

Original Article

Molecular identification of ligninolytic fungi from West Sumatra, Indonesia, using the Internal Transcribed Spacer (ITS) region

Eli Ratni^{1,*} , Lendrawati Lendrawati¹ , Muhammad Idris² , Fadilla Hefzi² 

1. Department of Animal Production Technology, Faculty of Animal Science, Universitas Andalas, Padang 25163, West Sumatra, Indonesia
2. Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang 25163, West Sumatra, Indonesia

*Correspondence: Eli Ratni (eratni@ansci.unand.ac.id)

Abstract

Objectives: This study aimed to isolate and identify ligninolytic fungi from decayed wood in West Sumatra, Indonesia, with a focus on their potential applications in animal production technology. The goal was to explore the potential of these fungi to improve lignocellulosic agricultural waste degradation, thereby enhancing the digestibility and nutritional value of ruminant feed.

Materials and Methods: Fungi were isolated from rotting wood samples collected in Padang and Solok. The Bavendamm test was used to screen for ligninolytic activity. DNA was extracted from purified isolates, and PCR amplification of the ITS region was performed using ITS1 and ITS4 primers. Amplified products were sequenced and analyzed using BLAST against the NCBI GenBank database for species identification.

Results: Two of four purified fungal isolates (P1 and S2) showed positive results in the Bavendamm test, indicating their ligninolytic potential. PCR amplification produced 574 bp (P1) fragments and 586 bp (S2). BLAST analysis identified P1 as *Trichoderma hamatum* and S2 as *Aspergillus flavus* with 100% similarity.

Conclusions: Identifying *T. hamatum* and *A. flavus* as ligninolytic fungi from West Sumatra provides valuable insight for applying these species in animal production technology. These fungi could contribute to biodelignification processes, improving the utilization of lignocellulosic waste as a ruminant feed source.

Keywords: *Aspergillus*; ITS; ligninolytic fungi; ruminant feed; *Trichoderma*

Article History

Received: November 15, 2025
Revised: January 20, 2026
Accepted: January 27, 2026
Published: March 10, 2026



© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

How to cite this article

Ratni E, Lendrawati L, Idris M, Hefzi F. Molecular identification of ligninolytic fungi from West Sumatra, Indonesia, using the Internal Transcribed Spacer (ITS) region. J Adv Vet Anim Res 2026; 13(1):140–146.

doi

10.5455/javar.2026.m1018

1. Introduction

Animal feed ingredients are expected to increasingly compete with human food and raw materials for bioenergy production [1, 2]. Therefore, future livestock production systems should utilize underutilized organic matter and agricultural waste, such as straw, rice husks, bagasse, peanut shells, palm oil, and sugarcane, to maximize their potential. In tropical countries, where these agricultural by-products are abundant, there is a significant opportunity to incorporate them into animal feed. However, lignin, which provides rigidity to plant cell walls, is resistant to degradation under anaerobic conditions, such as those in the rumen [3, 4]. As a result, lignocellulosic biomass from tropical crops has limitations in supporting optimal rumen fermentation, thereby affecting digestibility, intake rate, and production performance in livestock. Feed ingredients should be selected for their high digestibility and reduced methane emissions [5], as well as their potential to utilize common tropical feed resources to enhance livestock productivity and mitigate environmental impacts.

Rumen microorganisms can treat lignocellulosic biomass in various ways to increase the accessibility of cellulose and hemicellulose, including physical, physicochemical, and chemical treatments. However, biological treatment is a more economical and environmentally friendly alternative [3]. One potential approach is the cultivation of fungal mycelium on the substrate, increasing the nutritional value of crop waste as ruminant feed. Fungi have a unique ability: they are the only organisms that can degrade the complex lignin structure of plant cell walls.

Fungal microorganisms capable of producing extracellular ligninolytic enzymes, such as laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP), can degrade lignin [6, 7]. These enzymes have non-specific substrate

preferences and play an important role in the breakdown of lignin compounds [8, 9]. Naturally, there are three groups of fungi known to degrade lignin: white-rot, brown-rot, and soft-rot fungi [9, 10]. This grouping is based on the weathering products: brown-rot fungi leave brown residues, while white-rot fungi leave white residues.

Fungal resources with ligninolytic activity are abundant in tropical countries, such as Indonesia, which have high species diversity. West Sumatra is one of the provinces rich in such resources. Therefore, identifying fungal species that can degrade lignin is an important step. Morphological characters typically guide the identification of fungal species. However, this approach often encounters obstacles, especially at the species level, because closely related species tend to exhibit high morphological similarity [11].

