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Qualitative methods to identify potential strains for partial degradation of oil palm mesocarp fibre

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ABSTRACT

Chemical and physical treatments for cellulosic wastes degradation currently suffer several setbacks that limit its application in the management of palm oil-related wastes. This study isolated and screened fungal strains capable of degrading raw oil palm mesocarp fibre (OPMF). The fungal strains were identified by using the polymerase chain reaction amplification followed by phylogenetic studies. Three fungal strains namely *Mucor irregularis* (AZ1), *Pestalotiopsis microspora* (AZ2) and *Trichoderma harzianum* (AZ3) were selected to investigate the degradability of OPMF. The OPMF, being a lignocellulosic biomass was partially degraded via ligninolytic and xylanolytic enzymes secreted by AZ1, AZ2 and AZ3, respectively. All three strains gave positive effects to xylanolytic and ligninolytic activities. The qualitative screening for lignocellulose degradation indicated that 36% lignin and 79% hemicellulose components of OPMF were completely degraded following fungi treatment. The presence of hollow zones on the screening plates affirmed the ability of these strains in hydrolyzing raw OPMF.

Keywords: Fungi; Oil palm mesocarp fibre; Lignocellulosic biomass; Polymerase chain reaction

1. Introduction

Palm trees generate approximately 10 times more oil per hectare than other food crops such as soybean or rapeseed. Malaysia is currently the world's largest exporter but the second-largest producer of palm oil after its neighbouring counterpart, Indonesia. The suiting climatic environment of Malaysia with regards to its fertile soils, rainfalls, humidity and temperature renders the industrialization of oil palm plantations a great success for the country.

At present, most of the palm oils produced in Malaysia are channelled for the synthesis of non-food related products and oleochemicals such as methyl esters, fatty alcohols, glycerine and fatty acids. Owing to the positive drive for health supplements and food nourishments, Malaysia is encouraging the expansion of high-value food crops and palm oil-based medicinal supplements that contain palm phytonutrients. The large amount of palm oil production produces and equally large accumulation of various wastes including cellulosic wastes which originate from the palm fruits oil extraction process. These cellulosic wastes are known to constitute severe environmental hazards especially when accumulated without proper management [1].

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Previous studies on the chemical and physical pre-treatments for cellulosic wastes degradation suffered several setbacks that currently limit its application in the management of these wastes. According to Ang et al. [2], these setbacks include the high cost of system maintenance and the harsh process required to disrupt lignin layers within the cellulosic components of the waste.

To overcome these limitations, biological pre-treatment was found as the most feasible and prominent alternative. In biological pre-treatment, microorganisms such as white-, brown- and soft-rot fungi have been widely used for lignin and hemicelluloses degradation [3]. Brown- and soft-rot fungi are commonly used to degrade cellulose and lignin, while white-rot fungi are mostly effective for lignin degradation in lignocellulosic materials [4]. Amongst other white-rot fungi, oyster mushroom is reported to be the most efficient for the degradation of lignocellulosic biomass found in oil palm mesocarp fibre (OPMF) [5]. However, the low rate of hydrolysis and longer residence times are required to attain partial degradation of OPMF by fungi [6].

Molecular approaches are receiving increased attention to develop a more advanced polymerase chain reaction (PCR)-based specific diagnostic assays for organisms such as bacteria and fungi [7]. Most of these molecular approaches provide useful information and act as an alternative for culture-based detection and identification methods. The study of ribosomal ribonucleic acid (rRNA) and the RNA component of the ribosome essential for protein synthesis in all living organisms is currently one of the best methods for identifying bacteria, fungal and other organisms [8].

The application of DNA amplification techniques on rRNA sequences for identifiable organisms are known as PCR. PCR is a nucleotide amplification process that engineers the replication of selected genomic structures prior to sequencing. PCR is widely used for detection, quantification and amplification of microorganisms in various environmental samples including air, soils, landfills, water, etc. [9]. Although PCR amplification procedures could be directly performed on various microbial cultures, DNA purification is also crucial to eliminate cellular impurities such as humic acids which can reduce DNA quality and further interfere with the PCR amplification process [9]. With these procedures, specific species of mushrooms can be identified and screened via PCR techniques. The molecular technique is a sensitive means to detect soil-borne fungi and internal transcribed spacer (ITS) of nuclear rDNA were used to analyze molecular differences amongst different fungi species, because interspecific and intraspecific variations are known to serve as embedded signatures within the ITS of related fungal species [10].

Fungi strains' identification and gene characterization involves key processes such as isolation of genomic DNA, inoculum preparation, PCR and phylogenetic analysis [2,11]. According to Singh et al. [12], DNA extractions and PCR amplification are the two important processes essential for identifying fungi species. However, phylogenetic studies as well as sequence alignment analysis must also be completed to understand the evolutionary trend of the identified fungus species.

