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Selection of Cellulase and Xylanase-Producing Fungi for Rice Straw Digestion in Ruminants

J. Noolaong[a], J. Kaewmanee[b], & A. Sukkaew[a,*]
[a]Faculty of Sciences Technology and Agriculture, Yala Rajabhat University,
[b]Faculty of Public Health and Allied Health Sciences, Yala Rajabhat University,
Sateng Sub-District, Muang Yala District, Yala Province, 95000 Thailand
*Corresponding author: adulsman.s@yru.ac.th
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ABSTRACT

The efficient degradation of lignocellulosic biomass is essential for sustainable bioenergy production and the enhancement of ruminant feed quality. This study aimed to isolate and characterize cellulase and xylanase-producing fungi from soil and decayed wood samples collected in the Hala-Bala Forest, Yala Province, Thailand, and to evaluate their potential for rice straw fermentation. A total of 15 fungal isolates were obtained through selective culturing and identified by internal transcribed spacer (ITS) region sequencing. The identified species included *Trichoderma reesei, Aspergillus niger, Chaetomium globosum, Penicillium oxalicum, Fusarium oxysporum, Trichoderma viride, Aspergillus flavus,* among others. Enzymatic activity was screened using plate assays with carboxymethyl cellulose (CMC) and xylan substrates. *T. reesei* demonstrated the highest enzymatic potential, with clear zones of 5.2 cm for cellulase and 6.8 cm for xylanase. Six top-performing strains (*T. reesei, A. niger, F. oxysporum, A. flavus, C. globosum,* and *T. viride*) were selected for rice straw fermentation trials using a completely randomized design. Fermentation quality was assessed through pH, lactic acid content, and organoleptic properties (color, odor, and texture). Results revealed that rice straw fermented with *A. niger, A. flavus,* and *T. viride* exhibited optimal pH, high lactic acid levels, and favorable physical characteristics. Additionally, chemical composition analysis showed that rice straw fermented with *A. niger* and *T. viride* had significantly higher crude protein content and improved fiber fractions (neutral detergent fiber, acid detergent fiber, cellulose, hemicellulose), indicating improved nutritional value for ruminants. These findings emphasize the possible use of indigenous fungal isolates for bioconversion of lignocellulosic biomass into value-added products for sustainable agriculture and energy applications.

Keywords: cellulase; fungi; rice straw; xylanase

INTRODUCTION

The economic expansion of the three southern border provinces and farmer employment will influence the 20-year national strategy plan and the sufficiency of the economy's vision of security and development. Promoting animal husbandry can boost local income. Animal breeds and feed quality must improve to boost livestock productivity. The cost of producing animal feed will boost farmer profits. We need to discover recipes that improve animal feed, including using microbes to ferment and increase protein and nutrients. Using microbes, such as *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Aspergillus niger*, *Lactobacillus delbruckii*, and *Bacillus spp.*, may increase protein and nutritional contents, enhancing animal feed use. Thai farmers in the southern border regions can use *Trichoderma harzianum* and *Rhizopus spp.* on fibrous feed sources to produce high-quality and value-added livestock products. This is essential for local economic growth.

Ullah *et al.* (2023), Dabul *et al.* (2025), and Evangelista *et al.* (2025) found that yeast-fermented cassava chips (*S. cerevisiae*) increased protein content by 47.5%, improving animal feed quality and protein nutritional value. The fermentation of cassava peels with *S. cerevisiae* over three days raised protein content from 4.4% to 10.9% and lowered cyanide toxin levels from 21.3 to 9.5 mg/kg, which is significant given that 50–100 mg can kill or sicken animals. As a high-quality protein source rich in amino acids like lysine and histidine, yeast has 40%–60% protein in its cells. Animals get vitamins, minerals, and proteins via yeast cell decomposition. Mixtures of microorganisms improve animal feed protein (Rodriguez *et al.*, 2015; Murata *et al.*, 2021); the protein content of soybean meal increased from 48.6% to 70.6% after three days of co-fermentation with yeast and lactic acid bacteria. Additive combinations affect the quality of rice straw silage; 5 treatment approaches were evaluated across 4 silo density levels (200, 300, 400, and 500 kg/m^3). The treatments included lactic acid bacteria (LAB) inoculants, molasses (M), cellulosic enzymes (E), and two combination treatments (M+LAB and E+LAB). The research revealed that single additives (molasses

or crude enzymes) increased dry matter losses at lower densities, presenting risks for silage quality. However, the LAB related to treatments, particularly combination approaches, demonstrated superior performance. At a silo density of 300 kg/m³, acetic acid produced by LAB treatments effectively inhibited harmful yeast and mold populations (Du *et al.,* 2025). The fermentation of palm oil cakes investigated using a mixed culture of *S. cerevisiae* and *Bacillus subtilis* (50:50 ratio) over periods of 0, 15, 30, and 45 days was found that the fermentation of palm kernel meal with yeast at a fermentation period of 45 days increased the protein content from 7.75% to 12.36%, representing an increase of 22.92% compared to the group not prepared with *S. cerevisiae* and *B. subtilis* (Nopparatmaitree *et al.,* 2014). This treatment was particularly intended to improve the nutritive value of palm oil by-products as feed for ruminant animals such as cattle, goats, and sheep (Tefera *et al.,* 2014). Gunun *et al.* (2023) investigated the nutritional value of cassava peel by fermentation with yeast (*S. cerevisiae*) or effective microorganisms (EM) for use as a concentrate replacement in goat diets. After 14 days, crude protein increased from 2.1% to 13.8%. Feeding trials showed no significant differences in intake, digestibility, rumen fermentation, or growth performance among treatments. However, replacing 50% of the concentrate with yeast-fermented cassava peel reduced feed cost per gain by up to 32%. Fermenting cassava with yeast (Y), effective microorganisms (EM), and EMY increases its protein content to 42.1%, 44.2%, and 45.3% of dry matter (Polyorach *et al.,* 2017). Microorganisms use carbohydrates as a carbon source for development during rumen fermentation, boosting protein content in animal feed. Ruminants consume fibrous feeds, mostly cellulose, hemicellulose, and lignin. Animal feed has strong cell walls that rumen bacteria cannot penetrate. A typical method is to use microbes to change animal feed fibrous materials to boost cellulolytic bacteria. These microorganisms include fungi and bacteria (Polyorach *et al.,* 2013; Tefera *et al.,* 2014). The yeast medium solution (YMS) applied for twenty-one days on fermenting rice straw of various lengths lowered NDF and ADF concentrations (Djunu, 2022).

