Gene

PCR amplification of the 16S rRNA gene was carried out using the bacterial-specific primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1429R (5'GAATTACCGCGGCGCGGCG-3') (Lane, 1991). The reaction mixture contained 2 ng of genomic DNA, 1 µL of each primer (10 µM), 2 µL of dNTPs (2 mM), 10 µL of 10X PCR buffer, 1 µL of Taq polymerase (5U/µL), and 84 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 1 min), with a final extension at 72°C for 10 minutes. The PCR conditions were adapted from the method described by Sambrook and Russell (2001) and modified for optimal amplification in our study (Shinde et al., 2017).

Sequencing of PCR Product

The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced at AgriGenom Labs Private Ltd., Cochin, using the ABI 3730XL automated sequencer. Both forward and reverse sequencing reactions were performed using the same primers (27F and 1429R) to ensure high-quality sequence data. Sequence assembly and primer trimming were performed using the Clustal W program. The consensus sequence was aligned and compared against the NCBI database using BLAST for species identification (Altschul et al., 1990). This methodology has been successfully employed in several microbial identification studies (Sreena et al., 2015).

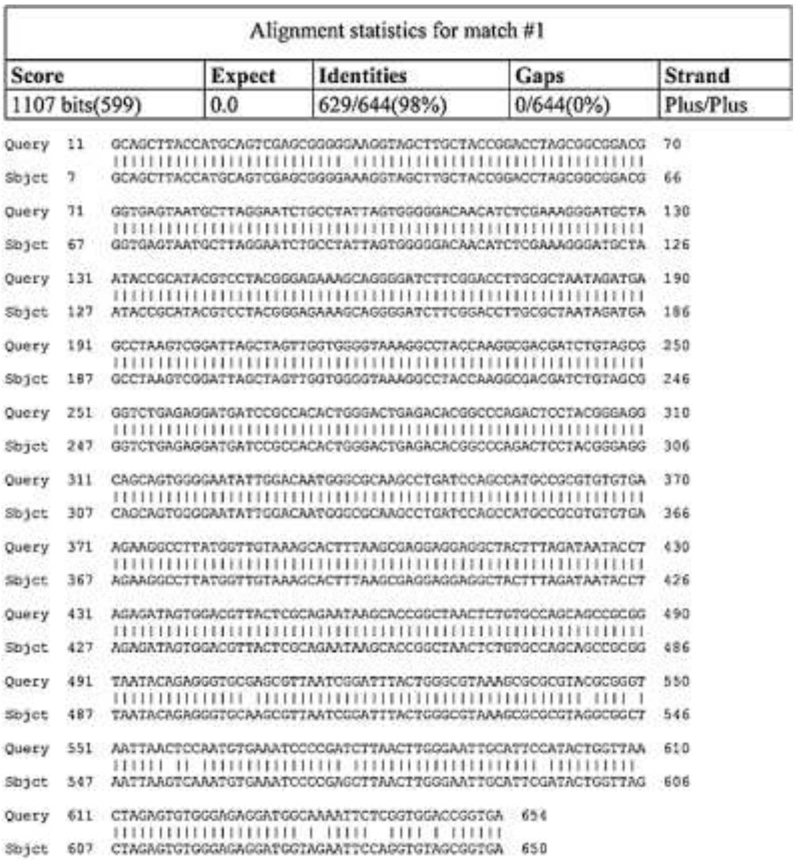
4. RESULT

Termites from the species *Odontotermes* were collected from their natural habitats and were carefully surface-sterilized using ethanol to prevent external contamination. Once sterilized, the termites were dissected under sterile conditions to extract their gut contents. These contents were then disrupted and homogenized to facilitate the isolation of microorganisms. The homogenized gut material was cultured on Carboxymethyl Cellulose (CMC) agar plates, supplemented with 1% CMC to encourage the growth of cellulolytic bacteria. These plates were incubated at 30°C for 48 to 72 hours, during which bacterial colonies that exhibited distinct morphological features were isolated for further study.

To evaluate the cellulolytic activity of the isolated bacteria, Congo red staining was performed. The staining process involved applying a 0.1% Congo red solution to the colonies, followed by counterstaining with a 1 M NaCl solution. This method helped identify the breakdown of cellulose, indicated by the formation of clear zones around bacterial colonies. Additionally, the ability of the isolates to hydrolyze starch was tested by growing the bacteria on starch agar plates and flooding them with iodine after incubation. A clear zone around the colony indicated the presence of amylase activity, confirming starch hydrolysis.