Phylogenies and molecular evolutionary analysis based on ITS for bacteria and fungi are usually conducted using Molecular Evolutionary Genetics Analysis (MEGA) software [13]. These phylogenetic studies provide an array of microbial populations belongs to the same community structure based on the proximate genomic relationship that exists amongst them. With phylogenetic studies, understanding how morphologically different species can be phylogenetically related to each other is no more a mystery [14]. According to Liu et al. [15], phylogenetic studies can also evaluate the importance of morphological characters based on ITS sequence analysis and this technique has been applied on several white-rot fungi species.

Owing to the fact that biological treatment does not exert high energy demand for the hydrolysis of lignin, providing safer environments and higher treatment efficiencies than physical and chemical treatment processes [3], this study aims to employ PCR techniques to isolate and identify potential fungi strains capable of partially degrading OPMF.

2. Materials and methods

2.1. Microbial characterization

The three fungal strains characterized in this study were isolated from a local recreational park at the Universiti Teknologi Malaysia (UTM), Johor [GPS data: 1.558018, 103.642109]. The samples exhibiting visible fungi mycelium cells were collected and mixed with sterile distilled water prior to streaking onto potato dextrose agar (PDA) plates. Further separation was done until pure cultures were obtained. The spores synthesized from the three pure fungal cultures assigned as AZ1, AZ2 and AZ3 were collected and stored in Protect Bacterial Preserves (Technical Service Consultants, Heywood, UK) at –80°C for long-term storage. After PCR amplification and phylogenetic studies, these strains AZ1, AZ2 and AZ3 having nucleotide sequence similarities >98% were identified as *Mucor irregularis* JN20615, *Pestalotiopsis microspora* HM190153 and *Trichoderma harzianum* KC819133, respectively.

2.2. Inoculum preparation

The selected strains of fungi were cultured on PDA plates for 7 d, and incubated at 27°C. Then, 1% (v/v) of sterile Tween-80 solution was used to collect the matured fungal spores, followed by centrifugation at 4,000 rpm for 20 min. The resulting pellet following the centrifugation process was mixed with sterile distilled water to produce the inoculum.

2.3. Identification of fungi species

DNA extraction was initiated by physically disrupting the cell walls of frozen fungi spores. This was conducted by grinding the fungi spores with cetyltrimethylammonium bromide buffer using a sterilized mortar and pestle. Crude DNA extract was then collected and further purified according to the modified Promega Wizard Genomic DNA purification kits protocols. The samples were incubated at 37°C for 1 h after being mixed with nucleus lysis solution. The purified DNA was used as the template for the PCR amplification of fungi ITS sequences. The primer set (forward and reverse primer) that encodes for the amplification of ITS region for the three fungi strains is ITS1F (5″-CTT GGT CAT TTA GAG

GAA GTA A-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′), respectively.

The PCR amplification of this ITS region was performed with a total reaction volume of 50 μL, which consisted of 25 μL Vivantis PCR Master Mix, 5 μL of DNA template, 1 μL of each forward and reverse primer, 5 μL 10× bovine serum albumin, 12 μL of Vivantis nuclease-free water and 1 μL of magnesium chloride ($MgCl_2$). Amplification was conducted using Eppendorf PCR Master Cycler by subjecting the sample to a cycle of initial denaturation at 94°C for 5 min, 35 cycles amplification and finally a cycle of final extension at 72°C for another 10 min. The protocols for amplification cycles adopted in this study were ITS1F–ITS4 (94°C for 1 min, 55°C for 40 s and 72°C for 1 min) for strains AZ1 and AZ2. However, the PCR amplification of strain AZ3 was conducted with initial denaturation at 95°C for 5 min, 30 cycles amplification and a final extension at 72°C for another 10 min. The amplification cycles adopted for this strain was ITS1F–ITS4 (95°C for 1 min, 53°C for 1 min and 72°C for 1 min).

The intensity and approximate sizes of the synthesized amplicons were examined using 1% (w/v) of agarose gel electrophoresis with ethidium bromide (EtBr) staining. The electrophoresis procedure was done at 80 V in 55 min. Amplified DNA was purified and sequenced at First Base Laboratories Sdn. Bhd. (Malaysia). The sequenced output data were analyzed using Heracle BioSoft Chromatogram Explorer, and nucleotide blast with GenBank database.