The development of molecular biology techniques enables the determination and identification of fungal isolates to the species level with greater accuracy [12, 13]. Polymerase chain reaction (PCR) techniques are typically used for molecular identification using markers such as the internal transcribed spacer (ITS) [14–17]. ITS is a repetitive region of non-coding DNA sequences located in the coding region of ribosomal RNA molecules [15].

The knowledge gap in this field stems from the limitations of conventional morphological and biochemical identification methods, which often fail to distinguish closely related indigenous white-rot fungi. Although a preliminary study [18] identified several potential candidates, there is an urgent need for more precise species characterization to ensure the reliability of lignin-degrading applications. This research addresses this necessity by employing molecular identification based on the Internal Transcribed Spacer (ITS) region, widely recognized as the universal DNA barcode for fungi. Compared to other markers such as *18S rRNA*, the ITS region offers superior taxonomic resolution and greater interspecific variation, enabling more accurate identification of local isolates through comparison with extensive global databases. Consequently, the objective of this study was to isolate and identify indigenous ligninolytic fungal species from West Sumatra using ITS markers and to evaluate their potential for the biodelignification of lignocellulosic waste, ultimately enhancing the efficiency and sustainability of ruminant feed solutions.

2. Materials and Methods

2.1. Ethical approval

Ethical approval was not necessary for this study because it did not involve live animals, human participants, or any invasive procedures. All activities were conducted in compliance with applicable institutional and national guidelines for research and publication ethics.

2.2. Study area

We sampled two locations in West Sumatra: Padang (P), a low-altitude area, and Solok (S), a high-altitude area. Sample testing was conducted at Labor Biota Sumatra and Genetics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang.

2.3. Sample collection

Sampling was based on direct observation of rotting wood infected with fungi. We collected three different wood tissues from each location, so the total number of woods collected was six. We placed the collected samples into paper envelopes and labeled them P1, P2, P3, S1, S2, and S3. Further, we stored them at room temperature until the isolation process began.

2.4. Fungi isolation and purification

The isolation process commenced with the preparation of Potato Dextrose Agar (PDA) media. PDA powder (39 gm) was weighed and dissolved in 1000 ml of distilled water within an Erlenmeyer flask. The solution was heated and boiled on a hot plate stirrer until homogenized. Subsequently, the media were sterilized in an autoclave at 121°C for 15 min. Once the sterilized media reached a warm temperature, it was amended with chloramphenicol (Kemicetin) to prevent bacterial contamination. The media was then poured into Petri dishes under laminar airflow and allowed to solidify before the isolation procedure.

We took the infected wood parts, cut them into square shapes, washed them with distilled water, and then dried them using paper towels for the isolation process. We placed the dried wood pieces on PDA media, with three replicates, and incubated them for 3–4 days on a culture rack. Periodically, we observed the growth of the formed fungal colonies. We then purified the growing fungal colonies by transferring them to new PDA media. We kept the purified isolates as culture stock for further test preparation.

2.5. Primary screening test for ligninolytic activity

We conducted primary screening using the Bavendamm Test, which aims to identify the ligninolytic ability of fungi. We perform the Bavendamm test by adding 0.1% tannic acid to PDA media. We grew purified isolates on the media and

incubated them for 7–10 days. We determine the test results based on the color change of the media. If no brown color is formed, the Bavendamm test is negative (-), indicating that the fungus is not ligninolytic. On the other hand, the formation of brown color yields a positive (+) Bavendamm test result, indicating that the fungus is ligninolytic.

2.6. DNA isolation and amplification

The fungal DNA isolation process used the Quick-DNA Magbead Plus Kit. The isolated DNA was then amplified using the PCR technique by mixing reaction components such as 12.5 µl MyTaq HS Red Mix Bioline (containing ten mM dNTPs, 50 mM MgCl₂, and 1-unit Taq DNA Polymerase), 3.5 µl nuclease-free water, 2 µl of ITS1 forward primer (5'-TCC GTA GGT GAA CCT GCG G-3'), 2 µl of ITS4 reverse primer (5'-TCC TCC GCT TAT TGA TAT GC-3'), and 5 µl of the DNA isolate.

We visualized the amplified DNA using electrophoresis on a 1% agarose gel. 2.5 µl of amplified DNA was inserted into each well of the agarose gel. We ran the electrophoresis at 100 V, 200 mA, and 20 W power for 55 min. After electrophoresis, the gel was placed on a UV transilluminator to document the amplification results.

2.7. DNA sequencing

We purified the PCR products and sent them to the 1st BASE Sequencing Services Laboratory (Selangor, Malaysia) for bidirectional sequencing.

