วารสารวิทยาศาสตร์บูรพา ปีที่ 28 (ฉบับที่ 3) กันยายน – ธันวาคม พ.ศ. 2566
BURAPHA SCIENCE JOURNAL Volume 28 (No.3) September – December 2023 บทความวิจัย

การแยกและคัดเลือกเชื้อราที่ผลิตเอนไซม์เซลลูเลส เพื่อใช้ย่อยสลายวัสดุเหลือใช้ทางการเกษตร

Isolation and Screening of Cellulase-Producing Fungi for the Degradation of Agricultural Residues

สันธยา บุญรุ่ง*, ปวีณา สุมะหิงพันธ์ และ ศิริลักษณ์ สุดศรี
Santhaya Boonrung*, Paweena Sumahingphan and Siriluck Sudsri
สาขาวิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยราชภัฏบุรีรัมย์ ประเทศไทย
Biology Program, Faculty of Science, Buriram Rajabhat University, Thailand

Received : 11 December 2022
Revised : 9 June 2023
Accepted : 23 June 2023

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความสามารถของเชื้อราที่ผลิตเอนไซม์เซลลูเลสในการย่อยสลายวัสดุเหลือใช้ทางการเกษตร โดยสามารถแยกเชื้อราบริสุทธิ์จากดิน ฟางข้าวและเศษใบอ้อยที่เน่าเปื่อยได้ทั้งหมด 36 ไอโซเลท จากนั้นคัดเลือกเชื้อราที่มีความสามารถในการผลิตเอนไซม์เซลลูเลสบนอาหาร carboxymethyl cellulose (CMC) agar ด้วยวิธี Gram's iodine และหาค่าดัชนีการผลิตเอนไซม์โดยวัดอัตราส่วนของเส้นผ่าศูนย์กลางของการเกิดบริเวณใสต่อเส้นผ่าศูนย์กลางของโคโลนี ผลการศึกษาพบว่า มีเชื้อราจำนวน 3 ไอโซเลท ที่มีกิจกรรมของเอนไซม์เซลลูเลสสูงสุด ได้แก่ ไอโซเลท MLP02, MLP05 และ NLP06 โดยมีค่าดัชนีการผลิตเอนไซม์เซลลูเลสเท่ากับ 1.57±0.06, 1.53±0.06 และ 1.47±0.06 ตามลำดับ จากการระบุชนิดของเชื้อราในเบื้องต้นโดยการศึกษาลักษณะทางสัณฐานวิทยา พบว่า เชื้อราทั้ง 3 ไอโซเลท มีลักษณะสอดคล้องกับเชื้อราในสกุล *Aspergillus* และเมื่อนำเชื้อราผลิตเอนไซม์เซลลูเลสที่คัดแยกได้มาศึกษาการผลิตเอนไซม์ย่อยสลายลิกโนเซลลูโลสในวัสดุเหลือใช้ทางการเกษตรชนิดต่าง ๆ พบว่า เชื้อรา *Aspergillus* sp. MLP02 มีค่าดัชนีการผลิตเอนไซม์บนอาหารทดสอบชนิดต่าง ๆ ไม่แตกต่างกันทางสถิติ (p>0.05) ส่วนเชื้อรา *Aspergillus* sp. MLP05 และ *Aspergillus* sp. NLP06 มีค่าดัชนีการผลิตเอนไซม์บนอาหารทดสอบชนิดต่าง ๆ แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p<0.05) โดยเชื้อรา *Aspergillus* sp. MLP05 มีค่าดัชนีการผลิตเอนไซม์บนอาหารวุ้นรำข้าว อาหารวุ้นแกลบ และอาหารวุ้นฟางข้าว ที่สูงกว่าอาหารวุ้นชานอ้อยและอาหารวุ้น CMC และเชื้อรา *Aspergillus* sp. NLP06 มีค่าดัชนีการผลิตเอนไซม์บนอาหารวุ้นรำข้าว อาหารวุ้นชานอ้อย และอาหารวุ้น CMC ที่สูงกว่าอาหารวุ้นแกลบและอาหารวุ้นฟางข้าว

คำสำคัญ : เชื้อราผลิตเอนไซม์เซลลูเลส ; เซลลูเลส ; *Aspergillus* sp. ; เอนไซม์ย่อยสลายลิกโนเซลลูโลส

