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RESEARCH ARTICLE
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# Isolation and Identification of Lignin Cellulolytic Fungi from Oil Palm Empty Fruit Bunches and the Soil Around Palm Trees in South Kalimantan, Indonesia

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## ABSTRACT

The integration of oil palm plantations and cattle farming is currently being intensively developed in South Kalimantan. Both sectors can mutually benefit each other, especially in addressing waste issues in both sectors. Oil palm empty fruit bunches are one of the most abundant byproducts of palm oil processing with high lignin content. Fungi have been found to have the ability to alter biomass components and can be naturally found in empty fruit bunches during the decaying process, while the cattle excretion process can increase the fungal diversity. This study aimed to isolate and identify the fungi present in oil palm empty fruit bunches and the soil within palm oil plantations, and to identify the fungi's ability to degrade fiber. After the isolated fungi were identified through morphological and DNA sequencing, the results revealed the presence of two potential species of fungi, *Lasiodiplodia theobromae* and *Acremonium sp.*, which possess the ability to degrade oil palm empty fruit bunches fiber fraction and enhance its digestibility. The type of fungi and incubation period were found to significantly alter the Neutral Detergent Fiber (NDF) and lignin levels ($P<0.05$). The fungi were also found to significantly alter the In Vitro Digestibility (IVTD) and Digestible Neutral Detergent Fiber (dNDF) levels ($P<0.05$), indicating digestion by fungal activity. Consequently, it can be concluded that the isolated fungi, especially *L. theobromae* from soils possess the best ability to degrade fiber and enhance the digestibility of biomass.

**Keywords:** fungi, Integrated farming, Oil palm, Empty fruit bunches, Soil, Cattle

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## INTRODUCTION

The grazing activity by large herbivores, such as cattle, can indirectly affect the soil composition in grazing areas. Grazing alters the soil's physicochemical characteristics and eventually alters the composition of soil microbial communities (Jordon, 2021; Liu et al., 2023). The microorganism community is one of the keys to plant nutrient availability and organic matter decomposition. Because of the benefit, merging livestock and plant production may be one of the ways to apply sustainable agriculture.

Integrating the livestock and plant production sector may be beneficial, as Indonesia boasted over 14 million hectares of oil palm land in 2020, making it the country with the largest oil palm plantations worldwide (Pusat Data dan Sistem Informasi Pertanian, 2020). The data from Databoks (Annur 2023) indicates that the region accounts

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for a substantial portion of the country's oil palm land, roughly 5,706,033 hectares. Additionally, Tanah Bumbu has approximately 1929.2 $km^2$ of oil palm land as of 2020 (Rasyidah et al., 2022). In South Kalimantan, researchers and farmers are actively developing integrated oil palm and cattle farming systems. Cattle can play a role in managing oil palm waste by utilizing waste such as palm fronds and empty fruit bunches as their feed, as these materials have the potential to serve as ruminant feedstuff (Jayanegara et al., 2019; Nur-Nazratul et al., 2021; Zailan et al., 2023). On the other hand, the cattle excrete their feces and urine, which contain a lot of carbon in the soil and may increase the variety of microbial communities in soils and plant roots. This eventually also helps with the oil palm waste biomass decomposition process. Undoubtedly, efficient waste management is essential to successfully implement the integrated oil palm-cattle farming system, including oil palm waste.

The conversion of palm oil into various products results in several types of by-products, including empty fruit bunches (EFB), which constitute a significant portion of the total waste generated. It is estimated that EFB accounts for approximately 23% of the total biomass (Rahayu et al., 2019). Traditionally, most crop residue has been managed by burning or burying it in the ground (Khoul et al., 2022). While some of it is used to generate steam for mills or returned to plantations for mulching, EFB is often regarded as waste and remains unutilized (Obada et al., 2023). Applying EFB to the soil surface or burial at low depths can lead to an increase in horn beetle populations, which are the primary pest in oil palm plantations (Fauzana et al., 2019). Currently, the processing of EFB waste is not optimal. Improper management of EFB waste can have negative implications for sustainable industrial applications. Therefore, processing palm oil waste in EFB is essential to maximize its benefits and minimize its negative environmental impacts.