2.8. Data analysis

The DNA sequencing results were contiguous when using the DNASTAR program. Furthermore, the DNA sequences were compared with sequences registered in public databases using the BLAST (Basic Local Alignment Search Tool), which can be accessed via the NCBI (National Center for Biotechnology Information) website at <http://www.ncbi.nlm.nih.gov>. The BLAST program was used to determine the similarity (similarity) level of the sequences with the data listed on the site. We then selected sequence data from the BLAST results in GenBank for alignment, phylogenetic tree construction, and genetic distance calculation using MEGA 11 [19].

3. Results and Discussion

3.1. Fungal isolates

Four fungal isolates that successfully grew on the medium were obtained from weathered wood in Padang and Solok, West Sumatra (Figure 1). Based on the observation results, the color of the isolated Colonie S is white, and there are hyphae. Fungi grow within their substrate, making their species diversity challenging to observe. Weathered or dead wood serves as the substrate for ligninolytic fungi. Researchers have documented more than 2500 species of fungi, including both basidiomycetes and ascomycetes, growing on dead wood [20–22].

3.2. Primary screening test for ligninolytic activity

The results of the Bavendamm test on four purified isolates showed that two isolates, P1 and S2, were positive and characterized by the formation of a brown color in the media (Figure 2). This indicates that both isolates belong to the group of ligninolytic fungi.

The Bavendamm test is used to determine a fungus's ability to produce extracellular phenol oxidase [23]. In addition, this test is also used to detect the activity and growth of extracellular polyphenol oxidase on lignocellulosic substrates [24].

3.3. DNA quantification

DNA isolation of ligninolytic fungi was successfully carried out using the Quick-DNA Magbead Plus Kit. After isolation, DNA quantification was performed using a Nanodrop spectrophotometer at 260 nm and 280 nm to determine the concentration and purity of the sample (Table 1).

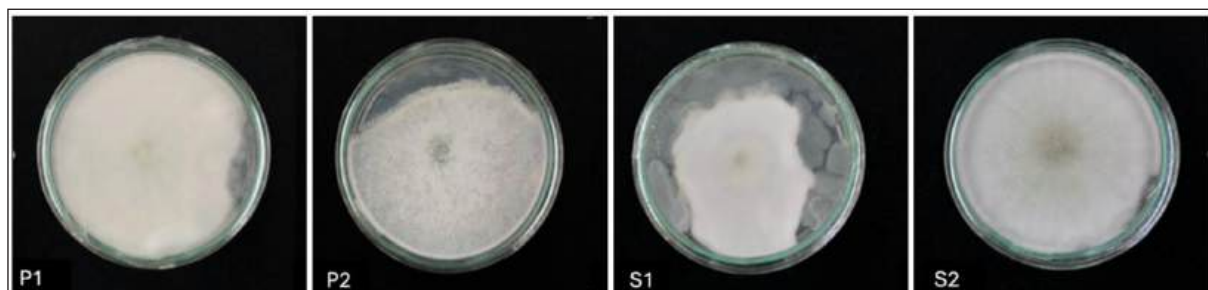


Figure 1. Ligninolytic fungi isolates (P = Padang, S = Solok).

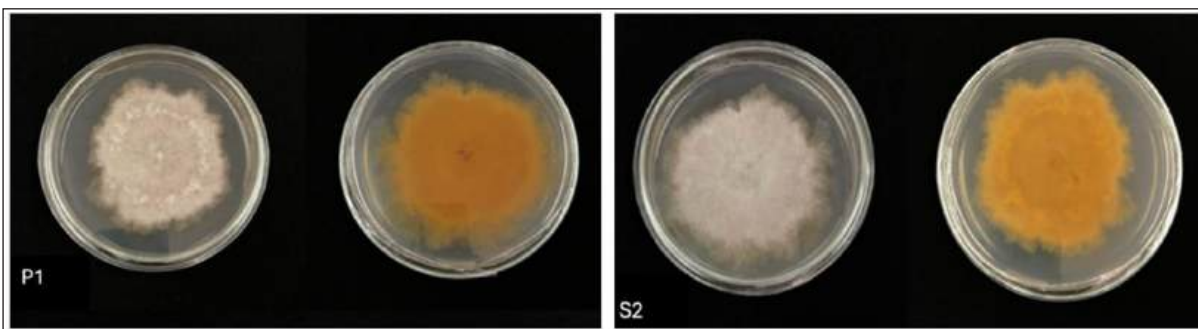


Figure 2. Bavendamm test results of fungi isolates that show positive results.

Table 1. Concentration and purity of isolated ligninolytic fungi DNA.

