การคัดแยก การจำแนกชนิด และลักษณะของเชื้อราที่สามารถย่อยลิกโนเซลลูโลสจากฟางข้าวและตอยูคาลิปตัสผุ

Screening, identification and characterization of lignocellulolytic fungi from decomposing rice straw and eucalyptus stump

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บทคัดย่อ

เชื้อราจากฟางข้าวและตอยูคาลิปตัสผุในเขตอำเภอเมือง จังหวัดหนองคายได้รับการคัดเลือกด้วยวิธี agar diffusion และพบเชื้อรา 32 สายพันธุ์ที่สามารถย่อยสลายลิกโนเซลลูโลสได้ เชื้อเหล่านั้นเป็นสายพันธุ์ที่สามารถย่อยเซลลูโลสและเฮมิเซลลูโลส แต่ไม่พบสายพันธุ์ที่สามารถออกซิไดซ์ลิกนิน เชื้อ 3 ไอโซเลตจากตอยูคาลิปตัส ได้แก่ NKU1-5, NKU1-7 และ NKU2-9 ให้ผลในการย่อยเฮมิเซลลูโลสสูงสุด อุณหภูมิที่เหมาะสมต่อการเจริญของเชื้อ NKU1-5, NKU1-7 และ NKU2-9 คือ 35, 35 และ 25 °C ตามลำดับ นอกจากนี้ พบว่าที่ 25 °C เชื้อทั้ง 3 มีอัตราการเจริญใกล้เคียงกัน จากการเปรียบเทียบกิจกรรมของเอนไซม์ไซลาเนส และศักยภาพในการย่อยเฮมิเซลลูโลสในฟางข้าว ที่ 25 °C ความชื้น 50% (w/v) ด้วยวิธี solid state fermentation พบว่าเชื้อ NKU2-9 เป็นสายพันธุ์ที่มีกิจกรรมของเอนไซม์ไซลาเนส (4.48 IU/ml) และความสามารถในการย่อยเฮมิเซลูโลส (8.29%) สูงสุด เมื่อทำการบ่งชี้ชนิดด้วยลำดับเบส 5.8S rDNA พบว่าเชื้อที่คัดเลือกทั้ง 3 ไอโซเลต เป็น *Aspergillus* sp. strain NKU1-5 (KY405016), *Aspergillus* sp. strain NKU1-7 (KY405017) และ *Curvularia* sp. strain NKU 2-9 (KY405018) เชื้อรากลุ่มนี้มีศักยภาพในการย่อยสลายวัสดุเหลือใช้ทางการเกษตรเป็นผลิตภัณฑ์ที่เป็นประโยชน์ และเป็นการจัดการของเสียจากอุตสาหกรรมเกษตรแบบไม่ทำลายสิ่งแวดล้อม

ABSTRACT

The fungi from decomposing rice straw and rotten eucalyptus stumps in Amphoe Mueang, Nongkhai Province were screened using agar diffusion technique. There were 32 isolates that could hydrolyze lignocelluloses. Those isolates hydrolyzed cellulose and hemicellulose, but lignin oxidation was not found. Among the fungi isolate from rotten eucalyptus stumps, NKU1-5, NKU1-7 and NKU2-9 showed highest ability to hydrolyze hemicelluloses. The optimal temperatures for those isolate were 35, 35 and 25 $^{\circ}$C, respectively. In addition, their growth rates were similar at 25 $^{\circ}$C. The xylanase activity and hemicelluloses hydrolysis ability of those isolate, rice straw as substrate, were compared at 25 $^{\circ}$C and 50 % moisture content on the model of solid state fermentation. The NKU2-9 showed the highest xylanase activity (4.48 IU/ml) and hemicellulose degradation (8.29%). Those 3 isolates were identified as *Aspergillus* sp. strain NKU1-5 (KY405016), *Aspergillus* sp. strain NKU1-7 (KY405017) and *Curvularia* sp. NKU 2-9 strain (KY405018) as assessed by partial 5.8S rDNA sequences. These lignocellulolytic fungi have the potential to degrade agricultural waste to produce valuable products and to handle the waste without environmental harmful.