Oil Palm Empty Bunches are lignocellulosic biomass obtained from fresh palm fruit bunches. The cell walls of EFB contain three structural components: cellulose, hemicellulose, and lignin (Mohammad et al., 2020; Tang et al., 2020). The processing of EFB can be optimized by breaking down the EFB plant cell walls using microorganisms with the necessary degrading capability. The decomposition of lignocellulosic waste by fungi is of significant capability, particularly in depolymerizing lignin (Sarma et al., 2022). Fungi possess two types of extracellular enzymatic systems, hydrolytic and ligninolytic, which enables them to break down lignocellulose into simpler components such as oligomers and monomers. Fungi can break lignocellulosic biomass by producing lignocellulolytic enzymes (Nargotra et al., 2023). This study aimed to isolate and identify the most abundant fungi with lignocellulolytic activity. Screening and isolating fungi from EFB substrates in their natural form is essential to discovering fungi that can effectively compost various lignocellulosic materials. By combining efficient lignocellulolytic fungi with the most effective treatment during the composting process, it is predicted that the isolated fungi will be useful for treating lignocellulose waste.

## MATERIALS & METHODS

### Sample Collection

A total of thirty soil samples from the soil around palm tree roots were collected from three distinct locations, including palm oil plantations that were grazed by cattle (14 samples), palm oil plantations that were not grazed by cattle (14 samples), and soil surrounding piles of empty bunches (two samples) to improve the likelihood of obtaining the isolate and the variety of isolates. Samples were collected from PT Buana Karya Bhakti, Tanah Bumbu, and South Kalimantan (Fig. 1). Furthermore, 27 empty fruit bunches samples with different stacking periods were collected, comprising 1 d, 1 week, 1 month, 2 months, and 6 months. A considerable amount of each sample, approximately 500-1000g, was collected and placed in clean plastic bags, stored in a cool box for transportation to the laboratory. Concurrently, microclimatic conditions, including temperature and soil pH, were recorded during sampling (Table 2).

### Fungi Isolation and Lignin Digestibility Screening

Fungal isolation and cellulose degradation were studied using the modified dilution method (Vishnu et al., 2021; Wróbel et al., 2023). 10g of sample was diluted into 100mL of 0.85% NaCl solution and homogenized, resulting in $10^{-1}$ dilution. Two additional serial dilutions were conducted in a test tube for this investigation: one at a dilution factor of $10^{-6}$ for empty bunches and another at a dilution factor of $10^{-8}$ for soil samples. From each dilution, 0.1mL of the solution was poured and spread onto Petri dishes containing Potato Dextrose Agar (PDA) media and incubated at room temperature for 7 days. The fungal isolates were purified if they were grown in a mixed culture.

The assessment of fungal lignin degradation was performed by the methodology described by M'barek et al. (2019). The investigation utilized media Czapek as the primary carbon source to gauge the rate of lignin degradation. The Czapek media is characterized by a high carbon-to-nitrogen ratio, making it an appropriate growth medium for a wide range of fungi with unspecified nutritional requirements. This study used two types of Czapek media: Czapek dox and Czapek lignin. The fungal isolates demonstrating lignin degradation will exhibit a significant growth rate in selective media and will be the preferred candidate for further observation. The fungi isolate preferred in the study was the fungi with great growth diameter, high hyphae density, and high lignocellulolytic index. A nominal indicator for hyphae formation was determined using four descriptors as described in Table 1. In addition, the lignocellulolytic index was determined using the following equation:

$$LC\ Index\ (\%)_{(Lignin\ or\ cellulose\ \ media\ )} = \frac{Colony\ diameter_{(selective\ \ media\ )}}{Colony\ diameter_{(control\ )}} \times 100$$

The fungal isolate exhibiting the most superior lignocellulosic ability was subsequently observed through a microscope.

**Table 1:** Nominal indicator for hyphae formation

| Notation | Hyphae formation descriptor | Description |
|---|---|---|
| 0 | No observed growth | No hyphae were found |
| 1 | Thin hyphae filaments | The hyphae layer has a relatively thin structure, but it has already initiated its spreading process. |
| 2 | Fair hyphae filaments | The hyphae dense layer comprised less than 50% of the surface area. |
| 3 | Thick, bulky hyphae filaments | The dense hyphae layer constituted at least half of the entire surface area, amounting to at least 50% of the total space |

