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Research

Identification and Evaluation of Locally Isolated Fungi Through Rapid Screening for Potential Mycelium-Based Biofoam Application in Malaysia

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ABSTRACT

Expanded polystyrene foam (EPS) contributes to environmental problems due to its inability to decompose in nature within a short period. In addition, petroleum as a core source for EPS is now depleting and new biological and environmentally friendly approaches are encouraged. As an alternative to EPS, mycelium-based biofoam (MBF) is a new foam technology formed of agricultural biomass and mycelium as a binding matrix is introduced. However, based on previous literature, the fungal strains used are mainly highlighted as one of the main factors which affect the final properties of MBF. Thus, this study aims to evaluate the most potential fungus used for MBF application using OPEFB, biomass from the palm oil industry as novel substrate using rapid screening. Twelve local fungi isolated from a local forest in Selangor, Malaysia were cultivated on Potato Dextrose Agar and OPEFB plate before being screened on agar containing four different types of dye indicators, which are methylene blue, guaiacol, Remazol Brilliant Blue R and azure B. As a result, *Phanerochaete concrescens* isolate LYN-UPM S1 and *Perenniporia subtephropora* isolate LYN-UPM S9 have shown the ability to produce ligninolytic enzymes and high content of chitin, which will be useful for the fabrication of mycelium-based biofoam.

Key words: Fungal screening, ligninolytic enzymes, mycelium-based composite, white rot fungus

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INTRODUCTION

Expanded polystyrene foam (EPS), or styrofoam is typically used in the manufacturing of packaging materials for the shipping and packaging industry. It is a lightweight cellular plastic consisting of small spherical-shaped particles, which contain approximately 98% air (Ramli Sulong *et al*., 2019). Despite the advantages EPS serves, its waste has caused many environmental problems due to its properties that cannot be decomposed in nature (Ramli Sulong *et al*., 2019). (Arifin & Yusuf, 2013) emphasize it is more economical to provide alternatives rather than recycle them. Hence, mycelium-based biofoam has been proposed for years as a potential alternative to EPS foam (Haneef *et al*., 2017; Bruscato *et al*., 2019; Ghazvinian *et al*., 2020). The selection of fungal strains and their end-product properties are getting attention among researchers and have been widely studied recently (Jiang *et al*., 2016; Appels *et al*., 2019; Bruscato *et al*., 2019).

White rot fungi play an important role in ecology as decomposers of rotten wood due to their capability of delignification. The principal mechanisms responsible for lignin degradation by white rot fungi involve ligninolytic enzymes, mainly including laccase, manganese peroxidase

(MnP), and lignin peroxidase (LiP) (Ferhan *et al*., 2013). The type of ligninolytic enzymes, produced during secondary metabolism is fungal strain-dependent (Madadi & Abbas, 2017). These oxidative enzymes catalyze the formation of radical intermediates from high molecular weight lignin. Lignin peroxidase degrades non-phenolic lignin units (Falade *et al*., 2017), whereas laccase works in conjunction with lignin peroxidase and manganese peroxidase to completely degrade lignin (Madadi & Abbas, 2017). Lignin peroxidase catalyzes the oxidation of phenolic units in lignin and phenolic compounds and aromatic amines to radicals.

Recently, renewable mycelium-based composite has been discovered as an alternative to polystyrene as a packaging material (Appels *et al*., 2019). Mycelium-based composites result from the growth of filamentous fungi on organic materials such as agricultural wastes specifically lignocellulosic biomass. Mycelium binds the lignocellulosic biomass by hyphal micro-filaments in natural biological processes to manufacture both low-value materials and high-value composite materials such as packaging materials and ultrahigh-molecular-weight polyethylene (UHWPE), respectively (Islam *et al*., 2018). Low-grade agricultural and forestry residues such as sawdust and straw are often suitable for the manufacturing of foam-like mycelium composite (Jones *et al*., 2018; Ghazvinian *et al*., 2020). Existing research proposed that the end product depends on fungal species used for inoculation, substrates used, growth conditions, and forming techniques (Jones *et al*., 2017; Appels *et al*., 2019; Bruscato *et al*., 2019). Among various fungi that were used for mycelium-based composites application, *Trametes multicolor* and *Pleurotus ostreatus* are the most common fungi used under current research (Appels *et al*., 2019). Malaysia is known for its tropical climate with a high rate of rainfall which prompts to high chance to obtain local fungi from its rainforest (Kusai *et al*., 2018). In this study, we specifically aimed to screen local white rot fungi and local biomass, oil palm empty fruit bunch, for mycelium-based biofoam production to substitute EPS.

MATERIALS AND METHODS

Collection of fungal samples

Sultan Idris Shah Forestry Education Centre (SISFEC) is located at Puchong, Selangor, Malaysia, with the coordinates (3° 0' 22.64" N 101° 38' 35.82" E). Local peninsular Malaysian fungi were sampled based on their characteristics such as fruiting bodies, rotten wood covered with whitish mycelia, and tree stumps. A fruiting body was the primary indication of the presence of fungus. The pieces of tree bark that were covered with fungal mycelium and fruiting bodies were collected and placed into paper bags. The samples were separated and labeled accordingly. Both positive controls for this study were collected separately. *Schizophyllum commune* was obtained from the Microbial Culture Collection Unit (UNiCC), Institute of Bioscience, Universiti Putra Malaysia, meanwhile, *Pleurotus ostreatus* (oyster mushroom) was acquired from a local supplier in Pekan, Pahang, Malaysia.