No.	Isolate	Nucleic Acid Conc. (ng/μl)	A260/280	Volume (μl)
1	P1	121.9	2.02	50
2	S2	120.9	2.02	50

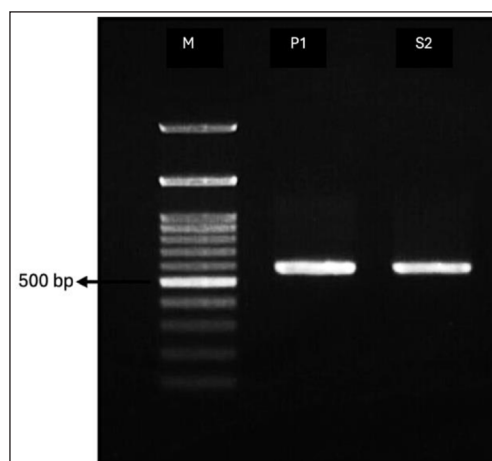


Figure 3. Visualization results of PCR products on 1% agarose gel (M = 100 bp DNA ladder).

Based on [Table 1](#), isolate P1 has a DNA concentration of 121.9 ng/μl with a purity of 2.02, while isolate S2 has a DNA concentration of 120.9 ng/μl with the same purity of 2.02. These values indicate that the DNA obtained from both isolates is of good quality, with high concentration and purity. Wood [25] defined good-quality DNA as having a concentration above 100 ng/l and a purity between 1.8-2.0. DNA purity values below 1.8 indicate the presence of contaminants, such as phenols or proteins, carried over during the isolation process, whereas values above 2.0 indicate possible contamination by RNA [26].

3.4. PCR amplification

DNA amplification of the two ligninolytic fungal isolates using ITS1 forward and ITS4 reverse primers produced products with fragment sizes of 574 bp for isolate P1 and 586 bp for isolate S2 ([Figure 3](#)). ITS1 and ITS4 are universal primers that amplify the ITS1 and ITS2 regions, including the 5.8S rDNA [27]. Fujita et al. [28] reported that amplification with ITS1 and ITS4 primers in fungi can yield amplicons ranging from 350 bp to 880 bp. Appiah et al. [29] also reported similar results, with fragment sizes of 400 bp for *P. ostreatus* and 600 bp for *S. commune* using ITS1 and ITS4 primers. Then, Apollos et al. [30] reported fragment sizes ranging from 450 to 650 bp in *Tricholoma robustum*. This difference in fragment size may be due to variations in the quality of DNA used in PCR [31–33]. Additionally, variability within the fungal ITS region, as well as differences in primer combinations used in amplification, can also affect the size of the resulting DNA bands [28, 34].

3.5. DNA sequencing

We purified and sequenced the amplification products using ITS primers to determine the nucleotide base sequence. The sequence of isolate P1 is 574 bp, while isolate S2 is 586 bp. [Figure 4](#) displays the complete nucleotide base sequence of both isolates.

1	CTGCGGAGGG	ATCATTACCG	AGTTTACAAC	TCCCAAACCC	AATGTGAACG	TTACCAAAC
61	GTTGCCCTCGG	CGGGGTCACG	CCCCGGGTGC	GTAAAAGCCC	CGGAACCAGG	CGCCCGCCGG
121	AGGAACCAAC	CAAACTCTTT	CTGTAGTCCC	CTCGCGGACG	TATTTCTTAC	AGCTCTGAGC
181	AAAAATTCAA	AATGAATCAA	AACTTTCAAC	AACGGATCTC	TTGGTTCTGG	CATCGATGAA
241	GAACGCAGCG	AAATGCGATA	AGTAATGTGA	ATTGCAGAAT	TCAGTGAATC	ATCGAATCTT
301	TGAACGCACA	TTGCGCCCGC	CAGTATTCTG	GCGGGCATGC	CTGTCCGAGC	GTCATTTCAA
361	CCCTCGAACC	CCTCCGGGGG	ATCGGCGTTG	GGGATCGGGA	CCCCTCACCG	GGTGCCGGCC
421	CTGAAATACA	GTGGCGGTCT	CGCCGCAGCC	TCTCCTGCGC	AGTAGTTTGC	ACAACTCGCA
481	CCGGGAGCGC	GGCGCGTCCA	CGTCCGTAAA	ACACCAACT	TCTGAAATGT	TGACCTCGGA
541	TCAGGTAGGA	ATACCCGCTG	AACTTAAGCA	TATC		
(a)						
1	CTTCCGTAGG	TGAACCTGCG	GAAGGATCAT	TACCGAGTGT	AGGGTTCCTA	GCGAGCCCAA
61	CCTCCCACCC	GTGTTTACTG	TACCTTAGTT	GCTTCGGCGG	GCCCGCCATT	CATGGCCGCC
121	GGGGGCTCTC	AGCCCCGGGC	CCGCGCCCGC	CGGAGACACC	ACGAACCTTG	TCTGATCTAG
181	TGAAGTCTGA	GTTGATTGTA	TCGCAATCAG	TTAAACTTTT	CAACAATGGA	TCTCTTGTTT
241	CCGGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAACTAGT	GTGAATTGCA	GAATTCCGTG
301	AATCATCGAG	TCTTTGAACG	CACATTGCGC	CCCCTGGTAT	TCCGGGGGGC	ATGCCTGTCC
361	GAGCGTCATT	GCTGCCCATC	AAGCACGGCT	TGTGTGTTGG	GTCGTCGTCC	CCTCTCCGGG
421	GGGGACGGGC	CCCAAAGGCA	GCGGCGGCAC	CGCGTCCGAT	CCTCGAGCGT	ATGGGGCTTT
481	GTCACCCGCT	CTGTAGGCC	GGCCGGCGCT	TGCCGAACGC	AAATCAATCT	TTTCCAGGTT
541	GACCTCGGAT	CAGGTAGGGA	TACCCGCTGA	ACTTAAGCAT	ATCAAT	
(b)						

