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Review Article

A Review of the Isolation of Cellulose-Degrading Bacteria from Termite Gut

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Abstract

Background: Cellulose is the most abundant structural polysaccharide in plant biomass and a major renewable carbon resource, but its crystalline structure resists degradation. Termites are efficient decomposers of lignocellulose through specialized associations with gut microorganisms, including bacteria that produce cellulolytic enzymes. **Methods:** This narrative review draws on published journal articles, textbooks and research reports to examine how cellulose-degrading bacteria are isolated, screened, identified and characterized from termite guts. **Results:** Bacteria from diverse groups, including Bacillus, Paenibacillus, Lysinibacillus, Stenotrophomonas, Enterobacter, Streptomyces and other taxa, have shown cellulolytic activity. Common approaches involve aseptic gut dissection, enrichment in cellulose-containing media, serial dilution and plating on carboxymethyl cellulose (CMC) agar, followed by Congo red screening. Quantitative CMCase, filter-paper cellulase (FPase) and β-glucosidase assays give more reliable estimates of activity than plate halos, and 16S rRNA sequencing supports identification. Genomic and metagenomic studies indicate substantial unexplored enzymatic potential. Reported applications include biofuel production, animal feed, pulp, paper and textile processing, and waste biodegradation. Cultivation bias, anaerobic requirements, variation among termite species and gut regions, and the weak correlation between halo size and enzyme yield remain challenges. **Conclusion:** Termite guts are valuable reservoirs of cellulolytic bacteria. Integrating culture-dependent methods with metagenomics, genome mining and enzyme engineering should accelerate the discovery of novel enzymes.

Keywords: *Cellulolytic Bacteria; Cellulase; Congo Red; Termite Gut*

Introduction

Cellulose is a linear polysaccharide composed predominantly of glucose units joined by β-1,4-glycosidic bonds. It is a major structural component of plant cell walls and of lignocellulosic biomass (Morohoshi, 1991; Lynd *et al*., 2002). Although cellulose is an abundant renewable resource, its crystalline organization and association with hemicellulose and lignin make it resistant to degradation. Efficient conversion therefore requires specialized enzymes and microorganisms capable of breaking down its complex structure (Lynd *et al*., 2002).

Termites are important biological decomposers of lignocellulosic materials. Their ability to use wood, leaf litter and other plant-derived materials is strongly associated with their digestive physiology and their interactions with gut microorganisms (Breznak & Brune, 1994; Ohkuma, 2003). Termite guts function as specialized microbial ecosystems in which bacteria and, depending on termite lineage, other microorganisms contribute to the degradation and fermentation of plant polymers (Brune, 2014). The termite gut is therefore a valuable reservoir for discovering microorganisms with cellulose-degrading properties. Cellulolytic bacteria have been isolated from several termite genera, and these isolates

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produce endoglucanases, exoglucanases, β-glucosidases and other enzymes involved in lignocellulose degradation (Ramin *et al*., 2009; Sreena *et al*., 2015).

Interest in termite gut bacteria has increased because cellulases have considerable industrial importance. Efficient cellulolytic microorganisms could contribute to the conversion of agricultural residues and other lignocellulosic materials into fermentable sugars and value-added products, including biofuels and biochemicals (Maki *et al*., 2009). This review summarizes the isolation, screening, identification and characterization of cellulose-degrading bacteria from termite guts, discusses their biotechnological significance, and identifies challenges and future research directions.

Methodology

This narrative review was compiled from journal articles, textbooks, research reports and indexed papers on cellulolytic bacteria from termite guts.

Results and Discussion

Cellulose and Its Structure

Cellulose is a homopolysaccharide of long glucose chains linked by β-1,4-glycosidic bonds. Individual chains form hydrogen bonds with one another, producing microfibrils with crystalline and amorphous regions. This organization gives cellulose considerable mechanical strength and resistance to chemical and enzymatic attack. In natural plant biomass, cellulose does not occur alone; it is embedded within a matrix of hemicellulose, lignin, pectin and other components (Morohoshi, 1991). Biological degradation of plant biomass therefore generally requires several complementary enzymatic activities. Cellulose degradation is primarily associated with three major classes of cellulolytic enzymes: (i) endoglucanases, (ii) exoglucanases (cellobiohydrolases) and (iii) β-glucosidases. Their combined action converts cellulose into soluble sugars, particularly glucose (Lynd *et al*., 2002).