Abstract

The objective of the present study was to investigate the ability of cellulase-producing fungi to degrade agricultural residues. A total of thirty-six fungal isolates were isolated from soil, decaying rice straw and decaying sugarcane leaf litter. Cellulase production of the fungal isolates were determined using Gram's iodine on CMC agar. The ratio of clear zone diameter to colony diameter was calculated and expressed as the enzymatic index. Among them, the isolate MLP02, MLP05 and NLP06 showed the highest cellulase activity with cellulolytic index of 1.57±0.06, 1.53±0.06 and 1.47±0.06, respectively. Based on morphological characteristics data, the three fungal isolates were identified as belonging to the genera of *Aspergillus*. In addition, cellulolytic fungi were investigated for the ability to produce lignocellulose-degrading enzymes during cultivation using different agricultural residues. The result found that the enzymatic index of *Aspergillus* sp. MLP02 from cultivation on different agar media tested were not significantly different ($p>0.05$). In contrast, the enzymatic index of *Aspergillus* sp. MLP05 on rice bran agar, rice husk agar and rice straw agar were significantly higher than that on sugarcane bagasse agar and CMC agar ($p<0.05$). Whilst the enzymatic index of *Aspergillus* sp. NLP06 on rice bran agar, sugarcane bagasse agar and CMC agar were significantly higher than that on rice husk agar and rice straw agar ($p<0.05$).

Keywords : cellulase-producing fungi ; cellulase ; *Aspergillus* sp. ; lignocellulose-degrading enzymes

***Corresponding author. E-mail** : Santhaya.br@bru.ac.th

Introduction

Thailand is an agricultural country and many agricultural wastes are available every year. These raw materials such as rice straw, corn stover, sugarcane bagasse, sawdust contain high amounts of lignocelluloses (Badhan *et al*., 2007). The major component of lignocellulosic materials is cellulose, along with hemicellulose and lignin. Cellulose is a linear polymer of D-glucose units linked together by β-1,4-glycosidic bonds and found as a major component of plant biomass. This polymer makes up about 45% of the dry weight of wood. (Pérez *et al*., 2002; Sohail *et al*., 2009). The hydrolysis of cellulose requires the synergistic action of three enzymes, namely, endoglucanase (*endo*-1,4-β-D-glucanase, EC 3.2.1.4), cellobiohydrolase (*exo*-1,4-β-D-glucanase, EC 3.2.1.91) and β-glucosidase (1,4-β-D-glucosidase, EC 3.2.1.21). Cellulases hydrolyze cellulose and produce as primary products glucose, cellobiose and cello-oligosaccharides (Deswal *et al*., 2011; Gao *et al*., 2008; Singhania *et al*., 2010). Therefore, those agricultural wastes are used as an excellent carbon source for cellulase production by microorganism. Cellulases can be produced by a number of fungi, bacteria and yeast that can use lignocelluloses as a primary carbon source. However, fungi are potential microorganisms for production of these enzymes, because, they produce a wide range of extracellular enzymes that enable them to degrade complex lignocelluloses substrates into soluble substances and their enzyme levels are much higher than those of yeast and bacteria (Sari *et al*., 2017; Wen *et al*., 2005).

Cellulases have been widely used in several industries including biofuel, food, animal feed, beverages, textile, pulp and paper, pharmaceutical, agricultural, etc (Sari *et al*., 2017; Bajaj & Mahajan, 2019; Sibanda *et al*., 2019). Concerning agricultural application, cellulolytic fungi can be applied to the compost production using agricultural wastes. Inoculation of cellulolytic fungi into the compost production process can improve the quality of composting process as it can increase the agricultural waste degradation. (Sakpetch *et al*., 2017; Sari *et al*., 2017). In addition, the compost production from agricultural wastes is an important alternative method for sustainable waste management. For application of enzymes in the pulp and paper industry, alkaliphile enzymes shows great activity at alkaline pH, giving it great potential for application in the dewatering and refining steps, and for bleaching processes without necessitating changes in pH (Muthezhilan *et al*. 2007).

Therefore, the objective of the present study was to isolate and screen the cellulase-producing fungi from soil and decayed agricultural waste. Moreover, cellulolytic fungi were investigated for the ability to produce lignocellulose-degrading enzymes during cultivation using different agricultural residues.

Methods

Isolation of fungi

Fungi were isolated from soil, decaying rice straw and decaying sugarcane leaf litter in Buriram Province, Thailand. The samples were 10-fold serially diluted and spread onto potato dextrose agar (PDA; HiMedia Laboratories Pvt. Ltd., India) plates containing with tetracycline (200 ppm), pH 9.0. The plates were incubated at 37˚C for 3-5 days. Morphological characteristics of different colonies appeared on the plates were purified on a PDA.

Screening of cellulase-producing fungi

Cellulase activity of the isolated fungi were determined using Gram's iodine. A 0.8 cm diameter agar plug of a 3-day-old mycelial culture of the purified isolates was aseptically cut with cork borer and placed at the center of CMC agar [0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% carboxymethylcellulose (CMC) sodium salt, 0.02% peptone, and 1.7% agar] (Kasana *et al*., 2008), pH 9.0. The plates were incubated at 37˚C for 3-5 days. After incubation, the plates were flooded with Gram's iodine for 3 to 5 minutes (Kasana *et al*., 2008). The diameter of colony and clear zone around the colony were measured. The ratio of clear zone diameter to colony diameter was calculated and expressed as enzymatic index (EI), according to equation (1) (Bundidamorn *et al*., 2021; Choudhary *et al*., 2016; Mardetko *et al*., 2021):

$$\text{Enzymatic index (EI)} = \text{diameter of hydrolysis zone (clear zone)/diameter of colony} \quad (1)$$

The isolated fungi showed the highest value of the enzymatic index were cultivated in PDA at 37˚C for further study.