Figure 4. Complete ITS sequences of ligninolytic fungi isolates, (a) isolate P1, (b) isolate S2.

Table 2. BLAST search results showing the similarity.

P1 Isolate					S2 Isolate				
No.	Species	Query Cover	E-value	Percentage (%) Similarity	Accession	Species	Query Cover	E-value	Percentage (%) Similarity
1	<i>Trichoderma hamatum</i>	100%	0.0	100%	ON927127.1	<i>Aspergillus flavus</i>	100%	0.0	100%
2	<i>Trichoderma hamatum</i>	100%	0.0	100%	ON927099.1	<i>Aspergillus flavus</i>	100%	0.0	100%
3	<i>Trichoderma hamatum</i>	100%	0.0	100%	OR435188.1	<i>Aspergillus flavus</i>	100%	0.0	100%
4	<i>Trichoderma</i> sp.	100%	0.0	100%	OM760666.1	<i>Aspergillus flavus</i>	100%	0.0	100%
5	<i>Trichoderma</i> sp.	100%	0.0	100%	MW760777.1	<i>Aspergillus flavus</i>	100%	0.0	100%
6	<i>Trichoderma hamatum</i>	100%	0.0	100%	OR553890.1	<i>Aspergillus oryzae</i>	100%	0.0	100%
7	<i>Trichoderma</i> sp.	100%	0.0	100%	MK870109.1	<i>Aspergillus flavus</i>	100%	0.0	100%
8	<i>Trichoderma hamatum</i>	100%	0.0	100%	DQ109530.1	<i>Aspergillus oryzae</i>	100%	0.0	100%
9	<i>Trichoderma hamatum</i>	100%	0.0	100%	PP422096.1	<i>Aspergillus flavus</i>	100%	0.0	100%
10	<i>Trichoderma hamatum</i>	100%	0.0	100%	MN264503.1	<i>Aspergillus flavus</i>	100%	0.0	100%

There was also a BLAST search of the complete ITS sequence of each isolate using the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST>) to find the homologous sequence of the sample DNA sequence with the nucleotide database in GenBank. This confirmed the similarity and allowed the type of ligninolytic fungus from the sample to be identified. Table 2 displays the top 10 BLAST results compared to the NCBI database.

Based on Table 2, the BLAST analysis shows that the isolated P1 is dominated by *Trichoderma hamatum*, with a similarity percentage of 100%. In contrast, isolate S2 is dominated by *Aspergillus flavus*, with 100% of the isolates. Therefore, we identified isolate P1 as *T. hamatum* and isolate S2 as *A. flavus*. Although *A. flavus* is commonly recognized as a potential aflatoxin producer, it was included in this study due to its robust ligninolytic enzyme system, which warrants further investigation regarding its safe application in bio-delignification processes. This decision is supported by Li et al. [9], who successfully isolated the *A. flavus* F-1 strain and demonstrated its superior ability to cleave lignin into smaller fragments, making it a promising candidate for transforming crop waste biomass. Consequently, these findings warrant further investigation into the enzymatic potential of isolate S2 while carefully considering its safe application in bio-delignification processes.