The bacterial isolates underwent detailed morphological and biochemical characterization. Gram staining was performed to determine the bacterial shape and the structure of the cell wall. The Gram reaction was examined under an oil immersion microscope at 100x magnification. To assess motility, the hanging drop method was employed, revealing that the isolate was non-motile. A series of biochemical tests, including Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, and Catalase tests, were conducted to further characterize the metabolic properties of the isolates, following standard protocols outlined by Cappuccino and Sherman (2005).

For molecular identification, DNA was extracted from the selected bacterial isolates. Polymerase Chain Reaction (PCR) was employed to amplify the 16S rRNA gene using the 27F forward primer and the 1492R reverse primer. The resulting amplicons, approximately 1220 base pairs in length, were then sequenced. Sequence analysis and comparison to known sequences in public databases confirmed the identity of the bacterium as *Acinetobacter calcoaceticus*, with 100% sequence similarity (Fig. 02).



Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1107 bits(599)	0.0	629/644(98%)	0/644(0%)	Plus/Plus

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Query  11   GCAGCTTACCATGCAGTCGAGCGGGGGAAGGTAGCTTGCTACCGGACCTAGCGGCGGACG  70
            |||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  7    GCAGCTTACCATGCAGTCGAGCGGGGAAAGGTAGCTTGCTACCGGACCTAGCGGCGGACG  66

Query  71   GGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATCTCGAAAGGGATGCTA  130
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   GGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATCTCGAAAGGGATGCTA  126

Query  131  ATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCGCTAATAGATGA  190
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  ATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCGCTAATAGATGA  186

Query  191  GCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCG  250
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  GCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCG  246

Query  251  GGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG  310
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  GGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG  306

Query  311  CAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGA  370
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  CAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGA  366

Query  371  AGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGATAATACCT  430
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  AGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGATAATACCT  426

Query  431  AGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGG  490
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  AGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGG  486

Query  491  TAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTACGCGGGT  550
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||| |||| |
Sbjct  487  TAATACAGAGGGTGCAAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTAGGCGGCT  546

Query  551  AATTAACTCCAATGTGAAATCCCCGATCTTAACTTGGGAATTGCATTCCATACTGGTTAA  610
            |||||| || |||||||||||||||| ||||||||||||||||||||| ||||||||||
Sbjct  547  AATTAAGTCAAATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTGGTTAG  606

Query  611  CTAGAGTGTGGGAGAGGATGGCAAAATTCTCGGTGGACCGGTGA  654
            ||||||||||||||||||||| | |||||  |||| | ||||||
Sbjct  607  CTAGAGTGTGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGA  650
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Fig. 02: Nucleotide Blast result SH01 strain shows 100% similarity to *A.calcoaceticus*

5. DISCUSSION

The present study sought to identify and characterize cellulolytic bacteria from the gut of termites, specifically *Odontotermes* species, which play a critical role in breaking down lignocellulosic material in their natural habitat. The use of standard microbiological techniques, including Congo red staining and biochemical assays, enabled the isolation and identification of a bacterium capable of cellulose degradation. This bacterium was identified as *Acinetobacter calcoaceticus* through both phenotypic (morphological and biochemical) and genotypic (16S rRNA sequencing) analyses.

The bacterial isolate exhibited typical characteristics of Gram-negative, non-motile rods, which was consistent with previous findings by Wenzel et al. (2002) on similar termite-associated bacteria. The positive results for the Indole, Voges-Proskauer, Citrate Utilization, and Catalase tests suggest a versatile metabolic profile, further supporting the isolate's classification as *A. calcoaceticus*. This bacterium's ability to hydrolyze starch (indicated by clear zones on starch agar) and its positive cellulolytic activity on CMC plates highlight its potential in breaking down complex carbohydrates, which is a crucial function in the termite's digestive system for accessing nutrients from lignocellulosic material.

The identification of the isolate as *A. calcoaceticus* was definitively confirmed by 16S rRNA gene sequencing, where the isolate showed 100% similarity to known *A. calcoaceticus* strains. This aligns with earlier studies (Garrido et al., 2023) where *A. calcoaceticus* was found to be prevalent in diverse environments, including termite guts. Furthermore, the sequencing results of the SH01 strain (1220 bp) confirm that this bacterium belongs to the *Acinetobacter* genus, which is known for its diverse ecological and metabolic capabilities. This result supports the bacterium's role in aiding cellulose degradation in the termite gut, a finding that has significant implications for understanding symbiotic relationships in termite colonies.

The formation of clear zones around bacterial colonies on CMC plates, as well as the starch hydrolysis test, corroborates the cellulolytic nature of the isolate. This cellulolytic activity aligns with findings from Peristiwati et al. (2018) and Kumar and Velayutham (2014), who identified similar strains capable of breaking down cellulose. The ability of *A. calcoaceticus* to degrade cellulose is of particular interest for potential applications in biomass conversion, waste management, and biofuel production (Fig. 03).

