The potential of cellulose-degrading fungi at various peat maturities in Teluk Bakung Peat Area, Kubu Raya District, Indonesia

SITI KHOTIMAH[1,2,♥], SUHARJONO[1], TRI ARDYATI[1], YULIA NURAINI[3]

[1]Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. Jl. Prof. Dr. H. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia,
Tel.: +62-561-577963, ♥email: siti1990khotimah@gmail.com
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[3]Department of Soil Science, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

Abstract. *Khotimah S, Suharjono, Ardyati T, Nuraini Y. 2021. The potential of cellulose-degrading fungi at various peat maturities in Teluk Bakung Peat Area, Kubu Raya District, Indonesia. Biodiversitas 22: 1981-1990.* Fungi play important roles in degrading cellulose in the maturation process of peat. This study aimed to evaluate the potential of cellulose-degrading fungi at various peat maturities in the forest. Peat soil samples were collected from Teluk Bakung Village, Kubu Raya District, West Kalimantan using a random sampling method. Peat soil samples were obtained at six sampling sites in forests and palm oil plantations. The fungi were cultured by the pour plate method on 1% CMC media. The cellulose-degrading fungi were determined based on a clear zone formation around the colony in CMC agar media. The cellulolytic fungal population in the fibric, hemic, and sapric peats of the secondary forest were 3.2×10^3, 4.6×10^3, and 5.6×10^3 CFU/g, respectively, whereas at fibric, hemic, and sapric-of the shrubs-were 5.6×10^4, 3.9×10^5 and 4.7×10^4 CFU/g, respectively. Among the 25 cellulolytic fungal isolates, 5 isolates (HS1.5, HS3.4.1, HB2.2, SS2.4.1, and SB2.5.1) showed high average clear zone diameter (21-28 mm). The HS3.4.1 and HS1.5 isolates had cellulase enzyme activity of 132.16 and 127.3 U/mL, respectively. These isolates can grow and degrade cellulose at 30°C to 35°C and pH of 2, 3, 4, and 5. The HS3.4.1 and HS1.5 isolate were closely related to *Penicillium citrinum* based on the phylogenetic tree analysis.

Keywords: Cellulase activity, fungi, ITS region, peat

Abbreviations: CMC: Carboxymethylcellulose; PDA: Potato Dextrose Agar; DNS: Dinitrosalicylic Acid, ITS: Internal Transcribed Spacer; PCR: Polymerase Chain Reaction

INTRODUCTION

One of the peatland forests in West Kalimantan Province is located in Teluk Bakung village, Ambawang Subdistrict, Kubu Raya District with an area of 10.139.909 ha, comprising of peat forests, mixed estate and plantations, palm oil plantations, and open land (shrubs) (Wahyunto et al. 2010). The peat in this area has a depth of >300 cm with three-peat maturity levels: fibric, hemic, and sapric. To date, there are no studies on the role of microbes in overhauling the organic material in peat soils of this area.

Tropical peatlands have low mineral content, but high organic matter content (>90%), low total N content, and high C/N ratio resulting in unavailability of these elements to plants. The microelements content, especially Cu, B, and Zn, is considerably low. Peat soils exhibit significantly high cation exchange capacity (CEC; 90–200 me/100 g), but considerably low base saturation (KB) (Wahyunto 2015). It causes a reduction in the availability of nutrients, especially K, Ca, and Mg. The fertility of peat soils can be improved by introducing organic decomposing microbes.

Peat formation and maturation occur through three processes which are biological, physical, and chemical maturation. Soil microbe activity supports the biological maturation of peat. Bacteria and fungi degrade wood decay by cellulase enzyme activity. A higher peat maturity level

usually associated with many species of decomposing fungi (Saragih 2009). *Aspergillus* sp. and *Mucor* sp. are found in fibric peat. Hemic peat usually has a high of *Penicillium chrysogenum*, *Mucor* sp., *Penicillium digitatum*, *Curvularia* sp., and *Penicillium* sp. *Aspergillus* sp., *Fusarium* sp., and *P. chrysogenum* are found in sapric peat (Saragih 2009).