คำสำคัญ: รายย่อยลิกโนเซลลูโลส การย่อยสลายเฮมิเซลลูโลส การย่อยลิกโนเซลลูโลส กิจกรรมเอนไซม์ไซลาเนส
Keyword: Lignocellulolytic fungi, Hemicellulose degradation, Lignocelluloses hydrolysis, Xylanase activity

INTRODUCTION

Rice straw and eucalyptus stumps become major postharvest wastes in Thailand currently (Ministry, 2014). Both of them primarily compose of cellulose, hemicellulose and lignin, or generally known as lignocelluloses with varied contents. The presence of cellulose and hemicelluloses, glucose polymer, could be found at 57-74% in rice straw (Lee et al., 2011) and 63-67% in eucalypt (Pereira et al., 2012). Whereas lignin, an aromatic polymer derived from phenylpropanoid, composed of 3-24% in rice straw and 28-31% in eucalypt (Lee et al., 2011; Pereira et al., 2012). Hemicellulose, secondary plant cell wall composition, composed of 40-50% in monocot and 20-30% in dicot (Rytioja et al., 2014). Xylan is the main composition (Scheller and Ulvskov, 2010), which is hydrolyzed by xylanase enzyme and resulted in xylose, one of the industrial interest substances (Haltrich et al., 1996; Chang et al., 2012; Anasontzis and Christakopoulos, 2014).

Both postharvest wastes could be naturally decayed by lignocellulolytic fungi or

wood-rooting fungi. These fungi are divided into three groups; white rots, brown rots, and soft rots fungi (Yuan et al., 2014). Ninety percent of the white rot fungi are basidiomycetes (Gilbertson, 1980) that able to degrade cellulose, hemicellulose and oxidize lignin (Rileya et al., 2014; Cragg et al., 2015); for instance, *Phanerochaete chrysosporium* (Vanden Wymelenbre et al., 2006), *P. carnosa* (Suzuki et al., 2012), *Ganoderma lucidum* (Kaur et al., 2016). They are commonly isolated from hardwoods. The brown rot fungi are basidiomycetes that commonly degrade polysaccharide but not lignin in plant cell wall, thereby brownish wood is left be after the degradation (Hatakka and Hammel, 2010). They are isolated from softwood especially conifers (Rytioja et al., 2014), for instance, *Postia placenta* (Zhanga et al., 2016), *Gloeophyllum trabeum* (*Lenzites trabea*) (Herr et al., 1978). The last group, soft rot fungi are ascomycetes and deuteromycetes (Camassola and Dillon, 2009; Hatakka and Hammel, 2010). They may degrade all lignocelluloses, especially carbohydrates in low lignin hardwoods (Worrall et al., 1997), which can be found in non-living trees in form diamond-shaped cavities (Cragg et al., 2015). The examples are *Aspergillus terreus* ATCC 74135, (Jahromi et al., 2011), *Aspergillus niger* and *Penicillium chrysogenum* (Bhavsar et al., 2008; Abdelkader and Hamed, 2013).

In Nong Khai province, Thailand, many rice and eucalyptus growing areas have been intercropped. This study aimed to isolate local fungi from decomposing rice straw and eucalyptus stumps and characterize their ability to degrade the agricultural waste.

MATERIALS AND METHODS

Sample collection and preparation

Decomposing rice straw and rotten eucalyptus stump were collected randomly (100 g/ each) with soil from 4 places of paddy and eucalyptus fields within the Nongkhai Province during dry season of Thailand, February – April 2014. Ambient soil pH and soil temperature were measured. The samples were stored at ambient condition until used.

Fungal isolation and classification

Each sample was immersed in sterile water at the ratio of 25:225 (w/v) and diluted to optimal dilution for fungal isolation using spread plate technique on Potato dextrose agar (HiMedia, India). Each plate was incubated at 33 ^{o}C for 5 - 7 days to observe the single colonies from various fungi. Each morpho-logically different colony was transferred onto a PDA-slant and stored at 4 ^{o}C. Fungal classification was carried out based on colony morphology and structural characteristics using the slide culture technique (Humber, 1997). Sizes, shapes of conidiospores and their arrangement were compared with fungi identification manual (Navi et al., 1999).

Screening of lignocellulolytic fungi by agar diffusion method

Stored fungi were sub-cultured onto PDA plate for 5 - 7 days. Each 2-cm diameter of fungal colony was transferred onto another agar plate containing either cellulolysis basal medium (CBM), xylanolysis basal medium (XBM) or tannic acid agar (Pointing, 1999), and incubated at 33 ^{o}C for 5 - 7 days.

Cellulose hydrolysis was analyzed by staining CBM agar medium with 2% (w/v) aqueous Congo red for 15 min, washed with distilled water and 1 M NaCl for 15 min, respectively. Hydrolysis of cellulose was shown in yellow-opaque area.

Hemicellulose hydrolysis was detected by staining XBM medium with 0.25% (w/v) aqueous iodine for 5 min. Hydrolysis of hemicellulose was shown in yellow-opaque area in blue reddish purple color for undegraded xylan.