Isolation of fungal samples

Fruiting bodies were surfaced and sterilized by soaking them with 70% ethanol for two minutes, followed by 5.0% sodium hypochlorite treatment for ten minutes in a sterile conical flask (Okereke *et al*., 2017). Each fungal sample was cut by using a sterile surgical blade into approximately 2 cm and placed in the middle of the PDA medium. The fruiting bodies were adhered to the medium surface to increase the probability of mycelium growth. Each of the samples was duplicated, and they were incubated inverted in the dark at 30 °C for 7 days. Each fungal growth was observed every 3, 5, and 7 days.

Morphological identification of newly isolated fungi

Fungal isolate was observed under a light microscope (Nikon YS100, New York) to identify the morphology of mycelium. A few drops of distilled water were put onto a glass slide. By using a loop, the tip of fungal mycelium was smeared into the droplets of distilled water. A drop of 0.1% (w/v) methylene blue was added after that and covered with a cover slip (Moemen *et al*., 2015). The morphology of fungi mycelium was observed in 40x, 100x, 400x, 1000x magnification.

Molecular identification of fungal samples

Fungal grown on agar was cut into 0.5 cm × 0.5 cm with a sterilized blade and placed in an empty 1.5 mL centrifuge tube. The samples were immediately sent to the Apical Scientific Sdn. Bhd. for polymerase chain reaction (PCR) and sequencing analysis at room temperature. Primers used in the analysis were ITS 1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 (5' TCCTCCGCTTATTGATATGC 3'). After obtaining the raw sequences of ITS region 18S ribosomal RNA for respective fungi, the sequences were then assembled into complete sequences by using Chromas software. Each completed fungal sequence's similarity was determined by using the nucleotide-nucleotide Basic Local Alignment Search

online tool on the National Center for Biotechnology Identification (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast). Next, the sequences were aligned using the ClustalW program, and the phylogenetic tree was constructed using a neighbor-joining algorithm in MEGA X software (Kumar *et al*., 2018).

Fungal growth on oil palm empty fruit bunch

Fungal mycelia development was conducted for selected fungi that gave positive results during rapid screening. OPEFB were washed, sun-dried, ground, and sieved into an average of 1-2 mm length fibers size. Five grams of unsterilized OPEFB was weighed and placed into a glass petri dish. Each sample preparation was done in triplicate. The synthetic culture media was prepared (g/L); 2 g of yeast extract, 0.8 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 0.005 g $FeSO_4.7H_2O$, 4 g NH_4NO_3, 0.75 g $K_2HPO_4.7H_2O$, 0.02 g $ZnSO_4.7H_2O$ and 0.06 g $CaCl_2.2H_2O$ (Zutz *et al*., 2013). The pH of the synthetic culture was adjusted to 6 before autoclaving together with a glass petri dish containing OPEFB at 121 °C for 15 min. The following steps were performed under a sterilized condition in laminar airflow. Synthetic culture media (10 mL) was added into 5 g of sterilized OPEFB in the glass petri dish and 5 plugs of mycelium from 14 days of culture were inoculated onto the OPEFB. The moisture content set was at 80%. Fungal mycelia development was conducted at 30 °C under static conditions for 24 days in the oven incubator (Labwit, Shanghai). Fungal mycelium development was observed at 3-day intervals for 14 to 20 days.

Rapid screening of fungal-producing ligninolytic enzymes

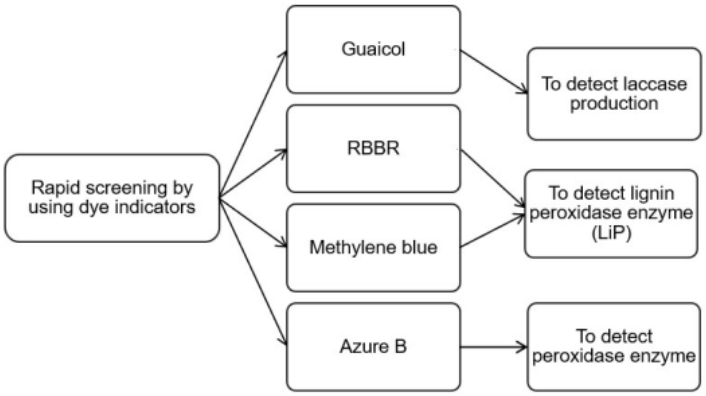

Fig. 1. Rapid screening method using four different dyes as indicators for fungi producing ligninolytic enzymes (Kiiskinen *et al*., 2004; Ghebreslasie *et al*., 2016; Saito *et al*., 2018).

Four different dyes were used as indicators for fungi producing ligninolytic enzymes (Figure 1). The method for screening guaiacol was based on Kiiskinen *et al*. (2004). Guaiacol was added to the PDA media before autoclaving. The plates were then incubated at 30 °C. After 7 days, lacasse production will be detected by visual inspection based on the brownish-red appearance of the agar. The ability of fungi to decolorize the anthraquinone dye Remazol Brilliant Blue R (RBBR) was evaluated by using PDA plates supplemented with 500 mg L^{-1} of the dye (Saito *et al*., 2018). Meanwhile, 0.02% methylene blue was prepared in malt extract agar (MEA). Chloramphenicol (200 µg/mL) was filtered and sterilized before being added into each medium before cooling. Each fungal disc of 1 cm diameter from the PDA plate was placed in the center of the plate. In addition, un-inoculated plates containing dye were used as the negative control. The plates were then incubated at 30 °C for 7 to 10 days. Both RBBR and methylene blue dye will help in detecting the presence of lignin peroxidase enzyme (LiP) through the decolorization of solid agar plates (Saito *et al*., 2018). The plates were examined on days 3, 5, and 7 for the visual disappearance of color. Peroxidase enzymes were screened by using 0.01% of azure B (Ghebreslasie *et al*., 2016).

