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Research

Isolation and Characterization of Cellulolytic Fungi From Decomposing Rice Straws

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ABSTRACT

Rice straw is an agricultural waste that is normally produced after the paddy is harvested. Rice straw, which is high in cellulose content, makes it difficult to degrade. It is burnt away by most farmers as this method saves time and labor. However, the burning of rice straws could have a serious impact on the environment and human health in general. To overcome this, biodegradation using fungi should be applied in degrading the cellulosic waste. In this study, rice straws from Bagan Serai, Perak were collected. Decomposing of rice straws was prepared by adding coffee residue, cow and chicken dung, and phosphate fertilizer. A total of 18 isolates were isolated and purified from the sample and the isolates were brought further to macroscopic by observing the morphology. Morphological and microscopic characterization using a microscope was performed where the structure of the isolate was observed and their respective genus level was deduced. Among the 18 isolates, most of them were *Aspergillus*. Next, cellulase screening was done using carboxymethylcellulose (CMC) agar with Gram iodine staining. Isolate C7 showed the largest diameter of the halo zone at 48 h of incubation whereas isolate 4D has the most significant increase of halo zone in 24-h duration.

Key words: Biodegradation, cellulase, cellulolytic fungi, rice straws

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INTRODUCTION

Rice straws or paddy straws are one of the vegetative parts of rice. During the production of paddy, 60% of rice crops are made up of rice straws (Harun, 2017). This type of residue is often used as animal feed or plowed into the soil. Rice straws have 40% cellulose, 35% Hemicellulose, 10% lignin, and 5% silica (Kahar, 2013). However, processing rice straws can take a long period and labor, hence, farmers do not prefer to degrade the rice straws as this method have the potential to breed pathogen and pest because of the moist environment that favors the growth of pathogen. The breed of pathogen might affect the soil quality in terms of safety for the crops. Hence, most farmers prefer cost-effective and simple methods to handle the waste such as through direct combustion (Obi *et al*., 2016).

Burning rice straws has its benefits but this method could bring environmental harm and cause adverse effects to human health through the emission of greenhouse gases and toxic gases. In India, open-field straw burning contributes up to 0.05% of the total greenhouse gas emissions (Gadde *et al*., 2009). When the rice straw is burned it releases greenhouse gases, for instance, nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4). The release of the gas affects the atmosphere and environment and leads to global climate change due to the incomplete combustion of rice straw (Gupta *et al*., 2004; Gadde *et al*., 2009). Therefore, a more environmentally friendly and sustainable method is needed to degrade the rice straw instead of burning it away. The degraded rice straw can then be used as a biofertilizer to return the nutrients to the soil. The treated rice straws could also be used as a raw material for animal feeds as these paddy residues still contain an adequate amount of nutrients

that are beneficial for livestock such as cattle, goats, pigs, and sheep. Besides, rice straws can also be composted and used as a nutrient source for certain species of mushrooms. (Zakhary *et al*.,1984).

Microorganisms have been widely applied to degrade agricultural wastes. There had been successful practice in degrading the rice straws using cellulolytic fungi. According to studies, there are about 14,000 species of cellulolytic fungi (Dashtban *et al*., 2010). *Trichoderma viride* and *Aspergillus niger* have been used for the biodegradation of rice straw (Kausar *et al*., 2010). *Trichoderma* sp. is the commonly used species for the production of cellulase for rice straw degradation. In another report, *Humicola insolens* was also reported to produce biomass-degrading enzymes for the hydrolysis of rice straw (Kogo *et al*., 2017). In addition, *Pleurotus ostreatus* and *Penicillium* sp. were isolated from the soil and have shown promising results in rice straw degradation (Pedraza-Zapata *et al*., 2017).

The current study aimed to isolate, characterize, and perform the cellulase screening activity of cellulolytic fungi isolated from decomposing rice straws. The isolates were first obtained from the rice straws. Their morphology and microscopic characteristics were observed and their genus were deduced. The cellulase activity of isolates was screened on CMC agar by measuring the diameter of the halo zone at 24 hours and 48 hours.

MATERIALS AND METHODS

Preparation of rice straws by composting

Post-harvest rice straws from *Oryza* sp. were collected from the farmers in Bagan Serai, Perak. The rice straw samples were cut and separated into 3 batches one was added with cow dung and coffee residue, the second batch was added with chicken dung and phosphate fertilizer, and the third batch was prepared without any organic matter (as control). 1 g of the enrichment materials was added by applying layers with the rice straws (approximately 1.5 g). The cow dung and chicken dung were obtained from a small-sized farm in Jalan Parit, Selangor, and Pontian, Johor. The phosphate fertilizer was purchased from QL Eco-Green fertilizer. The cow dung and chicken dung were dried before being used. The three batches of samples were incubated for 37 days at room temperature.