Lignin hydrolysis was determined on tannic acid agar medium. Oxidized tannin was shown in brown zone around colonies.

All of hydrolytic capacities (HC) of cellulose, hemicelluloses and lignin were expressed as ratio of hydrolyzed diameter (cm) to colonial diameter (cm) in mean ± SD from triplicate experiment (Huang et al., 2012).

Optimal temperature for mycelial growth

The selected isolates were cultured on PDA agars and incubated at 25, 35, 45 and 55 ^{o}C. Diameters of fungal mycelia were measured daily for 8 days.

Solid State Fermentation (SSF) and enzyme extraction

Rice straw obtained from a paddy field in Nong Khai Province was chopped to 0.5-cm size and dried at 105 ^{o}C for 3 h for SSF substrate. The dried substrate (2 g) was mixed with 1-ml sterile distilled water on a Petri dish. Two-cm diameter of PDA-grown fungal isolate was placed at the center and incubated at 25 ^{o}C for 15 days (Li et al., 2008). This experiment was performed in triplicates and the negative control was only wet substrate. After 15 days, the substrate residue was collected for crude xylanase extract (Chang et al., 2012) and hemicelluloses degradation analysis (Van Soest et al., 1991).

Hemicellulose degradation analysis

SSF residue substrate (0.5 g) without fungal plaque and mycelium was dried at 105 ^{o}C for 2 h. The neutral detergent fiber (NDF; cellulose, hemicellulose and lignin) and acid detergent fiber (ADF; cellulose and lignin) were extracted by detergent method (Van Soest et al., 1991).

NDF was extracted by boiling a thimble containing 0.5 g sample with 50 ml of neutral detergent (3% sodium lauryl sulphate, 1% Triethylene glycol, 0.912% (w/v) Na_2HPO_4, 1.36% (w/v) $Na_2B_4O_7.10H_2O$, 3.72% (w/v) EDTA in distilled water, pH 6.9 - 7.1), for 60 min

using fiber analyzer (VELP Scientifica, Italy). The sample residue was washed 3 times with boiling water, rinsed twice with cold acetone, dried for 8 h at 105 °C and left cool in a desiccator. Dry weight residue of NDF was weighed.

ADF was subsequently extracted by boiling NDF residue with 100-ml of acid detergent (20 g of CTAB ($(C_{16}H_{33})N(CH_3)_3Br$)) in 1 N H_2SO_4) for 60 min using Fiber analyzer. Then, it was washed with hot water and cold acetone, respectively. dried weigh of the left sample was weighed. The amount of NDF and ADF were calculated following equation 1 and 2, respectively.

$$\%NDF = \frac{W_{NDF}}{W_0} \times 100 \quad (1)$$

$$\%ADF = \frac{W_{ADF}}{W_0} \times 100 \quad (2)$$

Were, W_{NDF} and W_{ADF} are weight of residue after treating NDF and ADF solution, respectively. W_0 is dry weight of initiation of substrate.

Hemicellulose degradation (HD, %) of SSF residue substrate were calculated following equation 3 and 4, respectively.

$$\%HD \text{ of dry weight} = (\%NDF - \%ADF) \quad (3)$$

$$\%HD \text{ of total hemicellulose} = \frac{(W_{NDF}-W_{ADF})_{Ne} - (W_{NDF}-W_{ADF})_{s}}{(W_{NDF} - W_{ADF})_{Ne}} \times 100 \quad (4)$$

Where, S is inoculated experiment. Ne is negative control (uninoculated sample).

Xylanase assay

Crude xylanase was extracted from SSF substrate residue (0.5 g) with 5-ml of 0.5 M acetate buffer, pH 4.5, by shaking at 120 rpm for 3 h at 12 °C. The suspension was centrifuged at 9000 rpm at 4 °C for 10 min to collect the soluble crude xylanase. Xylanase activity was assayed by adding diluted crude enzyme solution (2.5 ml) to 2.5 ml of 1% xylan solution and left at 37 °C for 1 h. Reducing sugar product was determined by dinitrosalicylic acid (DNS) method (Negrulescu et al., 2012) using xylose as standard. Enzyme activity is defined as unit enzyme, the amount of enzyme that released 1 µmol of reducing xylose equivalent per minute. Protein concentration was determined by Lowry method (Waterborg, 2002) using bovine serum albumin (BSA) as standard protein. Specific activity of enzyme was expressed as IU/g protein and IU/g of substrate.

