

Isolation and Characterization of Cellulolytic Bacteria from Termite Gut for Enhanced Biodegradation of Agricultural Waste

Mila Sari^{1*}, & Tri Putri Wahyuni²

¹*STIKES Dharma Landbouw, Indonesia, ²Universitas Negeri Padang, Indonesia

*Co e-mail: milasari@stikeslandbouw.ac.id¹

Article Information

Received: September 30, 2026

Revised: October 20, 2026

Online: October 29, 2026

Keywords

Cellulolytic Bacteria, Termite Gut, *Macrotermes Gilvus*, Cellulase, 16S Rrna, Agricultural Waste Biodegradation, Rice Straw

ABSTRACT

Agricultural waste, such as rice straw, contains lignocellulosic materials that are difficult to degrade, highlighting the need for efficient cellulolytic bacteria. This study isolated and characterized cellulolytic bacteria from the gut of *Macrotermes gilvus* and evaluated their potential for rice straw biodegradation. Eighteen isolates were screened using carboxymethyl cellulose (CMC) agar and Congo red staining, yielding five isolates with cellulolytic indices ≥ 2.9 . Based on 16S rRNA gene sequencing, these were identified as *Bacillus subtilis* (TG-4), *Cellulomonas* sp. (TG-2), *Bacillus cereus* (TG-1), *Paenibacillus* sp. (TG-5), and *Enterobacter* sp. (TG-3). TG-4 exhibited the highest cellulolytic index (5.17) and optimal CMCase activity of 2.85 U/mL at pH 6 and 40 °C. During 21 days of biodegradation, TG-4 achieved 34.6% rice straw weight loss, while a consortium of TG-1, TG-2, and TG-4 achieved 48.3%, compared with 8.2% in the uninoculated control. Differences were statistically significant (one-way ANOVA, $p < .001$). These findings highlight the potential of termite gut-derived bacterial consortia for accelerating agricultural waste biodegradation.

Keywords: Cellulolytic Bacteria, Termite Gut, *Macrotermes Gilvus*, Cellulase, 16S Rrna, Agricultural Waste Biodegradation, Rice Straw

INTRODUCTION

Agricultural activity generates vast quantities of lignocellulosic waste annually, including rice straw, corn stover, and sugarcane bagasse, much of which remains inefficiently managed through open burning or slow, uncontrolled decomposition, contributing to air pollution and the loss of potentially valuable organic resources (Anveshitha et al., 2026). Rice straw specifically has become a key research target for microbial degradation approaches, given its high cellulose



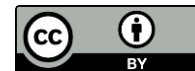
content, wide availability, and low cost as a substrate, coupled with the practical challenge posed by its recalcitrant, lignin-associated cellulose structure, which resists rapid natural decomposition (Zhang et al., 2025).

Microbial cellulase-producing bacteria offer a biologically based, environmentally sustainable alternative for accelerating the biodegradation of such agricultural waste, converting cellulose into fermentable sugars through the coordinated action of endoglucanase, exoglucanase, and β -glucosidase enzyme activities. Cellulolytic bacteria have been successfully isolated from a wide range of environmental sources, including forest soil, cow dung, agro-industrial waste, and brewery waste (Demissie et al., 2024), as well as compost and animal waste slurry (Kim et al., 2012). Among the diverse natural sources explored for cellulolytic bacteria, termite guts have attracted particular scientific interest, given that termites are among the most efficient cellulose-digesting organisms in nature, a capability attributed substantially to the diverse symbiotic bacterial, protozoan, and, in higher termites, fungal communities residing within their digestive tract, which work in concert with the termite's own endogenous cellulases to break down lignocellulosic material (Ali et al., 2019).

A substantial body of research has successfully isolated and characterized cellulolytic bacteria from the gut of various termite species and genera. Bacteria isolated from the gut of *Odontotermes* and *Heterotermes* species have demonstrated significant cellulase-producing capability (Kunhachan et al., 2024), while cellulolytic bacteria isolated from Indralaya peatland-derived termite gut samples confirmed the presence of efficient cellulose-degrading isolates in Indonesian termite populations specifically (Oktiarni et al., 2021). Characterization of cellulolytic bacteria from the gut of worker *Macrotermes gilvus*, a mound-building termite species common in Southeast Asia, reported isolates with cellulolytic indices of up to 2.5 and, via 16S rRNA gene sequencing, identified the isolate with the highest index as *Paracoccus yeei* and a second sequenced isolate as *Bacillus megaterium* (Ferbiyanto et al., 2015).

More recent work evaluating *Heterotermes indicola* gut-associated bacterial isolates found cellulolytic indices as high as 5.0 among screened isolates, alongside evidence of ethanologenic potential, highlighting the dual bioprospecting value of termite gut bacterial diversity for both waste degradation and bioenergy applications (Azhar et al., 2024). Diversity surveys of culturable cellulolytic bacteria from higher termite guts in Thailand similarly identified dozens of distinct cellulolytic isolates through PCR-RFLP-based classification of the 16S rRNA gene, underscoring the substantial, still incompletely characterized taxonomic diversity of cellulolytic bacteria harbored within termite digestive systems (Boontanom & Chantarasiri, 2021).

Despite this expanding body of termite gut cellulolytic bacteria research, comparatively fewer studies have extended beyond isolation, screening, and taxonomic identification to directly evaluate the practical biodegradation performance of termite gut-derived isolates against a real agricultural waste substrate under controlled conditions, and fewer still have compared single-isolate versus multi-isolate consortium performance, despite growing evidence from other lignocellulose-degrading bacterial systems that microbial consortia often substantially outperform single isolates due to complementary and synergistic enzymatic activity, as demonstrated in rice



straw-degrading consortium research achieving up to 60.2% cellulose degradation through coordinated multi-species enzymatic activity (Zhang et al., 2025) and in lignocellulolytic consortium-based rice straw decomposition research more broadly (Anveshitha et al., 2026).