The findings suggest that supplementing animal feed with yeast and beneficial bacteria can enhance the protein content of cassava, thereby improving its value as cattle feed. Fermentation of boiled cassava root with teff flour provides a suitable substrate for microbial activity, while the microorganisms involved in cassava fermentation contribute to improved nutritional quality and facilitate biochemical synthesis (Halake & Chinthapalli, 2020). Although several studies have examined microbial fermentation for feed improvement, limited research has focused on exploring indigenous fungal strains from unique ecosystems, such as the Hala-bala forest, which may harbor novel cellulolytic and xylanolytic species with superior enzymatic efficiency. The objective of this study was to isolate and screen fungi from soil and decayed wood samples collected from Hala-bala forest for their cellulase and xylanase production capabilities. The primary objective was to identify fungal strains with superior enzymatic efficiency for rice straw decomposition. Subsequently, the selected high-performance fungal isolates were evaluated for their potential in rice straw fermentation as a strategy for enhancing feed quality for ruminant nutrition.

MATERIALS AND METHODS

Soil Sampling for Fungal Isolation

Fungal screening for cellulase and xylanase production from soil and rotten wood focuses on areas with diverse vegetation, wetlands, high biodiversity, and accumulated plant debris. Soil samples were collected from Hala Bala Forest at Latitude: 5°50′- 5°57′ N and Longitude: 101°49′ -101°51′ E, Than To District, Yala Province. Thailand. The sampling process involved randomly collecting soil using a hand shovel at approximately 10-15 cm depth. About 300 grams of soil were collected and placed in zip-lock bags, which were then sealed. For the decayed wood, pieces of decomposing bark were collected and placed in zip-lock plastic bags. Each site was sampled in triplicate, resulting in a total of 12 individual samples. Samples were labeled with site-specific codes (HB-S1 to HB-S6 for soil, and HB-W1 to HB-W6 for wood) and transported under cooled conditions (4-10°C) to the laboratory within 24 hours.

Isolation of Fungi from Soil and Decayed Wood

The isolation of fungi from soil and decayed wood samples was conducted using two methodologies. Ten grams of soil were suspended in 90 mL of sterile distilled water and thoroughly homogenized. Serial dilutions were prepared by transferring 10 mL of the initial suspension to subsequent bottles containing 90 mL of sterile distilled water, achieving final concentrations of 10^{-2}, 10^{-3}, $10^{-4,}$ and 10^{-5}. One milliliter of aliquots from the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were pipetted onto 9 cm diameter petri dishes, with five replicates for each dilution. Three distinct culture media were employed: 1) Gochenaur's glucose ammonium agar (GAN) supplemented with Rose Bengal and Streptomycin, 2) Half-strength potato dextrose agar (½PDA), and 3) Water agar (WA). After gentle homogenization, the plates were incubated at 25-28 °C in darkness for 3-7 days. Emergent fungal colonies were subsequently transferred using a sterile needle to PDA slants to establish pure cultures for subsequent morphological and molecular characterization, identification, and preservation (Abuajah *et al.,* 2022; Sukkaew *et al*., 2025; Wu *et al.,* 2022).

Fungal Identification from Soil and Rotten Wood in Hala-Bala Forest, Than To District, Yala Province, using Polymerase Chain Reaction (PCR)

Utilizing sterile, contamination-free tools, samples of soil and decomposing wood were collected from the Hala-Bala forest in Yala province and stored in sterile plastic bags or containers. To make DNA extraction easier, the samples were mixed with a buffer. Utilizing the appropriate tools, DNA was extracted from soil and decayed wood, and the DNA was then separated from

other materials by filtering. Using gel electrophoresis, the quality of the extracted DNA was assessed after being measured with a spectrophotometer. Utilizing the polymerase chain reaction (PCR) technique, PCR mixtures comprising template DNA, dNTPs, Taq polymerase, specific fungal primers, and a buffer were prepared. Through the use of thermal cycling, DNA was amplified by the thermocycler. To separate and confirm the size of the amplified DNA, the PCR results were examined by subjecting the PCR products to gel electrophoresis. The results were recorded and visualized by the gel documentation system, allowing PCR product sizes to be compared to fungal databases or established standards. After being subjected to accurate fungal identification, the nucleotide sequences were examined using bioinformatics software to align them with database sequences.

Analysis of Cellulase Enzyme Production

The cellulase enzyme production was analyzed from 15 fungal isolates that were grown on a solid medium containing carboxymethyl cellulose (CMC) substrate. Firstly, culture fungi in sterile Petri plates using CMC agar medium. After that, the fungal isolates were cultivated for seven days at room temperature (25 to 30 **°C**) to check for fungal contamination. The staining of unhydrolyzed cellulose, the agar surface, and fungal colonies were soaked in 5% Gram's iodine solution after the incubation period and allowed to rest for 5 minutes. After that, the iodine solution was carefully decanted. A clear zone encircling the fungal colonies suggested that cellulase activity was hydrolyzing the cellulose. For additional analysis, the clear zone diameter was measured in centimeters (cm) (Abuajah *et al.,* 2022; Wu *et al.,* 2022).

Preparation of Medium for Testing Xylanase Enzyme Production Using Xylan Agar

To prepare the medium, 100 mL of xylanolysis basal medium (XBM) with 4 g of xylan and 1.6 g of agar were mixed, followed by sterilization in an autoclave at 121 °C for 20 minutes. After sterilization, the medium was cooled slightly and well-blended, then poured into Petri dishes. For the fungal test, the test fungi were cultured on potato dextrose agar (PDA). Using a cork borer with a 0.5 cm diameter, a plug of fungal mycelium was cut from the edge of the colony, placed at the center of the xylan agar plate, and incubated until the fungal colony reached a diameter of approximately 3 cm. Once the desired colony size was achieved, the entire surface of the medium and fungal colony was stained with Gram's iodine solution. After 5 minutes, the iodine was poured, and the plate was rinsed with sterilized distilled water. Finally, the diameter of the clear zone (zone of hydrolysis) in centimeters was measured to evaluate xylanase activity (Shrestha *et al.,* 2022; Wu *et al.,* 2022).