Molecular identification of fungi based on ITS sequences

Fresh fungal mycelia (1 g) were extracted for DNA using E.Z.N.A. Forensic DNA Isolation Kit (Omega, Bio-Tek, US) as described in manufacturer's instruction. 5.8S rDNA harboring ITS1 and ITS4 regions were amplified in a 50-µl reaction containing 1X buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer (ITS1 5'-TCCGTAGGTGAACCTGCGG-3', ITS4:5'-TCCTCCGC TTATTGATATGC-3'), and 1 U

Taq DNA polymerase. The PCR temperature profile began with an initial denaturation at 96 °C for 2 min, followed by 35 cycles of 96 °C for 1 min, 53 °C for 1 min and 72 °C for 1:30 min. The final extension was carried out for 10 min at 72 °C. The PCR products were separated by 1% agarose gel electrophoresis and collected for sequence analysis of both directions using an automated DNA sequence (Macrogen Inc., Korea). The nucleotide sequences were assembled using Cap contig assembly program, and analyzed by BioEdit Program (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html). The strains were identified and compared with other fungal nucleotide sequence database in National Center for Biotechnology Information (NCBI).

Statistical analysis

All values were means ± SD of triplicate. Analysis of variance was performed by One-way ANOVA with confidence level at 95%. Mean were compared using Least Significant Different (LSD) and Duncan multiple range to assess significant differences at 95% of probability.

RESULTS

Screening of lignocellulolytic fungi

Samples collecting from rice straw and eucalyptus stumps shared relatively similar ambient temperature (32.36 ± 1.6 and 34.10 ±1.2°C, respectively), moisture content (51.24 ± 13.5% and 50.02 ± 14.4%, respectively) and pH (6.63 ± 0.3 and 6.25 ± 0.0, respectively). Thereby, averaged temperature, moisture content and pH of sampling areas at 33°C, 50.5% and pH 6.4 were used for isolation and screening of lignocellulo-lytic fungi in this study.

Fungi could grow on PDA and lignocellulolytic fungi were screened by agar diffusion method and found to be 32 isolates (Table 1). There were 27 isolates showed clear zone for cellulose hydrolysis on CBM agar. In addition, 22 isolates exhibited of hemicellulose hydrolysis by showing clear zone on XBM agar. Finally, 17 isolates showed both hydrolytic activities. None of them showed lignin oxidation on tannic acid agar. Based on microscopic analysis, the fungi were classified into genera of *Aspergillus* (17 isolates, 53%) and *Penicillium* (7 isolates, 22%), in division Ascomycota, and unknown (8 isolates, 25%). Among those isolates, 3 of them showed highest hemicellulose hydrolysis capacity. NKU1-5 exhibited highest hydrolysis of hemicellulose with modulate of cellulolytic activity, while NKU1-7 showed high activity on only hemicellulose hydrolysis. The NKU2-9 also showed highest cellulolytic activity. Therefore, NKU1-5, NKU1-7 and NKU2-9 were selected for further studies.

Optimal temperature of mycelial growth of NKU-5, NKU-7 and NKU2-9

Growth of NKU1-5, NKU1-7 and NKU2-9 at temperature ranged 25 – 55 °C was determined to obtain the optimal temperature of selected lignocellulolytic fungi. Mycelial growth of NKU1-5 and NKU1-7 was optimal at 35 °C (Fig. 1 A-B), which is higher than NKU2-9 (25 °C, Fig. 1C). All selected isolates grew at neither 45 nor 55 °C. Nevertheless, NKU1-5 and NKU1-7 could re-grow after transferring to 25 °C (data not shown). However, at 25 °C, similar growth rates were observed for those 3 isolates. Therefore, the 25 °C was selected as a condition for determining the degradation of rice straw by solid state fermentation.

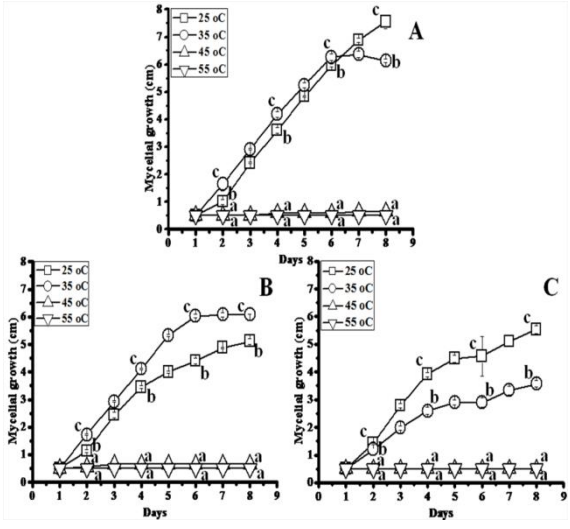

Fig. 1 Mycelial growth of fungal isolates (A) NKU1-5 (B) NKU1-7 (C) NKU2-9 at different temperature. Different superscripted letter(s) were statistically different (P<0.05).