Isolation of fungi

After the incubation for 37 days, 1 g of rice straw samples from each batch of fermentation was added to 9 mL of 0.8% sodium chloride solution. Ten-fold serial dilution was performed. Czapek dox agar was prepared with the addition of shredded Whatman No.1 filter paper as the sole carbon source. 100 µL of dilution samples from dilutions 10^{-3} and 10^{-4} were plated, and the inoculated plates were then incubated at 30 °C for 5 to 7 days. Fungal strains that grew on the agar were then isolated.

Subculture of fungi

A potato dextrose agar plate was prepared by adding 10% of streptomycin. The isolated fungus was subcultured and incubated under 30 °C for five days. The fungi cultures were sub-cultured periodically by obtaining the mycelia margin to maintain a pure culture. The pure culture was stored in 80% glycerol at 4 °C.

Morphological identification of cellulolytic fungi

Pure cultures of fungi were characterized by visualization and observation under a light microscope. The morphology and structure of the fungi were observed by first identifying the form, colors, and margin of the fungi. The pigmentation and opacity of the fungi culture were taken into observation. In preparing the microscopic slides, a thin layer of mycelia was cut by using a sharp razor blade or torn off a strip using a pair of needle-nosed tweezers and placed the specimen on a slide. If the fungi culture was translucent, the fungi, therefore, must be stained with methylene blue reagent which gives color depending on the species. Then with a cover slip, slight pressure was applied to flatten the section. The slide was observed under the microscope at ×4 magnification before switching to higher magnification. To obtain clearer images at ×100 magnification, an oil immersion lens was used.

The screening for cellulase activity

The isolated fungi were subcultured onto the CMC agar plate. After the plates were incubated at 30 °C for 24 h, several drops of iodine solution were dropped onto the CMC plate and waited for 2 min. The iodine solution was disposed of by using Pasteur pipettes and washed with distilled water. The presence of a clear zone was observed around the plug and the diameter of the clear zone was measured. The plates were incubated at 30 °C and observed after 48 h to view the increase in the diameter of the clear zone.

RESULTS AND DISCUSSION

Isolation of fungi from rice straws

The paddy straws obtained from Bagan Serai, Perak were prepared in 3 batches in sample bottles. The first one was added with cow dung and coffee residue, the second batch was added with chicken dung and QL phosphate fertilizer and the third batch was untreated. The treated rice straw was prepared based on composting method which was arranged in layers. The treated and untreated rice straws were degraded for 37 days under room temperature and the paddy straws from the first and second batches were shown to be darkened in color and soft in texture after the degradation. The untreated rice straw was used as a control. The importance of this step was to enrich the paddy straws which can provide the optimal environment and nutrient for the cellulolytic microbiota to degrade the rice straws. According to McDonald *et al*. (2012), the cellulose in rice straws is covalently bonded to lignin and forms hydrogen bonds with hemicelluloses which will make the microbial degradation difficult. Hence, the hemicelluloses that occupied the lignin space should be partly degraded before cellulolytic hydrolysis begins. During the degradation process, water was added to ensure moisture and optimal temperature for the microbes and the compost should be placed under a cool and dark environment to prevent overheating and kill the microorganism.

Macroscopic and microscopic characterization of the isolates.

To isolate cellulolytic fungi from paddy straws, diluted samples of rice straw were plated on modified Czapek dox agar with shredded Whatmann No.1 filter paper added. A total of 18 isolates were obtained, they were named according to the rice straw samples (Table 1). After isolation and purification, the morphology of fungal colonies on the agar plate was observed. The margin, size, shape, and color of the isolates were studied (Figures 1 & 2). The microscopic characteristics were observed by using the light microscope method. The fruiting bodies, hyphae, and spores of isolates were also observed under various magnifications.

Out of a total of eighteen isolates, 6 of them (Isolate C1, C6, C7, P4, 4D, & 5D) were deduced to belong to the genus *Aspergillus* due to their similarity in morphology or microscopic observation. Isolate C1 and C7 had similar green pigmented mycelium but isolate C7 (Figure 3g) was found to have conidiospore while C1 (Figure 3a) has chlamydospore. Isolate C6 (Figure 3f) has conidiophores with aseptate hyphae and the absence of a metula which is a structure that holds the conidiospore that is only present in *Penicillum*. Isolate P4 (Figure 4e) has conidiospore and lateral branching was observed from the hyphae (Figure 4d), the genus was deduced by referring to the hyphae growth in previous studies (Trinci, 1974). Isolate 4D (Figure 4i) has sporangiospore and round fruiting bodies while isolate 5D (Figure 4j) has chlamydospore, They were both deduced to belong to the genus *Aspergillus* based on their morphology where isolate 4D had a white, yellowish green mycelium while isolate 5D had a black filamentous margin.