Fig.03: Clear zone formed after applying Congo red and Nacl

Ecological Significance: The termite gut, with its diverse microbial community, serves as a unique habitat for cellulolytic and hemicellulolytic bacteria, as shown in studies by Tokuda et al. (1997).

The current study adds to the growing body of knowledge suggesting that *Acinetobacter* species, particularly *A. calcoaceticus*, play a significant role in breaking down plant polymers such as cellulose and hemicellulose in symbiosis with their termite hosts. This metabolic process is essential for termites to digest their woody, cellulose-rich diet, and provides insights into how termite gut bacteria contribute to nutrient cycling in terrestrial ecosystems.

Industrial and Biotechnological Implications: The cellulolytic enzymes produced by *A. calcoaceticus* could have wide-ranging industrial applications, particularly in the bioconversion of lignocellulosic waste into valuable products like biofuels, bioplastics, and other bio-based chemicals. The bacterium's ability to efficiently degrade cellulose makes it a promising candidate for use in industrial processes that require the breakdown of plant biomass. Furthermore, the study highlights the potential of termite-associated bacteria as a source of novel enzymes for biotechnological applications, including environmental sustainability through waste valorization and biomass processing.

6. CONCLUSION

The identification of *A. calcoaceticus* from termite gut bacteria reinforces the role of these microorganisms in cellulose degradation and opens the door to further exploration of their biotechnological potential. Future studies should focus on the functional characterization of cellulolytic enzymes produced by *A. calcoaceticus* and other termite gut bacteria, with an emphasis on optimizing their use for industrial and environmental applications.

REFERENCES

[1] Breznak, J. A., & Brune, A. (1994). Role of microorganisms in the digestion of lignocellulose by termites. *Annual review of entomology*, *39*(1), 453-487.

[2] Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, *12*(3), 168-180.

[3] Hongoh, Y. (2010). Diversity and genomes of uncultured microbial symbionts in the termite gut. *Bioscience, biotechnology, and biochemistry*, *74*(6), 1145-1151.

[4] Ohkuma, M. (2003). Termite symbiotic systems: efficient bio-recycling of lignocellulose. *Applied microbiology and biotechnology*, *61*(1), 1-9.

[5] Wenzel, M., Schönig, I., Berchtold, M., Kämpfer, P., & König, H. (2002). Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. *Journal of applied microbiology*, *92*(1), 32-40.

[6] Brune, A., & Ohkuma, M. (2011). Role of the termite gut microbiota in symbiotic digestion. *Biology of termites: a modern synthesis*, 439-475.

[7] Mathew, G. M., Sindhu, R., Huang, C. C., Pandey, A., & Binod, P. (2022). Microbial diversity in termite gut ecosystem and their role in lignocellulosic degradation. In *Microbial Diversity in Hotspots* (pp. 155-175). Academic Press.

[8] Pester, M., & Brune, A. (2007). Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts. *The ISME Journal*, *1*(6), 551-565.

[9] Täyasu, I., Sugimoto, A., Wada, E., & Abe, T. (1994). Xylophagous termites depending on atmospheric nitrogen. *Naturwissenschaften*, *81*, 229-231.

[10] Tokuda, G., Watanabe, H., Matsumoto, T., & Noda, H. (1997). Cellulose digestion in the wood-eating higher termite, *Nasutitermes takasagoensis* (Shiraki): distribution of cellulases and properties of endo-β-1, 4-gIucanase. *Zoological Science*, *14*(1), 83-93.

[11] Ni, J., & Tokuda, G. (2013). Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnology advances*, *31*(6), 838-850.

[12] Tsegaye, B., Balomajumder, C., & Roy, P. (2019). Isolation and characterization of novel lignolytic, cellulolytic, and hemicellulolytic bacteria from wood-feeding termite *Cryptotermes brevis*. *International Microbiology*, *22*, 29-39.

[13] Upadhyaya, S. K., Manandhar, A., Mainali, H., Pokhrel, A. R., Rijal, A., Pradhan, B., & Koirala, B. (2012). Isolation and characterization of cellulolytic bacteria from gut of termite. In *Rentech Symposium Compendium* (Vol. 1, No. 4, pp. 14-18).

[14] Watanabe, H., & Tokuda, G. (2010). Cellulolytic systems in insects. *Annual review of entomology*, *55*(1), 609-632.

[15] Mikaelyan, A., Dietrich, C., Köhler, T., Poulsen, M., Sillam-Dussès, D., & Brune, A. (2015). Diet is the primary determinant of bacterial community structure in the guts of higher termites. *Molecular ecology*, *24*(20), 5284-5295.