Cellulose is the most abundant renewable biomass in nature, with 7.2×10^{10} tons synthesized through photosynthesis annually. Cellulose is a homogeneous polysaccharide with the cellobiose unit linked by β-1,4-glycosidic bonds (Voriskova and Baldrian 2013). Hydrolysis of cellulose to D-glucose requires endo-1,4-β-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Zhang et al. 2014). Endo-1,4-β-glucanase randomly cuts the cellulose chain, producing the cellulose end. Cellobiohydrolase acts on the cellulose chain from the reducing or non-reducing end, produces cellobiose. β-glucosidase hydrolyzes cellobiose and cello-oligosaccharides to form glucose (Kjoller and Struwe 1982). Due to the chemical structure complexity, natural cellulose is difficult to degrade. Moreover, cellulose is present with lignin and hemicellulose structures. In nature, cellulose was mainly degraded by microbial cellulase. Fungi are the primary decomposer microorganisms due to their ability to produce various

extracellular enzymes. It efficiently decays the recalcitrant, that difficult to be decomposed by other organisms. Several previous studies reported the ability of fungi to degrade leaves under laboratory conditions (Kjoller and Struwe 1982; Osono 2005; Zhang et al. 2008; Osono et al. 2009; Baldrian et al. 2011; Zhang et al. 2014).

Cellulolytic fungi play important roles in the forest ecosystem, transforming cellulose from various plant materials using their secreted enzymes even in extreme environments. In industrial, fungi can be isolated easily, and different strains had been stored in appropriate subcultures (Bahrami et al. 2010; Kunitake and Kobayashi 2017). Peat soil consists of organic material derived from plants, and the soil structure undergoes several changes due to decomposition. Moreover, the degree of maturity of peat to fibric, hemic, and sapric peat is determined by the level of organic decomposed material, such as cellulose content. Each level of peat maturity is inhabited by a group of fungi that can degrade cellulose. In this study, we isolate indigenous cellulolytic fungi and determine their potential to degrade cellulose to accelerate the process of peat maturity and increase peat fertility.

MATERIALS AND METHODS

Soil sampling

The geographical location of Teluk Bakung Village is 0°20′43″ latitude and 109º15′48″ east longitude (Figure 1). The village's climatic conditions were as follows: average temperature (27.1°C); average humidity (86%); air pressure (1012.20 mb); wind speed (4 knots); rainfall (2991 mm).

Peat soil samples were taken at six sampling sites in the forest and palm oil plantation in Teluk Bakung Village, Ambawang Subdistrict, Kubu Raya District, West Kalimantan Province, Indonesia based on peat maturity level.

Peat soil samples were collected randomly. It was collected three samples from the same site, then mixed as composite samples. The grass was cleared from the ground surface of sampling sites. Soil samples were collected using cylinder drill with 3.2 mm of diameter. The peat soil samples were transferred to plastic bags and labeled with depth, date, and acquisition location (Agus et al. 2011). Peat soil characteristics were analyzed based on soil maturity, chemical constituents, and physical structure. Soil chemicals were performed by determining N total levels, available P, organic C, C/N ratio, CEC, pH, and temperature.