Table 1. Fungal strains isolated from rice straw samples

Isolate	Rice straw samples
C1	Rice straw with cow dung and coffee residue
C2	Rice straw with cow dung and coffee residue
C3	Rice straw with cow dung and coffee residue
C4	Rice straw with cow dung and coffee residue
C5	Rice straw with cow dung and coffee residue
C6	Rice straw with cow dung and coffee residue
C7	Rice straw with cow dung and coffee residue
C8	Rice straw with cow dung and coffee residue
C9	Rice straw with cow dung and coffee residue
P1	Rice straw with chicken dung and phosphate fertilizer
P2	Rice straw with chicken dung and phosphate fertilizer
P3	Rice straw with chicken dung and phosphate fertilizer
P4	Rice straw with chicken dung and phosphate fertilizer
1D	Rice straw only
2D	Rice straw only
3D	Rice straw only
4D	Rice straw only
5D	Rice straw only

Table 2. The macroscopic and microscopic characteristics of the 18 fungal isolates

Isolates	Colony texture, margin, and color	Types of spore	Probable organism (-spp.)
C1	Greenish mycelium and the mycelium is ciliated	Chlamydospore	*Aspergillus*
C2	Yellowish-white mycelium with black dots covered	sporangiospore	*Rhizopus*
C3	White cottony mycelia with black dots covering it	sporangiospore	*Rhizopus/ Aspergillus*
C4	Pale ash-colored colony	Sporangiospore	*Rhizopus/Mucor*
C5	Light brownish mycelia	Sporangiospore	*Rhizopus/ Mucor*
C6	White scattered colony	Conidiospore	*Aspergillus*
C7	Scattered white and faint greenish mycelium	Conidiospore	*Aspergillus*
C8	Velvety-black and ciliate margin with the submerged mycelium	Condiospore	*Alternaria/Curvularia*
C9	White irregular with raised margin	Chlamydospore	*Fusarium*
P1	White and woolly margin	Sporangispore	*Rhizopus*
P2	White mycelium that has a round colony	No spore observed	*Sclerotium*
P3	Dark green mycelium (aged) and light pink mycelium with white margins	Sporangiospore	*Trichoderma*
P4	Light brown with white margins	Conidiospore	*Aspergillus*
1D	White, fluffy, and thick mycelia	Chlamydospore	*Phythium/ Candida*
2D	Scattered green turf and filamentous margin	Sporangiospore	*Rhizopus/Mucor*
3D	Black and filamentous margin	Sporangiospore	*Rhizopus*
4D	White, yellowish-green tinge mycelium	Sporangiospore	*Aspergillus*
5D	Black margins that submerged and filamentous	Chlamydospore	*Aspergillus*

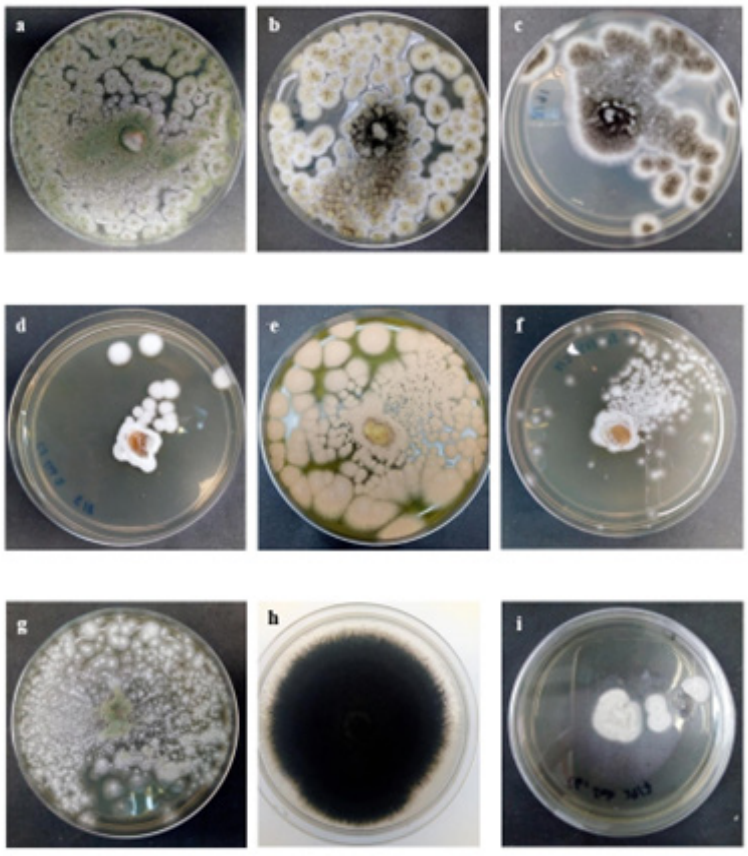

Fig. 1. Fungal isolates grown on potato dextrose agar after 5 days of incubation at 30 °C. (a) Isolates C1; (b) Isolate C2; (c) Isolate C3; (d) Isolate C4; (e) Isolate C5; (f) Isolate C6; (g) Isolate C7; (h) Isolate C8; (i) Isolate C9.