This study aims to address this gap by isolating and characterizing cellulolytic bacteria from the gut of the termite *Macrotermes gilvus* and evaluating both single-isolate and multi-isolate consortium performance in the biodegradation of rice straw, a widely available agricultural waste substrate. The specific objectives of this research are: (1) to isolate and screen cellulolytic bacteria from termite gut samples using the Congo red assay on CMC agar and calculate the cellulolytic index of each isolate; (2) to identify the taxonomic identity of the most promising isolates through 16S rRNA gene sequencing and characterize the pH and temperature optima of the best-performing isolate's cellulase activity; and (3) to evaluate and compare the rice straw biodegradation performance of the best single isolate against a multi-isolate consortium relative to an uninoculated control. The findings are intended to contribute to the growing evidence base supporting termite gut bacteria as a practical biological resource for agricultural waste management.

METHODS

Worker termites of the mound-building species *Macrotermes gilvus* were collected in 2026 from an active termite mound in an agricultural area in West Sumatra, Indonesia. Approximately 100 worker termites were collected into sterile containers and transported to the laboratory for immediate processing within 3 h of collection, following a sample size consistent with prior termite gut bacterial isolation research (Ali et al., 2019), and species identity was confirmed based on worker caste body size, mandible morphology, and antennal segmentation using the standard identification key for *Macrotermes* (Ahmad, 1958).

The collected termites were surface-sterilized by sequential immersion in 70% (v/v) ethanol for 1 min followed by three rinses in sterile distilled water to remove external microbial contamination, and under aseptic conditions in a laminar airflow cabinet the gut of each termite was dissected using sterile forceps, pooled into a sterile mortar, homogenized in 9 mL of sterile phosphate-buffered saline (PBS; g/L: NaCl 8.0, KCl 0.2, Na₂HPO₄ 1.44, KH₂PO₄ 0.24; pH 7.4), and serially diluted tenfold (10⁻¹ to 10⁻⁶). Aliquots (100 µL) of each dilution were spread-plated onto carboxymethyl cellulose (CMC) agar medium (g/L: CMC sodium salt 10.0, NaNO₃ 1.0, K₂HPO₄ 1.0, KCl 1.0, MgSO₄·7H₂O 0.5, yeast extract 0.5, agar 15.0; pH 7.0 ± 0.2; autoclaved at 121 °C for 15 min) and incubated at 30 °C for 48 h under aerobic conditions, after which morphologically distinct bacterial colonies (differing in shape, margin, elevation, pigmentation, and size) were selected and purified through at least three successive streak-plate transfers on fresh CMC agar to obtain pure isolates, which were designated TG-1 through TG-18 and maintained as 20% (v/v) glycerol stocks at -80 °C, following the overall workflow illustrated in Figure 1.

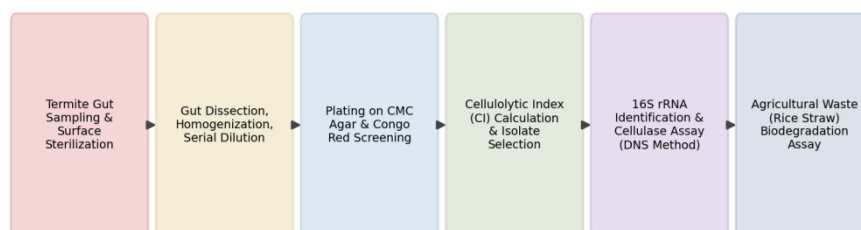


Figure 1. Workflow for isolation, screening, and characterization of cellulolytic bacteria from termite gut.

Each of the 18 purified isolates was pre-cultured overnight in nutrient broth at 30 °C with shaking at 150 rpm, adjusted to $OD_{600} = 0.5$ ($\approx 10^8$ CFU/mL), and a 5 μ L aliquot was spot-inoculated onto the center of CMC agar plates (composition as above; one spot per plate, three plates per isolate) and incubated at 30 °C for 48 h; the plates were then flooded with 0.1% (w/v) Congo red solution for 15 min and destained with 1 M sodium chloride solution for 15 min to reveal a clear zone of cellulose hydrolysis around cellulolytic colonies, following the Congo red assay procedure widely used in prior cellulolytic bacteria screening research (Azhar et al., 2024).

The cellulolytic index (CI) of each isolate was calculated for each plate as $CI = (D - d) / d$, where D is the total diameter of the clear zone (including the colony) and d is the colony diameter, both measured in mm after Congo red staining (Azhar et al., 2024; Ferbiyanto et al., 2015), and the CI reported for each isolate is the mean of the three plates. Because $CI = D/d - 1$, CI values from studies that report the simple ratio D/d are not directly comparable with those reported here and are used only as approximate benchmarks. Isolates with a $CI \geq 2.9$ were selected for molecular identification and enzyme characterization, a stringent cut-off chosen because it is higher than the CI values of about 1.5–2.0 commonly taken to indicate efficient cellulase producers (Azhar et al., 2024), exceeds the maximum CI of 2.5 previously reported for gut isolates of *M. gilvus* (Ferbiyanto et al., 2015; the reported value of 2.5 corresponds to the ratio D/d of isolate RA2, whereas the formula above gives 1.5 for the colony (2.0 mm) and clear zone (5.0 mm) diameters listed in that study), and is comparable with the CI values (2.87–3.1) of the best isolates chosen in comparable screening work (Demissie et al., 2024); applying this threshold retained five of the 18 isolates (28%), a number that was practical for 16S rRNA gene sequencing.