Table 1 Hydrolysis capacity of cellulose and hemicellulose by agar diffusion method

Isolation codes	Morphology classification	Hydrolysis capacity			
		Cellulose		Hemicellulose	
		Mean	SD	Mean	SD
NKR1-1	*Aspergillus* sp.	1.14^{b}	0.02	1.07^{abc}	0.01
NKR1-2	*Aspergillus* sp.	0	0	1.16^{ef}	0.01
NKR1-3	*Aspergillus* sp.	1.15^{ab}	0.07	1.06^{ab}	0.02
NKR1-5	Unknown	1.09^{ab}	0.07	1.10^{bcde}	0.07
NKR2-1	*Aspergillus* sp.	1.07^{a}	0.02	1.09^{abcd}	0.13
NKR2-2	*Penicillium* sp.	1.09^{ab}	0.02	1.09^{abcd}	0.02
NKR3-1	*Aspergillus* sp.	0	0	1.14^{def}	0.05
NKR3-2	*Penicillium* sp.	0	0	1.08^{abcd}	0.02

Table 1 Hydrolysis capacity of cellulose and hemicellulose by agar diffusion method (continues)

Isolation codes	Morphology classification	Hydrolysis capacity			
		Cellulose		Hemicellulose	
		Mean	SD	Mean	SD
NKR3-3	*Aspergillus* sp.	1.46^{ef}	0.04	1.24^{h}	0.06
NKR3-4	*Aspergillus* sp.	1.15^{ab}	0.04	1.11^{bcde}	0.02
NKR3-6	*Aspergillus* sp.	1.10^{ab}	0.02	1.36^{abc}	0.92
NKR3-7	Unknown	1.12^{ab}	0.01	0	0
NKR4-1	*Aspergillus* sp.	1.24^{bcd}	0.02	0	0
NKR4-2	*Aspergillus* sp.	1.22^{abc}	0.01	1.17^{fg}	0.01
NKR4-3	*Aspergillus* sp.	1.39^{de}	0.09	1.21^{gh}	0.04
NKR4-4	Unknown	1.06^{a}	0.01	1.04^{a}	0.00
NKU1-1	*Aspergillus* sp.	1.22^{abc}	0.04	1.33^{i}	0.04
NKU1-3	Unknown	1.32^{cde}	0.01	1.38^{jk}	0.03
NKU1-4	*Penicillium* sp.	1.21^{abc}	0.09	0	0
NKU1-5	*Aspergillus* sp.	**1.33^{cde}**	0.05	**1.43^{l}**	0.02
NKU1-6	*Penicillium* sp.	1.69^{g}	0.23	1.12^{bcde}	0.01
NKU1-7	*Aspergillus* sp.	0	0	**1.41^{kl}**	0.05
NKU2-9	Unknown	**1.97^{h}**	0.07	**1.35^{ij}**	0.09
NKU2-10	Unknown	1.15^{ab}	0.02	0	0
NKU3-11	*Aspergillus* sp.	1.93^{h}	0.21	0	0
NKU3-12	*Penicillium* sp.	1.42^{e}	0.05	0	0
NKU3-14	*Aspergillus* sp.	0	0	1.12^{cde}	0.02
NKU3-15	*Penicillium* sp.	1.59^{fg}	0.27	0	0
NKU3-16	*Penicillium* sp.	1.59^{fg}	0.10	0	0
NKU3-18	Unknown	1.12^{ab}	0.01	0	0
NKU4-19	*Aspergillus* sp.	1.18^{abc}	0.02	1.11^{bcde}	0.04
NKU4-20	Unknown	1.33^{cde}	0.08	0	0

NK = Nong Khai, R = Rice straw, U = Eucalyptus stump,

First number = site source, Second number = isolate number.

Values were means of three replicates with ±SD.

a-k means homogeneous subsets by Duncan at the 95% confidence level

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Solid state fermentation (SSF) of NKU1-5, NKU1-7 and NKU2-9

NKU1-5, NKU1-7 and NKU2-9 were tested for their rice straw degradation capability using rice straw as substrate. The white mycelium grew over the substrate, causing a soft decay. Then, masses of conidia eventually form grey-black to black colour (Fig. 2). This result showed, at 25 ^{o}C, NKU1-7 and NKU2-9 faster grew than NKU1-5.