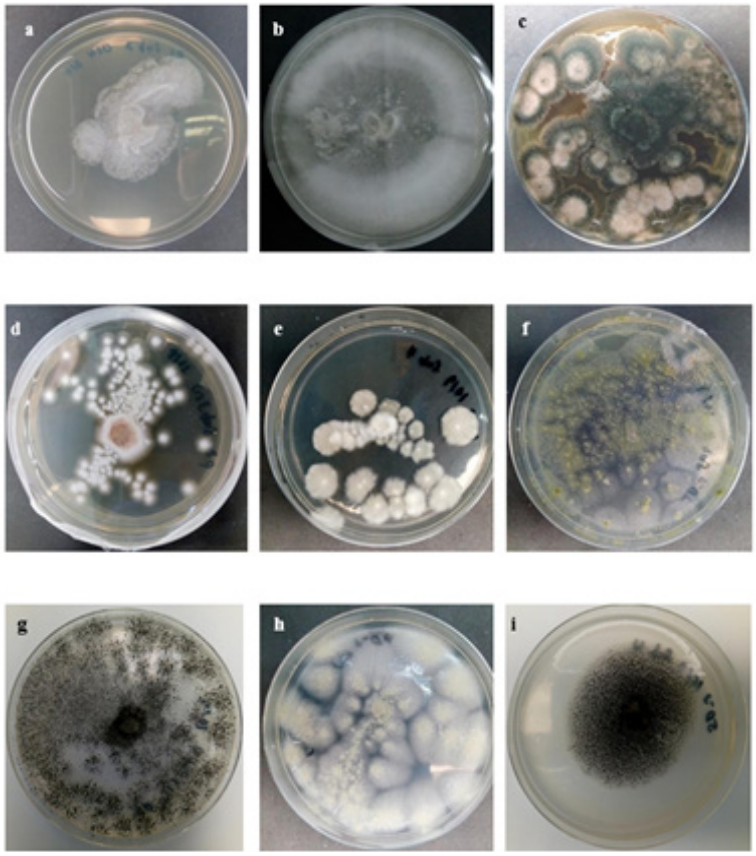

Fig. 2. Colony morphology of isolates on potato dextrose agar after 5 days of incubation under 30 °C. (a) Isolates P1; (b) Isolate P2; (c) Isolate P3; (d) Isolate P4; (e) Isolate 1D; (f) Isolate 2D; (g) Isolate 3D; (h) Isolate 4D; (i) Isolate 5D.

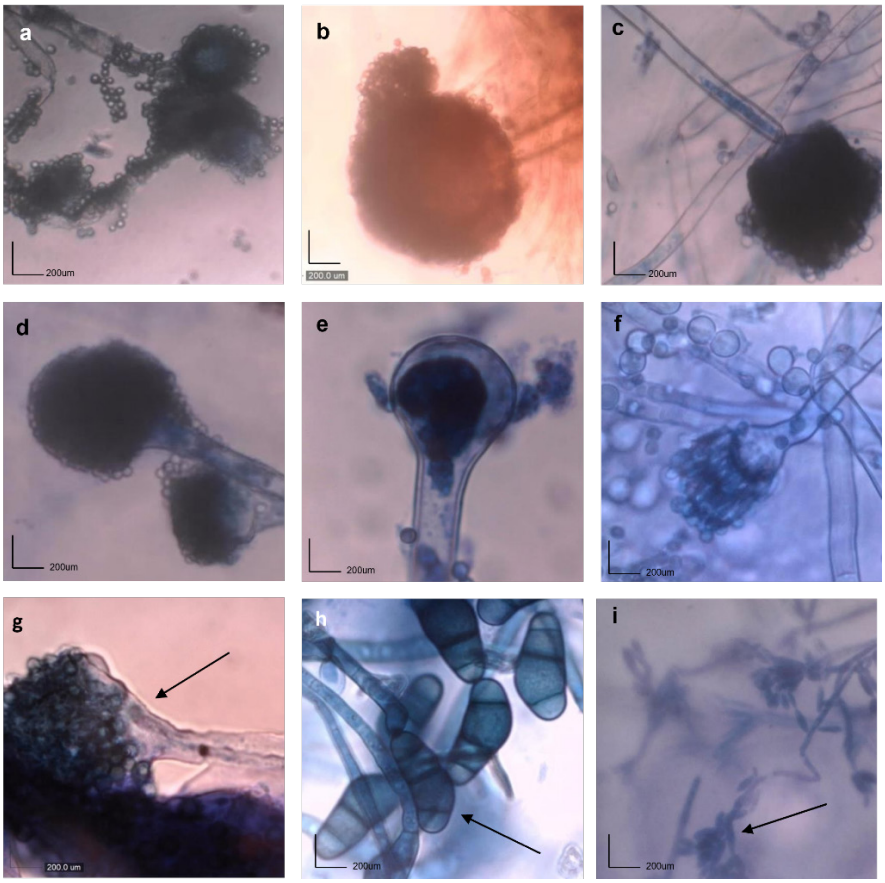

Fig. 3. Observation of isolates at 400× and 1000x magnifications. (a) Isolate C1 shows the presence of round fruiting bodies. (b) Isolate C2 with sporangiospore detected. (c) Isolate C3, round fruiting bodies were found. (d) Isolate C4, the fruiting bodies, and round spore were observed. (e) Isolate C5 with the presence of dense sporangiospore around columella. (f) Isolate C6, conidiospore was found. (g) Isolate C7, arrow indicates conidiophore. (h) Isolate C8, arrow indicates conidiospore that was distinctly septate. (i) Isolate C9 with the structure of hyphae and macroconidia (arrow). The scale bar represents 200 µm.