Chitin assay

Fungal chitin content was determined by biomass estimation. According to Babitha *et al*. (2007), fungal chitin can be detected by determining N-acetyl glucosamine released via acid-hydrolysis. The acid hydrolysis sample (1 mL) was mixed with 1 mL acetylacetone reagent and incubated in a boiling water bath for 20 min. After cooling, 6 mL of absolute ethanol was added to the mixture, followed by 1 mL of Ehrlich reagent, and incubated at 65°C for 10 min in an oven incubator (Labwit, Shanghai). After cooling, the absorbance was measured at 530 nm using a spectrophotometer (Thermo Fisher, United States). N-acetyl glucosamine was used as the standard for this assay.

RESULTS AND DISCUSSION

Fungal isolation and collection

In this study, the biodiversity of isolated fungal species was observed among the geographical locations. All fungi were isolated at different coordinates of the same forest area, and this demonstrates the random dispersion of the fungi (Figure 2). Some species such as the *Perenniporia subtephropora* and *Lasiodiplodia theobromae* were isolated and identified from two different locations around SISFEC, whereas other fungal species such as *Hexagonia glabra*, *Trametes elegans*, *Daldinia korfii*, *Fusarium solani* and *Cunninghamella echinulata* were isolated only from one location (Figure 3). According to (Zakaria *et al*., 2011; Alsohaili & Bani-Hasan, 2018), the biodiversity of fungi mainly relates to the physiochemical properties of the environment such as the temperature, pH of the soil, and humidity. The highest number of fungal species discovered was in the families of *Basidiomycota*, followed by *Ascomycota* at 42% and *Mucoromycota* at 8%. A higher fraction in a fungal population helps in deepening the knowledge of their identity, morphology, and favor in host and habitat. For instance, *Polyporales*, one of the most common groups of *Basidiomycota* studied among researchers (Bolhassan *et al*., 2012; Hattori *et al*., 2012). Several fungal species are found in lowland rain and montane forests located in Malaysia including the genus of *Perenniporia* and *Trametes* (Hattori *et al*., 2012). Nevertheless, the identified species do not suffice for any conclusion of fungi diversity in this location.

Fig. 2. The area of sampling is located at Sultan Idris Shah Forestry Education Centre (SISFEC), Puchong, Selangor, Malaysia, with the coordinate (3° 0' 22.64" N 101° 38' 35.82" E.) Source: Google Earth.

The morphological examination and identification of fungi are useful for the identification of fungal isolates up to the family or genus level (Yao *et al*., 2016). Figure 4 shows 12 fungal species isolated from nature and cultured on a PDA plate. In this study, the isolated fungal species were examined based on cultural and macro morphological characteristics. The fungal isolates were first identified to a genus level using a morphological examination depending on the colors of the colony formed after being cultured on the PDA plate under controlled conditions. The morphology of the fungal colony on day 7 was compared with their morphological structure in the natural environment. From the observation, several fungal species such as S1, S2, S5, S8, and S19 show different colors of the colony after being cultured on PDA compared to their original color in the natural environment. However, some of the fungal isolates such as S3, S9, S13, S14, S18, and S21 show identical colour of the colony after being cultured on PDA. Based on previous studies, filamentous fungi are capable of producing a pigment-producing color as a consequence of the production of secondary metabolite during the growth on a synthetic medium (da Costa Souza *et al*., 2016). In terms of the shape of the colony on PDA, most of the fungal isolates were in filamentous form except for S18 and S21 which were in circular and rhizoid form, respectively.

Fungal mycelia usually radiate outwards as nutrients are acquired at hyphal tips which then will be transported into interior regions to support further development and formation of the next generation of spores (Harris, 2019). Under the light microscope, the fungal hyphae were stained by methylene blue against the background (Figure 5). Based on Figure 5a and Figure 5b, fungal hyphae of S1 and S9 with cross walls (septa) and a mesh of fungal mycelium can be observed. Septa divides the hypha into

individual cells, each with its nucleus. This allows the fungus to transport nutrients and other materials more efficiently. In addition, a mesh of fungal mycelium is a network of hyphae that form a tangled mass that provides a large surface area for the fungus to absorb nutrients from the environment (Appels *et al*., 2019). Lateral branching, whereby new branches developed from each cross wall was observed during the growth period of *Pleurotus ostreatus* (control) which allows the fungus to spread out and colonize a larger area. Several phyla have shown the same pattern including members of the *Saccharomycotina* (*Geotrichum candidum*), zygomycetes (*Basidiobolus ranarum*), and basidiomycetes (*Coprinus species*) (Harris, 2008). The hyphae of mycelium act as fungal glue or mycelial glue which allows the mycelium to bind together loose particles or substrates, such as agricultural waste, sawdust, or straw by penetrating and envelope the particles, forming a cohesive network that holds the material together. This binding property is crucial for the formation of mycelium-based biofoam. Furthermore, mycelium has the remarkable ability to self-assemble and organize itself into complex structures. As the mycelium grows, it seeks out and connects with other mycelial strands, forming an integrated network that allows the mycelium to fill voids, gaps, and irregular shapes, making it adaptable for various applications, including insulation and packaging (Liu *et al*., 2019; Jones *et al*., 2020).