Termites as Efficient Cellulose Degraders

Termites exploit lignocellulosic resources through the interaction of termite-derived and microbial enzymes (Breznak & Brune, 1994; König *et al*., 2013). Termites have traditionally been divided into “lower” and “higher” groups, based partly on the presence or absence of gut flagellated protists. Lower termites generally possess diverse protists and bacteria, whereas higher termites lack the characteristic flagellated protists and have predominantly bacterial gut communities (Brune, 2014). Modern classification is more complex than this distinction, but it remains useful when discussing digestive symbiosis.

In lower termites, cellulolytic protists can play major roles in cellulose digestion, while bacteria contribute additional metabolic functions. In higher termites, bacterial communities make important contributions to lignocellulose processing. The contribution of bacteria varies with termite species, diet, gut compartment and microbial community structure. The hindgut is particularly important because it provides a densely populated microbial environment in which plant-derived polymers are further degraded and fermentation products are generated. Acetate is an important end product that the termite can use as an energy source (Brune, 2014).

Termite Gut Microbiota

The termite gut contains a complex community of bacteria, archaea, protists and, in particular termite groups, associated fungi. These microorganisms perform complementary functions in digestion and nutrient acquisition. Reported bacterial groups include members of the Firmicutes, Proteobacteria, Actinobacteria and Spirochaetota, among other lineages. A study of *Coptotermes formosanus* isolated 87 bacterial strains from different gut regions, most of which belonged to the Proteobacteria and Firmicutes; the hindgut yielded the highest proportion of cellulolytic isolates. The study also showed the importance of examining gut compartments separately, because microbial composition and function vary between the foregut, midgut and hindgut (Dar *et al*., 2022).

The diversity of the termite gut microbiota is considerably greater than what conventional cultivation can recover. Many gut microorganisms remain difficult or impossible to cultivate under routine laboratory conditions, and culture-independent methods have revealed a much broader diversity of potential lignocellulose-degrading organisms and genes (Hongoh, 2011; Zhou *et al*., 2019).

Cellulolytic Bacteria in the Termite Gut

Cellulolytic bacteria are microorganisms that produce enzymes capable of hydrolyzing cellulose or related substrates, and numerous studies have demonstrated them in termite digestive systems. Cellulolytic bacteria were isolated from *Coptotermes curvignathus* on media containing CMC and cellobiose (Ramin *et al*., 2009). Five cellulolytic isolates were recovered from the gut of *Psammotermes hypostoma* and identified by 16S rRNA sequencing as *Paenibacillus lactis*, *Lysinibacillus macroides*, *Stenotrophomonas maltophilia*, *Lysinibacillus fusiformis* and *Bacillus cereus* (Ali *et al*., 2019). An investigation of *Odontotermes* and *Heterotermes* termites identified several cellulase-producing isolates, including *Bacillus cereus*, *Staphylococcus* and *Enterobacter*; some produced endoglucanase, FPase and β-glucosidase activities (Sreena *et al*., 2015). Members of the genus *Bacillus* are frequent inhabitants of termite guts (König, 2006), and a cellulose-degrading *Bacillus velezensis* strain (BV-10) isolated from a termite gut has been characterized by whole-genome sequencing and tested as a silage additive (Zhang *et al*., 2023). Actinomycetes, most of them *Streptomyces*, have also been isolated from termite guts and can secrete cellulase during lignocellulose hydrolysis (Varma *et al*., 1994; Zhou *et al*., 2019).

Collection of Termites for Bacterial Isolation

The first stage of isolation is the collection of appropriate termite specimens. The choice of termite species is important because feeding behavior can influence the composition and function of the gut microbiota. Wood-feeding termites are particularly interesting because their diet provides abundant lignocellulosic substrates, although cellulolytic bacteria have also been recovered from termites with other feeding habits. Researchers should record the following:

- Termite species or morphotype and caste
- Collection location and date
- Feeding substrate and environmental conditions
- Approximate number of termites collected

Healthy and active individuals are generally preferable for microbiological investigations.

Surface Sterilization and Gut Dissection

To reduce contamination from microorganisms on the external surface, specimens are commonly surface sterilized before dissection. Published isolation studies have used aseptic procedures involving short exposure to disinfectants such as 70% ethanol, followed by careful removal of the digestive tract under sterile conditions. The dissected gut is then transferred to sterile buffered solution and homogenized. This step is important because the objective is to investigate microorganisms associated with the gut rather than those on the cuticle or in the surrounding environment.