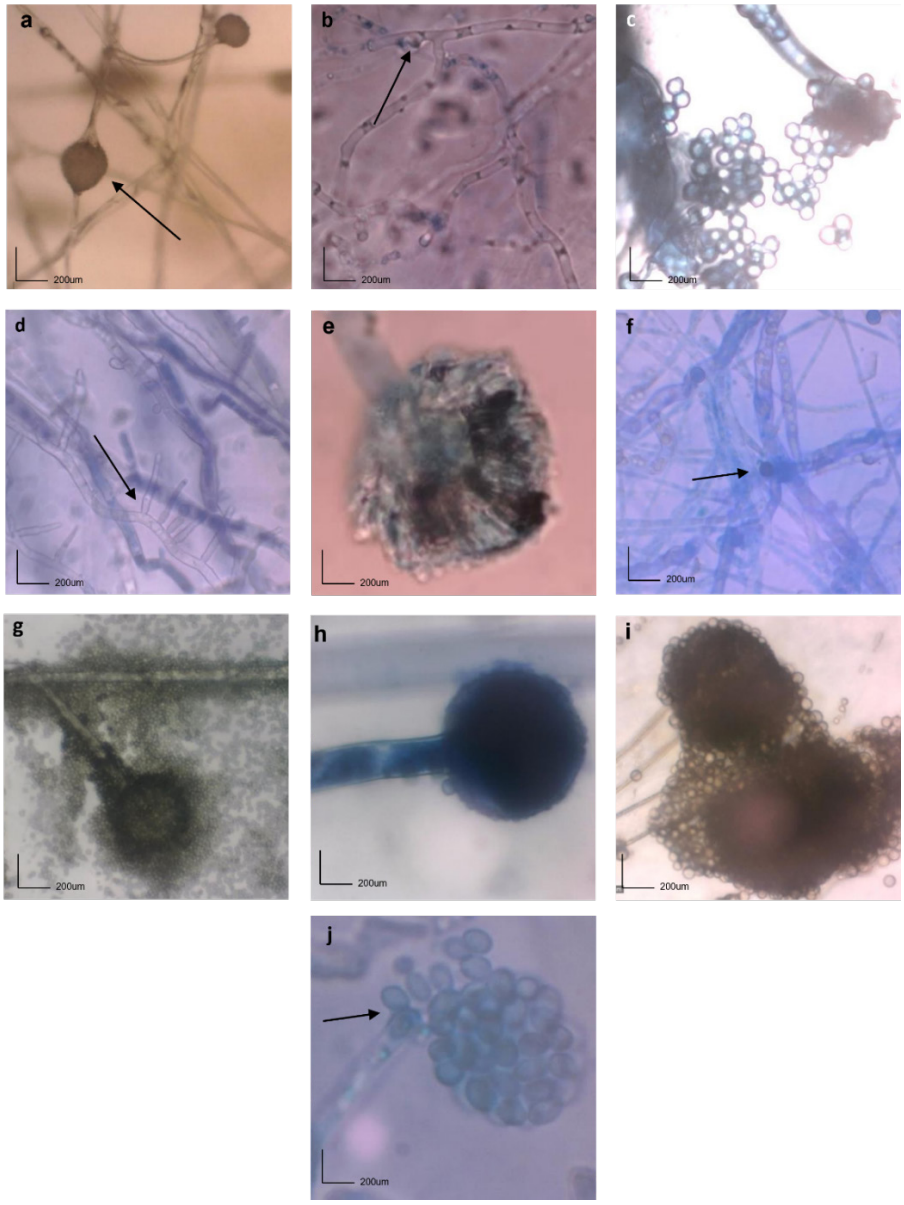

Fig. 4. Microscopic examination of isolates at 400× and 1000x magnifications. (a) Isolate P1 with the presence of sporangiospore (b) Isolate P2 has hyphae clamp connection (arrow); (c) Isolate P3 with its sporangiospore. (d) Isolate P4 with hyphae that have lateral branch initiation (arrow). (e) Conidiospore of P4. (f) Microscopic observation of Isolate 1D in which chlamydospore was observed. (g) The sporangium and sporangiospore of isolate 2D. (h) Isolate 3D with sporangium. (i) Microscopic slide of isolate 4D with its sporangium and sporangiospore; (j) Isolate 5D formed chlamydospore (arrow) and the septate hyphae were observed. The scale bar represents 200 µm.

Isolate C2 (Figure 3b), C3 (Figure 3c), C4 (Figure 3d), C5 (Figure 3e), P1 (Figure 4a), 2D (Figure 4g) and 3D (Figure 4h) were deduced to belong in genus *Rhizopus* according to their microscopic (the presence of sporangium and aseptate hyphae) and morphological characterization (Rogerson & Webster, 1981). Isolate P1 was not stained with methylene blue due to the staining procedure caused the fungal slide to have limited characteristics to observe. However, isolates C4, C5, and 2D, could belong to genus *Mucor* due to their similar structure such as sporangium and both genus have coenocytic hyphae.