The NDF, ADF and hemicellulose degradation were analyzed after 15 days as results shown in Table 2. Initial hemicellulose content in rice straw was 27.63 ± 0.73% of dry weight. NKU2-9 showed high hemicellulose degradation at 8.29 ± 0.42% (30.74 ± 1.56% of total hemicellulose) than NKU1-7 at 5.17± 0.77% (19.19±2.85% of total hemicellulose) and NKU1-5 at 2.43±0.82% (9.00±3.05% of total hemicellulose), respectively. This agreed well with xylanase activity that NKU2-9 showed the highest activity at 4.48 ± 0.08 IU/ml, while NKU1-7 and NKU1-5 were 3.59 ± 0.22 and 2.42 ± 0.09 IU/ml, respectively. Protein contents in NKU2-9, NKU1-7, and NKU1-5 were 0.84 ± 0.05, (0.73 ± 0.01, and 0.46 ± 0.01 mg/ml, respectively. Then, the specific activity of xylanase were calculated as shown in Table 2. The highest activity was NKU2-9, followed by NKU1-5 and NKU1-7, respectively.

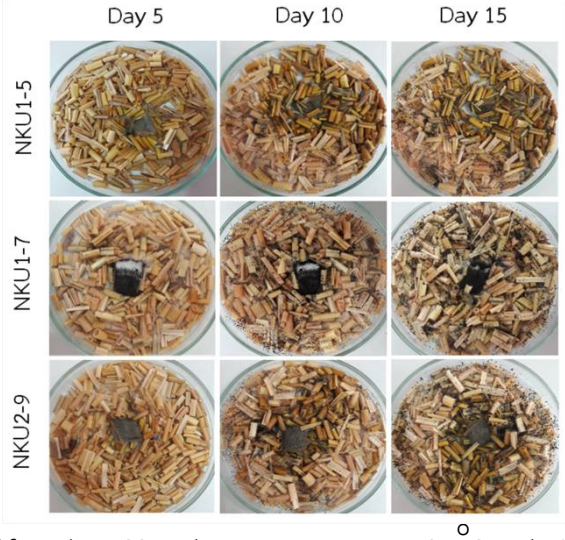

Fig. 2 Growth of fungal on SSF substrate rice straw at 25 °C and 50 % moisture content

Molecular identification of NKU1-5, NKU1-7 and NKU2-9

NKU1-5 and NKU1-7 were prior classified as *Aspergillus* sp. by their morphology, while NKU2-9 was unable to be classified. To verify species of those isolates, partial 5.8S rDNA harborion ITS1 and ITS4 regions, ITS sequence, were amplified fragments using the primers ITS4 were approximately as 500 bases. The BLASTn results of ITS sequences showed that isolates fungi matched 100% similarity with diverse fungal sequences in GenBank (Table 3). NKU1-5 highest %similarity with *Aspergillus niger* (KY378951.1) and *A. tubingensis* (KY378944.1). Isolate NKU1-7 similar with *A. niger* (KY378951.1) and *A. niger* (KY354579.1). Isolate NKU2-9 similar with *Bipolaris papendophii*

(JQ753972.1) and *Curvularia lunata* (KU85 6626.1) and *C. lunata* (KU856627.1).

The phylogenic analysis (Fig.3) indicated that NKU1-5, NKU1-7 and NKU2-9 were clustered into two families, Trichoco-maceae and Aspergillaceae. NKU1-5 and NKU1-7 closed with *A. niger* (KY354579.1) and *A. tublingensis*. Both isolates were identified as *Aspergillus* sp. strain NKU1-5 (KY405016) and *Aspergillus* sp. strain NKU1-7. In fact, NKU2-9 was in the cluster of *Bipolaris* sp. and *Curvularia* sp. strains in Aspergillaceae family. Since *C. papendorfii* is suggested to be *B. papendorfii* origin, belonging to division Ascomycota. In fact, NKU2-9 was clustered with *Bipolaris* sp. and *Curvularia* sp. strains in Aspergillaceae family. Since *C. papendorfii* is suggested to be *B. papendorfii* origin. It supposed to be division Ascomycota. Therefore, NKU2-9 was designed as *Curvularia* sp. strain NKU2-9 (KY405018) accession number KY405016, KY405017 and KY405018 as tabulated in Table 3.