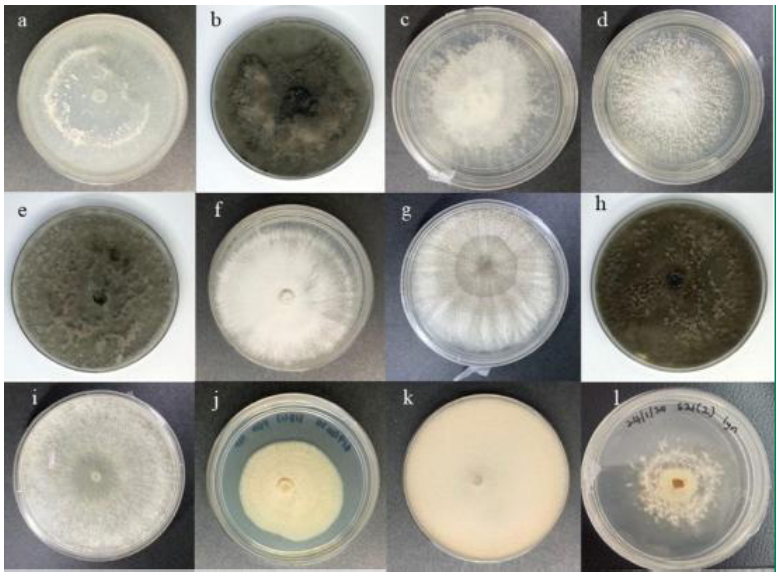

Fig. 3. A collection of local fungi isolated from the local forest in Selangor, Malaysia. (a) S1, (b) S2, (c) S3, (d) S5, (e) S8, (f) S9, (g) S10, (h) S13, (i) S14, (j) S18, (k) S19, (l) S21.

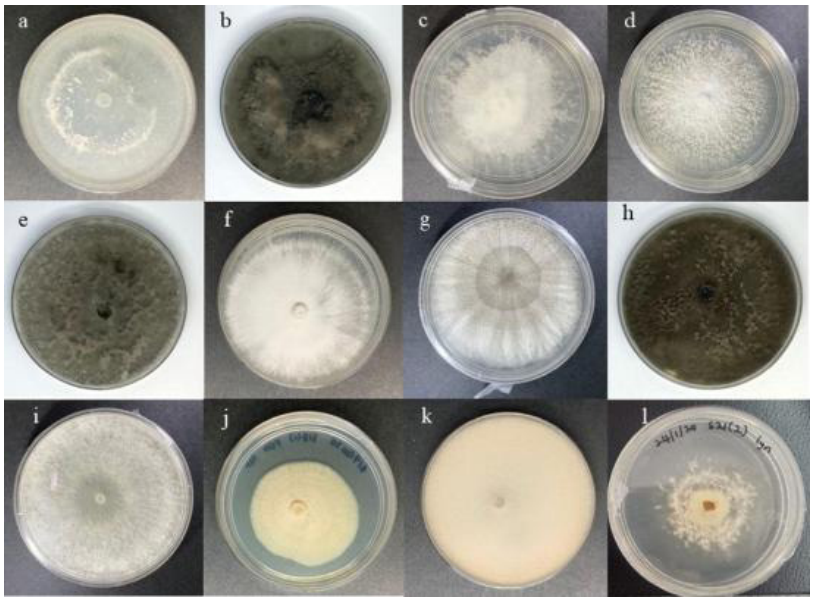

Fig. 4. Fungal growth observation on potato dextrose agar (PDA). (a) S1, (b) S2, (c) S3, (d) S5, (e) S8, (f) S9, (g) S10, (h) S13, (i) S14, (j) S18, (k) S19, (l) S21.

On the other hand, *Schizophyllum commune* was seen to produce fungal spores on day 14 of fungal growth. Generally, at this stage, the spore is a reproductive spore that contains a haploid nucleus as preparation for the next step, mating (Erdmann *et al*., 2012). In addition, spore formation is also contributed by environmental conditions such as changes in temperature, light, moisture, and nutrient availability. When the mycelium exhausts the available nutrients in its immediate surroundings, it may undergo a transition to spore formation. Nutrient depletion serves as a signal for the fungus to reproduce and disperse to seek new sources of nutrition (Krull *et al*., 2013). In the context of mycelium-based biofoam, the transition to spore formation can impact the stability and longevity of the material. Spores are typically smaller, lighter, and more fragile compared to mycelium. When spores are present within the mycelium-based biofoam, they may lead to reduced structural integrity and potentially affect its overall performance. By maintaining optimal conditions for vegetative growth and limiting factors that trigger spore formation, such as nutrient depletion or exposure to light cultivating mycelium needs to grow under optimal properties for mycelium-based biofoam formation (Islam *et al*., 2017).