The Development of Animal Feed Formulations Using Rice Straw as a Substrate Supplemented with Selected Fungi

A procedure was established for developing animal feed using rice straw. Rice straw was processed through a shredder to achieve uniform particle sizes of 2-3 cm. Fungal inoculants consisting of *T. reesei, A. niger, F. oxysporum, A. flavus, C. globosum,* and *T. viride* were applied at 10% (w/w) of the substrate weight. Molasses was incorporated at 4% (w/w) of the rice straw weight as a palatability enhancer and carbon source. The mixture was prepared with distilled water at a substrate-to-water ratio 1:1 (w/v) to the rice straw weight. The mixtures were thoroughly mixed and then transferred to 30-liter containers, where they were tightly compressed by using a pump to remove air before they were tightly sealed in the bags. This fermentation proceeded for 21 days in a shaded area. After fermentation, the physical characteristics of the resulting silage, including color, smell, and texture, were examined, and the pH of each treatment was measured. The container was then stored in a shaded area for 21 days. Post-fermentation analysis included assessment of physical characteristics (color, odor, and texture) and pH determination for each treatment. The experimental

Table 1. Physical quality evaluation of fermented rice straw silage enriched with selected fungi

Physical characteristics	
Smells	- Fragrant, similar to the smell of fermented fruit or vinegar (12 points)
	- Not fragrant, slightly pungent (8 points)
	- Very pungent and slightly unpleasant (4 points)
	- Rotten or moldy smell (0 points)
Fermented plant material	- Firm, with leaves and stems intact and no impurities (4 points)
	- Firm, with leaves and stems intact and no impurities (2 points)
	- Firm, with leaves and stems very decomposed and with impurities (1 point)
	- Slimy and very dirty (0 points)
Color	- Yellowish-green or khaki (3 points)
	- Greenish-yellow or dark green (2 points)
	- Golden brown (1 point)
	- Dark brown or black (0 points)
pH	- 3.5-4.2 (6 points)
	- 4.3-4.7 (4 points)
	- 4.7-5.1 (2 points)
	- > 5.1 (0 points)

Note: Source: Department of Livestock Development, 2004.

design are comprised seven treatments with three replications: T1 (control - rice straw without fungal inoculation), T2 (rice straw + *T. reesei*), T3 (rice straw + *A. niger*), T4 (rice straw + *F. oxysporum*), T5 (rice straw + *A. flavus*), T6 (rice straw + *C. globosum*), and T7 (rice straw + *T. viride*). Following the fermentation period, representative samples from each treatment were collected in beakers, thoroughly mixed, and subjected to organoleptic evaluation and pH measurement by a panel of five assessors according to the parameters outlined in Table 1.

Dried rice straw samples were ground through 5 and 1 mm sieves after drying at 65 °C to analyze their nutritional value via proximate analysis, including dry matter (DM), organic matter (OM), and crude protein (CP). The CP content was analyzed using the Kjeldahl method (Model KT 20s, Gerhardt, Germany), following the procedures of AOAC Official Method 954.01 (AOAC, 1990). Dry matter and organic matter were determined by gravimetric analysis, with DM assessed through oven drying at 105 °C (AOAC Official Method 930.15), and OM calculated by subtracting ash content obtained from ignition at 550 °C (AOAC Official Method 942.05) (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured with a fiber content analyzer (Fibretherm) model FT12, Gerhardt brand, according to the methods of Goering and Van Soest (1970). The pH of the silage was measured at every stage of the experiment using a pH meter according to the method of Bernardes *et al.* (2019). The quality of silage from different types of rice straw was studied for pH at every stage using the pH meter (Hanna Instruments HI2211) method of Bernardes *et al.* (2019). Organic acids in the silage were analyzed at 21 days of fermentation and then analyzed using Chromatography (Agilent 7890A/7890B GC System), according to Leventini *et al.* (1990).

The completely randomized design was conducted to determine significant differences between treatments (T1, T2, T3, T4, T5, T6, and T7) for each measured parameter. The averages were then calculated from the obtained data using the Microsoft Excel software, and analysis of variance (ANOVA) was used to analyze the statistical differences in the various values. Where the treatments showed a significant influence, Duncan's new multiple range test (DMRT) with the appropriate SAS® OnDemand for Academics (SAS, 2025).

RESULTS

Fungal isolation from soil and rotten wood in Hala-Bala forest, Yala Province, obtained 15 isolates as single colonies, as shown in Figure 1. In isolate 1 (A), the fungus grows in all directions from a spot in the petri dish. Fungal hyphae are dense in the core and thin out toward the edges. Some dark green hyphae include white rings, possibly suggesting spore production or growth phases. The fine hyphal surface spreads uniformly around the dish. The thick central hyphae have black patches, and the growth boundary is smooth and symmetrical. This fungus may be Trichoderma. Isolate 2 (B), the fungus fills virtually the entire petri dish, spreading outward from a center point. The white spot in the middle indicates beginning growth. Dark

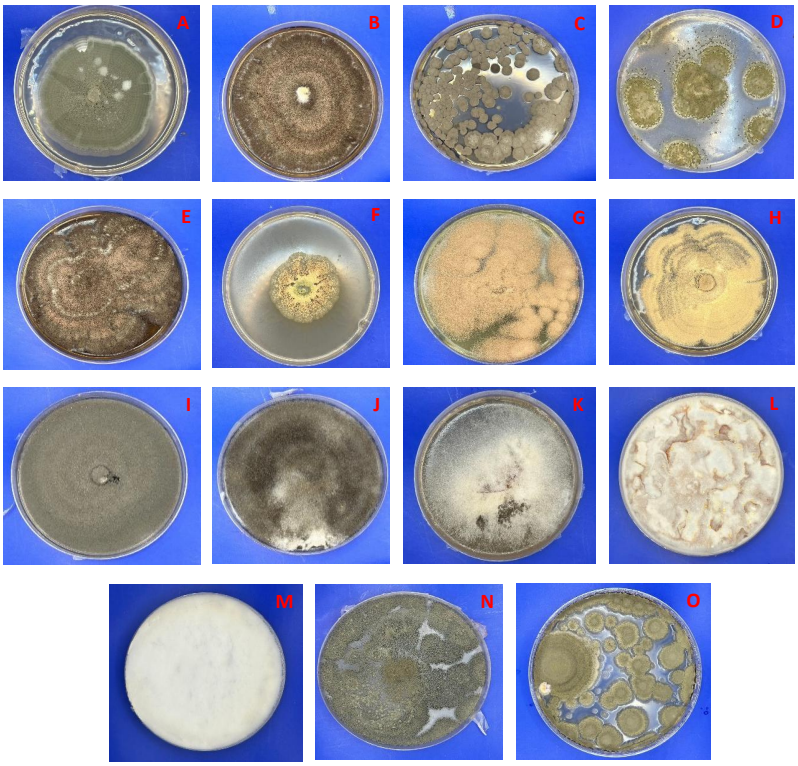

Figure 1. Characteristics of fungi isolated from soil in the Hala-Bala Forest, Than To District, Yala Province, Thailand. By isolate 1 is A; isolate 2 is B; isolate 3 is C; isolate 4 is D; isolate 5 is E; isolate 6 is F; isolate 7 is G; isolate 8 is H; isolate 9 is I; isolate 10 is J; isolate 11 is K; isolate 12 is L; isolate 13 is M; isolate 14 is N and isolate 15 is O.

brown to black hyphae may vary due to environmental changes. A fine hyphal surface has high density in the center and decreasing density at the edges. Smooth growth boundaries with clear growth rings indicate phases. Due to its extensive growth, black hyphae, and copious spore generation, this fungus is likely Aspergillus. Isolate 3 (C), multiple hyphal masses arise as the fungus clusters over the petri dish. Brown to dark brown hyphae indicate spore or fungal development and white rings indicate early growth. Dense hyphal masses have fine and dense surfaces with varied heights. The growth limit is patchy. Due to their clustered growth and abundant hyphal production, *Rhizopus* spp., *Mucor*, or *Chaetomium* are likely candidates.