From a taxonomic perspective, both *T. hamatum* and *A. flavus* are members of the phylum Ascomycota, although they diverge significantly at the ordinal and familial levels. *T. hamatum* is classified within the order Hypocreales and family Hypocreaceae, whereas *A. flavus* belongs to the order Eurotiales and family Trichocomaceae. Despite these phylogenetic differences, both genera are characterized by versatile metabolic pathways that enable them to thrive on lignin-

rich substrates. Their presence in diverse ecological niches further highlights their capacity to secrete a complex array of extracellular enzymes essential for the degradation of recalcitrant lignocellulosic materials.

The functional divergence between the two isolates is further reflected in their enzymatic profiles. Members of the *Hypocreaceae* family, such as *Trichoderma*, are predominantly recognized for their robust cellulolytic activity, though they also contribute to lignin modification through laccase secretion [35]. Conversely, the *Trichocomaceae* family, particularly the *Aspergillus* genus, exhibits a more diverse array of lignin-modifying enzymes (LMEs), including lignin peroxidase (LiP) and manganese peroxidase (MnP), which are essential for the complete depolymerization of recalcitrant lignin [36]. This enzymatic synergy explains the high potential of both isolates for bio-delignification of agricultural waste, as they target distinct structural components of the plant cell wall.

While vigorous ligninolytic activity is traditionally associated with Basidiomycetes, certain Ascomycetes genera, including *Trichoderma* and *Aspergillus*, have demonstrated significant potential for lignin degradation through distinct enzymatic mechanisms [37, 38]. Unlike the aggressive delignification performed by Basidiomycetes, Ascomycetes often employ a 'soft-rot' strategy, which involves the gradual modification of the lignin-carbohydrate complex (LCC) via the secretion of laccases and auxiliary enzymes (AA9 family) [39, 40]. This approach is particularly effective in tropical environments, where these genera exhibit high adaptability and rapid colonization of lignocellulosic waste, making them efficient candidates for localized bio-delignification processes.

4. Conclusions

Based on the study results, two of the four purified ligninolytic fungal isolates, P1 and S2, showed positive results in the Bavendamm Test, indicating that both belong to the group of ligninolytic fungi. Molecular identification using the ITS region produced amplification products of 574 bp for isolate P1 and 586 bp for isolate S2. The BLAST analysis identified isolate P1 as *Trichoderma hamatum* and isolate S2 as *Aspergillus flavus*. These fungi could improve the degradation of lignocellulosic crop residues, especially in tropical countries, thereby enhancing the digestibility and nutritional value of ruminant feed. Importantly, their application may provide an economically beneficial strategy by enabling the utilization of locally available agricultural by-products as low-cost feed resources, reducing dependence on expensive commercial concentrates, and potentially lowering overall feeding costs for farmers.

List of abbreviations: BLAST, basic local alignment search tool; DNA, deoxyribonucleic acid; ITS, internal transcribed spacer; Lac, laccase; LiP, lignin peroxidase; MnP, manganese-dependent peroxidase; MEGA, molecular evolutionary genetics analysis; NCBI, national center for biotechnology information; P, Padang city; PCR, polymerase chain reaction; PDA, potato dextrose agar; RNA, ribonucleic acid; S, Solok regency; sp., species.

Data availability: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgment: The authors would like to express their gratitude to the Research and Community Engagement Institute, Universitas Andalas, Padang, West Sumatra, Indonesia, for the research grant under the Skim Penelitian Dosen Pemula (PDP), contract number 66/UN16.19/PT.01.03/PDP/2024.

Conflicts of interest: The authors declare that they have no financial, personal, academic, or professional conflicts of interest that could have influenced the work reported in this manuscript.

Author contributions: ER designed the study, presented it for funding, collected field samples, interpreted the data, and finalized the manuscript. L accompanied ER to find funding resources and contributed to preparing and critically checking the manuscript. MI and FH collected samples, isolated and identified them under the supervision of ER, and were involved in drafting the manuscript.

References

- [1] Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. Biomass pretreatment: Fundamentals toward application. *Biotechnol Adv* 2011; 29(6):675–85.
- [2] Ahmed S, Warne T, Smith E, Goemann H, Linse G, Greenwood M, et al. Systematic review on effects of bioenergy from edible versus inedible feedstocks on food security. *NPJ Sci Food* 2021; 5:9. [Crossref]
- [3] Sarnklong C, Cone JW, Pellikaan W, Hendriks WH. Utilization of rice straw and different treatments to improve its feed value for ruminants: A review. *Asian-Australas J Anim Sci* 2010; 23(5):680–92. [Crossref]
- [4] Sufyan A, Ahmad N, Shahzad F, Embaby M, AbuGhazaleh A, Khan NA. Improving the nutritional value and digestibility of wheat straw, rice straw, and corn cob through solid state fermentation using different pleurotus species. *J Sci Food Agric* 2021; 102(6):2445–53. [Crossref]