**Table 2:** Oil Palm Empty Fruit Bunches OPEFB) and soil sample collection

| No | Total Sample | Sample | Media Code | pH level | Temperature (°C) | Isolate Collecting Area | Sample Condition moisture etc. |
|---|---|---|---|---|---|---|---|
| Non-grazing area | | | | | | | |
| 1 | 10 | Soil | A1-A10 | 4.97 | 30 | Around palm tree | Soil slightly damp |
| 2 | 4 | Soil | A11-A14 | 4.5 | 31 | Around the collecting area during harvest | Soil slightly damp |
| Cattle grazing area | | | | | | | |
| 3 | 8 | Soil | A15-A22 | 4.8875 | 29.75 | Around palm tree | Soil is slightly damp with stems remaining around it |
| 4 | 2 | Soil | A23-A24 | 5.4 | 31 | Under a pile of palm fronds | Soil slightly dry |
| 5 | 4 | Soil | A25-A28 | 5 | 31.5 | Around palm tree | Soil slightly damp |
| Around the pile of empty fruit bunches | | | | | | | |
| 6 | 2 | Soil | A29-A30 | 4.5 | - | Around the pile of empty fruit bunches | Soil slightly dry |
| Empty fruit bunches stacked for 6 months | | | | | | | |
| 7 | 2 | Empty fruit bunches | B1, B4 | 5.7 | - | Top Stack | Fiber part, the empty fruit bunches is slightly damp and covered with a few of white fungus |
| 8 | 2 | Empty fruit bunches | B2, B5 | 5.7 | - | Middle stack | Fiber part, empty fruit bunches slightly damp |
| 9 | 2 | Empty fruit bunches | B3, B6 | 5.7 | - | Bottom stack | Fiber part, empty fruit bunches mixed with soil |
| Empty fruit bunches stacked for 1 week | | | | | | | |
| 10 | 2 | Empty fruit bunches | B7, B10 | 6.5 | - | Top Stack | EFB is slightly dry, the fiber part |
| 11 | 2 | Empty fruit bunches | B8, B11 | 6.5 | - | Middle stack | EFB is slightly damp, and the fiber part |
| 12 | 2 | Empty fruit bunches | B9, B12 | 6.5 | - | Bottom stack | EFB is slightly damp and warm; the fiber part |
| Empty fruit bunches stacked for 1 month | | | | | | | |
| 13 | 2 | Empty fruit bunches | B13, B16 | 4.6 | - | Top Stack | EFB is slightly dry; the fiber part |
| 14 | 2 | Empty fruit bunches | B14, B17 | 4.6 | - | Middle stack | EFB is slightly damp, and the fiber part |
| 15 | 2 | Empty fruit bunches | B15, B18 | 4.6 | - | Bottom stack | EFB is slightly damp and warm; the fiber part |
| Empty fruit bunches stacked for 2 months | | | | | | | |
| 16 | 1 | Empty fruit bunches | B19 | 5.6 | - | Top Stack | EFB is slightly dry, the fiber part |
| 17 | 2 | Empty fruit bunches | B20, B23 | 5.35 | - | Middle stack | EFB is slightly damp, fiber part |
| 18 | 2 | Empty fruit bunches | B21, B24 | 5.35 | - | Bottom stack | EFB is slightly damp, fiber part |
| 19 | 1 | Empty fruit bunches | B22 | 5.1 | - | Top Stack | EFB was slightly dry, covered with white fungus, fungi noodles was found, fiber part |

### Empty Fruit Bunches Samples Processing

Empty fruit bunches were initially reduced in size to approximately 3-5cm. They were then dried using an oven set at 60°C for 48h and ground to 2mm particles using a hammer mill. Afterward, the empty bunches are subjected to heat treatment using an autoclave at 135°C and 2.3 atmospheric pressure for 2.5h. The empty fruit bunches media is then inoculated with fungal isolates grown on Potato Dextrose Agar media for 7 days at a dosage of 50mgkg$^{-1}$ (dry weight). The empty bunches media is then incubated at room temperature (25-30°C) and in the dark for 10, 20, and 30 days. The research design is based on a completely randomized method with 4 replications.