Isolate 4 (D), the fungus spreads in places and forms several clusters in the petri dish. These clusters contain dense, green to dark green hyphae from various fungus species. Green indicates metabolite or photosynthetic synthesis. Fine, dense hyphal surfaces are common in clusters. Smooth but spotty development borders form clusters. Clustered growth, green hyphae, and profuse spore generation suggest *Penicillium* spp. Isolate 5 (E), the fungus spreads across the petri dish in rings or layers, signifying different growth phases. Many fungi have dark brown or black hyphae that transition from the center to the edge. The hyphal surface is fine and dense, with peak density at the center and decreasing outward. Smooth growth boundaries with clear growth rings indicate phases. This fungus's extensive growth, black hyphae, and copious spore generation suggest *Cladosporium* spp. Isolate 6 (F), the fungus creates a thick mass in the petri dish center with extensive hyphal dissemination to the periphery. The hyphae are yellow to light brown, shifting color from center to edge, suggesting spore or metabolite accumulation. Hyphae have fine, dense surfaces, especially in the core, which generate dense masses. The growth border is smooth, and the hyphae spread widely. This fungus is reckoned to be *Aspergillus* or *Penicillium* based on its thick, clustered growth and similar hue.

Isolate 7 (G), the fungus spreads across the petri plate in clusters. The light brown to dark brown hyphae indicate spore or metabolite buildup, with color variation related to growth phases. In clusters, the hyphal surface is fine and thick, with various heights. The smooth growth boundary spans the dish. This fungus's clustered growth and abundant hyphal production suggest *Rhizopus* spp. or *Mucor*. Isolate 8 (H), This isolate exhibited concentric layers across the Petri plate, with distinct color variations ranging from golden yellow to dark brown. Such pigmentation likely reflects the accumulation of spores or secondary metabolites within the hyphae. The central zone presented a dense and fine hyphal texture, while the colony margin remained smooth, with uniform hyphal distribution. The colony's broad spread and characteristic yellow-to-brown hues suggest affinity to *Aspergillus flavus* or *Penicillium* spp. Isolate 9 (I), growth initiated from the colony center and expanded radially. The mycelium displayed a darkened central area with paler margins, giving the colony a gradient of dark gray to black. The central hyphal surface appeared smooth, and the growth boundary was gentle and uniform. Based on its extensive growth and dark pigmentation, the isolate is likely a member of the genus *Aspergillus*.

Isolate 10 (J), this isolate colonized the Petri plate from the center outward, with hyphae exhibiting dark gray to black pigmentation. A pronounced dark center contrasted with lighter margins. The colony margin was smooth and extended across the plate. The morphological traits, including filamentous growth and consistent black pigmentation, suggest possible identification as *Aspergillus niger* or *Cladosporium* spp. Isolate 11 (K), the Petri dish was covered in stratified layers of fungal growth. Hyphae ranged from white to light gray, with scattered darker or brown patches indicative of sporulation or metabolite accumulation. Hyphal density was especially evident in the darker regions. The colony margin was smooth, uniformly extending across the plate. The combination of abundant growth and white-to-light-gray pigmentation suggests probable classification as *Trichoderma*. Isolate 12 (L), colonies displayed irregular growth patterns with grouped hyphae extending unevenly across the Petri dish. Hyphae were predominantly whitish to light gray, with darker patches indicating areas of spore or metabolite buildup. Compact and fine hyphal surfaces were observed in zones of denser growth. Margins were typically smooth and extended to the dish edge, although uneven distribution was evident. The morphological profile is consistent with *Rhizopus* or *Mucor* spp.

Isolate 13 (M) uniformly colonized the plate, with dense, smooth, and fine hyphae distributed evenly throughout. The colony appeared completely white, lacking observable pigmentation. The growth margins were smooth and continuous. Based on its dense, pure white hyphae with uniform coverage, this isolate is likely attributable to *Penicillium* or *Geotrichum*, both known for producing compact, unpigmented colonies. Isolate 14 (N), growth was radial, covering the plate from the center outward. White speckling was observed across the colony, indicative of spore formation. Hyphae exhibited green to dark green pigmentation, while the central region contained densely packed, fine hyphae. The smooth margin extended to the edge of the dish. The characteristic green pigmentation and morphology are strongly indicative of *Penicillium* spp. Isolate 15 (O), colonies formed multiple concentric clusters, producing layered or ring-like patterns across the plate. Hyphae displayed a striking emerald to dark green coloration, interspersed with white dots associated with spore or metabolite accumulation. The hyphal surface appeared compact and finely textured, especially along the concentric rings. Margins were smooth and extended across the entire Petri dish. Given the broad colony spread and distinctive green pigmentation, the isolate is most consistent with *Penicillium* or *Aspergillus* spp.

Fungal Identification from Soil and Rotten Wood in Hala-Bala Forest, Than To District, Yala Province

The PCR analysis for fungal identification revealed 15 isolates with the following fungi: *T. reesei*, *A. niger*, *C. globosum*, *P. oxalicum*, *C. cladosporioides*, *A.versicolor*,

F. oxysporum, *A. flavus*, *A. terreus*, *C. sphaerospermum*, *T. viride*, *R. oryzae*, *G. candidum*, *P. chrysogenum*, and *P. expansum*, as presented in Table 2.

Enzyme Activity Testing for Cellulase and Xylanase from Identified Fungi

The diameter of the zone of clear area (cm) was determined in the cellulase and xylanase enzyme activity tests, indicating the ability to produce cellulase and xylanase from the fungal isolate of 15 fungi. The isolates tested were *T. reesei*, *A. niger*, *C. globosum*, *P. oxalicum*, *C. cladosporioides*, *A. versicolor*, *F. oxysporum*, *A. flavus*, *A. terreus*, *C. sphaerospermum*, *T. viride*, *R. oryzae*, *G. candidum*, *P. chrysogenum*, and *P. expansum*. The study showed statistically significant differences in the production of cellulase and xylanase enzymes ($p<0.05$), with $F= 20.18$ and $p= 0.000111$. Among the 15 fungal isolates tested, the highest cellulase activity was exhibited by *T. reesei*, *A. niger*, and *T. viride* (Figure 2). The average diameter of clear zones for these strains ranged from 4.8 to 5.2 cm. For xylanase activity, a clear zone of 6.8 cm was shown by *T. reesei*, followed by *A. flavus* and *C. globosum.*