All sequences obtained in this work were aligned and analyzed with Chromas© Lite Software Informer 2.0 (Technelysium Pty Ltd., Australia, 1998–2004). Identified nucleotide sequences were compared with the GenBank database using the Basic Local Alignment Search Tool – nucleotide (BLASTn) algorithm (Benson et al. [16]) via www.ncbi.nlm.nih.gov/Blast.

2.4. Qualitative screening of potential strains

The selected fungi were isolated and observed for their level of cellulolytic, xylanolytic and ligninolytic enzymes produced under solid-agar screening. Selective solid agar was chosen based on readability and rapidness. The qualitative screenings for all fungi were conducted for 3 d in carboxymethyl cellulose (CMC) and birchwood-xylan plates. Successful strains will produce clearing zones on the agar plate after 3 d.

Agar plated with CMC was prepared. Strains AZ1, AZ2 and AZ3 were then streaked onto these CMC plates and incubated at 37°C for 3 d. After 3 d, the plates were then flooded with 0.1% of congo red for 30 min. Then, all plates were washed with 1 M sodium chloride (NaCl). The appearance of holozones against the red colouration of the congo red staining agent indicates that the cellulolytic enzymes of these strains are actively expressed.

For xylanolytic activities, agar plated with xylan were prepared and strains AZ1, AZ2 and AZ3 were streaked on the xylan agar for 3 d. The plates were kept in an incubator at 37°C to accommodate for the growth of fungus. After 3 d, all plates were flooded with iodine stain and left for 30 min. Thereafter, the iodine stains were discarded and washed off using deionized water (dH_2O). The appearance of a yellow opaque area against the blue/purple colouration of the iodine staining agent indicates the strains can degrade the hemicellulose components within lignocellulosic biomass.

Agar plates with $FeCl_3$ and $K_3[Fe(CN)_6]$ were prepared for testing ligninolytic activities. The fungal strains were streaked on the agar plates for 3 d. Potential strains with actively expressed ligninolytic enzymes stains the bluish green colour of the agar while generating clear zones around its colonies. The percentage of these clear zones was measured using grid method, by determining the percentage ratio of the area of fungi growth (clear zone), A and the total area of plate, B. This is defined as 100(A/B). Since the agar was not transparent, the area was determined by placing the grid on the top side of petri dish cover as illustrated in Fig. 1.

2.5. Identification of potential fungal strains in degrading OPMF

Twenty (20) samples of mushroom were exposed to remazol Brilliant Blue R (RBBR) test for identification of its ability to hydrolyze OPMF. All the samples were isolated and cultured in individual agar plate mixed with RBBR dye for 14 d. Plates were incubated at 30°C until they were totally colonized. The plates were monitored for fungi growth and appearance of decolourization/clear zones. The presence of these clear zones depicts that the identified fungi strains actively expresses lignin peroxidase for the enzymatic hydrolysis of OPMF and this results in the decolourization of the RBBR dye to generate clear zones [4].

3. Results and discussion

3.1. PCR amplification, sequencing of ITS genes and identification of fungi

The genomic DNA of three lignocellulose degrading fungi was successfully isolated. Amplification of ITS region was essential to disclose the identity of the fungi species used in this study. One set of primer (ITS1–ITS4) was used to specifically amplify the ITS region.

In sequential order, 16 loading wells were inscribed onto the agarose gel prior to electrophoretic analysis. Slot 1 was assigned for DNA ladder and slots 3–9 were assigned for the metagenomic DNA extract of strains AZ1, AZ2 and AZ3 before undergoing PCR amplification. The amplified DNA of these strains was loaded into slots 11–16 on the allotted agarose gel prior to electrophoresis.

As shown in Fig. 2, the length of the amplified nucleotides after PCR experiments ranges from 500 to 1,000 bp for all fungi strains. According to the size-based speciation of the 1 kB DNA

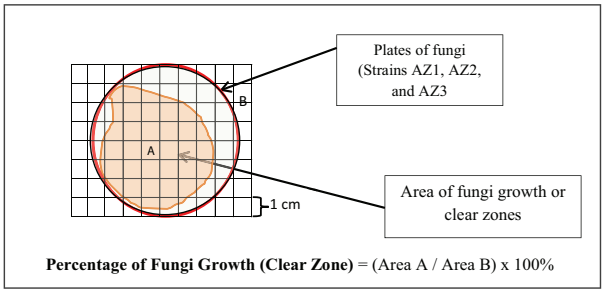

Fig. 1. Grid method for determining the percentage of clear zones appeared.

ladder, the PCR product of strain AZ1 lies within the line of 500 to 1,000 bp while AZ2 and AZ3 had nucleotide sizes above 500 bp after PCR amplification. According to Valente et al. [17], PCR amplification of the ITS region helps to delineate the species of fungi detected and the length of the ITS amplified products is a characteristic tool for delineating fungi groups. The DNA size-based electrophoretic bands for strains AZ1, AZ2 and AZ3, as shown in Fig. 2, exhibited some fade thickness due to the low concentration of DNA deposited in the PCR tubes. However, this did not interfere with the accuracy of the sequencing result because only the PCR amplicons with the highest DNA quality were selectively deposited for sequencing at First Base Laboratories Sdn. Bhd. (Malaysia).