Morphological identification of cellulase-producing fungi

Cellulase-producing fungi were identified using evaluation of colony characteristics and microscopic features. Macroscopic features of the fungal isolates were determined by observing the colony color, elevations, texture and margin on PDA plates. Microscopic observations were performed by the slide culture technique and staining with lactophenol cotton blue. The morphological characteristics of cellulase-producing fungi were carried out according to the manual of taxonomy and classification of fungi (Nyongesa *et al*., 2015; Samson *et al*., 2014).

Determination of agricultural residues degradation by cellulase-producing fungi

The ability of cellulase-producing fungi to produce lignocellulose-degrading enzymes was determined using modified agar media containing agricultural residues. Agricultural residues, namely rice straw, sugarcane

bagasse, rice husk and rice bran were used as substrates. They were collected from area around Buriram Province, Thailand, and the small pieces (1-2 cm) of each waste product were soaked in 0.5 N NaOH for 2 hrs. Thereafter, they were washed with tap water, and then dried at 50˚C. The dried materials were ground into powder using a blender. The modified agar media were prepared as described by Kasana *et al*. (2008) with some modifications. Media were prepared with agricultural residues powder instead of CMC in CMC agar medium as follows: alkali-treated agricultural residues powder 2.0 g, $NaNO_3$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.5 g, KCl 0.5 g, peptone 0.2 g, agar 17 g and distilled water 1000 mL. CMC agar medium was also used for comparative study of agricultural residues degradation. The pH of the medium was adjusted to 9.0 prior to sterilization (121˚C, 15 min). A 0.8 cm diameter agar plug of a 3-day-old mycelial culture of the purified isolates was aseptically cut with cork borer and placed at the center of each agar media. Each experiment was performed in triplicate. The inoculated plates at 37˚C for 3 days were flooded with Gram's iodine for 3 to 5 minutes. The evaluation of enzyme activity was performed by observing a clear zone formed around the fungal colony. Then the ratio of clear zone diameter to colony diameter (enzymatic index) was calculated.

Statistical analysis

The data were analyzed using the SPSS program for one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used for significant differences ($p<0.05$) between treatments.

Results

Isolation and screening of cellulase-producing fungi

A total of 36 isolates were isolated from different samples by serial dilution method and spread plating on PDA plates (data not shown). Cellulase activity of the isolated fungi was determined using Gram's iodine method. The results found that only 3 isolates namely MLP02, MLP05 and NLP06 showed high cellulase production. Cellulolytic index of the fungal isolates MLP02 and MLP05 isolated from decaying rice straw were 1.57±0.06 and 1.53±0.06, respectively. Whilst, cellulolytic index of the fungal isolate NLP06 isolated from decaying sugarcane leaf litter was 1.47±0.06 (Figure 1).

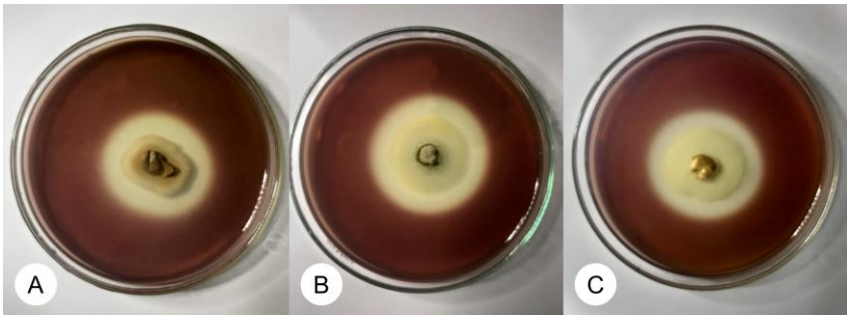

Figure 1 The clear zone around the fungal colony on CMC agar plates, pH 9.0. A, isolate MLP02; B, isolate MLP05; C, isolate NLP06

Morphological identification of cellulase-producing fungi

Cellulase-producing fungi were identified using evaluation of colony characteristics and microscopic features. The colony of isolate MLP02 on PDA was flat, white to pale yellow with white edges and a rough texture. Under a light microscope with a magnification of 400x, this isolate contained conidiophores with bulging ends that form vesicles which support phialides and conidia. Conidial heads were typically columnar-shaped. Conidia were globose in shape (Figure 2). The colony of isolate MLP05 on PDA was flat, white to pale yellow with white edges and often with radial grooves. Under a light microscope with a magnification of 400x, conidial heads with compact phialides were produced from conidiophores (Figure 3). Isolate NLP06 showed white to pale yellow color colony with white edges and floccose on PDA medium. Under a light microscope with a magnification of 400x, this isolate produced conidiophores with radiate conidial heads. Conidia were globose in shape (Figure 4). These morphological properties suggest that the three fungal isolates belonged to the genera *Aspergillus* and designated as *Aspergillus* sp. MLP02, *Aspergillus* sp. MLP05 and *Aspergillus* sp. NLP06, respectively.