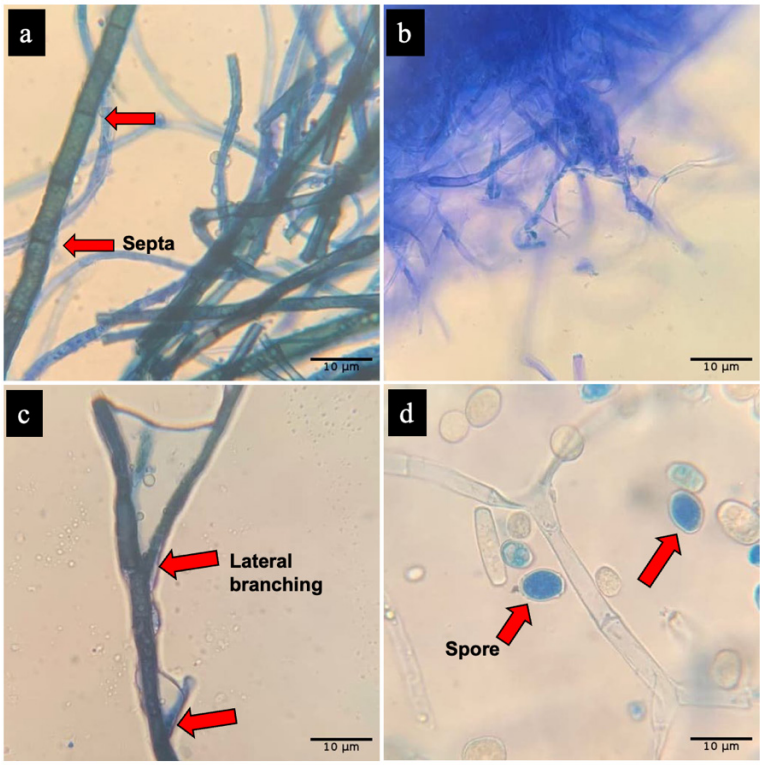

Fig. 5. Fungal hyphae observation using methylene blue stain under 1000x total magnification of light microscope. (a) S1; fungal hyphae with cross walls, (b) S9; mesh network of fungal mycelium, (c) *Pleurotus ostreatus;* hyphae branch, (d) *Schizophyllum commune;* spores.

Identification of fungal samples and their phylogenetic tree

In this study, twelve fungi were successfully isolated and grouped by using the Neighbor-Joining method (Saitou & Nei, 1987) (Figure 6). The phylogenetic tree formed three major clades: *Basidiomycota*, *Ascomycota,* and *Mucoromycota*. Meanwhile, *Allochytridium* has been selected as an outgroup to prune the phylogenetic tree. The number stated above and below branches represent the bootstrap values. Each fungal species was grouped under its respective phylum with a percentage of similarity above 99%, except for *Trametes elegans* isolate LYN-UPM S3 (MW157265) and *Phanerochaete laevis* isolate LYN-UPM S14 (MW157271), which the highest percentage of similarity was only 98% and 97% respectively. Four isolates were clustered under each of the same species. *Perenniporia subtephropora* isolate LYN-UPM S5 (MW157266) and *Perenniporia subtephropora* isolate LYN-UPM S9 (MW157268) were related to the same species, which is *Perenniporia subterphropora*, the same goes to *Lasiodiplodia theobromae* isolate LYN-UPM S2 (MW157264) and *Lasiodiplodia theobromae* isolate LYN-UPM S13 (MW157270) which were related to *Lasiodiplodia theobromae*. A total of ten different fungal species were identified via 18s rRNA small subunit ITS region in 500 to 700 base pair lengths (White *et al*., 1990). All identified fungal samples were submitted to the GeneBank database under accession numbers MW157263- MW157274.

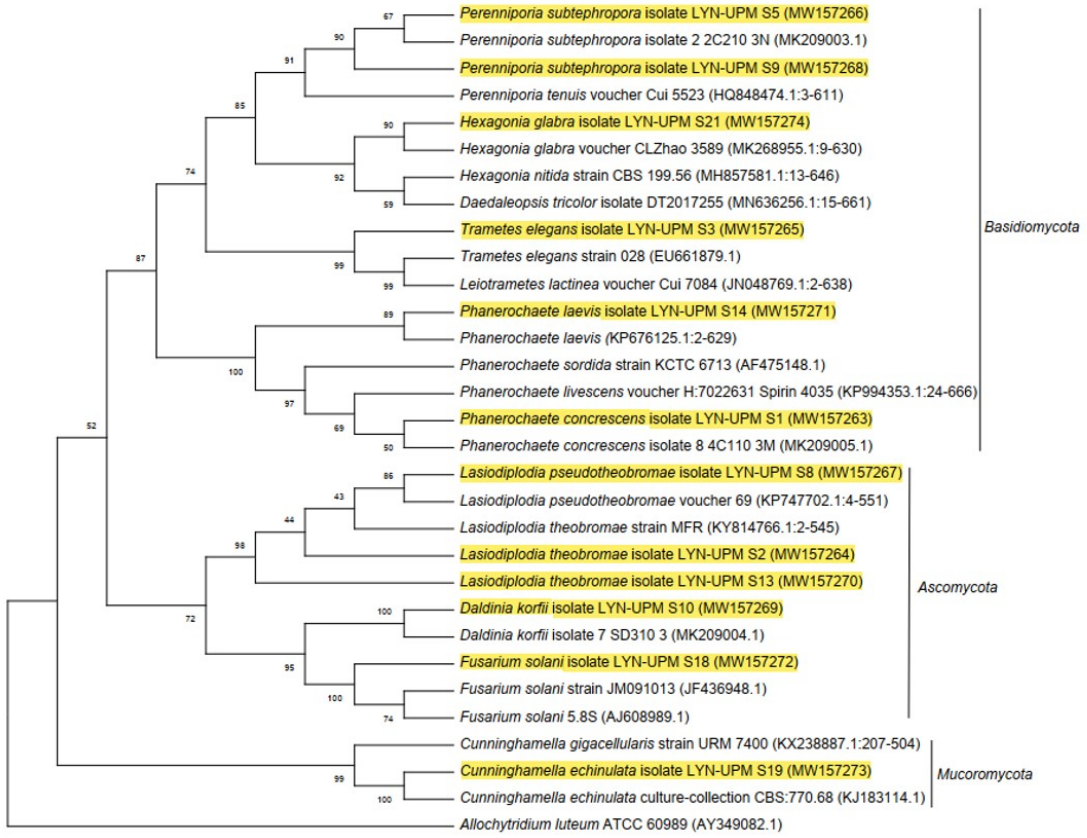

Fig. 6. Phylogenetic tree analysis between 18s rRNA gene of isolated fungal species using Neighbor-Joining method. Bootstrap supports values for 500 replicates.