The gut may also be separated into the foregut, midgut and hindgut, which allows researchers to determine whether cellulolytic organisms are concentrated in particular regions. Studies of *C. formosanus* demonstrate the value of investigating gut-region-specific microbial communities (Dar *et al*., 2022).

Enrichment of Cellulose-Degrading Bacteria

Enrichment culture is frequently used to increase the probability of recovering cellulose-utilizing bacteria. Homogenized gut material is introduced into a mineral or basal medium containing cellulose or CMC as the principal carbon source, so that bacteria able to use the substrate grow preferentially. Filter paper has also been used as the sole carbon source for isolating and testing cellulolytic bacteria from *C. formosanus*; in a Nigerian study, five isolates macerated the paper and degraded 75–95% of it

over 30 days (Egwuatu & Appeh, 2018). Enrichment applies selective pressure favoring microorganisms that use cellulose-derived substrates. However, it may also introduce bias, because fast-growing organisms can become overrepresented while slow-growing or nutritionally demanding organisms remain undetected.

Isolation on Cellulose-Containing Media

After enrichment, serial dilution and plating are commonly used to obtain individual colonies. Carboxymethyl cellulose (CMC) is frequently incorporated into agar as a substrate for screening cellulase-producing bacteria; it is a soluble cellulose derivative that endoglucanases can hydrolyze (Hendricks *et al*., 1995; Jahromi, 2008). A study of cellulose-degrading bacteria from termite guts and termitaria used CMC-containing medium followed by Congo red screening (Varghese *et al*., 2017), and CMC and other cellulose-containing media were used to isolate cellulolytic bacteria from *P. hypostoma*, where five of 33 gut isolates showed clear zones in the Congo red assay (Ali *et al*., 2019). Selective enrichment followed by solid-medium screening yields individual isolates for subsequent identification and enzymatic characterization.

Primary Screening Using Congo Red

Congo red staining is one of the most frequently reported qualitative methods for detecting cellulolytic microorganisms (Gupta *et al*., 2012; Khianngam *et al*., 2014). After growth on CMC-containing agar, the plate is treated with Congo red. Regions containing cellulose retain the dye, whereas areas in which cellulose has been hydrolyzed appear as clear or lighter halos around cellulase-producing colonies. A clear zone therefore provides preliminary evidence of extracellular cellulolytic enzymes. A commonly calculated parameter is the hydrolysis capacity (HC) ratio:

HC = Diameter of clear zone (mm) ÷ Diameter of colony (mm)

A larger ratio can serve as an initial indicator of stronger hydrolytic activity. However, halo size should not be interpreted as a definitive measure of enzyme productivity, because plate-based hydrolysis indices may correlate poorly with quantitative cellulase production (Liang *et al*., 2014). Positive isolates should therefore undergo quantitative enzyme assays (Table 1).

Table 1: *Methods for Detecting and Identifying Cellulolytic Bacteria*

Method	Principle	Strength	Limitation
Congo red on CMC agar	Clear halo where CMC is hydrolyzed	Simple, rapid primary screen	Halo size correlates poorly with enzyme yield
CMCase (DNS)	Reducing sugar released from CMC	Quantifies endoglucanase activity	CMC is soluble and amorphous
FPase	Reducing sugar released from filter paper	Reflects activity on insoluble cellulose	Measures total activity of the preparation
β-Glucosidase	Hydrolysis of cellobiose to glucose	Shows relief of cellobiose inhibition	Does not measure cellulose attack
16S rRNA sequencing	Gene sequence comparison	Reliable species-level identification	Does not show enzyme activity

Note: CMC, Carboxymethyl Cellulose; Fpase, Filter-Paper Cellulase.

Quantitative Determination of Cellulase Activity

Qualitative screening should be followed by quantitative determination of cellulase activity. The CMCase assay measures endoglucanase activity using CMC as substrate; the reducing sugar released is determined with the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959) and expressed in enzyme activity units, conventionally the amount of enzyme releasing 1 µmol of glucose equivalent per minute (Ghose, 1987).

