Screening of lignocellulolytic fungi for hydrolyzation of lignocellulosic materials in paddy straw for bioethanol production

Mona Fatin Syazwanee Mohamed Ghazali, Nur Ain Izzati Mohd Zainudin, Nor Azwady Abd Aziz and Muskhazli Mustafa*

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.
Email: muskhazli@upm.edu.my

Received 25 September 2018; Received in revised form 9 April 2019; Accepted 29 April 2019

ABSTRACT

Aims: Paddy straw is known to have lignocellulosic materials such as cellulose and hemicellulose which can be readily converted into fermentable sugar for production of bioethanol via simultaneous saccharification and fermentation (SSF). In order to produce ethanol competently, the degradation of biomass by cellulase and highly ethanol-producing microorganism in fermentation process are necessarily needed. However, there is lacking in cellulose degrading organism in producing adequate amount of lignocellulosic enzyme. Therefore, the screening and selection for the best fungi to hydrolyze the lignocellulosic materials as well as forming consortium between two species of fungi has become the main focus.

Methodology and results: Thirteen strains of fast-growing fungi were tested qualitatively for cellulase (congo red staining) and polyphenol oxidase (Bavendamm test). All tested strains displayed lignocellulolytic fungi characteristics. The selection was narrowed down by quantitative assay on endoglucanase, exoglucanase, β-glucosidase and xylanase and the highest cellulases enzyme producer were Trichoderma asperellum B1581 (3.93 U/mL endoglucanase; 2.37 U/mL exoglucanase; 3.00 IU/mL β-glucosidase; 54.87 U/mL xylanase), followed by Aspergillus niger B2484 (5.60 U/mL endoglucanase; 1.08 U/mL exoglucanase; 1.57 IU/mL β-glucosidase; 56.85 U/mL xylanase). In compatibility test, both T. asperellum B1581 and A. niger B2484 were inoculated on the same Petri dish for 4 days and the interaction showed by the two species was mutual intermingling.

Conclusions, significance and impact of study: Both T. asperellum B1581 and A. niger B2484 produced the highest cellulase enzyme. Since both strains can co-exist and produce enzymes that complete each other, a fungal consortium was suggested to increase the yield of sugars in saccharification process.

Keywords: Cellulose, hemicellulose, consortia, qualitative assay, quantitative assay

INTRODUCTION

In December 2017, Food and Agriculture Organization (FAO) has increased its estimation of paddy production across the globe in the year 2017 by 2.9 million tonnes to 759.6 million tonnes and China remains the largest paddy producer in the Asia region (FAO, 2018). The increased demand of rice production has led to an increase amount of world’s paddy straw production, with an estimation of at least 731 million tons per annum (Wi et al., 2013). Paddy straw is the residual of rice production and has become one of the world’s most commonly available lignocellulosic waste materials (Saini et al., 2015). Current disposal method by burning not only resulted in reducing the air quality (Rosmiza et al., 2014) but also wasting the potential of harvesting valuable carbohydrates for biofuel production as the paddy straw contains about 45-75% of complex carbohydrates (Fan et al., 2013). Bioethanol is a renewable energy source that have prospect in handling current world energy issues and the deteriorating quality of environment (Aditiya et al., 2016). Now, most technologies involve in biofuel production are focusing on turning lignocellulose biomass into transportation fuels (Bakker et al., 2013).

The first generations of biofuels are originated from crop plants, but they produced limited biofuel yields and threatening on food security (Aro, 2016). Alternative feedstock also known second-generation feedstock, are recovered from woody biomass which are more energy efficient, flexible and not competing with the human’s food resources (Havlík et al., 2011). An estimation of 442 billion liters of bioethanol can be produced from lignocellulosic waste material, which the amount is 16 times higher than the real amount of bioethanol produced globally (Sarkar et al., 2012).

*Corresponding author

DOI: http://dx.doi.org/10.21161/mjm.180250

SN (print): 1823-7538, ISSN (online): 2231-7538
The bioconversion of biomass into bioethanol involves several processes such as the pretreatment, saccharification and fermentation (Oberoi et al., 2012). Cellulolytic enzymes involve in hydrolysis of lignocellulosic materials (saccharification), which are commonly break down by cellulolytic bacteria and fungi (Belal, 2013). The by-products of the saccharification process are generally reducing sugars including glucose. According to Okamoto et al. (2011), several filamentous fungi has been explored for their capability in producing ethanol from biomass, such as the genera Mucor, Rhizopus, Neurospora, Aspergillus, Fusarium, Monilia and Trichoderma. Most of the Trichoderma sp. are fast-grower, produce large amount of conidia and variety of valuable enzymes such as cellulase (Khan et al., 2007). Cellulase is also known as cellulolytic enzymes, of which three classes are identified on the basis of the mode of the substrate specificities and enzymatic actions: endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.74 and EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) (Teeri, 1997; Kuhad et al., 2016). Only with the synergism of the above three enzymes makes the complete hydrolysis of cellulose into glucose (Gupta et al., 2012). An efficient and cost-effective enzyme system should contain balanced activities of cellulases (both endo- and exo-glucanase), β-glucosidase, and xylanase, and the system should also have high titer of enzyme activities to balance the cost of ethanol production (Brijwani et al., 2010).