Fungal growth on oil palm empty fruit bunch

For the first screening, fungi were cultured on OPEFB, which is a potential substrate source for mycelium-based biofoam. Out of twelve fungi, only five fungi were able to grow on oil palm empty fruit bunch after being observed for 14 to 20 days of cultivation. Each fungal mycelia diameter was measured at intervals of three days to observe the development until it was fully grown approximately 9 cm on the plate as shown in Figure 7. *Phanerochaete concrescens* isolate LYN-UPM S1, *Lasiodiplodia theobromae* isolate LYN-UPM S2, *Perenniporia subtephropora* isolate LYN-UPM S9, *Fusarium solani* isolate LYN-UPM S18 and *Cunninghamella echinulata* isolate LYN-UPM S19 were able to colonize on OPEFB and proved that they are capable to utilize OPEFB as a carbon source. Based on visual observation, the colonization of fungal mycelium on agar plates did not correlate with its thickness and density (Attias & Grobman, 2017), thus, a quantitative analysis such as chitin assay is needed to support the findings.

Schizophyllum commune isolates ENN1 and *Pleurotus ostreatus* were included as positive controls due to their originality and purposes, respectively. *Schizophyllum commune* isolates ENN1, an indigenous fungus isolated from oil palm empty fruit bunch also has the potential to be a source of mycelium-based biofoam (Arbaain *et al*., 2019). Meanwhile, several researchers have used *Pleurotus* strains as the matrix in respective biofoam due to the strength of the fungal mycelia network involved during the development of biofilm (Attias & Grobman, 2017; Bruscato *et al*., 2019; Ghazvinian *et al*., 2020).

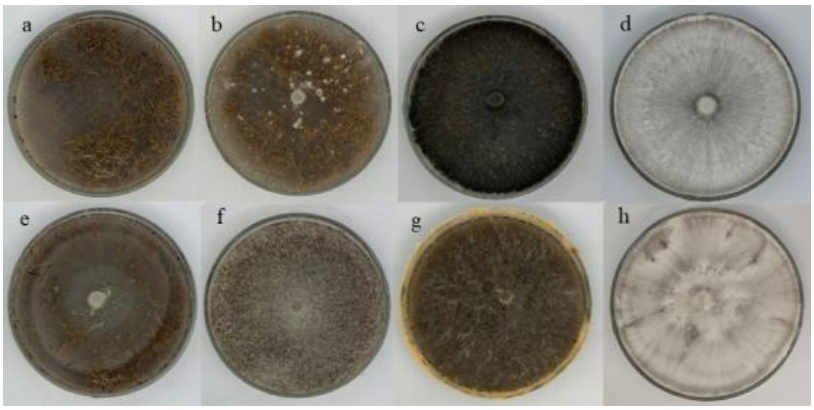

Fig. 7. Fungal mycelia growth on oil palm empty fruit bunch (OPEFB) supplemented with minimal media agar after 14 to 20 days of incubation. (a) Control, (b) S1, (c) S2, (d) S9, (e) S18, (f) S19, (g) *Schizophyllum commune*, (h) *Pleurotus ostreatus.*

Rapid screening of fungi producing ligninolytic enzymes

All seven fungi were then tested for lignin-degrading capability using four different types of dye indicators. They were inoculated onto PDA and MEA plates respectively containing guaiacol, RBBR, methylene blue, and azure B. Previous screening methods for lignin-degrading fungi have been developed using either the RBBR, methylene blue, or azure B but not a combination of all (Saito *et al*., 2018). Reactions of each fungal strain were compared based on visual inspection of the agar plates and labeled based on their outcomes (Table 1). Positive fungal strains were indicated by the appearance of holo-zone on agar containing RBBR, methylene blue, and azure B and reddish-brown zone formation around grown fungal mycelium on agar plates containing guaiacol (Figure 8). *Phanerochaete concrescens* isolate LYN-UPM S1 and *Perenniporia subtephropora* isolate LYN-UPM S9 exhibited positive results on RBBR and azure B agar plates due to the formation of holo-zone. RBBR decolorization correlates with the ligninolytic ability of the microorganisms (Ghebreslasie *et al*., 2016). Only *Phanerochaete concrescens* isolate LYN-UPM S1, *Schizophyllum commune* isolate ENN1, and *Pleurotus ostreatus* were able to decolorize methylene blue. Decolorization of RBBR, methylene blue, and azure B observed on the agar plate proved the secretion of peroxidases enzyme into the agar by the fungal mycelium. Meanwhile, on guaiacol plates, *Phanerochaete concrescens* isolate LYN-UPM S1, *Perenniporia subtephropora* isolate LYN-UPM S9, and *Pleurotus ostreatus* showed laccase production due to the formation of reddish-brown pigment along the fungal growth (Abd El Monssef *et al*., 2016). Less or no detectable reactions by *Lasiodiplodia theobromae* isolate LYN-UPM S2, *Fusarium solani* isolate LYN-UPM S18, and *Cunninghamella echinulata* isolate LYN-UPM S19 implies the unfavorable amount of ligninolytic enzymes have been produced.

Based on this screening combination results, *Phanerochaete concrescens* isolate LYN-UPM S1 and *Perenniporia subtephropora* isolate LYN-UPM S9 have been chosen for the next assay due to their potential for mycelium-based biofoam application. The control *Pleurotus ostreatus* strain gave strong positive reactions with most of the dye indicators.