- [5] Bashar MK, Haese E, Sultana N, Rodehutsord M. *In vitro* ruminal fermentation, methane emissions, and nutritional value of different tropical feedstuffs for ruminants. *J Adv Vet Anim Res* 2024; 11(4):924–35. [\[Crossref\]](#)
- [6] Gill M, Kocher G, Singh A. Evaluation of fungal consortium lignozyme for biodelignification of agricultural residues. *Biofuels Bioprod Biorefin* 2022; 16(6):1772–80. [\[Crossref\]](#)
- [7] Bautista-Zamudio PA, Flórez-Restrepo MA, López-Legarda X, Monroy-Giraldo LC, Segura-Sánchez F. Biodegradation of plastics by white-rot fungi: A review. *Sci Total Environ* 2023; 901:165950. [\[Crossref\]](#)
- [8] Cristy AM, Mumpuni A, Ratnaningtyas NI. Biopulping of bagasse using different types of white-rot fungi and different incubation times. *BioEksakta J Ilm Biol Unsoed* 2020; 2(3):403–10. [\[Crossref\]](#)
- [9] Li SF, Wang H, Chen JL, Yao RS, Wu H. Degradation and transformation of lignin by a fungus *Aspergillus flavus* strain F-1. *Iran J Biotechnol* 2020; 18(3):62–9. [\[Crossref\]](#)
- [10] Kipping L, Jehmlich N, Moll J, Noll M, Gossner MM, Bossche TVD, et al. Enzymatic machinery of wood-inhabiting fungi that degrade temperate tree species. *ISME J* 2024; 18(1):1–14. [\[Crossref\]](#)
- [11] Susila E, Maulina F, Emilda D. Characterization and identification of *Trichoderma* on shallots isolated from three elevation regions in West Sumatra, Indonesia. *Biodiversitas* 2023; 24(4):2064–71. [\[Crossref\]](#)
- [12] Bari E, Karimi K, Aghajani H, Schmidt O, Zaheri S, Tajick-Ghanbary MA, et al. Characterizations of tree-decay fungi by molecular and morphological investigations in an Iranian alamdard forest. *Maderas Cienc Tecnol* 2021; 23:33. [\[Crossref\]](#)
- [13] Ezeonuegbu BA, Abdullahi MD, Whong CMZ, Sohunago JW, Kassem HS, Yaro CA, et al. Characterization and phylogeny of fungi isolated from industrial wastewater using multiple genes. *Sci Rep* 2022; 12:2094. [\[Crossref\]](#)
- [14] Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci USA* 2012; 109(16):6241–6. [\[Crossref\]](#)
- [15] Badotti F, de Oliveira FS, Garcia CF, Vaz ABM, Fonseca PLC, Nahum LA, et al. Effectiveness of ITS and sub-regions as DNA barcode markers for identifying *Basidiomycota* (Fungi). *BMC Microbiol* 2017; 17:42. [\[Crossref\]](#)
- [16] Riaz A, Rasib KZ, Aslam H, Raza S. Molecular identification of *Aspergillus flavus* using internal transcribed spacer (ITS). *Biotechnol J Int* 2017; 20(4):1–8. [\[Crossref\]](#)
- [17] Mustafa K, Rostam SK. Molecular identification of local isolates *Aspergillus nidulans* from Erbil Province using internal transcribed spacers. *Int J Appl Biol* 2021; 5(2):24–42. [\[Source\]](#), accessed on 17 September 2025
- [18] Ratni E, Lendrawati L, Hefzi F, Muqarramah M. Isolation and identification of local white-rot fungi from West Sumatra and their potential for biodelignification of ruminant feed. *J Adv Vet Anim Res* 2025; 12(2):558–64. [\[Crossref\]](#)
- [19] Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol Biol Evol* 2021; 38(7):3022–7. [\[Crossref\]](#)
- [20] Stenlid J, Penttilä R, Dahlberg A. Wood-decay *Basidiomycetes* in boreal forests: Distribution and community development. *Br Mycol Soc Symp Ser* 2008; 28:239–62. [\[Crossref\]](#)
- [21] Stokland JN, Meyke E. The saproxylic database: An emerging overview of the biological diversity in dead wood. *Rev Ecol* 2008; 10:37–48. [\[Crossref\]](#)
- [22] Yang S, Poorter L, Sterck FJ, Cornelissen JHC, van Logtestijn RSP, Kuramae EE, et al. Stem decomposition of temperate tree species is determined by stem traits and fungal community composition during early stem decay. *J Ecol* 2024; 112(6):1240–55. [\[Crossref\]](#)