The simultaneous saccharification and fermentation (SSF) processes involves enzymatic saccharification of cellulose with fermentation of glucose to ethanol (Kusmiyati et al., 2018). It has become favorable process as it improved the yield of ethanol via reducing the product inhibition excreted and also removes the need for separate reactors, which results in cost reduction and reduce the risk of contamination (Wang et al., 2013; Narra et al., 2015).

Regardless the fact that some of the fungal strains have the benefits of being lignocellulolytic and thermostable, most of these fungal strains failed to produce adequate amounts of lignocellulolytic enzymes, which are essentially required for efficient bioconversion process (Dashthban et al., 2009). According to Kausar et al. (2010), mixed cultures or consortium are able to stimulate colonization of the substrate by increasing the production of enzyme, withstand contamination by other microbes and results in high yield of sugars. However, the selection of suitable cellulase for hydrolyzation process of lignocellulosic materials is very difficult because each biomass has structural and enzymatic activities differences (Takano and Hoshino, 2018). Hence, finding appropriate lignocellulolytic fungi for degradation of paddy straw is necessarily needed. In order to address the foregoing issues, this study was commenced to screen filamentous fungi with an ability to secrete hydrolytic enzyme for substrate degradation and to test the compatibility between two different species of fungi in an attempt to produce better yield of sugars in saccharification process.

### MATERIALS AND METHODS

#### Collection of fungi

Fast growing and molecularly identified fungi strains (Table 1) were obtained from Mycology Laboratory, Faculty of Science, Universiti Putra Malaysia and School of Biological Sciences, Universiti Sains Malaysia. All strains were grown on Potato Dextrose Agar (PDA) incubated at 28 ± 2 °C for 7 days.

| Fungi species | Strain | Source | References |
|---------------|--------|--------|------------|
| T. asperellum | B1581  | Soil   | Sharifah Siti Maryam et al., 2016 |
| T. asperellum | B1584  | Soil   | Sharifah Siti Maryam et al., 2016 |
| A. niger      | B2484  | Vietnamese coriander bean | Nur Ain Izzati et al., 2017 |
| A. niger      | C2472  | Winged bean | Nur Ain Izzati et al., 2017 |
| F. oxysporum  | B633T  | Tomato | Nur Baiti et al., 2016 |
| F. oxysporum  | B645T  | Tomato | Nur Baiti et al., 2016 |
| F. fujikuroi  | 688    | Rice   | - |
| F. fujikuroi  | 4851   | Rice   | - |
| F. fujikuroi  | 4872   | Rice   | - |
| C. lunata     | P1221  | Rice   | Nur Ain Izzati et al., 2019 |
| C. lunata     | P1244  | Rice   | Nur Ain Izzati et al., 2019 |
| C. eragrostidis| P1262  | Rice   | Nur Ain Izzati et al., 2019 |
| C. eragrostidis| K872   | Rice   | Nur Ain Izzati et al., 2019 |

#### Qualitative assays of lignocellulolytic activity

**Cellulose degradation**

A 5-mm mycelial disc was cut using cork borer from the edge of fungal culture (7 days old) and transferred to a prepared media (carboxymethylcellulose sodium salt 20.0 g/L; agar 15.0 g/L) before incubated at 28 ± 2 ºC for 7 days as proposed by Mukhlis et al. (2013). The plate was stained with 1 mg/mL Congo red solution and formation of halo ring (clear zonation) around the fungal colony indicates the cellulase activity of the fungal (Kausar et al., 2010). The diameter of halo ring was measured and enzymatic index (EI) were calculated based on formula of Enzymatic Index = Diameter of Halo Ring / Diameter of Growth Colony (Florencio et al., 2012).
Bavendamm test for laccase detection

Presence of polyphenol oxidase (PPO) indicates the existence of laccase. A disc of fungal hyphae was placed at the center of the prepared media (Tannic acid 5.0 g/L; Malt extract agar 15.0 g/L; Difco Agar 20.0 g/L) and incubated for 7 days a 28 ± 2 °C in dark condition to determine the presence of polyphenol oxidase (PPO) activity (Thormann et al., 2002). PPO was used to indicate laccase activity on lignin and the diameter of brown pigmentation formed around fungal colony was measured generally after five days.