Determination of agricultural residues degradation by cellulase-producing fungi

The ability of cellulase-producing fungi to degrade agricultural residues on agar media was determined and the results were summarized in Table 1 and Figure 5. The enzymatic index of *Aspergillus* sp. MLP02 from cultivation on five different agar media were not significantly different after 3 days of incubation at 37˚C (p>0.05). Whereas, *Aspergillus* sp. MLP05 and *Aspergillus* sp. NLP06 showed significantly differences of degradation ability of different agricultural residues. *Aspergillus* sp. MLP05 showed a high enzymatic index on rice bran agar, rice husk agar and rice straw agar when compared with sugarcane bagasse agar and CMC agar. Whilst, the enzymatic index of *Aspergillus* sp. NLP06 from the cultivation on rice bran agar, sugarcane bagasse agar and CMC agar were significantly higher than that on rice husk agar and rice straw agar after 3 days of incubation at 37˚C (p<0.05).

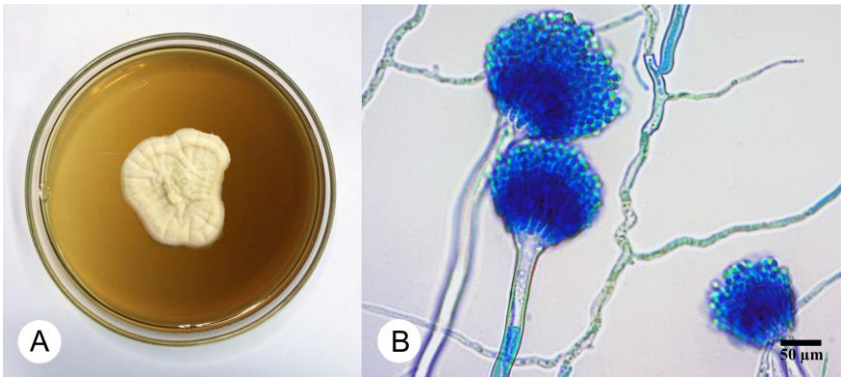

Figure 2 Morphological characteristics of *Aspergillus* sp. MLP02. A, Colony morphology after incubation for 5 days at 37˚C on PDA, pH 9.0; B, Microscopic features (400x)

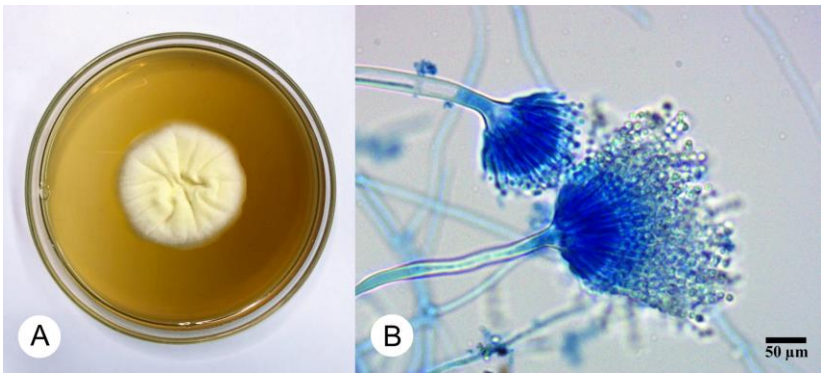

Figure 3 Morphological characteristics of *Aspergillus* sp. MLP05. A, Colony morphology after incubation for 5 days at 37˚C on PDA, pH 9.0; B, Microscopic features (400x)

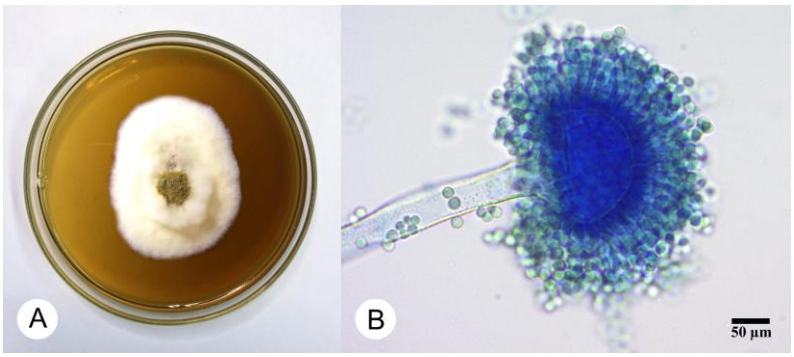

Figure 4 Morphological characteristics of *Aspergillus* sp. NLP06. A, Colony morphology after incubation for 5 days at 37˚C on PDA, pH 9.0; B, Microscopic features (400x)