### DNA Sequencing of Isolated Fungi

The identification of fungi using a molecular approach is based on single-pass DNA Sequencing. The identification process involved several steps, including DNA extraction, DNA purification, DNA concentration measurement, electrophoresis, PCR, and sequencing. Specifically, ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and ITS 4 (TCC TCC GCT TAT TGA TAT GC) primers were used for fungi identification during sequencing according to method described by Raja et al. (2017). The resulting data were then compared with the National Center for Biotechnology Information (NCBI) database using the BLAST (Basic Local Alignment Search Tool) nucleotide


**Fig. 1:** Study map area.

collection program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the classification of the fungi sample was established at least at the genera level.

### Empty Fruit Bunches Fermentation with Fungi Isolates

A total of 25g of chopped empty fruit bunches were placed in glass bottles. Subsequently, these empty fruit bunches underwent sterilization using an autoclave, subjected to heat at 121°C and pressure at 1 atm for 15min (Koesoemadinata et al., 2021). Next, the sterilized

empty fruit bunches were inoculated with 10%(g/v) of fungi isolate grown in liquid media with agitation, resulting in five distinct treatments with four replicates each. The fermentation process was allowed to proceed for 30 days, with samples being observed and analyzed every 10 days. The visual appearance of the empty bunches was documented during the observation process. To assess the fiber fraction of the empty bunches, analyses of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were performed. Additionally, in vitro true digestibility and digestible neutral detergent fiber were conducted to determine the level of digestibility of the empty bunches (Tassone et al., 2020; Gürsoy et al., 2021).

**Fiber Fraction and Digestibility Analysis**

After the incubation period, empty bunches media samples were collected, homogenized, and dried at 60°C. The amounts of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin were determined using the modified Van Soest method (Goering and Van Soest 1970) with the ANKOM Fiber Analyzer A200 (ANKOM Technology, NY, USA). Moreover, the digestible NDF and in vitro true digestibility (IVTD) were analyzed using the Daisy II incubator (ANKOM Technology, NY, USA). The rumen fluid inoculants used in the digestibility analysis were obtained from Ongole cattle at Cibinong Science Centre, Bogor (Tassone et al., 2020; Gürsoy et al., 2021).

**Data Analysis**

The data obtained were subjected to analysis of variance (ANOVA) using a Factorial Completely Randomized Design (CRD). This analysis was conducted using SAS programming for data science (<https://welcome.oda.sas.com/>). The Duncan Multiple Range Test (DMRT) was applied at a 5% probability level to determine the difference between the means of each treatment.

## RESULTS & DISCUSSION

**Oil Palm Empty Fruit Bunches (OPFEB) and soil samples**

A total of thirty soil samples were collected from three separate regions, including a palm plantation area grazed by cattle (14 samples), a palm plantation area not grazed by cattle (14 samples), and an area surrounding the pile of empty fruit bunches (2 samples). Additionally, 27 empty fruit bunches samples were gathered from palm oil trees, with varying storage durations (1 day, 1 week, 1 month, 2 months, and 6 months). Detailed information regarding the samples is presented in Table 1.

The various regions of an oil palm plantation display distinct characteristics in their samples. The pH level in Soil samples located in the soil surrounding the roots of the oil palm trees typically falls between 4.5 and 5.4. Meanwhile, the average temperature in these samples is generally between 29.75 and 31.5°C. Additionally, the Soil samples collected near the palm tree tend to be slightly moist, while those gathered from piles of palm fronds are usually somewhat dry.

The collected fruit bunches from the top stack were generally found to be slightly dry, except those that were stacked for 6 months. In contrast, the fruit bunches collected from the middle and bottom parts were observed to be slightly damp. This happens due to airflow and microbial activity. Temperature and humidity are transported to the higher, more internal parts of the pile via airflow, resulting in an increase in temperature through conduction and an increase in humidity through the condensation of gases and vapours (Cepeda, 2020). The lower portion of the stack may experience less airflow, resulting in a more humid environment that retains moisture (Hogland & Marques, 2003). In the central parts of the stack, the temperature increases rapidly during the initial weeks before stabilizing and then decreasing gradually. The presence of different microorganisms at various temperatures cannot be ruled out. As they metabolize nutrients, these microorganisms can release heat and consume moisture. As a result of the heat generated by the stack, fermentation will produce more lactic acid, acetic acid, water, and carbon dioxide (Cepeda, 2020). This activity may be more concentrated in the middle and lower parts of the stack, affecting moisture levels.