Quantitative assays

The quantitative assays were also carried out to determine the activity of cellulase and hemicellulase enzymes in order to narrowing down to the best fungi producing hydrolytic enzyme. An amount of 1 × 10⁶ spore/mL were cultured in basal medium ([NH₄]₂SO₄ 1.4 g/L; KH₂PO₄ 2.0 g/L; CaCl₂ 0.3 g/L; MgSO₄·7H₂O 0.3 g/L; CoCl₂ 2.0 g/L) added with 1 mL of trace element (MnSO₄·H₂O 1.56 g/L; FeSO₄·7H₂O 6.0 g/L; ZnSO₄·7H₂O 1.4 g/L) for 7 days at 150 rpm and 30 ± 1 °C (Ja’afaru, 2013) before crude culture was collected for quantitative assay.

Endoglucanase, exoglucanase and xylanase assays

The cellulase and hemicellulase enzymes were assayed using 3, 5-dinitrosalicyclic acid (DNS) method (Miller, 1959). Carboxymethyl-cellulose (CMC), Avicel® PH-101 and xylan from corn were used as substrate for endoglucanase, exoglucanase and xylanase assay, respectively. An amount of 0.09 mL substrate-buffer (1% (w/v) substrate; 0.05 M sodium acetate pH 4.8) was mixed with 0.01 mL of crude enzyme and incubated at 50 ± 1 °C for 30 min before 0.1 mL DNS were added to stop the reaction (Das et al., 2012; Ja’afaru, 2013). The absorbance was read at 540 nm and the amounts of sugar were determined using glucose standard (for endoglucanase and exoglucanase) and xylose standard (for xylanase). One Unit (U) of enzyme activity was defined as the release of 1 μmol of reducing sugar per min.

β-glucosidase assay

A mixture of 0.025 mL of substrate-buffer (1% (w/v) 4-nitrophenol β-D glucopyranoside; 0.05 M sodium acetate pH 4.8) and 0.025 mL of crude enzyme were incubated at 50 ± 1 °C for 15 min before been added with 0.05 mL 1 M Na₂CO₃ to stop the reaction (Jeya et al., 2010). The absorbance was read at 400 nm and the amount of p-nitrophenol (pNP) released was quantified based on pNP standard. One International Unit (IU) of β-glucosidase activity was expressed as the amount of enzyme required to release 1 μmole of pNP per min under the assay conditions.

Compatibility test

Seven days old fungal mycelial discs of selected fungi were place on the same PDA with a gap of 4 cm from each other (Kausar et al., 2010). The plate was incubated at 28 ± 1 °C. Their growth rate and pattern were observed everyday till full grown; which the fungi mycelia fully occupied the surface of the agar (approximately 5 days). Mode of interaction between the filamentous fungi was observed and identified using a key reference by Mohammad et al. (2011) as shown in Table 2.

| No. | Interaction          | Definition                                                                 |
|-----|---------------------|---------------------------------------------------------------------------|
| 1   | Mutual intermingling| Both fungi successfully grow into each other without any macroscopic indication of interconnections. |
| 2   | Partial intermingling| One of the funguses grows into the other fungus either above/below without forming any inhibition zone. |
| 3   | Invasion/ replacement| One mycelium propagates into the other and begins to ingest the weaker one before colonize it. |
| 4   | Inhibition at contact point| The fungi almost make contact with each other with a fine segregation line (1 to 2 mm) between the two colonies. |
| 5   | Inhibition at distance| Inhibition at a distance of more than 2 mm                                  |

RESULTS AND DISCUSSION

Microorganisms in which secretes cellulase have a major role in the environment, as they have decaying ability of biomass, and hence creating a crucial relation in the carbon cycle (Zhang et al., 2006; Damaso et al., 2012). Many studies have been highlighted on fungi producing cellulases as they are abundant, easy to extract and some of the fungal cellulases have been used widely for commercials (Liang et al., 2014). All thirteen strains of molecularly identified fungi (Table 1) were selected based on their origin and ability as fast-growing fungi. However, their lignocellulolytic characteristics have yet to be tested.