Identification of fungal strains was only performed on these three strains due to their enzymatic ability to hydrolyze OPMF after being screened via RBBR test and amplified through the PCR process. The evolutionary relationship of all the three strains AZ1, AZ2 and AZ3 was based on their ITS sequence and expounded via the phylogenetic tree constructions as shown in Figs. 3–5, respectively. Phylogenetic analysis of the partial ITS region was carried out between the three fungi clones with a bootstrap of 1,000.

Based on this phylogenetic study, strain AZ1 was most related to *M. irregularis* JN26151 with sequence similarity of 98% based on its ITS sequences. This similarity was obtained from NCBI Blast procedure. The nucleotide sequence of strain AZ1 has been deposited to GenBank and assigned accession number KM871188. From this study, *M. irregularis* can partially degrade raw OPMF. Further qualitative investigation on its enzymatic activities was carried out to confirm its ability to drive lignocellulosic degradation. No previous study has been reported with regards to the use of *M. irregularis* for partial degradation of OPMF.

Other species closely nested to AZ1 were *M. irregularis* JX976261, *M. irregularis* KC461926, *M. irregularis* JX976251, *M. nidicola* HQ913647, *M. hiemalis* FN650640, *M. luteus* JN206149 and *M. zonatus* KF313126, as shown in Fig. 3. A study conducted by Asachi and Karimi [18] reported that one of the *Mucor* family members – *M. indicus* is an ethanol-producing microorganism while mycelium of *M. indicus* was used as nutrient source in the fermentation processes by cultivating the fungus on wheat straw for ethanol production. It is worth to note that this enzymatic/hydrolytic process by *M. indicus* is carried out by β-glucosidase. This enzyme is responsible for the fermentation of wheat straw, degrading its biomass and synthesizing ethanol. It has also been reported that majority of studies on ethanol production by *M. indicus* from glucose or lignocellulosic biomass have utilized nutrient media containing complex growth supplements such as yeast extract to achieve high yield concentration of ethanol.

Another species identified with the ability to degrade lignocellulosic biomass was *M. circinelloides*. Saha [19] has also found that *M. circinelloides* could actively secrete three cellulase enzymes – endoglucanase, β-glucosidase and cellobiohydrolase, which can transform various cellulose substrates to glucose. Endoglucanase from *M. circinelloides* strain is capable of utilizing corn fibre xylan and helps in the hydrolysis of cellulosic substrates in corn fibre. *M. circinelloides* was also found to have the abilities to utilize lactose and cellobiose

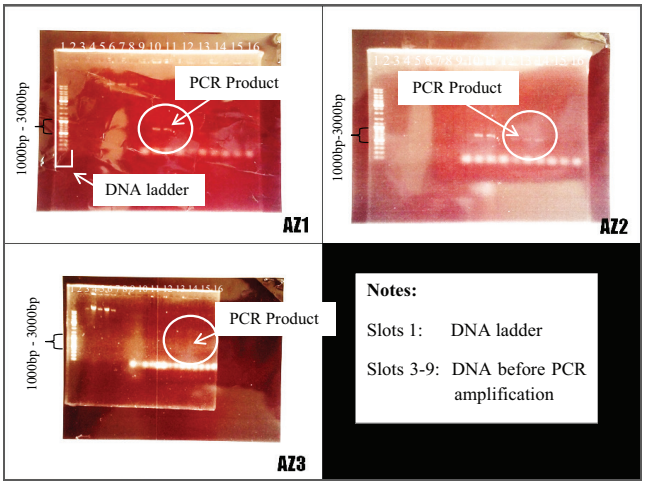

Fig. 2. PCR products of strains AZ1, AZ2 and AZ3.

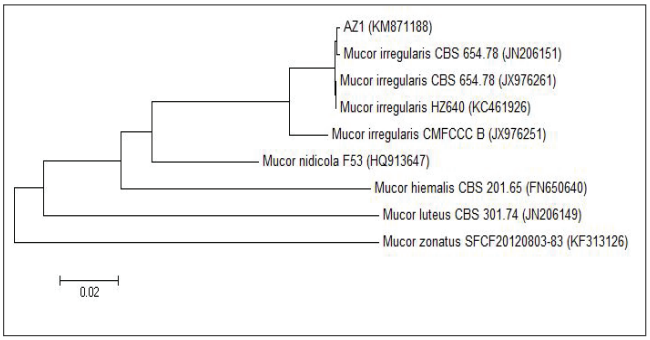

Fig. 3. Phylogenetic tree of strain AZ1.