The results of the experiment showed that high cellulase and xylanase enzyme production was exhibited by *T. reesei*, demonstrating its effectiveness in degrading cellulose and hemicellulose fibers. The lowest enzyme production, however, is shown by *C. sphaerospermum* and *C. cladosporioides*, which contribute less to the breakdown of organic matter than the other fungi. The findings of the study can be used to choose and create high-efficiency fungi strains for use in a variety of industrial applications, such as the production of biofuel, the breakdown of organic matter in the environment, and the production of meat. As in other studies where *T. reesei* is mentioned as a good producer of cellulose and hemicellulose for degrading enzymes, high productivity for both cellulase and xylanase was exhibited by *T. reesei*. High xylanase production was exhibited by *T. viride*, demonstrating its capacity to degrade hemicellulose. Low production of both enzymes was exhibited by *C. sphaerospermum* and *C. cladosporioides*, which may be due to genetic characteristics or unfavorable growth conditions. Due to their extensive enzyme production systems capable of degrading a variety of organic materials, it is suggested that several fungi, including *T. reesei* and *A. flavus*, could efficiently produce both cellulase and xylanase in the relationship between cellulase and enzymes. Overall, the study found that many fungi exhibited good enzyme production capabilities, particularly *T. reesei*, *A. niger*, *F. oxysporum*, *A. flavus*, *C. globosum*, and *T. viride*, indicating their potential to degrade a variety of organic compounds. This research can guide the selection and development of high-efficiency fungi for various industrial applications. Analyzing the enzyme production capabilities of different fungi will help researchers and industry practitioners choose suitable

Table 2. Fungal identification results from soil and rotten wood samples in Hala-Bala Forest, Than To District, Yala Province, Thailand

No.	Isolates	Organism	Sequence (5'-->3')	% Identity
1	A	*Trichoderma reesei*	CCCTAACCCTAACCCTAACCCTAACCCCTAAGCTATTAAAGGCCTA GGGCATGTTTTATAAACTTTATTAGCTATTACCT	99.8
2	B	*Aspergillus niger*	ATGTCAAATATAATTTCAATAATTCAAGGATTATTAGTTATTGTTCCT GCTTTAATATCTGTTGCTTTTG	99.6
3	C	*Chaetomium globosum*	GACAGCGGCGCAATTACGGGTGCAAAAGGGTCAGTGTCGTTTCCC TTATCAGCGCACCCCTTCT	99.4
4	D	*Penicillium oxalicum*	ATGGCGCGCCCAGATACAGAGTATGTTGTCGAGCCTGTGTTTAGTC TAAGGAAACAAAATCTGACAAAAC	98.9
5	E	*Cladosporium cladosporioides*	AAAAAAGGCATTCAGTTGCTGGTTCGCAACGCAGACGGCACCCTT CAGGTCAACAAGATTGCTGCGACAT	99.3
6	F	*Aspergillus versicolor*	ATCAAATCAAAACCATAAGAACAACCAGTGTTCTATTCTAAGCAA CATCGACTAAGGTCACATTTAATGT	99.5
7	G	*Fusarium oxysporum*	GAATTGCTGTAGAATTGCTGTAGAATTGCTGTAGAATTGCTGTAGAA TTGCTGTAGAATTGCTGTAGAATTGCTGTAGAA	99.7
8	H	*Aspergillus flavus*	GCAGGGTATCGAGCGGCAGAAAGCCAAACTGCACCCAGACATGC AGTCTGGCTGCAAAGTCCGTTGGGTCGAGAGTTTTG	99.2
9	I	*Aspergillus terreus*	CGACAAATCCTAAACATCTTTAATGATCTTAAACCCGGAATATAATC GATTACTACAAACCCTGAATACTATCGATTACT	99.0
10	J	*Cladosporium sphaerospermum*	CAGTAGCCGCTTGCCGTTAGCGCCGACTTGGGGTTAGCTGGGGATA AGTGTTCCCCTCCGCTGCTTTTTG	99.1
11	K	*Trichoderma viride*	TAATCTACTATTATGTCAAGACCATGTCTATAAAAAGCATGTAGGTA GTCAATTTAGGATAGATACTAAGAAGGTTGACA	99.6
12	L	*Rhizopus oryzae*	TATTATTATAAGGAAAATAAAGCGTGGCTCAGAGAGCATGTATCAC ATTTTCAGTCTTCGTTTCGTCAACATTAGTATTT	98.7
13	M	*Geotrichum candidum*	CAAAATTGACATTTTTACTCAGAAATGACAAAAAGACTTTTGCTGC TGACAAACCCATCATTCGGCCAGC	98.5
14	N	*Penicillium chrysogenum*	GAGAAGAGCGAGAATTCCGGGAAACTGATTTCAATAACCTCGACG ATGCATACATTGGGAGATTTGAAGCTGCTGCATAC	99.4
15	O	*Penicillium expansum*	AGGATGGTCTAAAATATAGATATCTTCCTCTATATTTTCGACTAAGCT AAACTTCTATATTTTTAACTATTAGGTTCTCT	99.1

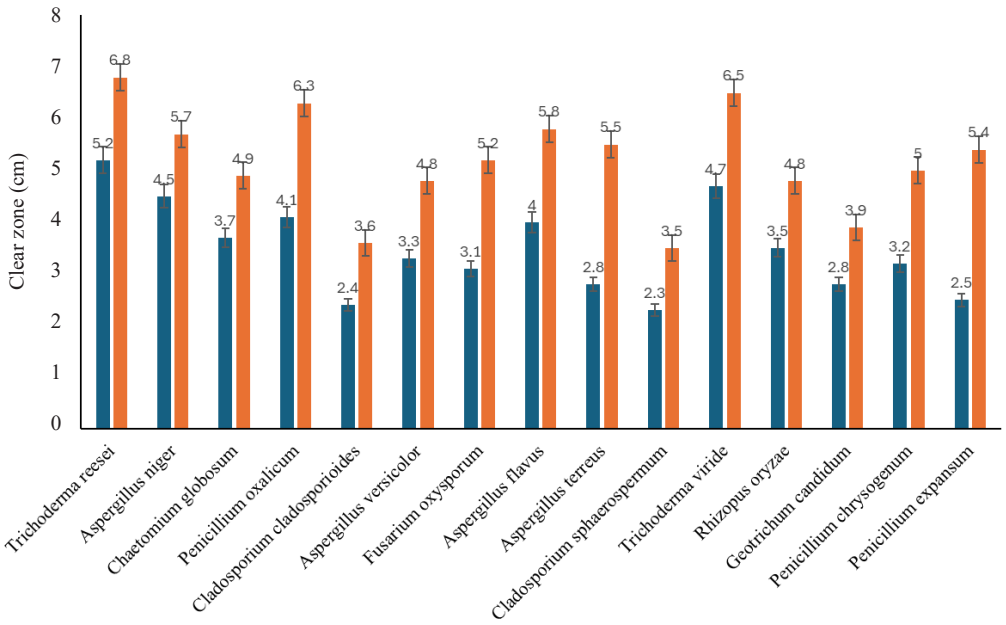

Figure 2. Results of cellulase and xylanase enzyme tests from identified fungi. ■ Cellulase; ■ Xylanase.

fungi and enhance enzyme production efficiency for industrial benefits.