The filter-paper cellulase (FPase) assay evaluates the ability of an enzyme preparation to degrade a relatively insoluble cellulose substrate (Ghose, 1987). It is a more functionally relevant measure than

CMC hydrolysis alone, because CMC is an amorphous, soluble cellulose derivative. The β-glucosidase assay measures conversion of cellobiose and related oligosaccharides into glucose. This activity is important because accumulated cellobiose can inhibit other cellulases. Studies of termite-derived cellulolytic bacteria have therefore evaluated combinations of endoglucanase, FPase and β-glucosidase activities (Sreena *et al*., 2015).

Identification of Cellulose-Degrading Bacteria

Morphological characterization is the first step and may include colony morphology, color, size, elevation and margin, as well as cell shape, Gram reaction and spore formation. These characters provide preliminary information but are generally insufficient for species identification. Biochemical tests, such as catalase, oxidase, carbohydrate utilization, indole production, citrate utilization, urease activity, nitrate reduction and motility, help differentiate bacterial groups.

16S rRNA gene sequencing is widely used for bacterial identification and phylogenetic analysis. Cellulolytic isolates from *P. hypostoma* were identified using biochemical characteristics and 16S rRNA sequencing (Ali *et al*., 2019), and cellulase-producing bacteria from *Odontotermes* and *Heterotermes* were similarly characterized (Sreena *et al*., 2015). Molecular identification is especially valuable when phenotypic characteristics do not allow reliable species-level identification.

Figure 1 depicts the workflow for isolation, screening and identification of cellulose-degrading bacteria from termite guts

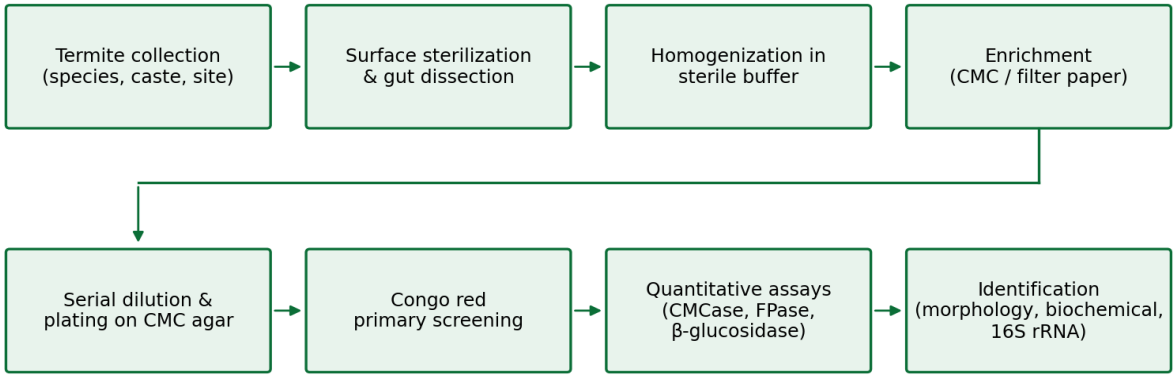

Figure 1: Workflow for isolation, screening and identification of cellulose-degrading bacteria from termite guts

Examples of Cellulolytic Bacteria Isolated from Termite Gut

Table 2 summarizes representative reports. Termite-associated cellulolytic bacteria are taxonomically diverse and are not restricted to one bacterial genus.

Table 2: *Representative Cellulolytic Bacteria Reported from Termite Guts*

Termite	Bacteria	Reported Activity	Source
Psammotermes hypostoma	*Paenibacillus lactis*, *Lysinibacillus macroides*, *Stenotrophomonas maltophilia*, *L. fusiformis*, *Bacillus cereus*	Five of 33 gut isolates cellulolytic (Congo red); CMCase 0.22–2.28 U/mL; 16S rRNA identification	Ali *et al*. (2019)
Odontotermes and *Heterotermes* spp.	*Bacillus cereus*, *Staphylococcus*, *Enterobacter*	Endoglucanase, FPase and β-glucosidase activities	Sreena *et al*. (2015)
Coptotermes curvignathus	*Enterobacter*, *Clavibacter* and others	Strong cellulolytic activity on CMC and cellobiose media	Ramin *et al*. (2009)
Coptotermes formosanus	87 strains in 27 species and 10 genera, mainly Proteobacteria and Firmicutes	Cellulolytic isolates most frequent in the hindgut	Dar *et al*. (2022)