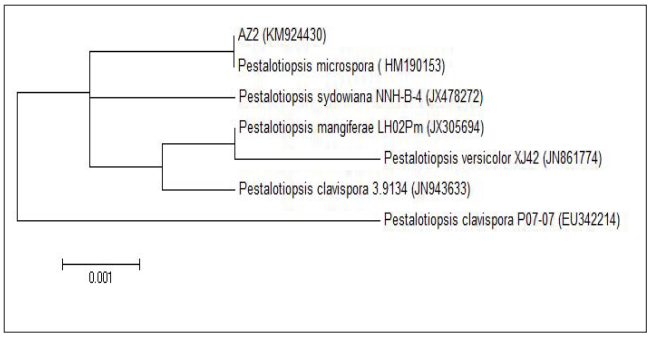

Fig. 4. Phylogenetic tree of strain AZ2.

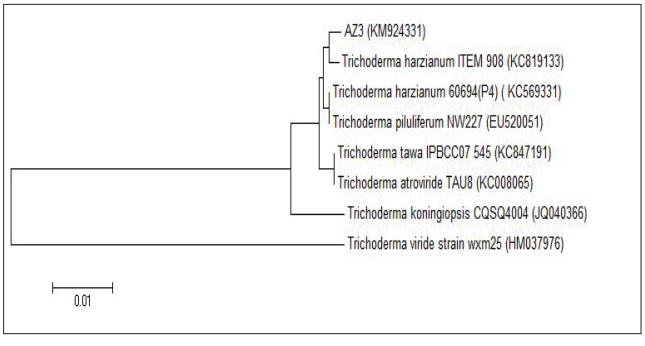

Fig. 5. Phylogenetic tree of strain AZ3.

as sole growth substrate [20]. These observations reported by Persson et al. [20], Saha [19] and Asachi and Karimi [18] agreed with the findings for *M. irregularis* AZ1 in this study. This is presumably due to them belonging to the same *Mucor* genus. AZ1 also showed positive clearing zones during qualitative assays on xylan and lignin plates as shown in Fig. 6. *M. irregularis* AZ1 is also capable of secreting xylanase and ligninase enzymes for decomposing the lignin and hemicellulose content within lignocellulosic biomass.

Based on the RBBR test, strain AZ2 can partially degrade lignocellulosic biomass and was closely nested to *P. microspora* HM190153 with sequence similarity of 99% based on ITS sequences, as indicated in Fig. 4. An accession number of KM924430 was assigned to it by GenBank. Other species identified to be closely nested to AZ2 were *P. sydowiana* JX478272, *P. mangiferae* JX305694, *P. versicolor* JN861774, *P. clavispora* JN943633 and *P. clavispora* EU342214. No previous study has been reported for *Pestalotiopsis* sp. with lignocellulosic degradation ability on raw OPMF. However, Xiaoyu et al. [21] found high laccase activity in *Pestalotiopsis* sp. J63 during the degradation of rice straw. Laccase enzyme is known to degrade the lignin component of rice straw into small molecular substrates such as ferulic acid and ρ-coumaric acid, which serves as laccase inducers. Thurston [22] states that fungal laccases were discovered to be involved in lignin degradation and in the removal of potential toxic phenols during delignification. Other researches including Hao et al. [23] have successfully isolated deuteromycete *Pestalotiopsis* sp. on a forest litter from oak tree (*Quercus variabilis*). This fungus demonstrates a strong laccase activity without any other inducers. Secretion of laccase enzymes in *Q. variabilis* was believed to come from the degradation of the biomass itself. The isolated deuteromycete *Pestalotiopsis* sp. also had decolourized 88% of the azo dye within 12 h. These findings support the preposition that the *Pestalotiopsis* genus identified for strain AZ2 in this study can degrade lignocellulosic biomass.