The pH levels in the empty fruit bunch samples varied widely, ranging from 4.1 to 6.5. The lowest pH level was recorded in the empty fruit bunches stacked for one month, while the highest pH level was observed in the empty fruit bunches stacked for one week. The pH levels in the different stacking periods exhibited fluctuations. However, it is worth noting that some of the sample pH characteristics in the soil samples collected from non-grazing areas, cattle grazing areas around palm trees, piles of empty bunches, and the empty fruit bunches stacked for one month are suitable for the degradation of lignin and cellulose by fungi, which is around 4-6 (Legodi et al., 2019; Suryadi et al., 2022).

**Isolation of Potential Fungi from Oil Palm Empty Fruit Bunches and Soil around Palm Tree Roots**

A total of 228 plates with media PDA and Czapek dox were used to isolate potential fungi from empty fruit bunches and soil. Upon initial observation, 27 plates were covered by fungi isolates after three days of isolation. After ten days, 55 Petri dishes displayed fungal growth. However, ten isolates were contaminated with bacteria and were not included in the analysis. Additionally, a selection and purification process were conducted for isolates that exhibit rapid growth rates.

The purification process was conducted on the selective media, Czapek Dox and Czapek Lignin, and was monitored every two days. Fifteen isolates, after the purification process, showed steady fungal growth. The initial measurement involved assessing the hyphae's diameter using a Calliper. The findings of the fungal growth on selective media have been compiled in Table 3. The soil sample exhibited varying hyphae area diameter values compared to the empty fruit bunches. The fungi derived from soil growth in czapek dox medium demonstrated area diameters ranging from 22.81 to 85.00mm, while those isolated from the empty fruit bunches exhibited diameters

ranging from 19.66 to 82.96mm. Moreover, the fungi from soil samples demonstrated growth on czapek lignin media with diameters ranging from 17.85 to 78.73mm, whereas those from the empty fruit bunches media ranged from 20.94 to 82.74mm. The lignocellulose (LC) index for soil samples exhibited values ranging from 33.75 to 94.55mm, whereas those for the empty fruit bunches ranged from 35.40 to 100.39mm.

According to the findings, a high LC index does not always correspond to a high nominal notation. In other words, fungi with a large diameter and a high LC index may not necessarily have dense hyphae (M'barek et al., 2019). Five fungi isolates, namely A11, A18, A24, A28, and B16, exhibited good growth on the selective media, as indicated by a high value of hyphae diameter on both media, a high nominal notation indicating that the hyphae are dense enough, and a satisfactory LC index percentage. The chosen isolates were then obtained for further tests on morphological observations, DNA sequencing, and fiber fraction tests.

The following text provides observations on the shape and color of various fungi isolates: B16, A11, A18, A24, and A28. Microscopic examinations of isolate B16 revealed that the fungi had a slender-sickle shape, were blue in color, and produced an abundance of macroconidia. In contrast, isolate A11 exhibited conidiophores with a blue-to-green hue, which were dense and branched. The fungi on isolate A18 featured septate, hyaline hyphae, and black, globose-shaped conidia. Isolate A24 was characterized by oval-to-spindle-shaped fungi with blue-to-purple coloration, single-celled conidia, and small colonies. Moreover, isolate A28 displayed fine blue-to-black threads and irregularly shaped globe-like spores.

To ensure accurate results, the isolated cultures were tested by DNA sequencing. Fig. 2 provides an overview of the fungal isolates based on macroscopic observations. Furthermore, DNA sequencing (Fig. 3) identified potential fungi, which revealed that four of the five samples collected from soil (A11, A18, A24, and A28) were consistent with *Lasiodiplodia theobromae*. The remaining sample, designated as media code F (B16) obtained from empty fruit bunches, was identified as *Acremonium sp*. (Table 4).