Table 1 The ratio of clear zone diameter to colony diameter of each isolate of cellulase-producing fungi grown on different culture media at 37˚C for 3 days

Fungi	Culture media	Clear zone diameter/Colony diameter ratio (Enzymatic index)
Aspergillus sp. MLP02	Rice bran agar	1.70 ± 0.20^{ns}
	Rice husk agar	1.55 ± 0.06^{ns}
	Rice straw agar	1.68 ± 0.08^{ns}
	Sugarcane bagasse agar	1.60 ± 0.04^{ns}
	CMC agar	1.70 ± 0.05^{ns}
Aspergillus sp. MLP05	Rice bran agar	1.47 ± 0.03^{a}
	Rice husk agar	1.46 ± 0.04^{a}
	Rice straw agar	1.48 ± 0.01^{a}
	Sugarcane bagasse agar	1.39 ± 0.01^{b}
	CMC agar	1.38 ± 0.01^{b}
Aspergillus sp. NLP06	Rice bran agar	1.62 ± 0.01^{a}
	Rice husk agar	1.55 ± 0.01^{b}
	Rice straw agar	1.51 ± 0.01^{b}
	Sugarcane bagasse agar	1.64 ± 0.04^{a}
	CMC agar	1.62 ± 0.02^{a}

Data are means ± standard deviation (SD). Data with different letters within the same column indicate a significant difference at $p<0.05$ according to Duncan's multiple range test (DMRT). ns, not significant.

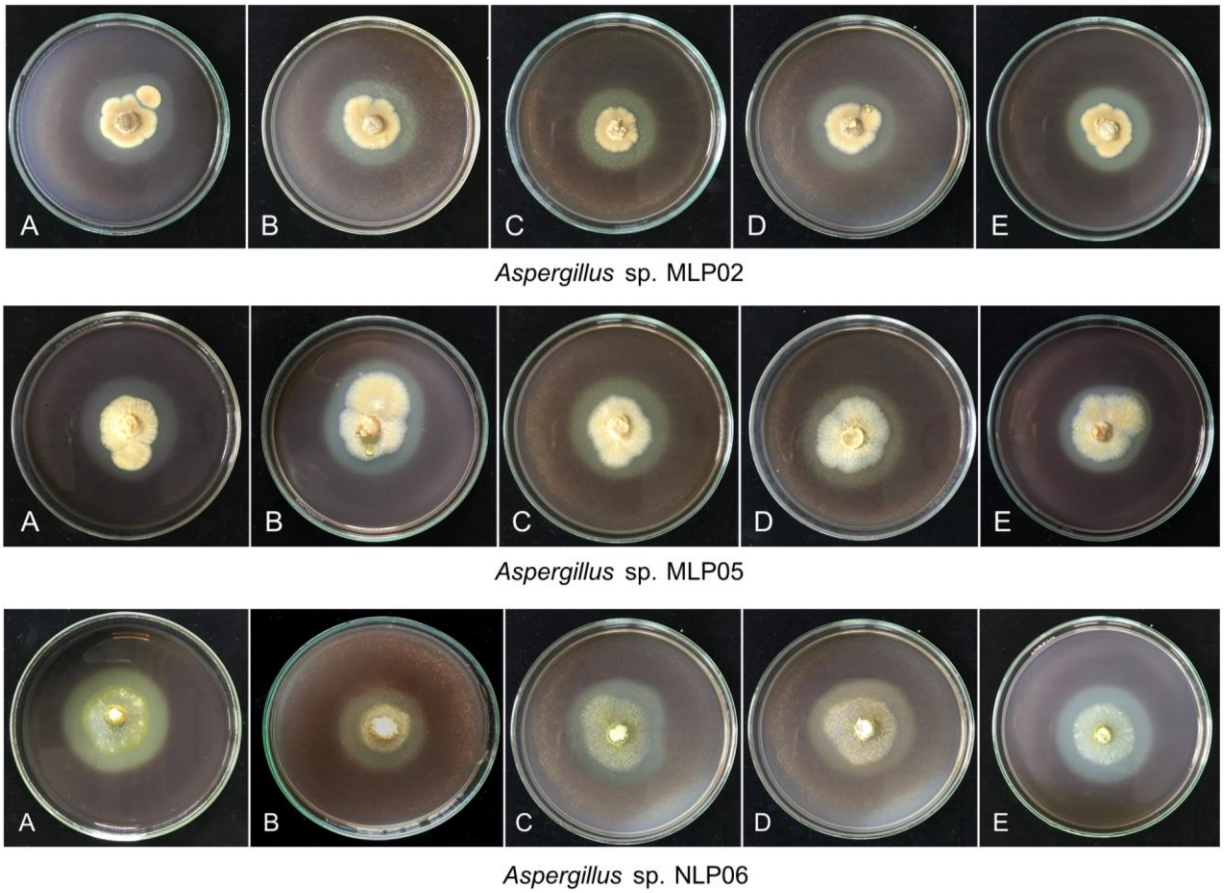

Figure 5 The clear zone around the fungal colony on different culture media, pH 9.0 at 37˚C for 3 days. A, rice bran agar; B, rice husk agar; C, rice straw agar; D, sugarcane bagasse agar; E, CMC agar