In addition, isolate C8 (Figure 3h) was unique among isolates from plate C because of its distinct spore shape observed in the microscopic slides. Besides, from its velvety-black margin on culture plates, it has a distinct septate conidiospore that was slightly bent. Hence, this isolate might belong to *Alternaria* or *Curvularia* which is a type of plant pathogen based on the characteristics.

For the rest of the isolates from plate P, isolate C9 (Figure 3i) has macroconidia that was in an older stage, and based on its white and raised margin, it was deduced to belong to the genus

Fusarium. Isolate P2 (Figure 4b) was deduced to belong to the genus *Sclerotium*. Even though there were no spores observed on the microscopic slide the deduction was based on the white zonation morphology and the presence of coenocytic hyphae observed in the isolate. Furthermore, isolate P2 has a formation of clamp connection in the slides. A clamp connection is a hook-like structure that is formed by growing hyphae cells, and this can form septa that separate the hyphae. Isolate P3 (Figure 4c) was deduced to belong to the genus *Trichoderma* because they have dark green mycelium in culture and sporangiospore was found in the microscopic slide at magnification of 1000×.

For the isolates from plate D, isolate 1D (Figure 4f) was deduced to belong to the genus *Phythium* or *Candida* due to their having round chlamydospore in a microscopic slide at magnification 400×. Furthermore, the isolate had white, fluffy mycelia in culture which supported the deduction.

Of the 18 isolates obtained, most of the isolates were deduced to belong to the genus *Aspergillus* (Table 2). This might be due to their ligninolytic ability (Conesa *et al*., 2000) and this genus is oligotrophic which means they can survive environments where nutrients are scarce (Bolu *et al*., 2014). Compared with previous studies, similar isolates were isolated from rice straws, and *Aspergillus* and *Trichoderma* consortium were found to be able to significantly decompose cellulose, hemicelluloses, and lignin during the biodegradation of rice straws among other isolates (Kausar, *et al*., 2010). *Aspergillus* was also found to be the dominant genera where other 14 species were isolated in fermented rice straws based on one study (Kim *et al*., 2013).

The other deduced genus that was found from these isolates was *Rhizopus*. *Rhizopus* is a genus of saprophytic fungi that are found in a wide variety of organic substances. Their hyphae are generally coenocytic and their fruiting body is almost similar to *Aspergillus*. *Rhizopus* has been found to have the ability to produce cellulase and hemicellulase enzymes (Ofongo *et al*., 2019). Based on another study, *Rhizopus* spp. can form lactic acid if cultivated in rice straw waste (Abedinifar *et al*., 2009). Most of the isolates belong in Ascomycota because there is no presence of Basidiomycota structure found in all of the isolates. In terms of reproduction, Zygomycota, a sexual structure was only found in isolate C2 while others showed an anamorph stage because only fruiting bodies were found. This might be due to the isolates being stored in an environment that is not favorable for their growth.

However, this identification of fungi may not be accurate because some spores and structures might be identical under the same genusHence, molecular identification was needed to identify their species. The deduction was based on the fungal morphology and microscopic observation with the help of references from previous fungal identification studies (Ravimannan *et al*., 2016). The deduction was done based on the morphology and microscopic characteristics found in the isolate and the results were summarized in a table (Table 2).

Screening of cellulase activity

After the macroscopic and microscopic characterization, the cellulase activity of 18 isolates was screened by using Carboxymethylcellulose agar with gram Iodine staining. Of all 18 isolates, 17 isolates showed positive results which formed a halo zone on CMC agar. The cellulose in the CMC agar was degraded to simpler sugar and hence gave the clear zone after staining.

Based on the result (Figure 5), isolate C7 has the largest halo zone diameter (40mm) after 48 hours whereas isolate 4D showed the highest cellulase activity among the isolates because there was a significant growth of clear zone which was 20 mm of increase in diameter in 24 h. Only isolate C9 didn't have any clear zone shown throughout the same 48 h of incubation. The fungal colony might grow well on the CMC agar but it doesn't show any cellulase activity because other nutrients in CMC agar support the fungal growth. Isolate 4D is a potentially effective cellulose producer even though this isolate did not have the largest halo zone after 48 h. However, the halo zone of this isolate increased significantly in the 24 hours, which means this isolate had a high cellulose activity in 24 h. This might be because this isolate grew slower in CMC agar and it took around 48 hours to produce cellulase. Hence, isolates C7, P1, and 4D can be the main isolates for further characterization.