**Table 3:** Nominal notation, colony diameter, and Lignocellulolytic LC) index of potential fungi

| No | Sample | Media Code | Nominal Notation | $Ø_C$ mm) | $Ø_{CL}$ mm) | LC Index %) |
|---|---|---|---|---|---|---|
| 1 | Soil | A3 | 1 | 54.55 | 24.55 | 45.00 |
| 2 | Soil | A5 | 1 | 43.14 | 17.85 | 41.38 |
| 3 | Soil | A6 | 1 | 82.20 | 27.74 | 33.75 |
| 4 | Soil | A11 | 2 | 85.00 | 44.75 | 52.65 |
| 5 | Soil | A12 | 1 | 22.81 | 18.47 | 80.97 |
| 6 | Soil | A18 | 2 | 57.39 | 25.76 | 44.89 |
| 7 | Soil | A24 | 2-3 | 56.28 | 25.97 | 46.14 |
| 8 | Soil | A28 | 2 | 83.27 | 78.73 | 94.55 |
| 9 | Empty Fruit Bunches | B5 | 1 | 83.5 | 82.28 | 98.54 |
| 10 | Empty Fruit Bunches | B7 | 1 | 59.16 | 20.94 | 35.40 |
| 11 | Empty Fruit Bunches | B16 | 2-3 | 19.66 | 18.06 | 91.86 |
| 12 | Empty Fruit Bunches | B17 | 1 | 48.26 | 31.25 | 64.75 |
| 13 | Empty Fruit Bunches | B21 | 1 | 82.96 | 31.25 | 37.67 |
| 14 | Empty Fruit Bunches | B23 | 1 | 82.42 | 82.74 | 100.39 |
| 15 | Empty Fruit Bunches | B27 | 1 | 82.74 | 82.66 | 99.90 |

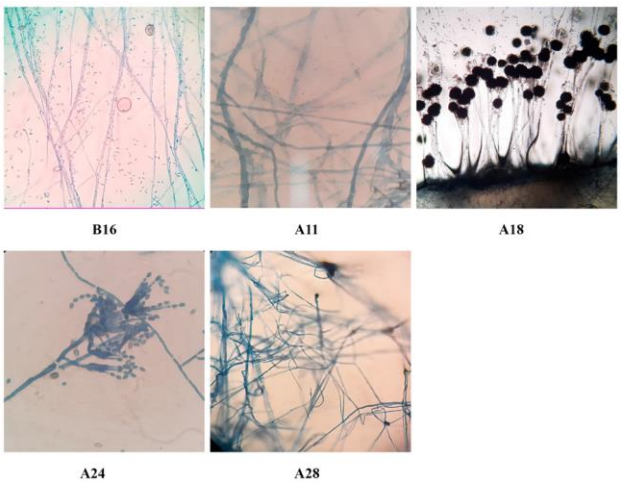


**Fig. 2:** Results of fungi isolation from EFB and soil on microscope.

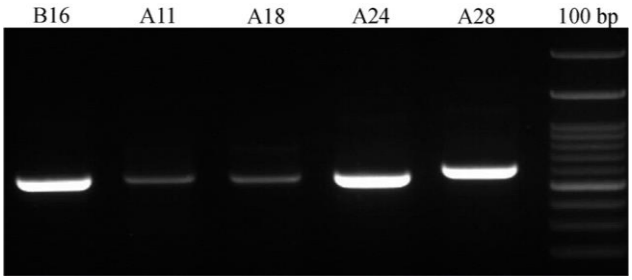


**Fig. 3:** PCR results of each fungi isolates.

Previous studies conducted by Li et al. (2019), found that *L. theobromae* secretes cell wall degradation enzymes, and it was investigated that the expression levels of genes encoding cell wall degradation proteins, including glycoside hydrolase family, extracellular aldonolactonase, and cellulose-binding domain protein were secreted in peach shoots during the development of peach gummosis. Peach gumosis is a disease in peach production caused by *Lasiodiplodia theobromae*. Fungi *L. theobromae* was also proven to release lignin-modifying enzymes, specifically peroxidases (LiP and MnP) and laccases (LAC), which are responsible for degrading BaP (Benzo[a]pyrene) (Cao et al. 2020). These enzymes are secreted outside the cells and compete for the same substrates to catalyze the formation of radicals through oxidation and the attack of molecules, destabilizing the bonds in the molecule. The formed radicals then interact with each other or fungal

metabolites, generating additional radicals that continue the process (Zhuo & Fan, 2021; Punetha et al., 2022).

There have been no reported instances of *L. theobromae* causing harm to oil palm plants. Consequently, it is reasonable to assume that the presence of *L. theobromae* in or around oil palm plantations will not have a detrimental effect on the plants and may even have beneficial effects. However, it should be noted that *L. theobromae* has been known to cause various diseases in other plants, such as root rot in date palms (*Phoenix dactylifera L*) and leaf blight in coconuts (*Cocos nucifera L*) (Mohamad & Arafat, 2013; Ramjegathesh et al., 2019; Santos et al., 2020; Coelho et al., 2022).