Physical Quality Assessment of Fungal-Fermented Rice Straw

The physical characteristics assessment revealed substantial differences among treatments based on a standardized scoring system (Figure 3). The control group and treatments with *A. niger*, *A. flavus* and *T. viride* achieved superior quality scores 21-23 point, characterized by desirable yellow to cinnamon coloration (3 points), pleasant fruity or vinegar-like aroma (12 points), intact structural integrity with preserved leaf and stem morphology (4 points), and optimal pH range of 4.50-4.99 (2-6 points). *C. globosum* fermentation resulted in moderate quality (15 points), while *T. reesei* and *F. oxysporum* treatments produced suboptimal fermentation products with scores of 3 and 4 points, respectively. These findings indicate that specific fungal species significantly influence the organoleptic properties and overall acceptability of fermented rice straw for feed applications.

Chemical Quality of Fermented Rice Straw

Fermentation with different fungal species significantly influenced the chemical composition of rice straw (Table 3). pH values ranged from 4.50 to 4.99 with significant variations observed among treatments ($p<0.01$). Organic acid profiles differed markedly between treatments ($p<0.01$). The control treatment (without fungal inoculation) exhibited the highest concentrations of lactic acid (2.57) and butyric acid (0.78), while *C. globosum* fermentation resulted in the highest acetic acid production (0.095). Conversely, *A. flavus* treatment showed the lowest lactic acid (0.28) and butyric acid (0.18) levels, whereas *A. niger* fermentation produced no detectable acetic acid.

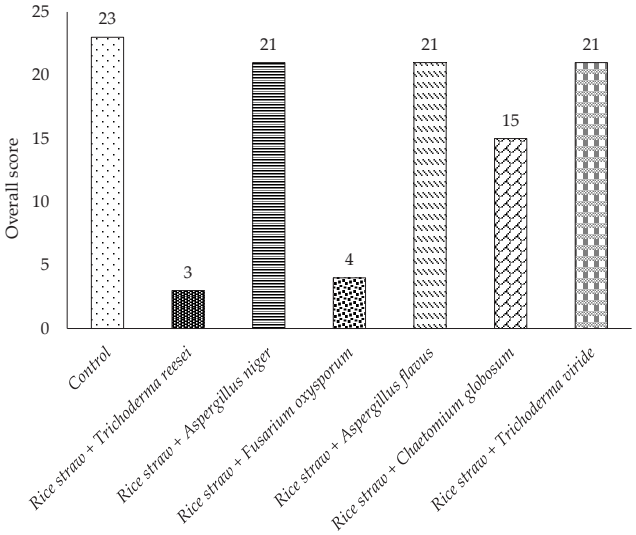

Figure 3. Physical properties of rice straw as a substrate supplemented with selected fungi. Score: 20-25 = excellent, 15-19 = well, 6-14 = fair, 0-4 = bad.

Chemical Composition of Fermented Rice Straw

Chemical composition analysis of rice straw following 21-day fermentation revealed significant differences among treatments for all measured parameters ($p<0.01$) (Table 4). Dry matter content was highest in *A. flavus*-fermented straw (68.48%), followed by *T. reesei* (65.74%) and *T. viride* (64.42%), with the latter two showing no statistical difference. Crude protein content varied significantly among treatments, with *A. flavus* achieving the highest concentration (68.48%), followed by *T. reesei* (65.74%) and *T. viride* (64.42%). The control treatment (unfermented straw) exhibited the lowest protein content (49.03%). Fungal fermentation substantially modified the fiber composition, including neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and

Table 3. Quality of the components of fermented rice straw as a substrate supplemented with selected fungi

Rice straw with selected fungi	Fermentative quality			
	pH	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)
Control	4.50^{C}	2.57^{A}	0.06^{AB}	0.78^{A}
Rice straw + *Trichoderma reesei*	4.75^{B}	0.73^{C}	0.02^{CD}	0.29^{ED}
Rice straw + *Aspergillus niger*	4.83^{AB}	0.50^{CD}	0.00^{D}	0.41^{CD}
Rice straw + *Fusarium oxysporum*	4.99^{A}	0.30^{D}	0.01^{CD}	0.53^{BC}
Rice straw + *Aspergillus flavus*	4.86^{AB}	0.28^{D}	0.05^{BC}	0.18^{E}
Rice straw + *Chaetomium globosum*	4.53^{C}	1.67^{B}	0.095^{A}	0.62^{B}
Rice straw + *Trichoderma viride*	4.98^{A}	0.40^{D}	0.01^{CD}	0.38^{D}
SEM	0.056	0.081	0.012	0.043

Note: $^{A, B, C}$: Means in the same column of the same comparison parameter factor with different superscripts differ significantly (p<0.01). Control: rice straw without fungal inoculation; SEM: Standard error of means.

Table 4. Chemical composition of rice straw as a substrate supplemented with selected fungi

Rice straw with selected fungi	Proximate chemical composition (%)										
	DM	CP	Ash	CF	EE	NFE	NDF	ADF	ADL	Cellulose	Hemi cellulose
Control	49.03^{D}	7.04^{B}	13.07^{E}	30.30^{D}	1.41^{C}	48.16^{A}	66.80^{E}	37.75^{D}	6.72^{A}	31.55^{E}	29.04^{D}
Rice straw + *Trichoderma reesei*	65.74^{B}	7.63^{A}	15.38^{B}	31.39^{B}	1.42^{C}	44.17D^{E}	68.28^{C}	39.06^{B}	6.39^{B}	32.67^{B}	29.21^{D}
Rice straw + *Aspergillus niger*	58.47^{BC}	6.94^{B}	15.20^{BC}	30.90^{C}	1.83^{A}	45.05^{CD}	65.88^{E}	38.55^{C}	6.33^{B}	32.22^{C}	27.32^{E}
Rice straw + *Fusarium oxysporum*	62.59^{BC}	7.18^{B}	15.93^{A}	31.77^{A}	1.41^{C}	43.70^{E}	71.33^{A}	39.52^{A}	6.08^{B}	33.44^{A}	31.81^{A}
Rice straw + *Aspergillus flavus*	68.48^{A}	7.11^{B}	15.02^{CD}	30.26^{D}	1.42^{C}	46.17^{B}	67.71^{D}	37.70^{D}	6.70^{A}	31.00^{F}	30.00^{B}
Rice straw + *Chaetomium globosum*	51.70^{C}	6.07^{C}	14.71^{D}	31.61^{AB}	1.63^{B}	45.96^{BC}	69.12^{B}	39.33^{AB}	6.72^{A}	32.61^{B}	29.21^{D}
Rice straw + *Trichoderma viride*	64.42^{B}	7.70^{A}	15.77^{A}	30.72^{C}	1.51^{BC}	44.28^{DE}	65.74^{F}	38.25^{C}	6.35^{B}	31.90^{D}	27.48^{E}
SEM	0.014	0.105	0.096	0.106	0.46	0.281	0.119	0.763	0.92	0.061	0.057
P-Value	<.0001	<.0001	<.0001	<.0001	<.002	<.0001	<.0001	<.0001	<.011	<.0001	<.0001