A positive reaction indicates the secretion of ligninolytic enzymes throughout the plate assay. The diameter of holo-zones was measured on the optimum incubation period (7th day of fungal growth), to evaluate the capability and quality of fungi producing ligninolytic enzymes (Table 2). This study has found that *Phanerochaete concrescens* isolate LYN-UPM S1 and *Perenniporia subtephropora* isolate LYN-UPM S9 were the best decolorizers due to clear formation of holo-zone surrounding the mycelia growth on each RBBR, methylene blue and azure B agar. The clear zones were observed to be smaller than the diameter of the corresponding outgrowth of the fungal mycelium. This is caused by the secondary metabolic activity of older mycelia (Levin *et al*., 2004). *Phanerochaete* sp., *Perenniporia* sp., and *Pleurotus* sp. which portrayed positive results in laccase, agreed with those obtained from other research (Jarosz-Wilkołazka *et al*., 2002; Madadi & Abbas, 2017). However, *Pleurotus ostreatus* from this study shows better results as only 1.9 ± 0.03 cm diameter of reddish-brown zone formation was cited for *Pleurotus* sp. laccase production (Saroj *et al*., 2018). In addition, both *Phanerochaete concrescens* isolate LYN-UPM S1 and *Perenniporia subtephropora* isolate LYN-UPM S9 have successfully degraded RBBR colour from light blue to colourless up to day 7 which demonstrates a larger average diameter of holo-zone per day (1.2 cm) as compared to *Phanerochaete* sp. strain with 0.54 cm diameter per day. (Ghebreslasie *et al*., 2016). Different fungal species may result differently due to different efficiency of oxidation and reduction reactions involved (Camarero *et al*., 1999).

Table 1. Comparison of reactions of the isolated fungal species, *Schizophyllum commune* isolate ENN1 and *Pleurotus ostreatus* with different dye indicators

Fungal species	RBBR	Guaiacol	Methylene Blue	Azure B
Phanerochaete concrescens isolate LYN-UPM S1	+	+	+	+
Lasiodiplodia theobromae isolate LYN-UPM S2	-	-	-	+
Perenniporia subtephropora isolate LYN-UPM S9	+	+	-	+
Fusarium solani isolate LYN-UPM S18	-	-	-	-
Cunninghamella echinulata isolate LYN-UPM S19	-	-	-	-
Schizophyllum commune isolate ENN1	-	-	+	-
Pleurotus ostreatus	-	+	+	+

+ Indicate positive reaction.
- Implies no detectable reaction.

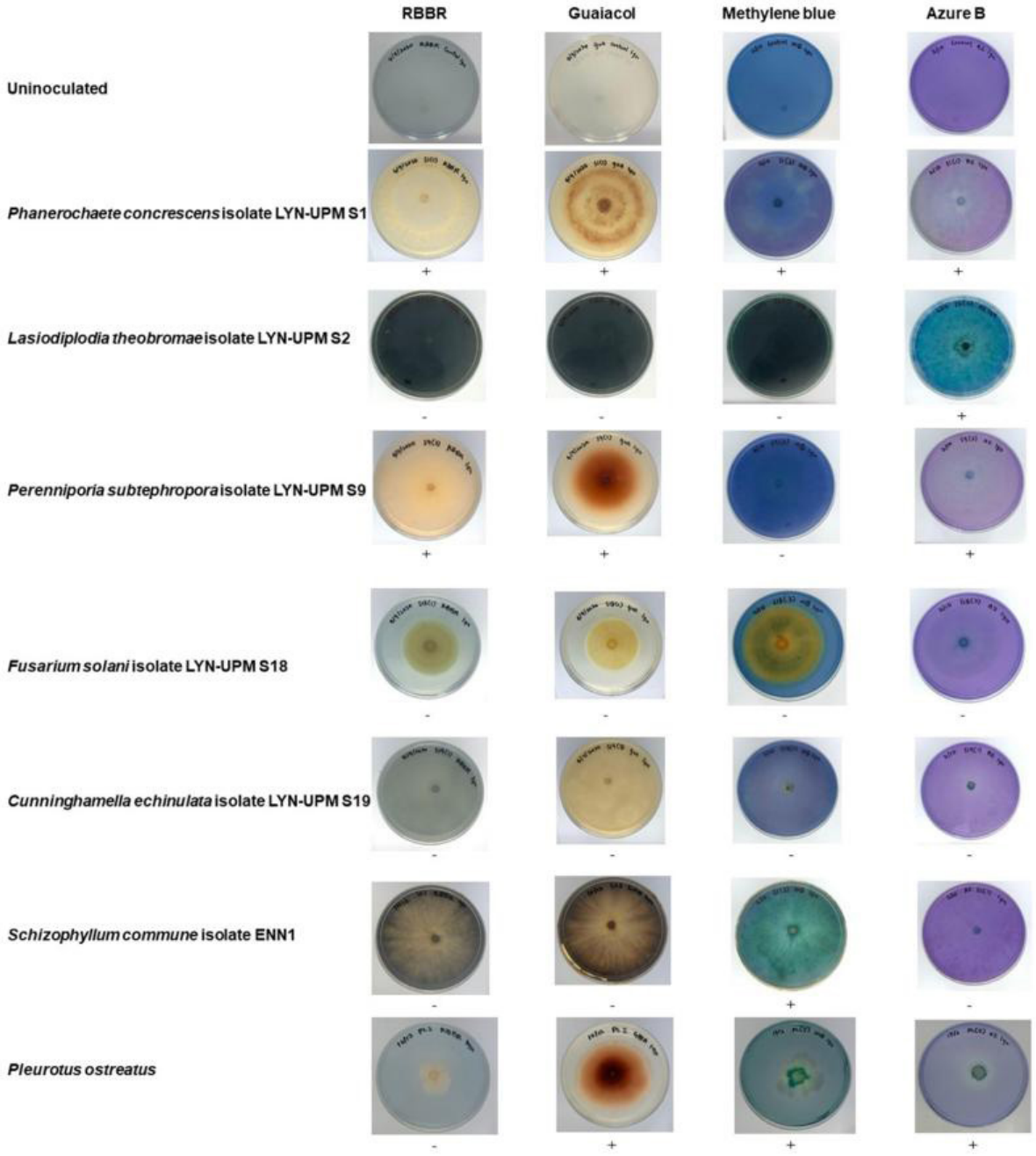

Fig. 8. Screening of fungi that degrade lignin using different types of dye indicators; guaiacol, Remazol Brilliant Blue R (RBBR), methylene blue, and azure B. Appearance of holo-zone and dye degradation were observed throughout fungal growth up to day 7.