Rice straws are a lignocellulolytic waste that normally comes in bulk size and they are not easily degraded due to the cellulose in the vegetative structure shielded by lignin together with the hemicellulose. However, they can act as a good substrate for microbial cellulase production. Cellulase is an enzyme that can convert cellulose into glucose. Normally, cellulase production requires high cost and this makes the industrial application in bioconversion of rice straws difficult (Khan *et al*., 2007). Hence, the cellulase activity of the isolates needed to be determined to identify their effectiveness in producing cellulolytic enzymes so that they could degrade the rice straws effectively. Isolate C7 was deduced to belong to the genus *Aspergillus*, isolate P1 was deduced to belong to the genus *Rhizopus* whereas isolate 4D was deduced to belong to the genus *Aspergillus*. Based on one of the studies conducted on fungi isolated from the rice straws, the fungal isolates belong to *Aspergillus*, *Trichoderma*, *Penicillium,* and *Curvularia*. *Trichoderma harzanium* was shown to have the highest total cellulase and cellulobiohydrase activities followed by *Aspergillus niger* (Lee *et al*., 2011). Cellulobiohydrase is a cellulase that cleaves 2 to 4 units from the end of cellulose.

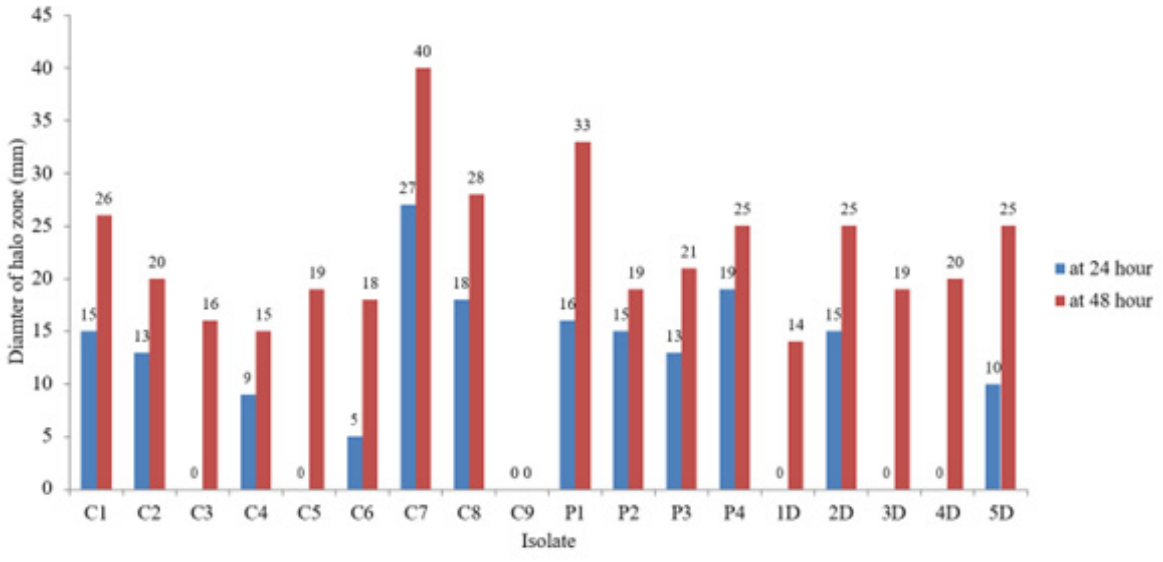

Fig. 5. Result of cellulase screening after 24 h and 48 h which showed the halo zone diameter at 24 h and 48 h.

CONCLUSION

In conclusion, 18 fungal isolates were isolated from the 3 samples of decomposing rice straw. Most of the isolates obtained were deduced to belong to *Aspergillus* and *Rhizopus* while some of the isolates were deduced to belong to plant pathogen genus such as *Curvularia*. As for cellulase production, 17 isolates showed a positive result in which the presence of a halo zone was detected on CMC agar. Isolate C7 showed the largest diameter of the halo zone followed by isolate P1. Isolate 4D showed the most significant increase of the halo zone in 24-h period incubation. Hence, these two isolates can be an effective additive in agriculture that can help farmers in solid and liquid waste management. Further molecular characterisation can be done to identify the isolates at the species level and chemotaxonomy can be done as well through GC-MS (Gas Chromatography-Mass Spectrometry) to classify the fungi based on their biochemical composition. Further study could also focus on increasing cellulase production, and gene overexpression through promoter engineering in fungi. These fungal isolates are potentially useful for rice straw degradation.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abedinifar, S., Karimi, K., Khanahmadi, M. & Taherzadeh, M.J. 2009. Ethanol production by *Mucor indicus* and *Rhizopus oryzae* from rice straw by separate hydrolysis and fermentation. Biomass and Bioenergy, 33(5): 828–833. https://doi.org/10.1016/j.biombioe.2009.01.003

Bolu, S.A., Elelu, R.N., Ahmed, F.E., Solaojo, K.F. Daramola, V.S. Omotosho, A.G. Atitebi, A. & Afeni, M. 2014. Effect of vitamins, amino acids and phyto-active biomolecules on *Aspergillus flavus* in poultry production. Pharmacology and Therapeutics, 21(2):23-44

Conesa, A., Van Den Hondel, C.A.M.J.J. & Punt, P.J. 2000. Studies on the production of fungal peroxidases in *Aspergillus niger*. Applied and Environmental Microbiology, 66(7): 3016–3023. https://doi.org/10.1128/AEM.66.7.3016-3023.2000

Dashtban, M., Schraft, H., Syed, T.A. & Qin, W. 2010. Fungal biodegradation and enzymatic modification of lignin. International Journal Biochemistry and Molecular Biology, 1(1):36-50.