Different from *L. theobromae*, previous research has shown that *Acremonium* sp. isolates obtained from different parts of alfalfa and subtropical forests are capable of degrading cellulose (Hao et al., 2006; Attia et al., 2020). The research findings indicate that *Acremonium* sp. produces ligninolytic and cellulolytic enzymes, including laccase, lignin peroxidase, manganese peroxidase, carboxymethylcellulose (CMCase), and filter paper activity (FPA). *Acremonium* sp. has excellent hydrolytic capabilities and is more effective in producing cellulolytic enzymes than *Penicillium spp.* (Adsul et al., 2020).

**Oil Palm Empty Fruit Bunches Inoculated with Potential Fungi Inoculation**

The empty bunches without any treatment (control) data were obtained as a baseline for this experiment. The initial fiber fraction data were NDF (68.99%), ADF (53.46%), and Lignin (13.17%), while the IVTD (38.59%) and dNDF (61.41%) were also obtained. The data obtained from this experiment, including the fiber fraction values from the empty fruit bunches incubated with fungal isolates, are presented in Table 5.

**Table 4:** Identification of selected fungal isolates

| Media Code | Closest Hit | Similarity (%) |
|---|---|---|
| B16 | *Acremonium sp.* | 99.83 |
| A11 | *Lasiodiplodia theobromae* | 99.63 |
| A18 | *Lasiodiplodia theobromae* | 99.81 |
| A24 | *Lasiodiplodia theobromae* | 99.81 |
| A28 | *Lasiodiplodia theobromae* | 99.62 |

The impact of the type of fungi on the NDF and lignin content of empty fruit bunches was found to be statistically significant ($P<0.005$). The incubation period was also found to be statistically significant in altered the NDF and lignin content ($P<0.005$). However, no significant interactions were observed between the type of fungi and incubation period for all parameters besides the lignin content. The impact of the type of fungi, incubation period, and interaction between fungi and incubation period on ADF content was found to be non-significant ($P<0.005$). The results showed that the addition of the incubation period led to a decrease in NDF and lignin levels, suggesting that the lignocellulose complex was successfully degraded. The decreasing levels of NDF and lignin happened because of the ability of fungi to dilute and use carbohydrates as their source of carbon which is essential for fungal microorganism cell wall synthesis (Yilkal, 2015; Adesogan et al., 2019; Bartnicki-Garcia et al., 2000).

The treatment of empty fruit bunches with heat and/or chopping before inoculation with fungi affects the reduction in fiber fraction. Heating accelerates the opening of the lignocellulose structure and makes it more accessible to the enzymes produced by the fungi. Previous studies have shown that empty fruit bunches treated with urea and heated using a special Fiber Cracking Technology (FCT) autoclave with a temperature of 135°C and a pressure of 2.3atm, as well as rice straw treated with urea and an autoclave temperature of 121°C and pressure of 1.4atm, were effective in reducing NDF, ADF, and lignin levels (Dewi et al., 2018; Jayanegara et al., 2019). Reducing

**Table 5:** Fiber fraction of empty bunches inoculated with potential fungi isolates

| Isolate Name | Observation Period (Days) | | | Average |
|---|---|---|---|---|
| | 10 | 20 | 30 | |
| NDF (%) | | | | |
| A11 | 66.35bc | 66.78bc | 65.25b | 66.13B |
| A18 | 64.69b | 64.95b | 60.25a | 63.30A |
| A24 | 66.31bc | 69.31c | 67.30bc | 67.64BC |
| A28 | 66.82bc | 66.58bc | 64.79b | 66.06B |
| B16 | 66.90bc | 69.00c | 67.75bc | 67.89C |
| Average | 66.21bc | 67.33c | 65.07a | |
| ADF (%) | | | | |
| A11 | 53.03b | 54.3a | 51.85b | 53.06A |
| A18 | 50.39b | 52.14b | 51.58ab | 51.37A |
| A24 | 52.90b | 55.08b | 53.17b | 53.72A |
| A28 | 51.90b | 52.44b | 54.09b | 52.82A |
| B16 | 53.70b | 53.78b | 53.39b | 53.63A |
| Average | 52.57a | 53.54a | 52.92a | |
| Lignin (%) | | | | |
| A11 | 14.34ab | 13.43ab | 13.44ab | 13.74AB |
| A18 | 12.86ab | 13.23ab | 12.93ab | 13.00A |
| A24 | 13.97ab | 16.42c | 13.71ab | 14.70CD |
| A28 | 12.76a | 17.99d | 14.42ab | 15.06D |
| B16 | 14.52b | 14.02ab | 13.47ab | 14.00BC |
| Average | 13.69 a | 15.02b | 13.59a | |