Table 2. Comparison of formation of holo-zone from three fungal species that have strong positive reactions: a) *Phanerochaete concrescens* isolate LYN-UPM S1, b) *Perenniporia subtephropora* isolate LYN-UPM S9 and c) *Pleurotus ostreatus* (as control)

Fungal species	Diameter of fungal holo-zone on day 7 (cm)			
	Guaiacol	Remazol Brilliant Blue R	Methylene Blue	Azure B
Phanerochaete concrescens isolate LYN-UPM S1	7.07 ± 0.29	8.50 ± 0.00	6.43 ± 0.8	6.43 ± 0.81
Perenniporia subtephropora isolate LYN-UPM S9	6.4 ± 0.4	8.50 ± 0.00	ND	6.85 ± 0.21
Pleurotus ostreatus (as control)	5.85 ± 0.07	ND	2.1 ± 0.09	2.07 ± 0.32

*ND means not determined

Fungal chitin content

The final screening was performed by determining the glucosamine content of each isolated fungi. Glucosamine contents were measured by using N-acetyl glucosamine as standard. *Perenniporia subtephropora* isolate LYN-UPM S9 yielded the highest glucosamine content on day 20; 13.9 mg/g, followed by *Schizophyllum commune* isolate ENN1; 9.82 mg/g (Figure 9). Glucosamine, an amino-monosaccharide, is a component of chitin and chitosan, whose chemical constitution is 2-amino-2-deoxy-D-glucose (Stoykov *et al*., 2015). Whereas chitin is a component of the fungal cell wall (Vega & Kalkum, 2012; Steinfeld *et al*., 2019). It is commonly known to serve multiple functions. Other than stabilizing the cell wall during hyphal growth, budding, and cell division, chitin supports the fungal cell wall as a fibrillar scaffold to support its extracellular matrices. According to (Sakurai *et al*., 1977), chitin content represents the abundance of fungal mycelium developed. However, different fungal groups possess different amounts of fungal chitin depending on their cellular activities.

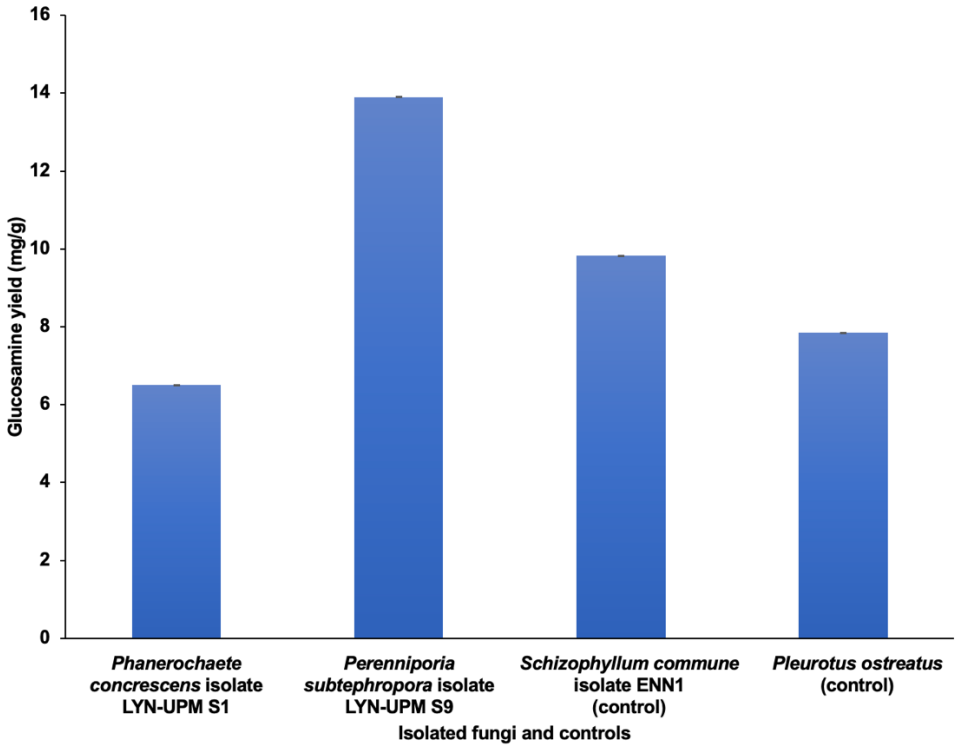

Fig. 9. Glucosamine yield from isolated fungi and control were determined on day 20 of fungal growth. Results represent the mean ± SD of triplicate samples.

CONCLUSION

Mycelium-based biofoam utilizes biological growth on biomass and has no disposal issue as it is completely biodegradable. Screening of local white rot fungi is beneficial to produce high-impact mycelium-based biofoam. The combination of the screenings helps in narrowing down the selection of

isolated white rot fungi for the application. Fungi's ability to grow and intertwine through the substrate is an important factor for good biocomposite production. This study found that the highest glucosamine yield by *Perenniporia subtephropora* isolate LYN-UPM S9 (13.9 mg/g), which indicates that local fungus can be discovered and has high potential as the natural binder in mycelium-based biofoam application using OPEFB as substrate.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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