Determination of soils peat maturity on sampling sites

Approximately 100 g of peat soil was squeezed into the palm. The retained fibers in the palms were observed and classified according to the following criteria: sapric peat, which was decayed to a great extent that its original material could not be recognized; dark brown to black color; and the fibers retained in the palm are less than one-third of the initial amount. Hemic peat was half-ripe peat, some of its original materials are recognizable, brown in color, and the fiber content retained is one-third or two-thirds of the initial amount. Fibric peat is undisturbed peat, and the original material could be identified, brown, and the fibers retained in the palm were more than two-thirds of the initial amount (Sumawinata et al. 2015).
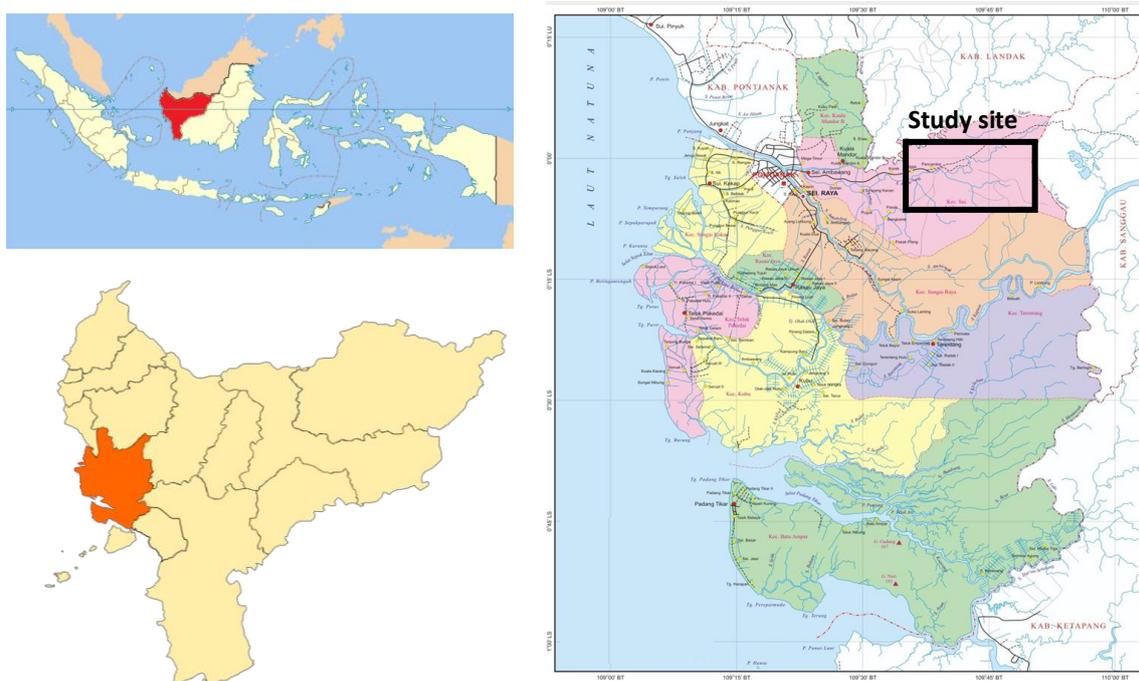
Figure 1. Study site in Teluk Bakung Village, Ambawang Subdistrict, Kubu Raya District, West Kalimantan Province, Indonesia

Determination of soils peat maturity in the laboratory

Peat soil sample (100 g) was transferred into a 10 mL syringe. The syringe pump was pressed, and the volume of peat soil after the syringe pump cannot be compressed was recorded. Peat soils were sieved using a 100-mesh sieve. Filter was sprayed with water to rinse the fine peat. The remaining crude fiber was transferred into the syringe and compressed, and its volume was recorded. Fiber content was calculated by using the formula (Sumawinata et al. 2015).

Fiber content = (V2/V1) × 100%

Where:
V1 : volume of non-filtered peat
V2 : volume of filtered peat.

Isolation and screening of cellulolytic fungi

Peat soil (25 g) was suspended in an Erlenmeyer flask with 225 mL of 0.85% sterile saline solution. The suspension was diluted by serial dilution method in 0.85% saline solution to 10^{-6} dilution. One mL of an aliquot of each suspension was transferred onto CMC agar media (1% carboxymethylcellulose powder, 1.5 g agar, and 100 mL mineral salt solution at pH 7.0 (g/L) [$(NH4)_2SO_4$ 2.0, KH_2PO_4 2.7, Na_2HPO_4 5.3, NaCl 0.2, $MgSO_4$ 0.2, and $CaCl_2$ 0.05] (Arenas-Castro et al. 2016). The plates were incubated at 30°C–31°C for 7 days. After the incubation period, the growing colonies of fungi were purified by culturing on PDA media. The physical (fiber content, water content, and bulk density) and chemical (concentrations of C, N, P, K, microelements, CEC, cation content base, and pH) properties of the peat soil was observed.

Bioassay of cellulose-degrading fungi

Semi-quantitative assay of cellulose-degrading fungi

Fungal colonies were streaked onto the surface of PDA media and incubated at room temperature for 12–18 h. A monospore was inoculated into the CMC 1% agar media and incubated at 30°C for 7 days. The CMC media was flooded with 1% Congo Red reagent for 15 min, followed by NaOH 1.0 M. The plates were incubated at room temperature for 10 min. The clear zone around the fungi colony was measured using a digital caliper. The ratio of the clear zone diameter to the diameter of the colony was calculated (Behera et al. 2014). The fungi demonstrating the highest value of the clear zone index were chosen for further treatment.