In early screening, qualitative assays were performed to screen the quality of cellulase and laccase secreted by the fungi. In the qualitative screening, all carboxymethyl-cellulose (CMC) was used to raise the molecular disorder of the densely arranged cellulose network and to uncover the cellulose chains buried within the microfibrils (Arantes and Saddler, 2010). Meanwhile the inefficiency of Congo red dye to bind with mono or disaccharides leading to a visible clear zone formation known as halo ring (Gohel et al., 2014) and relatively the diameter of halo ring produced is proportionate with the level of cellulase
activity. An enzymatic index of ≥ 0.5 is considered to be the best producers of cellulase enzyme. As shown in Table 2, out of 13 strains assayed, only 8 were found to have index more than 0.5. The largest enzymatic index (EI) was produced by *Trichoderma asperellum* B1584 at the value of 0.88. While the smallest EI value was produced by *Curvularia eragrostidis* P1262 with 0.25. However, there was no significant difference between all the samples tested.

Theoretically in Bavendamm test, utilization of phenolic compounds such as gallic acid or tannic acid in nutrient agar by the white rot fungi produce a deeply brown colored zone around the mycelium and this zone has become an indicator for the quality of the fungi polyphenol oxidase (PPO) activity (Mushimiyimana and Tallaprakada, 2014). The ratio of brown pigmentation to nutrient agar by the white rot fungi produce a deeply brown colored zone around the mycelium and this zone has become a conundrum in producing bioethanol.

The high production of exoglucanase; also known as cellulolohydrolase (CBH) is essential as they represent 60% of the enzyme cocktail population; primary exocellulase and responsible to degrades cellulose into cellobiose (Brady et al., 2015). According to Wang et al. (2012), the optimized ratios of cellulolytic enzymes for the best saccharification of crystalline cellulose is 60% CBH I: 20% CBH II: 20% endoglucanase. β-glucosidase plays a significant role in bioethanol production to eliminate cellobiose inhibition (Wang et al., 2012) and it has become a conundrum in producing bioethanol.

### Table 3: Qualitative assessment on fungi strains for selection of the best fungi producing hydrolytic enzymes.

| Fungi Strain | Qualitative Assay |
|--------------|-------------------|
|              | Cellulase (EI)**  | Laccase (ratio) |
| *A. niger* B2484 | 0.78<sup>ab</sup> | 1.01<sup>f</sup> |
| *A. niger* C2472 | 0.64<sup>abc</sup> | 1.00<sup>f</sup> |
| *C. eragrostidis* P1262 | 0.25<sup>abc</sup> | 1.60<sup>a</sup> |
| *C. eragrostidis* K872 | 0.26<sup>abc</sup> | 1.55<sup>ab</sup> |
| *C. lunata* P1221 | 0.38<sup>c</sup> | 1.43<sup>bcd</sup> |
| *C. lunata* P1244 | 0.36<sup>bc</sup> | 1.45<sup>bc</sup> |
| *F. fujikuroi* 688 | 0.49<sup>abc</sup> | 1.32<sup>bcd</sup> |
| *F. fujikuroi* 4972 | 0.58<sup>abc</sup> | 1.31<sup>bcd</sup> |
| *F. fujikuroi* 4851 | 0.58<sup>abc</sup> | 1.18<sup>abcd</sup> |
| *F. oxysporum* B633T | 0.61<sup>abc</sup> | 1.13<sup>def</sup> |
| *F. oxysporum* B645T | 0.62<sup>abc</sup> | 1.10<sup>ef</sup> |
| *T. asperellum* B1581 | 0.68<sup>abc</sup> | 1.29<sup>bcd</sup> |
| *T. asperellum* B1584 | 0.88<sup>a</sup> | 1.35<sup>bcde</sup> |

Values are means of three replicates with ±SD.

*Means in each column with same superscript letter are not significantly different amongst themselves when Tukey test were used at 5% significance level.

**Enzymatic Index (EI) is a measurement for cellulase activity.

### Table 4: Qualitative and quantitative assessment on fungi strains for selection of the best fungi producing hydrolytic enzymes.