- [23] Nishida T. Lignin biodegradation by wood-rotting fungi. *Japan Tappi J* 1989; 43(11):1071–84. [\[Crossref\]](#)
- [24] Badalyan SM, Szafranski K, Hoegger PJ, Navarro-González M, Majcherczyk A, Kües U. New Armenian wood-associated coprinoid mushrooms: *Coprinopsis strossmayeri* and *Coprinellus* aff. *radians*. *Diversity* 2011; 3(1):136–54. [\[Crossref\]](#)
- [25] Wood EJ. Molecular cloning. A laboratory manual: By T Maniatis, EF Fritsch and J Sambrook. pp 545. Cold Spring Harbor Laboratory, New York. 1982. *Biochem Edu* 1983; 11(2):82. [\[Crossref\]](#)
- [26] Wirajana IN, Yuliana DA, Ratnayani K. Isolasi DNA metagenomik dari tanah hutan mangrove Pantai Suwung Bali. *Jurnal Harian Regional* 2021; 7(1):3.
- [27] White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, USA, pp. 315–22, 1990. [\[Crossref\]](#)
- [28] Fujita SI, Senda Y, Nakaguchi S, Hashimoto T. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *J Clin Microbiol* 2001; 39(10):3617–22. [\[Crossref\]](#)
- [29] Appiah T, Agyare C, Luo Y. Molecular identification of some Ghanaian mushrooms using internal transcribed spacer regions. *Mol Biol* 2017; 6(3):1–5. [\[Crossref\]](#)
- [30] Apollon WP, Joshua VI, Musa HD, Gyang MS, Nyam DD. Molecular characterization and phylogenetic analysis of some *Agaricomycetes* (mushroom) fungi from Kogi State, central Nigeria. *Int J Sci Basic Appl Res* 2017; 35(2):276–92. [\[Source\]](#), accessed on 22 September 2025
- [31] Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp* 2012; 62:e3923. [\[Crossref\]](#)
- [32] Lorenz TC. Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *J Vis Exp* 2012; 63:e3998. [\[Crossref\]](#)
- [33] Kim SR, Yang J, An G, Jena KK. A simple DNA preparation method for high-quality polymerase chain reaction in rice. *Plant Breed Biotechnol* 2016; 4:99–106. [\[Crossref\]](#)
- [34] Gomes EA, Kasuya MCM, de Barros EG, Borges AC, Araújo EF. Polymorphism in the internal transcribed spacer (ITS) of the ribosomal DNA of 26 isolates of ectomycorrhizal fungi. *Genet Mol Biol* 2002; 25(4):477–83. [\[Crossref\]](#)
- [35] Rubeena M, Neethu K, Sajith S, Sreedevi S, Priji P, Unni K, et al. Lignocellulolytic activities of a novel strain of *Trichoderma harzianum*. *Adv Biosci Biotechnol* 2013; 4(2):214–21. [\[Crossref\]](#)
- [36] Hasanina SM, Darwesh OM, Matter IA, El-Saieda H. Isolation and characterization of non-cellulolytic *Aspergillus flavus* EGYPTA5 exhibiting selective ligninolytic potential. *Biocatal Agric Biotechnol* 2019; 17:160–7. [\[Crossref\]](#)
- [37] Garzón L, Fajardo JL, Rodríguez-Maecker R, Fernández ED, Cruz D. Thermo-mechanical and fungi treatment as an alternative lignin degradation method for *Bambusa oldhamii* and *Guadua angustifolia* fibers. *J Fungi* 2022; 8(4):399. [\[Crossref\]](#)
- [38] Ma Y, Liu L, Zhou X, Tian T, Xu S, Li D, et al. Optimizing straw-rotting cultivation for sustainable edible mushroom production: Composting spent mushroom substrate with straw additions. *J Fungi* 2023; 9(9):925. [\[Crossref\]](#)
- [39] Ferrari R, Gautier V, Silar P. Lignin degradation by ascomycetes. *Adv Bot Res* 2021; 99:77–113. [\[Crossref\]](#)
- [40] Brenelli L, Squina FM, Felby C, Cannella D. Laccase-derived lignin compounds boost cellulose oxidative enzymes AA9. *Biotechnol Biofuels* 2018; 11:10. [\[Crossref\]](#)

Disclaimer/Publisher's Note: The views and data expressed are solely those of the author(s) and contributor(s) and not of the publisher or editor(s). The publisher and editor(s) are not responsible for any injury or damage arising from the ideas, methods, instructions, or products mentioned.