Discussion

In this study, cellulase-producing fungi were successfully isolated from soil, decaying rice straw and decaying sugarcane leaf litter. The fungal isolate MLP02, MLP05 and NLP06 showed the highest cellulase activity on CMC agar plates. Cellulase-producing fungi were identified using morphological characteristic of colony and microscopic features (Nyongesa *et al*., 2015; Samson *et al*., 2014). The three fungal isolates were identified as *Aspergillus* sp. MLP02, *Aspergillus* sp. MLP05 and *Aspergillus* sp. NLP06. *Aspergillus* species are widespread throughout the agricultural environment, growing in soil, decaying organic matter, plants, and animals. They are known to be good producers of cellulases (Abrão *et al*., 2017; Atallah *et al*., 2022). These data were similar to that reported by Nhan *et al*. (2021), who found that cellulase-producing fungus, isolate N.S8 was isolated from soil and

classified into *Aspergillus oryzae*. According to Sari *et al*. (2017), cellulolytic fungi, isolate SLL03 and SLL05 were isolated from *Salacca* leaf litter and identified as belonging to the genera of *Aspergillus*. In addition, Ogbonna *et al*. (2018) reported that the two isolates of cellulase-producing fungi were isolated from decaying tubers and were identified as *Aspergillus* species I and *Aspergillus* species II.

Aspergillus sp. MLP02, *Aspergillus* sp. MLP05 and *Aspergillus* sp. NLP06 showed the highest cellulase activity with cellulolytic index of 1.57 ± 0.06, 1.53 ± 0.06 and 1.47 ± 0.06, respectively. Cellulolytic index of these isolates was higher than some of the previous published data from other cellulase-producing fungi. For instance, Mardetko *et al*. (2021) reported that *Fusarium oxysporum* and *F. verticillioides* showed cellulase activity with the enzymatic index of 1.1 and 1.09, respectively. Whereas, Bundidamorn *et al*. (2021) reported that *Fusarium* sp. PSA-3 produced cellulase enzyme with the enzymatic index of 1.1 ± 0.0. The observations of Wisdawati *et al*. (2021) showed that six fungal isolates showed cellulase activity with cellulolytic index of 1.16-1.66.

The three fungal isolates were able to grow and produce lignocellulose-degrading enzymes on all culture media tested with the enzymatic index values ranging from 1.38-1.70. The enzymatic index of *Aspergillus* sp. MLP02 from cultivation on different agar media were not significantly different ($p>0.05$). In contrast, the enzymatic index of *Aspergillus* sp. MLP05 on rice bran agar, rice husk agar and rice straw agar were significantly higher than that on sugarcane bagasse agar and CMC agar ($p<0.05$). Whilst the enzymatic index of *Aspergillus* sp. NLP06 on rice bran agar, sugarcane bagasse agar and CMC agar were significantly higher than that on rice husk agar and rice straw agar ($p< 0.05$). This result showed that all the tested fungi could grow on all lignocellulosic substrates and secrete enzymes into the medium for lignocellulose degradation to simple sugars as a primary carbon source. The conversion of lignocellulose to simple sugars is caused by the synergistic actions of various enzymes that belong to a complex system of cellulase and xylanase enzymes (Mardetko *et al*., 2021). However, biodegradation of plant residues by fungi is dependent on the plant and fungus species (Sinegani *et al*., 2005). According to Rajinipriya *et al*. (2018), rice husk is approximately composed of 45.0% cellulose, 19.0% hemicellulose and 19.5% lignin, while bagasse consists of 55.2% cellulose, 16.8% hemicellulose and 25.3% lignin. Also, rice straw contains 25-45% cellulose, 20-30% hemicellulose and 10-15% lignin (Sarkar & Aikat, 2012). In addition, rice bran is composed of 15.5% cellulose, 31.1% hemicelluloses and 11.5% lignin (Sunphorka *et al*., 2012). The high content of lignocellulose might be a reason for induction of cellulases and xylanase biosynthesis. However, pretreatments of lignocellulosic materials are usually required. Alkaline pretreatment of lignocellulosic materials with alkaline solutions such as sodium hydroxide (NaOH) increased the swelling of the biomass, reduced cellulose crystallinity and removed lignin

and some hemicellulose in plant cell walls, which improves the accessibility of cellulose to cellulase enzyme (Mafa *et al*., 2020; Mustafa *et al*., 2021).