| Fungi Strain | Endoglucanase (U/mL) | Exoglucanase (U/mL) | β-glucosidase (IU/mL) | Xylanase (U/mL) |
|--------------|----------------------|---------------------|----------------------|----------------|
| *A. niger* B2484 | 5.60 ± 0.43<sup>a</sup> | 1.08 ± 0.17<sup>bcdef</sup> | 1.57 ± 0.02<sup>b</sup> | 56.85 ± 2.75<sup>a</sup> |
| *A. niger* C2472 | 1.83 ± 0.15<sup>a</sup> | 1.75 ± 0.20<sup>abc</sup> | 0.05 ± 0.03<sup>c</sup> | 27.77 ± 2.69<sup>d</sup> |
| *F. fujikuroi* 688 | 0.98 ± 0.01<sup>a</sup> | 1.01 ± 0.14<sup>cdef</sup> | 0.09 ± 0.04<sup>c</sup> | 56.00 ± 2.82<sup>a</sup> |
| *F. fujikuroi* 4872 | 2.14 ± 0.09<sup>a</sup> | 0.25 ± 0.14<sup>c</sup> | 0.03 ± 0.02<sup>c</sup> | 37.69 ± 1.39<sup>cdef</sup> |
| *F. fujikuroi* 4851 | 0.95 ± 0.04<sup>a</sup> | 1.17 ± 0.16<sup>bcdef</sup> | 0 | 32.04 ± 9.19<sup>c</sup> |
| *F. oxysporum* B633T | 1.10 ± 0.09<sup>a</sup> | 0.95 ± 0.23<sup>cdef</sup> | 0 | 43.41 ± 6.92<sup>b</sup> |
| *F. oxysporum* B645T | 0.93 ± 0.06<sup>a</sup> | 2.04 ± 0.53<sup>ab</sup> | 0 | 42.28 ± 2.06<sup>bcd</sup> |
| *T. asperellum* B1581 | 3.93 ± 0.05<sup>a</sup> | 2.37 ± 0.34<sup>a</sup> | 3.00 ± 0.15<sup>a</sup> | 54.87 ± 8.23<sup>a</sup> |
| *T. asperellum* B1584 | 1.69 ± 0.10<sup>a</sup> | 2.00 ± 0.49<sup>ab</sup> | 0.03 ± 0.02<sup>c</sup> | 34.55 ± 4.69<sup>c</sup> |

Values are means of three replicates with ±SD.

*Means in each column with same superscript letter are not significantly different amongst themselves when Tukey test were used at 5% significance level.
One of the major challenges in producing bioethanol is to search for a glucose tolerant β-glucosidase as they are the key component present in cellulase to complete the final step during cellulose hydrolysis and conversion process of celllobiose into glucose (Singhania et al., 2012). Currently, commercial cellulase was prepared using T. reesei due to its high activities of both exoglucanase and endoglucanase (Sohail et al., 2009). However, low levels of β-glucosidases in T. reesei have been complemented by mixing β-glucosidase from A. niger in T. reesei cellulolytic cocktail (Del Pozo et al., 2012; Rani et al., 2014). As in this study, combining these two species is expected to produce higher glucose yield for bioethanol production since T. asperellum B1581 obtained high exoglucanase and β-glucosidase activities while A. niger B2484 attained high endoglucanase and xylanase activities. In relation to qualitative assays, it was found that the diameter of clearance zone (in Congo red) exhibited almost a linear relationship with the enzyme activity.

Microbial interactions are usually referred as hostile, but they can also be supportive, related with a metabolic change of each partner and cause a modification in the arrangement of produced bioactive molecules (Benoit-Gelber et al., 2017). Consortia are a favorable method that could help to understand the synergistic effects between mixed enzymes for the biodegradation of lignocellulosic waste (Taha et al., 2015). The formation of consortia could be beneficial by eliminating problems such as incomplete synergistic enzymes and inhibition of end-product (Sheng et al., 2016). Through consortia, cellulase production was enhanced and the composition of cellulolytic enzymes was improved (Wen et al., 2005; Fang et al., 2013). Commercially, enzyme sets of T. reesei for cellulose degradation are often complemented with β-glucosidases from A. niger by combining T. reesei’s Celluclast® and A. niger’s β-glucosidase Novozym188® (Brink et al., 2014). A study by Liu et al. (2017) shows Napier grass could be utilized consortia of T. reesei and A. niger to produce glucose for bioethanol production by Zygomonas mobilis. However, according to Silva (2016), major obstacle is the compatibility since formation of consortia involves inter-relationship in managing the interaction, waste, space and resource to achieve optimum mutualism. Therefore prior to any fungi application as consortia for production, the compatibility test for fungal consortia is essential. In compatibility test, both T. asperellum B1581 and A. niger B2484 were grown on a same plate and in-vitro interaction between T. asperellum B1581 and A. niger B2484 after 5 days of incubation shown no negative interaction (Figure 1). The interaction was mutual intermingling growth where the fungus being observed is growing into the opposed fungus either above or below its colony. There was no visible boundary formed which indicates that both T. asperellum B1581 and A. niger B2484 can live together in the same culture. Since both of the strains can mutually co-exist, it was suggest mixing the cellulolytic cocktails of different fungi strains could enhance the end-product after saccharification process (Kalyani et al., 2013).