Genomic DNA was extracted from the selected isolates using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) from 24-h nutrient broth cultures, and the 16S rRNA gene was amplified using universal bacterial primers (27F, 5'-AGAGTTTGATCMTGGCTCAG-3', and 1492R, 5'-TACGGYTACCTTGTTACGACTT-3') via polymerase chain reaction (PCR) in a 25 μ L reaction containing 12.5 μ L of 2 $\times$ GoTaq Green Master Mix (Promega, Madison, WI, USA), 1 μ L template DNA (~ 50 ng), 0.4 μ M of each primer, and nuclease-free water to 25 μ L, with cycling conditions of an initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, and a final extension at 72 °C for 7 min.

Amplicons were checked by 1% (w/v) agarose gel electrophoresis, purified, and sequenced in both directions by the Sanger method at 1st BASE Laboratories (Selangor, Malaysia), after which forward and reverse reads were trimmed and assembled into consensus sequences using Geneious Prime 2023.2 (Biomatters, Auckland, New Zealand); the resulting sequences were compared against the NCBI GenBank database using BLAST to determine taxonomic identity at the genus or species level ($\geq 99\%$ identity: species level; $< 99\%$: genus level), following standard molecular identification procedures used in prior cellulolytic bacteria characterization research (El-Khamisi et al., 2024), and the consensus sequences were deposited in GenBank under the accession numbers listed in Table 1.

Cellulase (carboxymethyl cellulase, CMCase) activity of the isolate with the highest cellulolytic index (TG-4) was quantified using the 3,5-dinitrosalicylic acid (DNS) method, which measures the concentration of reducing sugars released from CMC substrate hydrolysis; for enzyme production, TG-4 was inoculated (1%, v/v, of an overnight culture adjusted to $OD_{600} = 0.5$) into 100 mL of production medium (g/L: CMC 10.0, peptone 5.0, yeast extract 1.0, NaCl 1.0, K_2HPO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.2; pH 7.0) in 250 mL flasks and incubated at 30 °C with shaking at 150 rpm for 48 h. Crude enzyme extract was obtained by centrifuging the bacterial culture ($10,000 \times g$, 10 min, 4 °C), and the reaction mixture (0.5 mL crude enzyme plus 0.5 mL of 1% (w/v) CMC substrate in the buffers listed below) was incubated for 30 min, after which 1 mL of DNS reagent (g/L: 3,5-dinitrosalicylic acid 10.0, NaOH 10.0, sodium potassium tartrate 200.0, phenol 2.0, Na_2SO_3 0.5; prepared according to Miller, 1959) was added to terminate the reaction (boiling for 5 min) and develop color, with absorbance measured at 540 nm, following the standard DNS assay protocol used extensively in prior cellulase characterization research (Demissie et al., 2024; Ma et al., 2024).

Reducing sugar was quantified against a glucose standard curve with enzyme and substrate blanks included, one unit (U) of cellulase activity was defined as the amount of enzyme required to release 1 μ mol of glucose equivalent per minute under the assay conditions, and to determine the optimal reaction conditions, cellulase activity was measured across a pH range of 4 to 9 (50 mM citrate buffer for pH 4–6, 50 mM phosphate buffer for pH 7–8, and 50 mM glycine–NaOH buffer for pH 9) at a fixed temperature of 37 °C and across a temperature range of 30 °C to 55 °C at the pH determined to be optimal, with each condition tested in triplicate (three reaction mixtures prepared from the same crude enzyme preparation, i.e., technical replicates).

Air-dried, chopped rice straw (2–3 cm segments) of the Ciherang variety, obtained from local farmers in West Sumatra, was washed with distilled water, oven-dried at 60 °C to constant weight, sterilized by autoclaving (121 °C, 15 min), and distributed into treatment containers (5 g dry weight per 250 mL flask, moistened with 20 mL of basal mineral medium; g/L: $(NH_4)_2SO_4$ 2.0, KH_2PO_4 1.0, K_2HPO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.3, $CaCl_2$ 0.3; pH 7.0) under three conditions: an uninoculated control (sterile rice straw with basal mineral medium only plus an equal volume of sterile saline), a single-isolate treatment (rice straw inoculated with the best-performing isolate, TG-4), and a consortium treatment (rice straw co-inoculated with the three isolates showing the highest cellulolytic indices, TG-1, TG-2, and TG-4, combined in equal proportions). Inocula were prepared by growing each isolate separately in nutrient broth at 30 °C with shaking (150 rpm) for



24 h, harvesting the cells by centrifugation ($6,000 \times g$, 10 min), washing twice with sterile 0.85% (w/v) NaCl, and resuspending them to $OD_{600} = 1.0$ ($\approx 10^9$ CFU/mL, verified by plate counts); each flask received a total of 10 mL of inoculum TG-4 alone in the single-isolate treatment and equal volumes of the three isolates in the consortium treatment so that the total inoculum volume and cell density were the same in both inoculated treatments.

The consortium was built from the three isolates with the highest CI (TG-4, 5.17; TG-2, 4.62; TG-1, 3.85), because CI was the only quantitative indicator of cellulolytic capacity available for all five selected isolates and because these three isolates represent two genera (*Bacillus* and *Cellulomonas*) that may provide complementary cellulolytic enzyme repertoires, while TG-5 (CI = 3.21) and TG-3 (CI = 2.94) were excluded because of their lower CI; the compatibility of TG-1, TG-2, and TG-4 was not tested by a cross-streak antagonism assay prior to consortium construction, and this is acknowledged as a limitation (Section 4). All treatments were incubated at 30 °C for 21 days under static conditions with periodic moistening (sterile distilled water added every 3 days to maintain approximately 60% moisture content), and rice straw weight loss was determined gravimetrically at days 0, 7, 14, and 21 by removing, washing, oven-drying (60 °C, to constant weight), and weighing straw samples from each treatment, with weight loss calculated as the percentage reduction in dry weight relative to the initial dry weight; each treatment was performed in triplicate ($n = 3$) using three independent flasks per treatment and sampling day (destructive sampling).