Termite	Bacteria	Reported Activity	Source
Coptotermes formosanus (Nigeria)	*Pseudomonas mendocina*, *Burkholderia pseudomallei*, *Chryseobacterium luteola*, *Klebsiella oxytoca*, *K. terrigena*	75–95% filter paper degradation in 30 days	Egwuatu & Appeh (2018)
Coptotermes heimi	Anaerobic fibrolytic bacteria	Isolated in an anaerobic chamber; screened for ruminant feed use	Kumawat *et al*. (2025)
Termite gut (strain BV-10)	*Bacillus velezensis*	Cellulose degradation; whole-genome analysis; silage additive	Zhang *et al*. (2023)
Various termites	*Streptomyces*, *Micromonospora* and other actinomycetes	Lignocellulolytic potential; *Streptomyces* can secrete cellulase	Varma *et al*. (1994); Zhou *et al*. (2019)

Note: DNS, 3,5-dinitrosalicylic acid; other abbreviations as in Table 1.

The Cellulase Enzyme System

Cellulose degradation is not usually accomplished by a single enzyme; several enzymes cooperate (Lynd *et al*., 2002). Endoglucanases attack internal β-1,4-glycosidic bonds within cellulose chains, producing shorter fragments and increasing the number of chains ends available for further attack. Exoglucanases (cellobiohydrolases) act preferentially on chain ends and release cellobiose and related soluble products. β-Glucosidases hydrolyze cellobiose into glucose. The coordinated action of these enzymes makes conversion of cellulose more efficient, and termite gut studies have identified diverse carbohydrate-active enzymes associated with this process (König *et al*., 2013).

Factors Affecting Cellulase Production

Several environmental and nutritional factors influence bacterial cellulase production (Lee *et al*., 2008; Macedo *et al*., 2013):

- *Temperature:* production and activity are temperature dependent, and isolates differ in their optima. Some termite-derived isolates show strong activity at moderate temperatures, while others produce thermostable enzymes.
- *pH:* pH affects both bacterial growth and enzyme structure, and the optimum varies with the producing organism.
- *Carbon source:* CMC, cellulose, filter paper, agricultural residues and other lignocellulosic substrates influence enzyme production.
- *Nitrogen source:* organic and inorganic nitrogen sources may affect bacterial growth and enzyme synthesis.
- *Incubation time:* production changes through the growth cycle, so activity should be measured at several time points rather than at a single arbitrary incubation period.

Culture-Dependent and Culture-Independent Approaches

Traditional isolation techniques are valuable because they yield living strains that can later be studied and used for enzyme production. However, culture-dependent methods recover only a fraction of the total gut microbial community. Culture-independent methods, including 16S rRNA amplicon sequencing, metagenomics, metatranscriptomics, metaproteomics and functional metagenomic screening, can reveal microorganisms and enzyme genes that cannot readily be cultivated (Hongoh, 2011; Zhou *et al*., 2019). Metagenomic investigations have identified numerous carbohydrate-active enzymes in termite gut microbiomes, highlighting considerable unexplored enzymatic diversity. The ideal strategy is therefore to combine cultivation with molecular approaches.

Importance of Anaerobic Cultivation

The termite hindgut contains oxygen gradients and strongly reducing or anaerobic regions, so strictly aerobic cultivation may fail to recover important members of the microbiota (Brune, 2014). Anaerobic

fibrolytic bacteria have been isolated from the gut of *Coptotermes heimi* using an anaerobic chamber and screened for their potential to improve fiber digestibility in ruminants (Kumawat *et al*., 2025). Isolation studies designed to maximize bacterial diversity should therefore consider both aerobic and anaerobic cultivation.

Biotechnological Importance

Termite-associated cellulolytic bacteria have considerable biotechnological potential, because termites process lignocellulose efficiently and their microbial communities are considered promising sources of enzymes for biomass conversion (Maki *et al*., 2009). Applications include:

- *Biofuel production:* conversion of agricultural residues and other lignocellulosic materials into fermentable sugars, which can serve as substrates for bioethanol and other biofuels; termite gut methanogens have also been explored for biogas production (Kavitha *et al*., 2014).
- *Agricultural waste degradation:* biodegradation of crop residues, straw, sawdust, paper waste and other plant residues, which can facilitate composting and reduce accumulation of lignocellulosic waste.
- *Animal feed:* cellulases can assist in processing fibrous feed by modifying plant cell-wall components and potentially improving nutrient accessibility.
- *Pulp and paper industry:* cellulases can be used in selected processes, including fiber modification and recycling-related applications (Ko *et al*., 2007, 2010).
- *Textile industry:* cellulases are used in biological modification and finishing of cellulosic fabrics.
- *Biorefineries:* efficient cellulose hydrolysis is a key component of biorefineries, where plant biomass is converted into sugars, fuels, chemicals and other products.