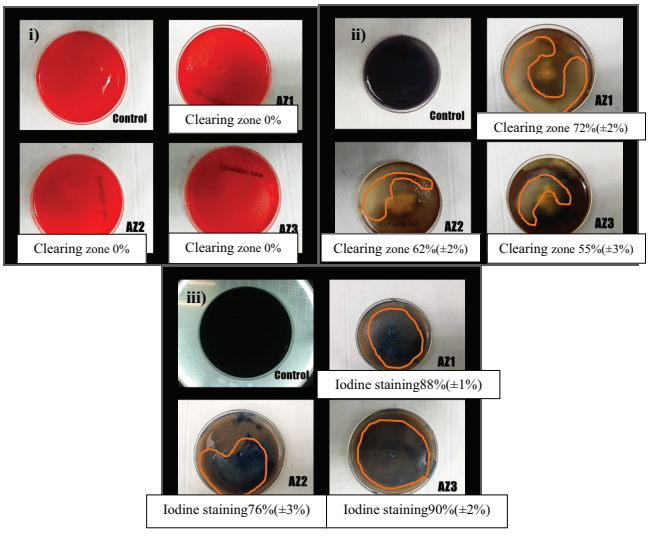

Fig. 6. (i) Qualitative screening for cellulolytic activities on CMC-agar plates with congo red staining. (ii) Qualitative screening for xylanolytic activities on agar plates. (iii) Qualitative screening for ligninolytic activity on agar plates with iodine staining agent.

The phylogenetic relationship for strain AZ3 is shown in Fig. 5. Based on this phylogenetic study, strain AZ3 was closely nested to *T. harzianum* with 99% similarity. AZ3 has an accession number of KM924331 assigned by GenBank. Other related strains include *T. pililuferum* EU520051, *T. tawa* KC847191, *T. atroviride* KC008065, *T. koningiopsis* JQ040366 and *T. viride* HM037976. Studies conducted by Delabona et al. [24,25] have reported that *T. harzianum* P49411 had biomass degradation ability and was used for decomposing wood sample from Amazon forest due to its ability to secrete xylanase enzyme. Xylanase enzyme secreted from *T. harzianum* P49411 improvises the cellulolytic hydrolysis of pre-treated sugarcane bagasse. Besides this function, *T. harzianum* IOC-4038 have the potential of producing cellulase and xylanase to degrade sugarcane bagasse for bioethanol production [26]. In addition to this, Saha [19] also reported that *T. koningii*, *T. reesei* and *T. viride* may synthesize endoglucanases for cellulose degradation in corn fibre xylan. The endoglucanase secreted from strain AZ3 identified as *Trichoderma* strains can efficiently degrade xylan substrates. However, no study has reported the use of *T. harzianum* for OPMF degradation.

3.2. Qualitative screening for lignocellulose degradation

Qualitative screening for the hydrolysis of lignocellulose was conducted as a confirmatory analysis to support the RBBR test. This analysis shows the abilities of fungal strains in degrading the cellulose, hemicellulose and lignin components contained within lignocellulosic biomass. Based on the observations made from this screening analysis, the white-rot fungi degraded more lignin substrate than cellulose substrate. The formation of a fungi growth area also referred to as the clear zones was monitored for an incubation period of 3 d on selective agar plates.

As shown in Fig. 6(i), strains AZ1, AZ2 and AZ3 did not produce cellulase enzyme, and therefore, no clearing zones surfaced on the culture plates with CMC after 3 d incubation period. CMC is the primary substrate that triggers endoglucanase activity in cells, and it was used as a suitable substrate for testing endoglucanase and β-glucosidase activities in this study. Pointing [27] reported that CMC is a good indicator of cellulolytic ability because endoglucanase is generally produced in larger titres by fungi. However, the identified fungi strains used in this study – *M. irregularis* AZ1, *P. microspora* AZ2 and *T. harzianum* AZ3 were not destaining the congo red colouration of the CMC plates after 3 d incubation as shown in Fig. 6(i). This result contradicts the findings by Ang et al. [2] who reported that *Trichoderma* species (identified as strain AZ2) showed larger clear zones on CMC plates. In this study, cellulose is not easily degraded by the strains AZ1, AZ2 and AZ3 because of the prior necessity to hydrolyze the lignin and hemicellulose components of the lignocellulose biomass in order to increase the bioavailability of its embedded cellulose components as well as the surface contacts for improved enzymatic digestibility.

Xylanase is the primary enzyme responsible for the degradation of the hemicellulose components in lignocellulosic biomass. From the xylanolytic plates, all the three fungi strains investigated in this study showed positive responses for hemicellulose degradation after 3 d incubation period.

The clear zones depicting fungi growth apparent on the xylanolytic plates indicate the presence of xylanase enzyme and the degradation of hemicellulose by the fungi strains. Fig. 6(ii) shows the qualitative screening for the xylanolytic activities of strains AZ1, AZ2 and AZ3. According to Techapun et al. [28] and Ang et al. [2], the degradation of hemicellulose produces high economic valued sugars essential for microbial fuel production. This study has demonstrated that the isolated strains AZ1, AZ2 and AZ3 can hydrolyze the intermolecular linkages existing between lignin and hemicellulose and can be applied in biofuel-related fields.