Our results indicated that three fungal isolates have strong degradation ability to pure cellulose materials and also to complex cellulose structure. They produced lignocellulose-degrading enzymes in culture media at alkaline conditions (pH 9.0). Therefore, three alkaliphilic isolates might be useful as source of key enzymes in the bioprocessing of pulp and paper industry. For pulp dewatering and refining step, mixtures of alkaline cellulase and xylanase are used to increase the drainage rate of mechanical pulp and secondary fiber (Godfrey & West, 1996). In addition, cellulase-producing fungi in this study might be useful as a source of inoculum for composting process of agricultural residues. According to Nhan *et al*. (2021), the use of cellulase-producing *Aspergillus oryzae* N.S8 for composting water hyacinth created an organic fertilizer with nitrogen, phosphorus and potassium contents of 3.35%, 0.43% and 0.74%, respectively after 45 days. Moreover, this study showed that modified agar media containing agricultural residues can replace Commercial CMC agar media for primary screening of lignocellulolytic fungi.

Conclusions

In the present study, cellulase-producing fungi were isolated from soil, decaying rice straw and decaying sugarcane leaf litter. The three fungal isolates, namely MLP02, MLP05 and NLP06 showed the highest cellulase activity on CMC agar plates, pH 9.0. These isolates were identified as belonging to the genera of *Aspergillus* by colony characteristics and microscopic features. The fungal isolates could grow and produce lignocellulose-degrading enzymes during cultivation using different agricultural residues as carbon source. Our results showed that three alkaliphilic fungal isolates with appreciable hydrolytic zones on culture media tested have good hydrolytic property of lignocellulosic materials. Therefore, these three fungal isolates can be further applied as a source of inoculum for composting process of agricultural residues. In addition, the enzymes from alkaliphilic fungal isolates might be useful as key enzymes in pulping, bleaching and deinking processes of the pulp and paper industry. However, purification and characterization of enzymes from these alkaliphilic fungi need further study. Furthermore, modified agar media containing agricultural residues in this study can replace Commercial CMC agar media for primary screening of cellulase-producing fungi or lignocellulolytic fungi.

Acknowledgements

We gratefully acknowledge the support of the Biology Program, Faculty of Science, Buriram Rajabhat University.

References

Abrão, F.O., Duarte, E.R., Pessoa, M.S., Santos, V.L., Junior, L.F.F., Barros, K.O., Hughes, A.F.S., Silva, T.D., & Rodriguez, N.M. (2017). Notable fibrolytic enzyme production by *Aspergillus* spp. isolates from the gastrointestinal tract of beef cattle fed in lignified pastures. *PLOS ONE*, *12*(8), 1-13.

Atallah, O.O., Mazrou, Y.S.A., Atia, M.M., Nehela, Y., Abdelrhim, A.S., & Nader, M.M. (2022). Polyphasic Characterization of Four *Aspergillus* Species as Potential Biocontrol Agents for White Mold Disease of Bean. *Journal of Fungi*, *8*, 626.

Badhan, A.K., Chadha, B.S., Kaur, J., Saini, H.S., & Bhat, M.K. (2007). Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. *Bioresource Technology*, *98*(3), 504-510.

Bajaj, P., & Mahajan, R. (2019). Cellulase and xylanase synergism in industrial biotechnology. *Applied Microbiology and Biotechnology*, *103*, 8711-8724.

Bundidamorn, D., Salaiphet, L., Vichitsoonthonkul, T., Ratanakhanokchai, K., & Phitsuwan, P. (2021). Screening of fungi isolated from damaged plant materials for the production of lignocellulolytic enzymes with decolorizing ability. *Asia-Pacific Journal of Science and Technology*, *26*(3), 1-10.

Choudhary, M., Sharma, P.C., Jat, H.S., Nehra, V., McDonald, A.J., & Garg, N. (2016). Crop residue degradation by fungi isolated from conservation agriculture fields under rice-wheat system of North-West India. *International Journal of Recycling of Organic Waste in Agriculture*, *5*, 349-360.

Deswal, D., Khasa, Y.P., & Kuhad, R.C. (2011). Optimization of cellulase production by a brown rot fungus *Fomitopsis* sp. RCK2010 under solid state fermentation. *Bioresource Technology*, *102*, 6065-6072.

Gao, J., Weng, H., Zhu, D., Yuan, M., Guan, F., & Xi, Y. (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. *Bioresource Technology*, *99*, 7623-7629.

Godfrey, T., & West, S. (1996). *Enzymology*. 2nd Edn. London: Macmillan Press Ltd.

Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., & Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Current Microbiology*, *57*, 503-507.

Mafa, M.S., Malgas, S., Bhattacharya, A., Rashamuse, K., & Pletschke, B.I. (2020). The Effects of Alkaline Pretreatment on Agricultural Biomasses (Corn Cob and Sweet Sorghum Bagasse) and Their Hydrolysis by a Termite-Derived Enzyme Cocktail. *Agronomy*, *10*, 1211.