Table 2 Hemicellulose degradation, xylanase activity in SSF

	Uninoculated		NKU1-5		NKU1-7		NKU2-9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Substrate dry weight (%)	100[a]		100[a]		100[a]		100[a]	
NDF (%)	95.62[b]	2.01	77.07[b]	2.34	74.45[ab]	1.99	69.83[a]	0.33
ADF (%)	67.98[b]	2.72	52.53[a]	1.61	52.66[a]	2.23	51.15[a]	0.63
Hemicellulose residues (%)	27.63[d]	0.72	24.53[c]	0.82	21.79[b]	0.77	18.67[a]	0.42
%HD of dry weight	0[a]	0.82	2.43[b]	0.82	5.17[c]	0.77	8.29[d]	0.42
Protein concentration (mg/ml)	0	0	0.463	0.01	0.73	0.01	0.84	0.05
xylanase activity (IU/ml)	0[a]	0	2.43[b]	0.09	3.59[c]	0.22	4.48[d]	0.08
xylanase specificity (IU/mg protein)	0[a]	0	5.25[b]	0.30	4.88[b]	0.34	5.36[b]	0.39
xylanase specificity (IU/g dry weigh substrate)	0[a]	0	485.70[b]	18.26	171.90[c]	44.43	896.4[d]	15.6

a-d means homogeneous subsets by Duncan at the 95% confidence level

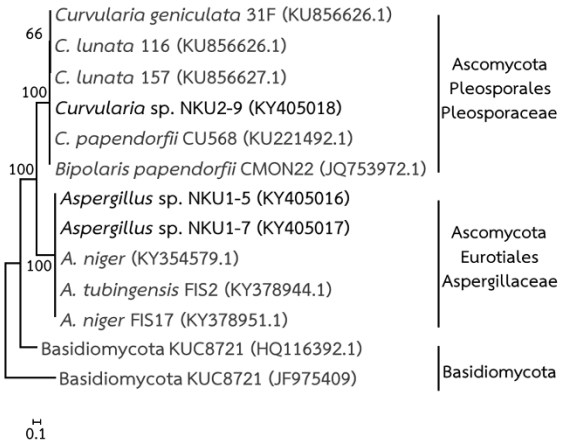

Fig. 3 Phylogenetic tree of isolates NKU1-5, NKU1-7, NKU2-9 and 16 Ascomycota. Numbers on the branches are bootstrap values. Strains used in this study are in bold type. Accession numbers are indicated in parentheses.

DISCUSSION

Recently, about 900 million tons of rice-straw are produced worldwide annually, more than 90% being produced in Asia (Jahromi et al., 2011). In Thailand, rice-straw is most agricultural waste, 10,727,682.14 tons/year. The 10.1% is used as fuel feed and other benefit, but residue is 9,640,908.02 tons/year (Energy, 2012). Normally, the remaining rice straw is disposed of by plowing or burning to clear land for faster crop rotation. These procedures are not suitable because the damage of soil structure and air pollutants are occurred (Phutela et al., 2011; Yuan et al., 2014). An aerobic bioconversion of biomass by microorganisms is an effective method. Due to, it is inexpensive, low-energy requirement, enhance biomineralization in soil, environmental-friendly and sustainable management. It enhance biomineralization in soil (Silva et al., 2013), reduces hardness soil, ultimately results in better plant growth and yield (Chimchana and Vangpikul, 2013) and produce useful byproduct in industries. This study attempted to isolate local lignocellulolytic fungi from rice straw and eucalyptus stump in postharvest season (February - April, 2014) of Nong Khai Province. The result showed that 32 fungi isolate produce polysaccharide and showed hydrolytic activities.

Table 3 Blast results of partial 5.8S rDNA sequences of NKU1-5, NKU1-7 and NKU2-9

isolate number	length (bp)	Species	accession number	Identity (%)	high identity
NKU1-5	579	*Aspergillus* sp. NKU1-5	KY405016	100	KY378951.1 KY378944.1
NKU1-7	577	*Aspergillus* sp. NKU1-7	KY405017	100	KY378951.1 KY354579.1
NKU2-9	545	*Curvularia* sp. NKU2-9	KY405018	100	KU856626.1 KU856627.1 JQ753972.1