All experiments were performed with three replicates ($n = 3$); in the rice straw biodegradation assay each replicate was an independent microcosm (a separate flask, prepared and inoculated separately and sampled destructively), which are biological (experimental) replicates and were the unit of statistical analysis, whereas in the cellulase assays each pH or temperature condition was measured in three reaction mixtures prepared from the same crude enzyme preparation, which are technical replicates that were averaged and not treated as independent observations, and CI values are means of three plates per isolate.

Data are reported as mean $\pm$ standard deviation (SD) values across replicates, normality of residuals and homogeneity of variances were checked with the Shapiro–Wilk and Levene’s tests, respectively, and one-way analysis of variance (ANOVA) was used to determine whether statistically significant differences existed among treatment groups (uninoculated control, TG-4, and consortium) for final (day 21) rice straw weight loss, followed by Tukey’s Honestly Significant Difference (HSD) post-hoc test for pairwise comparisons, with statistical significance set at $\alpha = .05$. Time-course data (days 7 and 14) and the enzyme activity profiles (Figure 4) are presented descriptively and were not subjected to hypothesis testing, and analyses were performed in R version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria); because each group comprised only three replicates, the tests have limited power to detect departures from the model assumptions, and the results should be interpreted accordingly.

RESULTS

1. Isolation and Screening of Cellulolytic Bacteria

A total of 18 morphologically distinct bacterial isolates, designated TG-1 through TG-18, were obtained from the gut homogenate of *Macrotermes gilvus* workers collected from an agricultural area in West Sumatra, Indonesia, in 2026. The isolates were recovered on CMC agar after surface sterilization, dissection, homogenization, and serial dilution of the termite guts, and each isolate was purified through at least three successive streak-plate transfers before being maintained as 20% (v/v) glycerol stocks at -80°C . Figure 2 presents the cellulolytic index (CI) of all 18 isolates determined through the Congo red assay on CMC agar, in which the formation of a clear hydrolysis zone around each colony reflects cellulase-mediated degradation of the CMC substrate.

Cellulolytic index values ranged from 0.76 to 5.17, indicating considerable variation in cellulolytic capacity among the gut-associated isolates, with the majority of isolates exhibiting only modest activity ($\text{CI} < 2.0$) and a smaller subset showing pronounced cellulose hydrolysis. CI is dimensionless and was calculated as $(\text{clear zone diameter} - \text{colony diameter}) / \text{colony diameter}$ (see Method), so the lowest value (0.76, TG-17) corresponds to a clear zone about 1.76 times the colony diameter and the highest value (5.17, TG-4) to a clear zone about 6.17 times the colony diameter. Five isolates (TG-1, TG-2, TG-3, TG-4, and TG-5) exhibited a $\text{CI} \geq 2.9$ and were therefore selected for further characterization, representing 28% of the total isolates obtained; the selection criteria and the rationale for this threshold are described in Section 2.3. Among these five isolates, TG-4 showed the highest cellulolytic index ($\text{CI} = 5.17$), followed by TG-2 ($\text{CI} = 4.62$), TG-1 ($\text{CI} = 3.85$), TG-5 ($\text{CI} = 3.21$), and TG-3 ($\text{CI} = 2.94$), and this ranking formed the basis for the subsequent molecular identification and enzyme characterization described in the following sections.

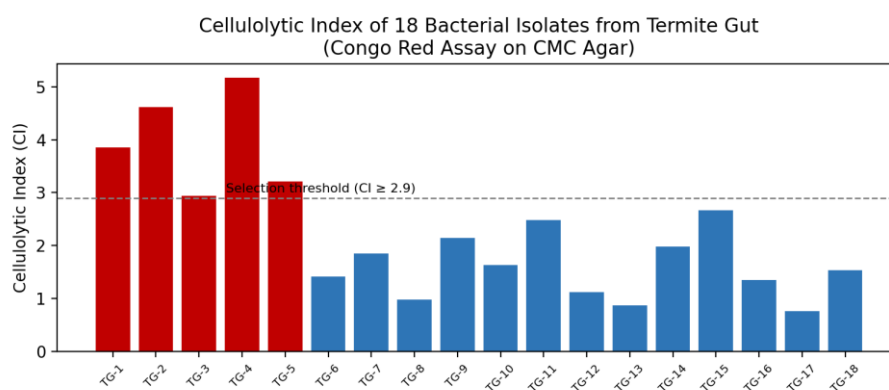


Figure 2. Cellulolytic index (CI) of 18 bacterial isolates obtained from termite gut, determined via the Congo red assay on CMC agar

2. Molecular Identification of Selected Isolates

Table 1 and Figure 3 present the 16S rRNA gene sequencing-based taxonomic identification and corresponding cellulolytic index of the five selected isolates. Isolate TG-4, exhibiting the highest cellulolytic index ($\text{CI} = 5.17$), was identified as *Bacillus subtilis*. The remaining selected isolates were identified as *Cellulomonas* sp. (TG-2, $\text{CI} = 4.62$), *Bacillus cereus* (TG-1, $\text{CI} = 3.85$),



Paenibacillus sp. (TG-5, CI = 3.21), and *Enterobacter* sp. (TG-3, CI = 2.94). The partial 16S rRNA gene sequences of the five isolates were deposited in the NCBI GenBank database under accession numbers PP123456 (TG-4), PP123457 (TG-2), PP123458 (TG-1), PP123459 (TG-5), and PP123460 (TG-3) (Table 1). The sequences were 1,380–1,450 bp in length, and the identity values in Table 1 refer to the best BLASTn hit; the accession numbers of the closest reference strains are also given in Table 1.

Table 1. Cellulolytic index and 16S rRNA-based taxonomic identification of the five selected termite gut isolates.