Advantages of the Termite Gut as a Source of Cellulolytic Bacteria

The termite gut has several characteristics that make it attractive for microbial discovery: (i) natural adaptation to lignocellulose, since associated microorganisms have evolved in an environment rich in plant polymers; (ii) microbial diversity, with complementary metabolic capabilities; (iii) enzyme diversity, including genes for cellulases, hemicellulases and other carbohydrate-active enzymes; and (iv) the potential for novel enzymes from uncultivated organisms with useful biochemical characteristics.

Challenges Associated with Isolation

Despite the considerable potential, several challenges affect isolation of termite gut cellulolytic bacteria:

- *Cultivation bias:* many gut bacteria cannot readily grow on conventional laboratory media.
- *Anaerobic requirements:* some organisms require oxygen-free conditions and specific reducing environments.
- *Complex microbial interactions:* some bacteria depend on metabolites or growth factors produced by other microorganisms.
- *Differences among termites:* cellulolytic communities vary with termite species, caste, diet, geographic location and gut region.
- *Difficulty reproducing the gut environment:* the pH, redox conditions, substrates and host-derived compounds of the gut are hard to reproduce in artificial media.
- *Plate-screening limitations:* a large hydrolysis halo does not necessarily mean the highest enzyme yield in liquid fermentation, so quantitative assays are necessary.
- *Biosafety:* several reported isolates, such as *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Enterobacter* and *Staphylococcus*, are opportunistic pathogens, so safety assessment is needed before industrial use.

Conclusion

Termite guts are important natural reservoirs of cellulose-degrading microorganisms. The capacity of termites to exploit lignocellulosic materials results from a complex association between the host and its gut microbiota, in which bacterial symbionts contribute cellulases and other carbohydrate-active enzymes. Studies of *Coptotermes*, *Psammotermes*, *Odontotermes*, *Heterotermes* and other termites have recovered cellulolytic bacteria from several groups, including *Bacillus*, *Paenibacillus*, *Lysinibacillus*, *Stenotrophomonas*, *Enterobacter* and actinobacteria.

CMC-based screening and Congo red assays are convenient preliminary methods, while CMCase, FPase and β-glucosidase assays give more meaningful quantitative information. 16S rRNA sequencing and genome-based analysis improve identification and reveal the genetic potential for lignocellulose degradation. Continued investigation of termite gut bacteria is relevant to agricultural-waste management, biomass conversion, enzyme production and renewable-energy technologies. Future research combining classical microbiological isolation with genomics, metagenomics and enzyme engineering is likely to reveal additional bacteria and enzymes with useful industrial properties.

Recommendations

Based on the literature reviewed, the following recommendations are made for future work on cellulolytic bacteria from termite guts:

- *Standardize reporting:* state medium composition, incubation conditions and the definition of the enzyme unit, so that activities can be compared across studies.
- *Rank isolates quantitatively:* follow Congo red plate screening with CMCase, FPase and β-glucosidase assays before selecting strains, because halo size alone is a poor predictor of enzyme yield.
- *Combine approaches:* pair aerobic and anaerobic cultivation with culture-independent methods (16S rRNA amplicon sequencing and metagenomics) to capture organisms and genes that cannot be cultured.
- *Sample systematically:* record termite species, caste, diet and collection site, and examine foregut, midgut and hindgut separately.
- *Assess biosafety early:* test promising isolates for pathogenicity and antibiotic resistance before any feed, food or industrial use, since several reported genera include opportunistic pathogens.
- *Share data and strains:* deposit 16S rRNA sequences in GenBank and strains in recognized culture collections to support reproducibility.
- *Test under realistic conditions:* evaluate candidate strains and enzymes on locally available lignocellulosic residues and under the temperature, pH and inhibitor conditions of the intended process.
- *Encourage regional surveys:* the only Nigerian isolation study cited here is Egwuatu and Appeh (2018), so surveys of termite fauna in north-eastern Nigeria and the wider Sahel would add useful new data.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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