Lowercase alphabets in rows and uppercase letters in different columns for each observed parameter indicate significant ($P<0.05$) effects. Neutral Detergent Fiber NDF) and Acid Detergent Fiber ADF); A1; A18; A24; A28 = *Lasiodiplodia theobromae*; B16 = *Acremonium* sp; Control= sterile empty fruit bunches incubated without the addition of fungi).

the particle size of empty bunches also enhances the cellulose hydrolysis activity carried out by fungi enzymes (Kayati et al., 2016; Fernandes et al., 2020). Among these fungi, empty fruit bunches inoculated with fungus A18 appeared to be the most effective in reducing the fiber fraction value. Based on DNA sequencing, the A18 was known as *Lasiodiplodia theobromae*.

The results of the digestion trial for the empty fruit bunches are shown in Table 6. The digestibility of NDF and IVTD was significantly affected by the type of fungi ($P<0.05$). However, there was no significant effect of incubation period on IVTD and dNDF ($P<0.05$). The interaction between the type of fungi and the incubation period was not found either. The results of the fungal inoculation treatment on empty bunches that had previously undergone chopping and heating demonstrated a notable increase in digestibility levels in the rumen. The chopping and heating process led to a more concentrated fungal activity in degrading lignocellulose, facilitating a faster enzymatic breakdown of lignin. By reducing lignin levels in empty fruit bunches, rumen microbes were better able to utilize cellulose. Research conducted previously has shown that heating pre-treatment using an autoclave on

empty bunches and straw can increase the digestibility of dry matter and organic matter in the rumen (Jayanegara et al., 2019; 2017). Consequently, the fermentation process carried out by *L. theobromine*, which secretes lignocellulosic enzymes capable of degrading lignin in empty bunches, is more effective when preceded by chopping and heating pre-treatment.

**Table 6:** Digestibility levels of empty bunches inoculated with potential fungi isolates

| Isolate Name | Observation Period Days) | | | Average |
|---|---|---|---|---|
| | 10 | 20 | 30 | |
| IVTD (%) | | | | |
| A11 | 34.35abc | 33.32a | 34.74abc | 34.14A |
| A18 | 34.66abc | 36.54abcde | 39.11bcde | 36.77AB |
| A24 | 35.36abcd | 40.35de | 39.63cde | 38.45BC |
| A28 | 34.07ab | 38.76bcde | 35.78abcde | 36.20AB |
| B16 | 41.14e | 38.86bcde | 39.42bcde | 39.81C |
| Average | 35.57a | 37.80a | 38.61a | |
| dNDF (%) | | | | |
| A11 | 65.65cde | 66.68e | 65.26cde | 65.86C |
| A18 | 65.34cde | 63.46abcde | 60.89abcd | 63.23BC |
| A24 | 64.64bcde | 59.65ab | 60.37abc | 61.55AB |
| A28 | 65.93de | 61.24abcd | 64.22abcde | 63.80BC |
| B16 | 58.86a | 61.14abcd | 60.58abcd | 60.19A |
| Average | 64.08a | 62.44a | 62.26a | |

Lowercase alphabets in rows and uppercase letters in different columns for each observed parameter indicate significant (P<0.05) effects. IVTD In vitro true digestibility); dNDF Neutral Detergent Fiber) digestibility; A1; A18; A24; A28 = *Lasiodiplodia theobromae*; B16 = *Acremonium* sp.; Control= sterile empty fruit bunches incubated without the addition of fungi).

## Conclusion

In this study, fungi were successfully isolated from oil palm empty fruit bunches and soil in the palm plantation area. Two types of fungi were identified as *Lasiodiplodia theobromae* and *Acremonium* sp. The results of this investigation suggest that these two types of fungi could degrade the lignocellulose complex, as evidenced by the reduction in fiber fraction, specifically on NDF and lignin levels. Additionally, these fungi were found to be effective in increasing the digestibility of oil palm empty fruit bunches.

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