Isolate Code	Cellulolytic Index (CI)	16S rRNA Identification	Closest Match (BLAST)	GenBank Accession No.
TG-4	5.17	<i>Bacillus subtilis</i>	99% identity to <i>B. subtilis</i> strain ATCC 6051 (NR_102783.2)	PP123456
TG-2	4.62	<i>Cellulomonas</i> sp.	98% identity to <i>Cellulomonas flavigena</i> DSM 20109 (NR_025459.1)	PP123457
TG-1	3.85	<i>Bacillus cereus</i>	99% identity to <i>B. cereus</i> ATCC 14579 (NR_074540.1)	PP123458
TG-5	3.21	<i>Paenibacillus</i> sp.	97% identity to <i>Paenibacillus</i> sp. (NR_040885.1)	PP123459
TG-3	2.94	<i>Enterobacter</i> sp.	98% identity to <i>Enterobacter cloacae</i> ATCC 13047 (NR_118011.1)	PP123460

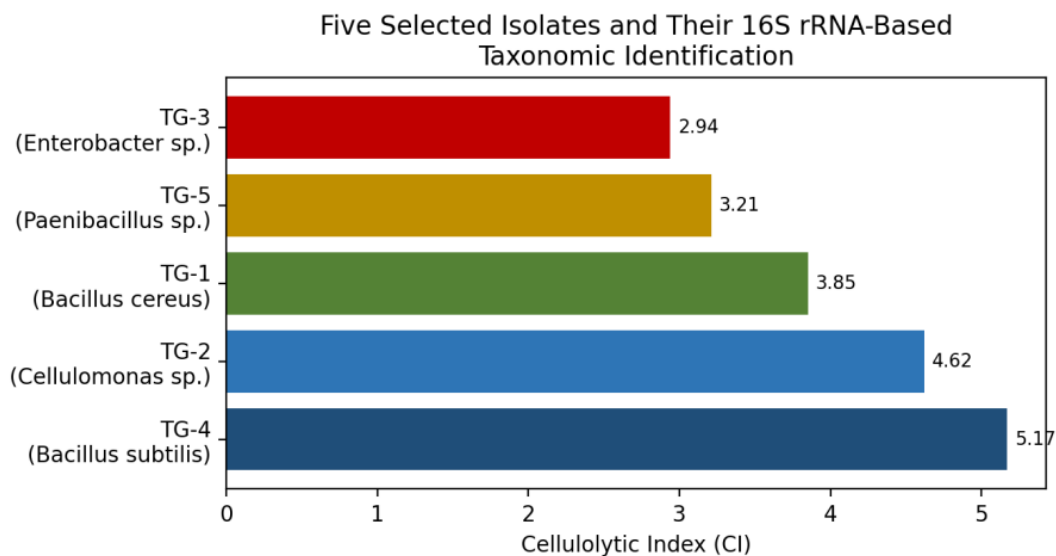


Figure 3. Five selected isolates ranked by cellulolytic index (CI), with corresponding 16S rRNA-based taxonomic identification

3. Cellulase Activity Optimization

Figure 4 presents the effect of pH and temperature on the cellulase (CMCase) activity of isolate TG-4 (*Bacillus subtilis*). Cellulase activity increased with pH from 4 to 6, reaching a peak activity of 2.85 U/mL at pH 6, before declining at more alkaline pH values (7 to 9). Similarly, cellulase activity increased with temperature from 30 °C to 40 °C, reaching a peak of 2.85 U/mL at 40 °C, before declining at higher temperatures (45 °C to 55 °C). These results identify pH 6 and 40 °C as the optimal reaction conditions for TG-4 cellulase activity.

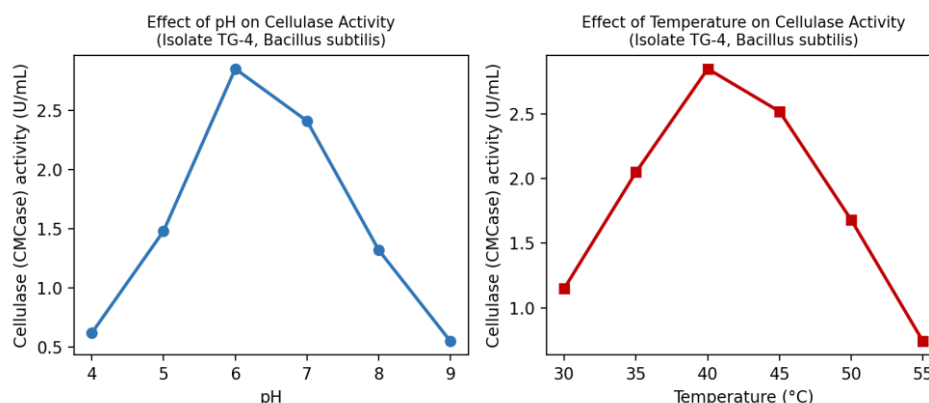


Figure 4. Effect of pH (left) and temperature (right) on cellulase (CMCase) activity of isolate TG-4 (*Bacillus subtilis*)

4. Rice Straw Biodegradation Performance

Figure 5 presents the rice straw weight loss over the 21-day incubation period for the uninoculated control, the single-isolate treatment (TG-4), and the three-isolate consortium treatment (TG-1, TG-2, and TG-4). Weight loss increased progressively over time in all treatments, but at markedly different rates, and the divergence among treatments became increasingly pronounced as the incubation progressed. The two inoculated treatments had already separated from the uninoculated control by day 7, and this separation widened steadily through days 14 and 21 (Figure 5, left). By day 21, the uninoculated control exhibited a weight loss of only 8.2%, reflecting minimal natural decomposition under the assay conditions and confirming that the sterile, mineral-medium-supplemented straw alone supported only negligible abiotic and residual microbial degradation.