However, lignin oxidation activity was not found. Most of them were classified in ascomycetes as *Aspergillus* and *Penicillium* genera. Based on partial 5.8S rDNA sequences, the top three of highest hemicellulose hydrolysis capacity were identified as *Aspergillus* sp. strain NKU1-5, *Aspergillus* sp. strain NKU1-7 and *Curvularia* sp. strain NKU2-9 (Table 2). According to previous study, *Aspergillus*, *Penicillium* and *Curvularia* were isolated from *Eucalyptus diversicolor* and showed brown rot symptoms (Davison and Tay, 2008). The hydrolytic ability of selected isolates suggested that the decomposing rice straw and eucalyptus stumps may involve in brown-rot or soft-rot symptom. Brown-rot degraded polysaccharides without lignin oxidation (Valaskova and Baldrian, 2006), while soft-rot fungi degraded polysaccharides with or without lignin oxidation (Hickman and Perry, 2010). Most of soft-rot fungi were ascomycetes, while member of brown-rot fungi were basidiomycetes (Hatakka and Hammel, 2010).Therefore, we suggested that the selected isolated were grouped in soft-rot fungi. Previous studies from different locations of rice straw showed that lignocellulolytic fungi were identified as *Fusarium moniliform* (Chang et al., 2012), *Aspergillus niger* KUC5183, *Mucor circinelloides* KUC6014, *Trichoderma harzianum* 1 KUC5182, *Aspergillus terreus* ATCC 74135 (Lee et al., 2011), *Phanerochaete chrysosporium, Phlebia brevispora* (Sharma and Arora, 2011). While, wood-rot fungi from eucalyptus were *Pycnoporous sanguineus* and *Ganoderma applanatum* (Andrade et al., 2012), *Hormonema* sp., *Pringsheimia smilacis*, *Ulocladium* sp., *Neofusicoccum luteum* and *N. austral* (Fillat et al., 2016). This suggested that the rice straw and eucalyptus stumps may need different groups of fungi for degradation.

The analyses of hemicellulose degradation and xylanase activity showed highest hemicellulose degradation with xylanase activity in NKU2-9, followed by NKU1-7 and NKU1-5, respectively (Table 3). The NKU2-9 xylanase activity was similar with SSF of *Curvularia intermedia* (KUC5194) that

grew at 27 °C and 300 % moisture content (Lee *et al*., 2011). Although xylanase specific activity of NKU1-5 and NKU1-7 grew on rice straw at 25 °C and 50% moisture content was low, this value was similar to *Aspergillus* sp.; KUC5183, KUC5204, KUC5201 and KUC5203 that grew at 27 °C and moisture content at 300% (Lee et al., 2011). Similarly, the specific activity of NKU1-5 (485.7 IU/g) and NKU1-7 (717.9 IU/g) were lower than *Aspergillus terreus* that culture under the same condition of temperature, 25 °C, in SSF. This may be due to the higher moisture content (100% w/w) with additional of N source from urea (Jahromi et al., 2011). In generally, enzyme activity is dependent on temperature and moisture content during fungal growth. The strains, NKU1-5 and NKU1-7, could be grown at the same optimal temperature (35 °C), similar to other *Aspergillus* sp., which was in range of 30 – 35 °C (Shehu and Bello, 2011). The optimal temperature for NKU2-9 was 25 °C, which corresponded with *Curvularia* sp. (optimal temperature in range of 10 - 40 °C) (Almaguer et al., 2013). This result may explain that why NKU2-9 has higher degradation than NKU1-5 and NKU1-7 in SSF. Likewise, the lower of enzyme activity of NKU1-5 and 1-7 than previous reported of *Aspergillus* sp. (Samanta et al., 2011), due to an inappropriate moisture content. The lowest humidity for growing of *Aspergillus* sp. was 32.5% and the higher relative humidity significantly increased the growth of the *Aspergillus* sp. Interestingly, the fungi isolated from eucalyptus degraded rice straw in a better extent than that isolated from rice straw. This result suggested that dicot fungi can degrade glucuronoarabinoxylan in monocots. Different types of xylan in monocots and dicots has been reported to be glucuronoarabinoxylan and glucuronoxylan, respectively (Scheller and Ulvskov, 2010).

CONCLUSIONS

Decomposing fungi from rice straw and eucalyptus stumps from Nong Khai Province were screen for lignocellulolytic fungi and found out of32 isolates. Most of them were ascomycetes in *Aspergillus* and *Penicillium* genera. The highest lignocellulolytic ability was examined to be 3 soft-rot fungi, *Aspergillus* sp. NKU1-5, *Aspergillus* sp. NKU1-7 and *Curvularia* sp. NKU2-9. They showed cellulose and hemicelluloses hydrolysis capacity. In addition, the moderate xylanase activity was found. The optimal temperature of those isolate was at 25 and 35 °C. This results suggested that co-culture fermentation might be of interested to further verify their decomposing capacity.

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