Note: $^{A, B, C, D}$: Means in the same column of the same comparison parameter factor with different superscripts differ significantly (p<0.01). Control: rice straw without fungal inoculation. DM: dry matter; CP: crude protein; Ash: Ash content; CF: crude fiber; EE: ether extract; NFE: Nitrogen-free extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin are visualized on a dry matter basis; SEM: Standard error of the mean.

hemicellulose fractions, demonstrating the efficacy of selected fungi in enhancing the nutritional profile of rice straw for ruminant feed applications.

Rice straw fermented with *F. oxysporum* had the highest ash (15.93%), which was not different from straw fermented with *T. viride* (15.77%), while straw without fungi (Control group) had the lowest ash content (13.07%). While rice straw fermented with *A. flavus* had the lowest crude fiber content (30.26%), rice straw fermented with *F. oxysporum* had the highest crude fiber (CF) (31.77%), no difference from rice straw fermented with *C. globosum* (31.61%). Rice straw fermented with *A. niger* had the greatest fat content (ether extract, EE) (1.83%), followed by rice straw fermented with *C. globosum* (1.63%). Rice straw without mold added (control group), rice straw fermented with *T. reesei*, *F. oxysporum,* and *A. flavus* all had the same fat content (1.41%-1.42%). While straw fermented with *F. oxysporum* had the lowest NFE concentration (43.70%), rice straw without fungus (Control group) had the greatest NFE content (48.16%), followed by straw fermented with *A. flavus* (46.17%). Fiber fraction analysis revealed treatment-specific effects: *T. viride* produced the most digestible profile with the lowest NDF (65.74%), while *F. oxysporum* resulted in the highest NDF (71.33%) and ADF (39.52%) values. Lignin content was minimized by *F. oxysporum* fermentation (6.08%), contrasting with the control and *A. flavus* treatments (6.70%-6.72%). Cellulose and hemicellulose fractions were optimized by *F. oxysporum* fermentation, achieving 33.44% and 31.81% respectively, while *A. niger* and *T. viride* treatments resulted in the lowest hemicellulose content (27.32%-27.48%).

DISCUSSION

The diversity of fungi with varying capacities for cellulase and xylanase enzyme production aligns with the ecological characteristics of the Hala-Bala forest, where high moisture levels and the continuous accumulation of organic matter promote the development of a rich microbial community (Singh *et al.,* 2018). The identification of well-recognized fungal genera – namely *Trichoderma, Aspergillus, Penicillium, Fusarium, Chaetomium,* and *Geotrichum* – was achieved through both morphological examination and molecular analysis of the 15 isolates. Their frequent association with lignocellulose decomposition highlights their ecological role in degrading plant residues in forest ecosystems (Qu *et al.,* 2025). *T. reesei* and *T. viride* were widely reported for high cellulase production in both natural habitats and industrial contexts (Cano y Postigo *et al.,* 2021). High enzymatic activity, especially from *T. reesei* and *A. niger,* was consistent with earlier findings (Zhang *et al.,* 2021), with the efficiency of *T. reesei* linked to its well-studied cellulase gene clusters. Conversely,

F. oxysporum displayed moderate activity, which may result from substrate specificity or enzyme repression. These results underscore the potential of native fungal strains for improving biomass utilization as ruminant feed.

Enzyme activity assays revealed significant differences ($p<0.05$) in cellulase and xylanase production among the isolates. *T. reesei* exhibited the highest activity for both enzymes, with clear zone diameters of 5.2 cm for cellulase and 6.8 cm for xylanase, demonstrating its superior lignocellulose-degrading potential. These observations reinforce prior reports that designate *T. reesei* as a benchmark species for industrial enzyme production due to its efficient regulation of gene expression and high secretion capacity (Shrestha *et al.,* 2022). Other noteworthy producers, including *A. niger*, *A. flavus,* and *F. oxysporum,* are valued for their diverse metabolic repertoire, while *T. viride* emerged as a promising candidate for hemicellulose degradation, with notable xylanase activity (6.5 cm). By contrast, *Cladosporium cladosporioides* and *C. sphaerospermum* displayed relatively weak activity, possibly constrained by genetic factors or unsuitable culture conditions. Despite their natural abundance, these species may not strongly express lignocellulolytic enzymes, reaffirming the importance of strain-specific selection for biotechnology. The co-production of cellulase and xylanase by *Trichoderma* and *Aspergillus* illustrates their robust enzymatic systems capable of hydrolyzing cellulose and hemicellulose simultaneously. Meanwhile, moderate activity in *Penicillium expansum* and *Geotrichum candidum* suggests opportunities for optimization through strain improvement or fermentation control. These results carry industrial implications, as species such as *T. reesei*, *A. niger,* and *P. oxalicum* hold promise for waste management, composting, feed additive production, and biofuel generation (Wu *et al.,* 2022). Moreover, the study highlights the significance of targeted screening of strains with desirable enzymatic traits and emphasizes the unexplored potential of ecosystems like the Hala-Bala forest for uncovering novel resources.

The potential of selected fungi was further validated by the fermentation trials with rice straw. Rice straw treated with *Aspergillus niger*, *Aspergillus flavus,* and *Trichoderma viride* exhibited favorable color, aroma, and texture, indicating high-quality fermentation suitable for animal feed, consistent with earlier descriptions (McDonald *et al.,* 1991). Recent research by Chen *et al.* (2019) similarly demonstrated that *T. viride* improved the nutritive value, and lignin and cellulose degradation ratios were 26.38% and 33.29%. In addition, *A. niger* exhibited higher enzyme activity on pretreated biomass when moistened with modified basal salt media (BSM) (Sharma *et al.,* 2012). By contrast, rice straw fermented with *T. reesei* exhibited inferior physical qualities, likely due to over-degradation that damaged its structural properties (Wanapat *et al.,* 2009). These outcomes stress the importance of carefully selecting fungal species to achieve the best compromise between nutritional enrichment and structural preservation in rice straw treatment. Concerning silage quality, previous guidelines recommend an optimal pH between 3.8 and 4.5 for long-term preservation (McDonald *et al.,* 1991). In this study, however, all treatments exceeded this threshold, potentially compromising stability. The closest values to the desired range were recorded for the control (4.50) and *C. globosum*-treated silage (4.53). Achieving sufficiently low pH is critical to inhibit undesirable bacterial growth (Cheli *et al.,* 2013). Interestingly, the untreated rice straw contained the highest lactic and butyric acid levels, which are key in reducing pH and suppressing spoilage organisms, thereby safeguarding silage quality (Weinberg & Muck, 1996). However, the control group's elevated butyric acid level could suggest *Clostridium* bacteria contamination or inadequate fermentation (McDonald *et al.,* 1991).