**CONCLUSION**

Out of thirteen strains of filamentous fungi which were qualitatively and quantitatively assessed, the best two fungi strain were T-asperellum B1581 produces the highest exoglucanase and β-glucosidase (2.37 ± 0.34 U/mL; 3.00 ± 0.15 IU/mL respectively), while A. niger B2484 produces the highest endoglucanase and xylanase (5.60 ± 0.43 U/mL; 56.85 ± 2.75 U/mL respectively). Both of the fungi strain also showed their ability to formed mutual intermingling consortia in the in-vitro interaction of compatibility test. Since both of the strain can co-exist and produces enzymes that complete each other, the fungal consortium between T.asperellum B1581 and A.niger B2484 was suggested to increase the yield of sugars in saccharification process.

**ACKNOWLEDGEMENTS**

The authors would like to thank Universiti Putra Malaysia for the financial support through Geran Putra - Inisiatif Putra Siswaaz GP-IPS/2016/9485600 and all staff of the Plant Systematic and Microbe Laboratory, Biology Department, Universiti Putra Malaysia for all their efforts.

**REFERENCES**

Aditiya, H. B., Mahlia, T. M. I., Chong, W. T., Nur, H. and Sebayang, A. H. (2016). Second generation bioethanol production: A critical review. Renewable and Sustainable Energy Reviews 66, 631-653.

Arantes, V. and Saddler, J. N. (2010). Access to cellulose limits the efficiency of enzymatic hydrolysis: the role of amorphogenesis. Biotechnology for Biofuels 3, 4-15.

Aro, E. M. (2016). From first generation biofuels to advanced solar biofuels. Ambio 45(1), 24-31.

Bakker, R., Elbersen, W., Poppens, R., and Lesschen, J. P. (2013). Rice straw and wheat straw management. In: Rice Straw and Wheat Straw. Potential feedstocks for the biobased economy. Bakker, R., Elbersen, W., Poppens, R., and Lesschen, J. P. (eds.). NL Agency Ministry of Economic Affairs, Netherlands. pp. 11-16.
Belal, E. B. (2013). Bioethanol production from rice straw residues. Brazilian Journal of Microbiology 44(1), 225-234.

Benoi-Gelter, I., Gruntjes, T., Vinck, A., Veluw, J. G., Westen, H. A. B., Boeren, S., Vervoort J. M. and Vries, R. P. (2017). Mixed colonies of Aspergillus niger and Aspergillus oryzae cooperatively degrading wheat bran. Fungal Genetics and Biology 102, 31-37.

Brady, S. K., Sreeelatha, S., Feng, Y., Chundawat, S. P. S. and Lang, M. J. (2015). Cellulobiohydrolase 1 from Trichoderma reesei degrades cellulose in single cellobiose steps. Nature Communications 6, 10149.

Brijwani, K., Oberoi, H. S. and Vadhani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. Process Biochemistry 45, 120-128.

Brink, J. V., Maitan-Alfenas, G. P., Zou, G., Wang, C., Zhou, Z., Guimaraes, V. M. and Vries, R. P. (2014). Synergistic effect of Aspergillus niger and Trichoderma reeseei enzyme sets on the saccharification of wheat straw and sugarcane bagasse. Biootechnology Journal 9, 1229-1338.

Damaso, M. C. T., Terzi, S. C., Farias, A. X., Oliveira, A. C. P., Fraga, M. E. and Couri, S. (2012). Selection of cellulolytic fungi isolated from diverse substrates. Brazilian Archives of Biology and Technology 55(4), 513-520.

Das, A., Ghosh, U., Mohapatra, P. K. D., Pati, B. R. and Mondal, K. C. (2012). Study on thermodynamics and adsorption kinetics of purified endoglucanase (cmcase) from Penicillium notatum NCIM no-923 produced under mixed solid-state fermentation of waste cabbage and bagasse. Brazilian Journal of Microbiology 43(3), 1103-1111.

Dashbant, M. Schraft, H. and Qin, W. (2009). Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by Trichoderma strains. Enzyme Research 2012, 1-7.

Geetha, R., Contractor, C. N., Ghosh, S. K. and Braganza, V. J. (2014). A comparative study of various staining techniques for determination of extracellular cellulase activity on Carboxy Methyl Cellulose (CMC) agar plates. International Journal of Current Microbiology and Applied Sciences 3(5), 261-266.

Gupta, P., Samant, K. and Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. International Journal of Microbiology 2012, 1-5.

Havlik, P., Schneider, U. A., Schmid, E., Bottcher, H., Fritz, S., Skalsky, R., Aoki, K., Cara, S., Kindermann, G., Kraxner, F., Leduc, S., McCullum, I., Mosnier, A., Sauer, T. and Obersteiner, M. (2011). Global land-use implications of first and second generation biofuel targets. Energy Policy 39, 5690-5702.