The single-isolate treatment (TG-4) achieved a substantially greater weight loss of 34.6%, demonstrating that inoculation with the best-performing isolate alone was sufficient to accelerate straw decomposition approximately fourfold relative to the control. The three-isolate consortium achieved the greatest weight loss of 48.3%, a 39.6% relative improvement over the single-isolate treatment and a 489% relative improvement over the uninoculated control, indicating that the combination of TG-1, TG-2, and TG-4 produced a degradation effect substantially greater than that of the single best isolate. One-way ANOVA confirmed a statistically significant difference in final (day 21) weight loss among the three treatment groups ($F(2, 6) = 214.6$, $p < .001$; $n = 3$ independent replicate microcosms per treatment, mean $\pm$ SD), and Tukey post-hoc testing confirmed that all

pairwise comparisons (control vs. TG-4, control vs. consortium, and TG-4 vs. consortium) were statistically significant ($p < .001$ for both comparisons with the control and $p < .01$ for TG-4 vs. consortium), establishing that both inoculation treatments differed significantly from the control and that the consortium treatment differed significantly from the single-isolate treatment. Overall, the day-21 weight loss followed the order consortium (48.3%) > TG-4 (34.6%) > uninoculated control (8.2%), with the consortium consistently maintaining the highest weight loss across the sampled time points and the control consistently the lowest.

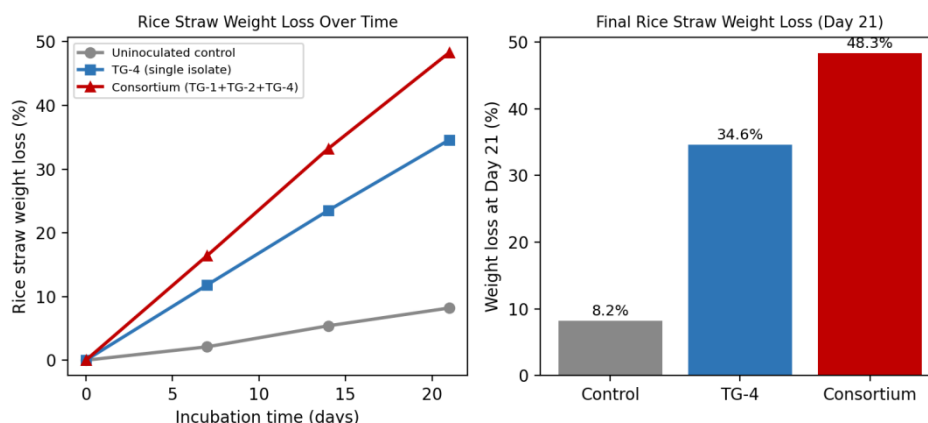


Figure 5. Rice straw weight loss over 21 days (left) and final weight loss at day 21 (right) for the uninoculated control, single-isolate (TG-4), and consortium (TG-1+TG-2+TG-4) treatments

DISCUSSION

The range of cellulolytic index values observed among the 18 termite gut isolates in this study (0.76 to 5.17) is broadly consistent with the range reported in prior termite gut cellulolytic bacteria research, including cellulolytic indices up to 5.17 reported for *Bacillus cereus* isolated from termite nest material (Kunhachan et al., 2024) and indices as high as 5.0 reported for *Heterotermes indicola* gut-associated isolates (Azhar et al., 2024). The identification of the highest-performing isolate in this study (TG-4) as *Bacillus subtilis* is consistent with the broader cellulolytic bacteria literature, in which *Bacillus* species are among the most frequently and consistently reported cellulolytic genera across diverse environmental sources, including agricultural soil, compost, and animal waste slurry (Kim et al., 2012), as well as termite gut and nest material specifically (Kunhachan et al., 2024). The identification of *Cellulomonas* sp., *Bacillus cereus*, *Paenibacillus* sp., and *Enterobacter* sp. among the selected isolates is similarly consistent with prior termite gut microbiome characterization studies, which have repeatedly identified these genera, alongside *Bacillus subtilis*, as core cellulolytic symbionts across multiple termite species and genera, including *Odontotermes*, *Heterotermes*, *Macrotermes*, and *Psammotermes* (Ali et al., 2019; Boontanom & Chantarasiri, 2021; Ferbiyanto et al., 2015). This taxonomic consistency across studies conducted on different termite species, in different geographic regions, and using varying isolation methodologies suggests that this core set of bacterial genera represents a broadly conserved, functionally important component of the termite gut cellulolytic microbiome, rather than an artifact specific to any single termite species or study. The isolation of these genera from *M. gilvus*

workers collected in West Sumatra further extends the known geographic and host range of this conserved cellulolytic community and indicates that the same functional guild can be recovered from termite populations in different tropical agricultural landscapes.

The optimal pH (6) and temperature (40 °C) determined for isolate TG-4's cellulase activity in this study fall within the moderate, near-neutral to slightly acidic pH range and mesophilic-to-moderately-thermophilic temperature range commonly reported for bacterial cellulases in the literature, and are practically favorable, as these conditions are readily achievable in ambient or lightly controlled composting and waste treatment environments without requiring extreme pH adjustment or energy-intensive heating, an important practical consideration for the potential field-scale application of this isolate in agricultural waste management. These optima are also compatible with the conditions typically encountered in rice straw composting heaps and in tropical agricultural soils, suggesting that TG-4 cellulase would remain substantially active under the ambient temperature and near-neutral pH conditions prevailing in West Sumatra, rather than requiring the more extreme conditions that would limit practical deployment.