The rice straw fermented with *C. globosum* had the second greatest lactic acid content (1.67) and the highest acetic acid level (0.095), which has the quality of suppressing the growth of yeast and fungus. Exposure to air helps the silage be more stable (Danner *et al.,* 2003). Rice straw fermented with *A. flavus* had the least butyric acid concentration (0.18), which is beneficial as butyric acid is usually linked with off-odor and silage degradation (McDonald *et al.,* 1991). On the other hand, one should consider *A. flavus's* possibility of producing aflatoxins, which could endanger animals (Pitt & Hocking, 2009).

This work supports Ike & Tokuyasu (2018), who indicated that *T. reesei,* the filamentous fungus, an anamorph of *Hypocrea jecorina*, is a well-known producer of hydrolytic enzymes for lignocelluloses, i.e., cellulases and hemicellulases. The work by Singh *et al.* (2018) also shows *T. reesei* is quite good at generating xylanase and cellulase enzymes. Current studies support the findings of Goda *et al.* (2024), who indicated that *A. niger* and *A. flavus* also have notable capacity for generating cellulase-degrading enzymes, hence corroborating the findings that these fungi display high enzyme production rates. High-efficiency fungi for several industrial uses, such as biofuel generation, environmental organic matter decomposition, and application in animal feed industries, will be chosen and developed using this study as a foundation. Examining the enzyme production potential of various fungi can help academics and business professionals select appropriate fungi and improve enzyme production efficiency for industrial use.

The rise in crude protein content in rice straw fermented with *T. viride* and *T. reesei* (7.70% and 7.63%, respectively) over the control (7.04%) could be caused by the fungus generating single-cell proteins throughout their development (Sharma & Arora, 2010). *Trichoderma* fungi can also create effective lignocellulose-degrading enzymes, which could free proteins attached to the lignocellulosic structure (Jafari *et al.,* 2007). Consistent with Cano y Postigo *et al.* (2021), the increase in crude protein in rice straw fermented with *Trichoderma* spp. was brought on by the formation of single-cell protein during the fungal growth, whose study suggested that solid fermentation with *T. fungus* could improve the nutritional profile of agricultural waste by successfully

increasing the protein content. Less lignin was visible in rice straw fermented with *F. oxysporum*, suggesting it can produce efficient lignin-degrading enzymes.

The fermentation with *F. oxysporum* produced the highest levels of NDF and ADF (71.33% and 39.52%, respectively) in rice straw, even though it produced the lowest lignin content (ADL) (6.08%), indicating that this fungus can degrade lignin. The results are consistent with the study that was carried out by Gupta and Chundawat (2020), who identified genes associated with lignin degradation through genomic analysis of *F. oxysporum*. Lignin is a tough component to decompose, and it impairs fiber digestion by ruminants. Therefore, its reduction is noteworthy (Zhong *et al.,* 2021). *T. viride* and *A. niger* produced the least NDF and hemicellulose levels in rice straw; in the meantime, supporting the findings of Rahman *et al.* (2022), who discovered that this group of fungi may generate very effective cellulase and xylanase enzymes. It lowers the cell wall makeup of rice straw. Xing *et al.* (2020) also found that these fungi may generate potent hemicellulose-degrading enzymes capable of efficiently destroying the fibrous structure of rice straw. Especially in fermentations with *F. oxysporum*, *T. viride,* and *T. reesei*, which had the lowest NFE content, the fungi may be using soluble carbohydrates as an energy source for growth, causing a decline in NFE content in rice straw fermented with fungi compared to the control group (Kogo *et al.,* 2017). Sukkaew (2021) found that before breaking more complicated polysaccharides, bacteria use easily degradable carbon sources, such as sugars and starch, which are the components of NFE, during the solid phase fermentation process. The rise in lipid content in rice straw fermented with *A. niger* (1.83%) and *C. globosum* (1.63%) relative to the control group (1.41%) could be caused by either the buildup of lipids in fungal cells or the generation of fatty acids during the fermentation process. This agrees with Andlar *et al.* (2018), who discovered that various fungal strains can generate and accumulate intracellular lipids during the breakdown of lignocellulosic materials. According to Patel *et al.* (2020), the rise in fermented materials' lipids could be caused by the generation of fungal lipase enzymes, which alter the lipid profile throughout the fermentation process. Despite extensive research on the application of fungal enzymes for lignocellulosic biomass degradation, few studies have systematically screened native fungal strains from unique ecological niches such as the Hala-Bala Forest, a biodiverse tropical rainforest in southern Thailand. Most previous works have focused on commercial or model strains under laboratory conditions, which may not reflect the adaptive capabilities of indigenous fungi in real-world fermentation systems. This study introduces a novel approach by isolating cellulase- and xylanase-producing fungi from undisturbed forest environments and evaluating their direct application in rice straw fermentation for ruminant feed. Unlike prior studies, this work integrates enzymatic activity screening, fermentation performance, and nutritional improvement analysis, providing a holistic assessment of fungal potential. Therefore, the novelty of this research lies in the use of native fungal biodiversity for agricultural bioconversion, which contributes to both sustainable livestock production and circular bioeconomy development.

CONCLUSION

This study successfully isolated and characterized 15 fungal species from soil and decaying wood samples in Thailand's Hala-Bala forest, with *Trichoderma reesei* demonstrating the highest cellulase and xylanase production activities (5.2 and 6.8 cm clear zone diameters, respectively), followed by *Aspergillus flavus* (4.0 and 5.8 cm, respectively). Rice straw fermentation trials revealed that *A. niger, A. flavus,* and *T. viride* produced superior fermented substrates with optimal organoleptic properties, favorable pH levels, and enhanced chemical composition suitable for ruminant nutrition. These indigenous tropical fungi demonstrate significant potential for improving livestock feed formulation through sustainable agricultural waste valorization, effectively converting rice straw into high-quality animal feed with improved nutritional value.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this article, the authors used ChatGPT to improve language and readability, and subsequently reviewed and edited the content to ensure accuracy and academic standards.

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