[16] Liu, N., Li, H., Chevrette, M. G., Zhang, L., Cao, L., Zhou, H., ... & Wang, Q. (2019). Functional metagenomics reveals abundant polysaccharide-degrading gene clusters and cellobiose utilization pathways within gut microbiota of a wood-feeding higher termite. *The ISME Journal*, *13*(1), 104-117.

[17] Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T., & Leadbetter, J. R. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, *450*(7169), 560-565.

[18] Hongoh, Y., Sharma, V. K., Prakash, T., Noda, S., Taylor, T. D., Kudo, T., ... & Ohkuma, M. (2008). Complete genome of the uncultured Termite Group 1 bacteria in a single host protist cell. *Proceedings of the National Academy of Sciences*, *105*(14), 5555-5560.

[19] Shinzato, N., Muramatsu, M., Matsui, T., & Watanabe, Y. (2005). Molecular phylogenetic diversity of the

bacterial community in the gut of the termite *Coptotermes formosanus*. *Bioscience, biotechnology, and biochemistry*, *69*(6), 1145-1155.

[20] Bourguignon, T., Lo, N., Šobotník, J., Ho, S. Y., Iqbal, N., Coissac, E., ... & Evans, T. A. (2017). Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. *Molecular Biology and Evolution*, *34*(3), 589-597.

[21] Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313-321.

[22] Purty, R. S., & Chatterjee, S. (2016). DNA barcoding: an effective technique in molecular taxonomy. *Austin J biotechnol bioeng*, *3*(1), 1059.

[23] Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*, *215*(3), 403-410.

[24] Bergey, D. H. (1994). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.

[25] Korsa, G., Beyene, A., & Ayele, A. (2023). Bacterial diversity from soil-feeding termite gut and their potential application. *Annals of Microbiology*, *73*(1), 38.

[26] Shinde, V. S., Agrawal, T., & Kotasthane, A. S. (2017). Molecular characterization of cellulolytic bacteria derived from termite gut and optimization of cellulase production. *Int J Curr Microbiol Appl Sci*, *6*(10), 2474-2492.

[27] Sreena, C., Resna, N., & Sebastian, D. (2015). Isolation and characterization of cellulase producing bacteria from the gut of termites (*Odontotermes* and *Heterotermes* species). *British Biotechnology Journal*, *9*(1), 1-10.

[28] Lane, D. J. (1991). 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*.

[29] Chauhan, A., Jindal, T., Chauhan, A., & Jindal, T. (2020). Biochemical and molecular methods for bacterial identification. *Microbiological methods for environment, food and pharmaceutical analysis*, 425-468.

[30] Devaraj, V., & Kesti, S. S. (2019). Isolation and molecular characterization of termite GUT microflora. *Int. J. Sci. Res. in Biological Sciences Vol*, *6*, 3.

[31] Makonde, H. M., Boga, H. I., Osiemo, Z., Mwirichia, R., Mackenzie, L. M., Göker, M., & Klenk, H. P. (2013). 16S-rRNA-based analysis of bacterial diversity in the gut of fungus-cultivating termites (*Microtermes* and *Odontotermes* species). *Antonie Van Leeuwenhoek*, *104*, 869-883.

[32] Peristiwati, Natamihardja, Y. S., & Herlini, H. (2018). Isolation and identification of cellulolytic bacteria from termites gut (Cryptotermes sp.). In *Journal of Physics: Conference Series* (Vol. 1013, p. 012173). IOP Publishing.

[33] Sambrook, J., & Russell, D. W. (2001). Detection of DNA in agarose gels. *Molecular Cloning, A Laboratory Manual, (3rd Ed.) Cold Spring Harbor Laboratory Press, New York*, 5-14.

[34] Yadav, P., Dixit, Y., Asnani, H., & Sharma, A. K. (2024). Exploration of ethanologenic bacteria from termite gut for bioethanol production. *Biomass Conversion and Biorefinery*, 1-14.

[35] Cappuccino, J. G., & Sherman, N. (2005). *Microbiology: a laboratory manual* (p. 507). San Francisco: Pearson/Benjamin Cummings.

[36] Kumar, R., & Velayutham, K. (2014). Isolation and characterization of cellulolytic bacteria from termite gut and their potential applications in biotechnology. *Environmental Science and Pollution Research*, 21(24), 13908-13916.

[37] Garrido, M., Veiga, J., Garrigós, M., & Martínez-de la Puente, J. (2023). The interplay between vector microbial community and pathogen transmission on the invasive Asian tiger mosquito, Aedes albopictus: current knowledge and future directions. *Frontiers in Microbiology*, *14*, 1208633.