Furthermore, these strains were tested for ligninolytic activities. Lignin, an aromatic compound which is structurally complex, non-carbohydrate and made up of phenylpropene units are complex structures that serve as a protective shield against biological and chemical attacks for the intracellular components of plant cells [29]. Ligninase is the key enzyme that degrades the lignin layers of a lignocellulosic biomass. In this study, the qualitative screening for lignin degradation shows that all the isolated strains AZ1, AZ2 and AZ3 synthesizes significant amounts of ligninase enzyme such as lignin peroxidase because the clear zones depicting fungi growth were apparent on the ligninolytic plates after 3 d incubation period. Likewise, the decolourization of the iodine staining agent indicates a positive drive for lignin degradation by these strains. Fig. 6(iii) shows the qualitative screening of strains AZ1, AZ2 and AZ3 for ligninolytic activities on agar plates with iodine staining indicator.

Due to the complex nature of the heteroenzymes secreted for different lignocellulosic biomass, different fungi strains might give different results for ligninolytic activities [30]. Ligninolytic enzymes may be non-specific on different lignin substrates and therefore different degradation rates may exist. This agrees with the qualitative study conducted by Ang et al. [2] for white-rot fungi's lignin degradation of wheat bran, rice straw, POME and bagasse.

The selected strains isolated from mushroom were selected to investigate their ability to utilize and pre-treat raw OPMF. Based on the qualitative assays, it was found that the strains *M. irregularis* AZ1 KM871188, *P. microspora* AZ2 KM924430 and *T. harzianum* AZ3 KM924331 have showed positive clearing zones on hemicellulose and lignin plates. This study affirms that white-rot fungi can be used as an efficient biological tool for treatment or partial degradation of raw OPMF.

4. Conclusion

Three fungal strains namely *M. irregularis*, *P. microspora* and *T. harzianum* were isolated and successfully demonstrated their abilities to partially degrade raw OPMF. This is affirmed by the presence of positive clear zones on the screening plates for ligninolytic and xylanolytic activities. These strains can hydrolyze the intermolecular linkages existing between lignin and hemicellulose. The results also indicated that the isolated fungi synthesize xylanase for the degradation of hemicellulose. Lignin and hemicellulose components of OPMF were found to be decreased after the fungal treatment; hemicellulose from 25.3% to 5.3% w/v and lignin from 25% to 16% w/v, respectively. Hollow zones formed on the screening plates were confirmed as an indication of positivity and the ability of these strains AZ1, AZ2 and AZ3 to partial degrade raw OPMF. The use of PCR, followed by fungi identification via RBBR dye decolourization after 15 d of incubation indicates the preparedness of these strains to be used in anaerobic co-digestion processes and treatment of OPMF.

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References

[1] S.H.A. Rahman, J.P. Choudhury, A.L. Ahmad, A.H. Kamaruddin, Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose, Bioresour. Technol., 98 (2007) 554–559.
[2] S.K. Ang, A. Yahya, S. Abd Aziz, M. Md Salleh, Isolation, screening, and identification of potential cellulolytic and xylanolytic producers for biodegradation of untreated oil palm trunk and its application in saccharification of lemongrass leaves, Prep. Biochem. Biotechnol., 45 (2015) 279–305.
[3] P. Kumar, D.M. Barrett, M.J. Delwiche, P. Stroeve, Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind. Eng. Chem. Res., 48 (2009) 3713–3729.
[4] G.Y. Mtui, Recent advances in pretreatment of lignocellulosic wastes and production of value added products, Afr. J. Biotechnol., 8 (2009) 1398–1415.
[5] M. Saidu, M.R. Salim, M.A.M. Yuzir, Biodegradation of palm oil mesocarp fibre by white rot fungi, Arch. Des. Sci., 64 (2012) 102–110.
[6] M. Harmsen, L. Yang, S.J. Pamp, T. Tolker-Nielsen, An update on *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal, FEMS Immunol. Med. Microbiol., 59 (2010) 253–268.
[7] P. Kelly, B. Good, R. Fitzpatrick, J.P. Hanrahan, T.D. De Waal, Development and application of a PCR diagnostic assay for the accurate and rapid identification of the nematophagous fungus *Duddingtonia flagrans*, Mycol. Res., 112 (2008) 1026–1030.
[8] W.G. Weisburg, S.M. Barns, D.A. Pelletier, D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study, J. Bacteriol., 173 (1991) 697–703.
[9] G.A. Płaza, R. Upchurch, R.L. Brigmon, W.B. Whitman, K. Ulfig, Rapid DNA extraction for screening soil filamentous fungi using PCR amplification, Polish J. Environ. Stud., 13 (2004) 315–318.
[10] Y. Terashima, K. Ogiwara, M. Kojima, C. Kubo, A. Seki, A. Fujiie, Primers based on specific ITS sequences of rDNAs for PCR detection of two fairy ring fungi of turfgrass, *Vascellum pratense* and *Lycoperdon pusillum*, Mycoscience, 43 (2002) 261–265.
[11] D. Deswal, R. Gupta, P. Nandal, R.C. Kuhad, Fungal pretreatment improves amenability of lignocellulosic material for its saccharification to sugars, Carbohydr. Polym., 99 (2014) 264–269.
[12] R. Singh, O.P. Ahlawat, A. Rajor, Identification of the potential of microbial combinations obtained from spent mushroom cultivation substrates for use in textile effluent decolorization, Bioresour. Technol., 125 (2012) 217–225.
[13] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol., 28 (2011) 2731–2739.
[14] R. Jeewon, E.C. Liew, K.D. Hyde, Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters, Mol. Phylogenet. Evol., 25 (2002) 378–392.