Mardetko, N., Trontel, A., Novak, M., Pavlecic, M., Ljubas, B.D., Grubisic, M., Tominac, V.P., Ludwig, R., & Santek, B. (2021). Screening of Lignocellulolytic Enzyme Activities in Fungal Species and Sequential Solid-State and Submerged Cultivation for the Production of Enzyme Cocktails. *Polymers*, *13*(21), 3736.

Mustafa, A.H., Rashid, S.S., Rahim1, M.H.A., Roslan, R., Musa, W.A.M., Sikder, B.H., & Sasi, A.A. (2022). Enzymatic Pretreatment of Lignocellulosic Biomass: An Overview. *Journal of Chemical Engineering and Industrial Biotechnology*, *8*(1), 1-7.

Muthezhilan, R., Ashok, R., & Jayalakshmi, S. (2007). Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicumin* solid state fermentation. *African Journal of Microbiology Research*, *1*, 20-28.

Nhan, N.T., Anh, D.L.H., & Tri, M.N. (2021). Isolation of cellulolytic fungi and their application for production of organic fertilizer from water hyacinth (*Eichhornia crassipes*). *Biotech Studies*, *30*(1), 16-21.

Nyongesa, B.W., Okoth, S., & Ayugi, V. (2015). Identification key for *Aspergillus* species isolated from Maize and Soil of Nandi County, Kenya. *Advances in Microbiology*, *5*, 205-229.

Ogbonna, C.N., Nnaji, O.B., & Chioke, O.J. (2018). Isolation of amylase and cellulase producing fungi from decaying tubers and optimization of their enzyme production in solid and submerged cultures. *International Journal of Biotechnology and Food Science*, *6*(1), 9-17.

Pérez, J., Muñoz-Dorado, J., de la Rubia, T., & Martínez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International Microbiology*, *5*, 53-63.

Rajinipriya, M., Nagalakshmaiah, M., Robert, M., & Elkoun, S. (2018). Importance of Agricultural and Industrial Waste in the Field of Nanocellulose and Recent Industrial Developments of Wood Based Nanocellulose: A Review. *ACS Sustainable Chemistry & Engineering*, *6*(3), 2807-2828.

Sakpetch, P., Kittikiun, A., & Chandumpai, C. (2017). Isolation and Screening of potential lignocellulolytic microorganisms from rubber bark and other agricultural residues. *Walailak Journal of Science and Technology*, *14*(12), 953-967.

Samson, R.A., Visagie, C.M., Houbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Susca, A., Tanney, J.B., Varga, J., Kocsub, S., Szigeti, G., Yaguchi, T., & Frisvad, J.C. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology*, *78*, 141-173.

Sari, S.L.A., Setyaningsih, R., & Wibowo, N.F.A. (2017). Isolation and screening of cellulolytic fungi from *Salacca zalacca* leaf litter. *Biodiversitas*, *18*(3), 1282-1288.

Sarkar, N., & Aikat, K. (2012). Alkali pretreatment of rice straw and enhanced cellulase production by a locally isolated fungus *Aspergillus fumigates* NITDGPKA3. *Journal of Microbiology and Biotechnology Research*, *2*(5), 717-726.

Sibanda, A., Ruzvidzo, O., Ncube, I., & Ncube, T. (2019). Diversity of cellulase- and xylanase-producing filamentous fungi from termite mounds. *Journal of Yeast and Fungal Research*, *10*(2), 15-29.

Sinegani, A.A.S., Emtiazi, G., Hajrasuliha, S., & Shariatmadari, H. (2005). Biodegradation of some agricultural residues by fungi in agitated submerged cultures. *African Journal of Biotechnology*, *4*(10), 1058-1061.

Singhania, R.R., Sukumaran, R.K., Patel, A.K., Larroche, C., & Pandey, A. (2010). Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. *Enzyme and Microbial Technology*, *46*, 541-549.

Sohail, M., Siddiqi, R., Ahmad, A., & Khan, S.A. (2009). Cellulase production from *Aspergillus niger* MS82: effect of temperature and pH. *New Biotechnology*, *25*(6), 437-441.

Sunphorka, S., Chavasiri, W., Oshima, Y., & Ngamprasertsith, S. (2012). Protein and Sugar Extraction from Rice Bran and De-Oiled Rice Bran using Subcritical Water in a Semi-Continuous Reactor: Optimization by Response Surface Methodology. *International Journal of Food Engineering*, *8*(3), 1-22.

Wisdawati, E., Kuswinanti, T., Rosmana, A., & Nasruddin, A. (2021). Screening and identification of cellulolytic fungi at rhizosphere of safira taro plant. *Earth and Environmental Science*, *807*, 1-5.

Wen, Z., Liao, W., & 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.