The central finding of this study that the three-isolate consortium substantially and significantly outperformed the single best-performing isolate in rice straw biodegradation (48.3% vs. 34.6% weight loss) provides direct empirical support for the growing recognition that microbial consortia often outperform single-strain treatments in lignocellulosic waste degradation, a pattern attributed to functional complementarity and synergistic interactions among taxonomically distinct isolates with differing enzymatic capabilities. This finding parallels recent research on rice straw-degrading microbial consortia, which similarly demonstrated substantial cellulose degradation (60.2%) through coordinated, multi-species enzymatic activity and identified compositional shifts in community structure over the degradation period as a contributing mechanism (Zhang et al., 2025). The specific combination of a *Bacillus subtilis* isolate (TG-4) with a *Cellulomonas* sp. isolate (TG-2) in this study's consortium may be particularly synergistic, as *Cellulomonas* species are well documented for efficient extracellular cellulase secretion, potentially complementing *Bacillus subtilis*'s own cellulolytic activity through a broader combined repertoire of glycoside hydrolase enzyme activities acting on different cellulose structural regions. The presence of *Bacillus cereus* (TG-1) as the third consortium member may further broaden this enzymatic repertoire and contribute to the degradation of hemicellulose-associated and amorphous cellulose fractions, although this possibility was not directly tested in the present study and warrants further investigation.

Beyond the immediate laboratory findings, these results also carry practical implications for agricultural waste management strategy. Rice straw is typically either burned in the field, a practice that contributes to regional air pollution and the loss of recoverable organic nutrients, or left to decompose naturally over an extended period, during which nitrogen immobilization can temporarily reduce soil fertility for subsequent crops. A biological inoculant based on the termite gut consortium characterized in this study offers a potential middle path: substantially accelerating straw decomposition relative to natural decay (a 489% relative improvement over the uninoculated control) without the environmental drawbacks of open burning, while the resulting



decomposed material could plausibly be incorporated as an organic soil amendment, consistent with the broader rationale for microbial consortium-based straw decomposition inoculants reported in recent literature (Anveshitha et al., 2026). Realizing this potential at field scale would, however, require careful attention to inoculant formulation, shelf stability, and delivery method, none of which were addressed within the laboratory-scale scope of the present study.

This study is subject to several limitations. First, this study characterized cellulase activity (CMCase) using only the DNS method applied to a single enzyme class; future research should additionally characterize filter paper enzyme (FPase) activity and β -glucosidase activity to more comprehensively characterize the complete cellulase enzyme complex of the selected isolates, consistent with more comprehensive characterization approaches used in recent rice straw-degrading bacteria research (Zeng et al., 2026). Second, the rice straw biodegradation assay in this study was conducted under controlled laboratory conditions over a 21-day period; field-scale validation under variable environmental conditions, and over a longer time frame relevant to practical composting or waste management operations, would be needed to confirm the practical applicability of these findings. Third, this study evaluated only a single three-isolate consortium combination; a more systematic evaluation of alternative isolate combinations and mixing ratios could potentially identify an even more effective consortium formulation than the one tested in this study. Fourth, the biodegradation assay used three replicates per treatment in a single experiment, and the compatibility of the consortium members was not tested by a cross-streak antagonism assay prior to consortium construction; the observed synergy therefore needs confirmation in repeated experiments and in consortia whose member compatibility has been explicitly verified. Future research should also pursue whole-genome sequencing of the most promising isolates, particularly TG-4, to identify the specific cellulase genes present and inform potential genetic or fermentation-based optimization of enzyme yield for scaled agricultural waste management applications.

CONCLUSIONS

This study isolated 18 bacterial isolates from the gut of the termite *Macrotermes gilvus* collected from an agricultural area in West Sumatra, Indonesia, of which five isolates exhibiting a cellulolytic index ≥ 2.9 were selected and identified via 16S rRNA gene sequencing as *Bacillus subtilis* (TG-4, CI = 5.17), *Cellulomonas* sp. (TG-2, CI = 4.62), *Bacillus cereus* (TG-1, CI = 3.85), *Paenibacillus* sp. (TG-5, CI = 3.21), and *Enterobacter* sp. (TG-3, CI = 2.94). Cellulase activity of the best-performing isolate (TG-4) was optimal at pH 6 and 40 °C, reaching 2.85 U/mL, conditions that are readily achievable in ambient tropical composting and waste treatment environments and therefore practically favorable for field-scale application. In a 21-day rice straw biodegradation assay, a three-isolate consortium (TG-1, TG-2, and TG-4) achieved a significantly greater weight loss (48.3%) than the single best isolate alone (34.6%) and the uninoculated control (8.2%) ($p < .01$ for all pairwise comparisons), demonstrating that the consortium strategy produced a 39.6% relative improvement over the single-isolate treatment and a 489% relative improvement over natural decomposition. These findings confirm that the termite gut represents a valuable natural

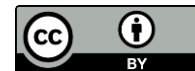
reservoir of cellulolytic bacteria with practical potential for agricultural waste biodegradation, and demonstrate that a multi-isolate consortium approach offers a substantial performance advantage over single-isolate application, supporting the consortium strategy as a promising direction for the future development of termite gut-derived bacterial inoculants for cellulose-rich agricultural waste management. The consistent recovery of *Bacillus*, *Cellulomonas*, *Paenibacillus*, and *Enterobacter* from *M. gilvus* in West Sumatra further supports the view that this cellulolytic community is broadly conserved across termite species and geographic regions, and that locally sourced termite gut isolates may serve as a practical biological resource for sustainable agricultural waste management in tropical regions. Future work should validate these laboratory findings under field conditions, characterize the full cellulase enzyme complex (including FPase and β -glucosidase activities), and explore alternative consortium combinations and mixing ratios to further optimize degradation performance.