[15] A.R. Liu, S.C. Chen, S.Y. Wu, T. Xu, L.D. Guo, R. Jeewon, J.G. Wei, Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in *Pestalotiopsis* taxonomy, Mol. Phylogenet. Evol., 57 (2010) 528–535.
[16] D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, D.L. Wheeler, GenBank. Nucleic Acids Research, 36 (2008) D25–D30.
[17] P. Valente, F.C. Gouveia, G.A. de Lemos, D. Pimentel, J.D. van Elsas, L.C. Mendonça-Hagler, A.N. Hagler, PCR amplification of the rDNA internal transcribed spacer region for differentiation of Saccharomyces cultures, FEMS Microbiol. Lett., 137 (1996) 253–256.
[18] R. Asachi, K. Karimi, Enhanced ethanol and chitosan production from wheat straw by *Mucor indicus* with minimal nutrient consumption, Process Biochem., 48 (2013) 1524–1531.
[19] B.C. Saha, Production, purification and properties of endoglucanase from a newly isolated strain of *Mucor circinelloides*, Process Biochem., 39 (2004) 1871–1876.
[20] I. Persson, F. Tjerneld, B. Hahn-Hägerdal, Fungal cellulolytic enzyme production: a review, Process Biochem., 26 (1991) 65–74.
[21] F. Xiaoyu, C. Huiying, X. Dongsheng, Y. Shanjing, Enhancement of laccase activity by marine-derived deuteromycete *Pestalotiopsis* sp. J63 with agricultural residues and inducers, Chin. J. Chem. Eng., 21 (2013) 1182–1189.
[22] C.F. Thurston, The structure and function of fungal laccases, Microbiology, 140 (1994) 19–26.
[23] J. Hao, F. Song, F. Huang, C. Yang, Z. Zhang, Y. Zheng, X. Tian, Production of laccase by a newly isolated deuteromycete fungus *Pestalotiopsis* sp. and its decolorization of azo dye, J. Ind. Microbiol. Biotechnol., 34 (2007) 233–240.
[24] P. Delabona, J. Cota, Z.B. Hoffmam, D.A.A. Paixão, C.S. Farinas, J.P.L.F. Cairo, J.G. da Cruz Pradella, Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and α-l-arabinofuranosidase, Bioresour. Technol., 131 (2013) 500–507.
[25] P.D.S. Delabona, C.S. Farinas, M.R. da Silva, S.F. Azzoni, J.G.D.C. Pradella, Use of a new *Trichoderma harzianum* strain isolated from the Amazon rainforest with pretreated sugar cane bagasse for on-site cellulase production, Bioresour. Technol., 107 (2012) 517–521.
[26] A.M. Castro, K.C.N.R. Pedro, J.C. da Cruz, M.C. Ferreira, S.G.F. Leite, N. Pereira Jr., *Trichoderma harzianum* IOC-4038: a promising strain for the production of a cellulolytic complex with significant β-glucosidase activity from sugarcane bagasse cellulignin, Appl. Biochem. Biotechnol., 162 (2010) 2111–2122.
[27] S.B. Pointing, Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi, Fungal Diversity, 2 (1999) 17–33.
[28] C. Techapun, N. Poosaran, M. Watanabe, K. Sasaki, Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review, Process Biochem., 38 (2003) 1327–1340.
[29] A. Breen, F.L. Singleton, Fungi in lignocellulose breakdown and biopulping, Curr. Opin. Biotechnol., 10 (1999) 252–258.
[30] C. Wan, Y. Li, Fungal pretreatment of lignocellulosic biomass, Biotechnol. Adv., 30 (2012) 1447–1457.