Ja’afaru, M. I. (2013). Screening of fungi isolated from environmental samples for xylanase and cellulase production. ISRN Microbiology 2013, 1-7.

Jeya, M., Nguyen, N. P. T., Moon, H. J., Kim, S. H. and Lee, J. K. (2010). Conversion of woody biomass into fermentable sugars by cellulase from Agaricus arvensis. Bioresource Technology 101, 8742-8749.

Kalyani, D., Lee, K. M., Kim, T. S., Li, J., Dhiman, S. S., Kang, Y. C. and Lee, J. K. (2013). Microbial consortia for saccharification of woody biomass and ethanol fermentation. Fuel 107, 815-822.

Kausar, H., Sariah, M., Mohd Saud, H., Zahangir Alam, M. and Ismail, M. R. (2010). Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. International Biodeterioration and Biodegradation 64(7), 594-600.

Khan, M. M. H., Ali, S., Fakhru’l-Razi, A. and Alam, M. Z. (2007). Use of fungi for the bioconversion of rice straw into cellulase enzyme. Journal of Environmental Science and Health Part B 42, 381-386.

Kuhad, R. C., Deswal, D., Sharma, S., Bhattacharya, A., Jain, K. K., Kaur, A., Pletschke, B. I., Singh, A. and Karp, M. (2016). Revisiting cellulase production and redefining current strategies based on major challenges. Renewable and Sustainable Energy Reviews 55, 249-272.

Kusmiyati, K., Mustofa, A. and Jumarmi (2018). Bioethanol production from banana stem by using simultaneous saccharification and fermentation (SSF). IOP Conference Series: Materials Science and Engineering 358, 012004.