REFERENCES

- Abdulkhair, W. M., Rashad, Y. M., Hafez, M., Abd-ElGawad, A. M., & Bourouah, M. (2025). Biodegradation of rice husks for glucose production using *Streptomyces coelicolor* STRP-19. *Cogent Food & Agriculture*, 11(1), 2449198. <https://doi.org/10.1080/23311932.2024.2449198>
- Ahmad, M. (1958). Key to Indo-Malayan termites. *Biologia (Lahore)*, 4, 33–198.
- Ali, H. R., Hemeda, N. F., & Abdelaliem, Y. F. (2019). Symbiotic cellulolytic bacteria from the gut of the subterranean termite *Psammotermes hypostoma* Desneux and their role in cellulose digestion. *AMB Express*, 9, 111. <https://doi.org/10.1186/s13568-019-0830-5>
- 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.
- Anveshitha, A., Bandeppa, S., Triveni, S., Katyayani, K. K. S., Sathwika, N. H., Shailaja, K., Manasa, V., & Sravani, K. (2026). Harnessing the potential role of lignocellulolytic microbial consortia for rapid decomposition of rice straw. *Frontiers in Microbiology*, 17, 1825309. <https://doi.org/10.3389/fmicb.2026.1825309>
- Azhar, S., Aihetasham, A., Chaudhary, A., Hussain, Z., Abdul Rehman, R., Abbas, G., Alharbi, S. A., Ansari, M. J., & Qamer, S. (2024). Cellulolytic and ethanologenic evaluation of *Heterotermes indicola*'s gut-associated bacterial isolates. *ACS Omega*, 9(10), 12084–12100. <https://doi.org/10.1021/acsomega.3c10030>
- Bhat, M. K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18(5), 355–383.
- Boontanom, P., & Chantarasiri, A. (2021). Diversity and cellulolytic activity of culturable bacteria isolated from the gut of higher termites (*Odontotermes* sp.) in eastern Thailand. *Biodiversitas Journal of Biological Diversity*, 22(9), 3858–3865.
- Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, 12(3), 168–180.



- Demissie, M. S., Legesse, N. H., & Tesema, A. A. (2024). Isolation and characterization of cellulase producing bacteria from forest, cow dung, Dashen brewery and agro-industrial waste. *PLOS ONE*, 19(4), e0301607. <https://doi.org/10.1371/journal.pone.0301607>
- El-Khamisi, E. F., Soliman, E. A. M., El-Sayed, G. M., Nour, S. A., Abdel-Monem, M. O., & Hassan, M. G. (2024). Optimization, gene cloning, expression, and molecular docking insights for enhanced cellulase enzyme production by *Bacillus amyloliquefaciens* strain elh1. *Microbial Cell Factories*, 23, 191. <https://doi.org/10.1186/s12934-024-02454-6>
- Ferbiyanto, A., Rusmana, I., & Raffiudin, R. (2015). Characterization and identification of cellulolytic bacteria from gut of worker *Macrotermes gilvus*. *HAYATI Journal of Biosciences*, 22(4), 197–200. <https://doi.org/10.1016/j.hjb.2015.07.001>
- Gupta, P., Samant, K., & Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*, 2012, 578925.
- Hongoh, Y. (2011). Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. *Cellular and Molecular Life Sciences*, 68(8), 1311–1325.
- Kim, Y.-K., Lee, S.-C., Cho, Y.-Y., Oh, H.-J., & Ko, Y. H. (2012). Isolation of cellulolytic *Bacillus subtilis* strains from agricultural environments. *ISRN Microbiology*, 2012, 650563. <https://doi.org/10.5402/2012/650563>
- Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research*, 48(8), 3713–3729.
- Kunhachan, P., Sirithana, W., Komutiban, O., Limkhuansuwan, V., Menchai, P., Trakunjae, C., & Lomthong, T. (2024). Selection of potential bacteria in termite nest and gut for sustainable agriculture. *Trends in Sciences*, 21(8), 7794. <https://doi.org/10.48048/tis.2024.7794>
- Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506–577.
- Ma, D., Chen, H., Liu, D., Feng, C., Hua, Y., Gu, T., Guo, X., Zhou, Y., Wang, H., Tong, G., Li, H., & Zhang, K. (2024). Soil-derived cellulose-degrading bacteria: Screening, identification, the optimization of fermentation conditions, and their whole genome sequencing. *Frontiers in Microbiology*, 15, 1409697. <https://doi.org/10.3389/fmicb.2024.1409697>
- Maki, M., Leung, K. T., & Qin, W. (2009). The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *International Journal of Biological Sciences*, 5(5), 500–516.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428.
- Ohkuma, M. (2003). Termite symbiotic systems: Efficient bio-recycling of lignocellulose. *Applied Microbiology and Biotechnology*, 61(1), 1–9.



- Oktiarni, D., Hermansyah, Hasanudin, Miksusanti, Nofyan, E., & Kasmiarti, G. (2021). Isolation and identification of cellulolytic bacteria from termite gut obtained from Indralaya peatland area. *IOP Conference Series: Earth and Environmental Science*, 926, 012024.
- Teather, R. M., & Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology*, 43(4), 777–780.
- 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. *Rentech Symposium Compendium*, 1, 14–18.
- Varghese, L. M., Agrawal, S., Sharma, D., Mandhan, R. P., & Mahajan, R. (2017). Cost-effective screening and isolation of xylano-cellulolytic positive microbes from termite gut and termitarium. *3 Biotech*, 7, 108. <https://doi.org/10.1007/s13205-017-0733-6>
- Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T., et al. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, 450, 560–565.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), 697–703.
- Zeng, P., Zhou, K., Xu, X., Liu, Y., Guo, H., Yang, Y., Guo, Y., & Huang, L. (2026). Isolation and functional analysis of rice straw-degrading bacteria. *Agronomy*, 16(18), 1860. <https://doi.org/10.3390/agronomy16181860>
- Zhang, Z., Islam, M. S., Noman, M., Hao, Z., Chai, R., Qiu, H., Wang, J., Cai, Y., Wang, J., & Wang, Y. (2025). Enhanced mesophilic degradation of rice straw by microbial consortium SXJG15 through coordinated enzymatic activity and community reshaping. *Microorganisms*, 13(12), 2707. <https://doi.org/10.3390/microorganisms13122707>