Gadde, B., Bonnet, S., Menke, C. & Garivait, S. 2009. Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environmental Pollution, 157(5): 1554–1558. https://doi.org/10.1016/j.envpol.2009.01.004

Gupta, P.K., Sahai, S., Singh, N., Dixit, C.K., Singh, D.P., Sharma, C., Tiwari, M.K., Gupta, R.K. & Garg, S.C. 2004. Residue burning in rice-wheat cropping system: Causes and implications. Current Science, 87(12): 1713–1717.

Harun, R. & Ariff, E.E.E. 2017. The Role of Institutional Support in Malaysia's Paddy and Rice Industry. Food and Fertilizer Technology Center Agricultural Policy Articles. pp. 1–7.

Kahar, P. 2013. Synergistic effects of pretreatment process on enzymatic digestion of rice straw for efficient ethanol fermentation. Environmental Biotechnology-New Approaches and Prospective Applications, Interchopen. pp. 73-77. https://doi.org/10.5772/54949

Kausar, H., Sariah, M., Mohd Saud, H., Zahangir Alam, M. & Razi Ismail, M. 2010. Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. International Biodeterioration and Biodegradation, 64(7): 594–600. https://doi.org/10.1016/j.ibiod.2010.06.012

Khan, M.H., Ali, S., Fakhru'l-Razi, A. & Alam, Z. 2007. Use of fungi for the bioconversion of rice straw into cellulase enzyme. Journal of Environmental Science and Health Part B, 42(4): 381-386. https://doi.org/10.1080/03601230701312647

Kim, D.H., Kim, S.H., Kwon, S.W., Lee, J.K. & Hong, S.B. 2013. Fungal diversity of rice straw for meju fermentation. Journal of Microbiology and Biotechnology, 23(12): 1654–1663. https://doi.org/10.4014/jmb.1307.07071

Kogo, T., Yoshida, Y., Koganei, K., Matsumoto, H., Watanabe, T., Ogihara, J. & Kasumi, T. 2017. Production of rice straw hydrolysis enzymes by the fungi *Trichoderma reesei* and *Humicola insolens* using rice straw as a carbon source. Bioresource Technology, 233: 67-73. https://doi.org/10.1016/j.biortech.2017.01.075

Lee, S., Jang, Y., Lee, Y.M., Lee, J., Lee, H., Kim, G.H. & Kim, J.J. 2011. Rice straw-decomposing fungi and their cellulolytic and xylanolytic enzymes. Journal of Microbiology and Biotechnology, 21(12): 1322–1329. https://doi.org/10.4014/jmb.1107.07022

McDonald, J.E., Rooks, D.J. & Mccarthy, A.J. 2012. Methods for the Isolation of Cellulose-Degrading Microorganisms. Methods in enzymology 510:349-374. https://doi.org/10.1016/B978-0-12-415931-0.00019-7

Obi, F., Ugwuishiwu, B. & Nwakaire, J. 2016. Agricultural Waste Concept, Generation, Utilization and Management. Nigerian Journal of Technology, 35(4): 957. https://doi.org/10.4314/njt.354.1204

Ofongo, R.T.S., Ohimain, E.I. & Iyayi, E. A. 2019. Cellulase and hemicellulase activity under submerged fermentation of rice mill feed by fungi. International Journal of Environment, Agriculture and Biotechnology, 4(1): 233-239. https://doi.org/10.22161/ijeab/4.1.35

Pedraza-Zapata, D.C., Sánchez-Garibello, A.M., Quevedo-Hidalgo, B., Moreno-Sarmiento, N. & Gutiérrez-Rojas, I. 2017. Promising cellulolytic fungi isolates for rice straw degradation. Journal of Microbiology, 55(9): 711-719. https://doi.org/10.1007/s12275-017-6282-1

Ravimannan, N., Sevvel, P. & Saarutharshan, S. 2016. Study on fungi associated with spoilage of bread. International Journal of Advanced Research. Biological Science, 3(4): 165-167.

Rogerson, C.T. & Webster, J. 1981. Introduction to Fungi. 3rd Ed. Cambridge University Press, Cambridge. 165 pp.

Trinci, A.P.J. 1974. A study of the kinetics of hyphal extension and branch initiation of fungal mycelia. Microbiology, 81(1): 225-236. https://doi.org/10.1099/00221287-81-1-225

Zakhary, J.W., El-Mahdy, A.R., Abo-Bakr, T.M. & Tabey-Shehata, A.M.E. 1984. Cultivation and chemical composition of the paddy-straw mushroom (*Volvariella volvaceae*). Food Chemistry, 13(4): 265–276. https://doi.org/10.1016/0308-8146(84)90090-6