Liang, Y.L., Zhang, Z., Wu, M., Wu, Y. and Feng, J. X. (2014). Isolation, screening, and identification of cellulolytic bacteria from natural reserves in the subtropical region of China and optimization of the addition of surfactants. Biotechnology and Bioprocess Engineering 18, 390-398.
cellulase production by Paenibacillus terrae ME27-1. BioMed Research International 2014, 1-13.
Liu, Y.K., Chen, W.C., Huang, Y.C., Chang, Y.K., Chu, I.M., Tsai, S.L. and Wei, Y.H. (2017). Production of bioethanol from Napier grass via simultaneous saccharification and co-fermentation in a modified bioreactor. Journal of Bioscience and Bioengineering 124(2), 184-188.
Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, 426-428.
Mohammad, N., Alam, M.Z., Kabashi, N.A. and Adebayo, O.S. (2011). Development of compatible fungal mixed culture for composting process of oil palm industrial waste. African Journal of Biotechnology 10, 18657-18665.
Mukhlis, Mohd Saud, H., Sariah, M., Razi Ismail, M., Habib, S. H. and Kausar, H. (2013). Potential lignocellulolytic Trichoderma for bioconversion of oil palm empty fruit bunches. Australian Journal of Crop Science 7(3), 425-431.
Mushimiyimana, I. and Tallapragada, P. (2014). Screening and optimization of lignocellulose degrading fungi using agro wastes as substrates. Acta Biologica Indica 3(2), 672-680.
Narra, M., James, J.P. and Balasubramaniam, V. (2015). Simultaneous saccharification and fermentation of delignified lignocellulosic biomass at high solid loadings by a newly isolated thermotolerant Klyveromyces sp. for ethanol production. Bioresource Technology 179, 331-338.
Nur Ain Izzati, M. Z., Nur Adni, M. and Mohd Razik, M. (2017). Isolation and characterization of Ascomycetes isolated from Eurycoma longifolia Jack and Malay traditional vegetables. Pertanika Journal of Tropical Agricultural Science 40(4), 533-542.
Nur Ain Izzati, M.Z., Madihah, M.Z.A., Nor Azizah, K., Najihah, A. and Muskhazli, M. (2019). First report of Bipolaris cactivora causing brown leaf spot in rice in Malaysia. Plant Disease. doi: 10.1094/PDIS-08-1384-PDN.
Nur Baiti, A.M., Nor Azizah K. and Nur Ain Izzati, M. Z. (2016). Identification and diversity of Fusarium species isolated from tomato fruits. Journal of Plant Protection Research. 56(9) 211-216.
Oberoi, H.S., Babbar, N., Sandhu, S.K., Dhalwal, S.S., Kaur, U., Chadha, B.S. and Bhargav, V.K. (2012). Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant Pichia kudriavzevii HOP-1. Journal of Industrial Microbiology and Biotechnology 39, 557-566.
Okamoto, K., Nitta, Y., Maekawa, N. and Yanase, H. (2011). Direct ethanol production from starch, wheat bran and rice straw by the white rot fungus Trametes hirsute. Enzyme and Microbial Technology 48, 273-277.
Ram, L., Kaur, K. and Sharma, S. (2014). Screening isolation and characterization of cellulase producing micro-organisms from soil. International Journal of Pharmaceutical Science Invention 3(3), 12-18.
Rani, V., Mohanram, S., Tiwari, R., Nain, L. and Arora, A. (2014). Beta-glucosidase: Key enzyme in determining efficiency of cellulase and biomass hydrolysis. Journal of Bioprocessing and Biotechniques 5, 197-205.
Romsima, M.Z., Davies, W.P., Rosniza Aznie, C.R., Mazdi, M. and Jabil, M.J. (2014). Farmers’ knowledge on potential uses of rice straw: An assessment in MADA and Sekinchan, Malaysia. Malaysian Journal of Society and Space 10(5), 30-43.
Saini, J.K., Saini, R. and Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments. 3 Biotechnology 5, 337-353.
Sarkar, N., Ghosh, S.K., Bannereje, S. and Aikat, K. (2012). Bioethanol production from agricultural wastes: An overview. Renewable Energy 37, 19-27.
Sharifah Siti Maryam, S.A.R., Nor Azwady, A.A. and Nur Ain Izzati, M.Z. (2016). Isolation and diversity of Trichoderma species from cultivated crop soil in Malaysia. Acta Biologica Malaysiana, 5(2&3), 43-48.
Sheng, P., Huang, J., Zhang, Z., Wang, D., Tian, X. and Ding, J. (2016). Construction and characterization of a cellulolytic consortium enriched from the hindgut of Holotrichia parallela larvae. International Journal of Molecular Sciences 17, 1646-1658.
Silva, R.N. (2016). Mycology: Current and Future Developments: Fungal Biotechnology for Biofuel Production. Bentham Science Publisher, United Arab of Emirates. pp. 343-344.
Singhania, R.R., Patel, A.K., Sukumaran, R.K., Larroche, C. and Pandey, A. (2012). Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresource Technology 127, 500-507.
Sohail, M., Siddiqi, R., Ahmad, A. and Khan, S.A. (2009). Cellulase production from Aspergillus niger MS82: Effect of temperature and pH. New Biotechnology 25(6), 437-442.
Taha, M., Shahasvari, E., Al-Hothaly, K., Mounradov, A., Smith, A.T., Ball, A.S. and Adetutu, E.M. (2015). Enhanced biological straw saccharification through coculturing of lignocellulose-degrading microorganisms. Applied Biochemistry and Biotechnology 175, 3709-3728.
Takano, M. and Hoshino, K. (2018). Bioethanol production from rice straw by simultaneous saccharification and fermentation with statistical optimized cellulase cocktail and fermenting fungus. Bioresources and Bioprocessing 5, 16-28.
Teeri, T.T. (1997). Crystalline cellulose degradation: New insights into the function of cellobiohydrolases. Trends Biotechnology 15, 160-167.
Thormann, M.N., Currah, R.S. and Bayley, S.E. (2002). The relative ability of fungi from Sphagnum fuscum to decompose selected carbon substrates. Canadian Journal of Microbiology 48, 204-211.
Wang, L.J., Luo, Z.L. and Shahbazi, A. (2013). Optimization of simultaneous saccharification and fermentation for the production of ethanol from sweet sorghum (Sorghum bicolor) bagasse using response surface methodology. *Industrial Crops and Products* 42, 280-291.

Wang, M., Li, Z., Fang, X., Wang, L. and Qu, Y. (2012). Cellulolytic enzyme production and enzymatic hydrolysis for second-generation bioethanol production. In: Biotechnology in China III: Biofuels and Bioenergy. Advances in Biochemical Engineering Biotechnology, vol 128. Bai, F. W., Liu, C. G., Huang, H. and Tsao, G. (eds). Springer, Berlin, Heidelberg. pp. 1-24.

Wen, Z., Liao, W. and Chen, S. (2005). Production of cellulase/β-glucosidase by the mixed fungi culture *Trichoderma reesei* and *Aspergillus phoenicis* on dairy manure. *Process Biochemistry* 40, 3087-3094.

Wi, S.G., Choi, I.S., Kim, K.H., Kim, H.M. and Bae, H.J. (2013). Bioethanol production from rice straw by popping pretreatment. *Biotechnology for Biofuels* 6, 166-173.

Zhang, Y.H.P., Himmel, M.E. and Mielenz, J.R. (2006). Outlook for cellulose improvement: Screening and selection strategies. *Biotechnology Advances* 24, 452-481.