Screening of Endoglucanase activity

The 7-days old of pure culture on PDA slant was suspended with 4 mL of 80% Tween 80 solution. The spore suspension was vortexed for 10 min and filtered using glass wool. The fungal spore suspension (5 mL) with a density of 10^7 spores/mL was inoculated into 95 mL of 1% CMC broth media. The culture was incubated in a shaker incubator at 150 rpm at room temperature for 7 days. The culture suspension was centrifuged at 10.000 rpm, 4°C for 5 minutes. The supernatant contains crude extract of endoglucanase enzymes. Endoglucanase was determined by incubating the mixture of 200 μL of cellulase enzyme and 1.0% of CMC in 1800-μL buffer phosphate (50 mM; pH 7.0) at 50°C for 30 min. Two mL of dinitrosalicylic acid (DNS) was added to the solution and heated in a water bath at 100°C for 5 min. The sample was cooled at room temperature, and its absorbance was measured at 540 nm. One unit of endoglucanase activity was the number of enzymes that can hydrolyze CMC and release 1 μg glucose per 1 min reaction at 50°C (Yin et al. 2010). The selected isolates (high endonuclease activity, high spore viability, and does not inhibit other fungal isolates) were cultured in the 1% CMC broth media at pH of 2, 3, 4, and 5 and temperatures of 30°C, 35°C, and 40°C.

Cellulase activity = C/t x V_{E+S}/V_E, (2)

Where:
C : glucose concentration (μg/mL)
V : volume (mL)
t : time reaction (min)
E : enzyme
S : Substrate

Phylogenetic tree

The selected isolates that can grow at various pH (2, 3, 4, and 5) and high temperatures of 35 °C were identified based on the ITS sequence similarity. The 3- to 4-day-old fungal isolates were conducted to total DNA isolation using the Zymo-Spin™ kit. The suspension mixture was vortexed at maximum speed for 5 min for hyphae lysis. It was followed by centrifugation on microcentrifuge at 10.000 g for 1 min. The supernatant (400 μL) was transferred into the Zymo-Spin ™ III-F filter containing a collection tube and recentrifuged at 10.000 g for 1 min.

Table 1. Composition of 50 μL PCR mix

Solution	Volume (μL)	Concentration
ddH$_2$O	19	
GoTaq®Green Master Mix	25	1x
Primer 1 f (ITS 4)	2	10 pmol/μL
Primer 2 f (ITS 5)	2	10 pmol/μL
DNA *template*	2	27.2 ng/μL

Table 2. 16S rDNA PCR reaction program

Step	Temp. (°C)	Time (seconds)	Cycle
Early denaturation	95	300	1
Denaturation	95	30	35
Annealing	52	45	35
Extension	72	90	35
Final extension	72	300	1

Filtrate collected in the tube added with 1,200 μL genomic lysis buffer. 800 μL of this mixture was transferred to the Zymo-Spin™ IIC column contain the collection tube and centrifuged at 10,000 g for 1 min. The filtrate was removed, and the previous steps were repeated. Subsequently, 200 μL DNA-prewash buffer was added to the Zymo-Spin™ IIC column containing a new set of collection tubes and centrifuged at 10,000 g for 1 min. The collection tubes were replaced with sterile microtubes, followed by 50 μL DNA elution buffer to the upper tube, which was recentrifuged at 10.000 g for 1 min. The chromosomal DNA was verified using 0.8% agarose gel electrophoresis. The ITS region of chromosomal DNA was amplified using polymerase chain reaction (PCR) with ITS 4 (5'-TCCTCCGCTTATTGATATGC-3 ') and ITS 5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') as primers (1st BASE 2015). The ITS region was amplified using the PCR mix in Table 1 and PCR program in Table 2. The amplicon of the ITS region was verified using 1.5% agarose gel electrophoresis.

The amplicon of ITS region was sequenced at the 1st Base DNA Sequencing Service, Malaysia. The sequences of the ITS region of each fungal isolate were combined using Bioedit software. It was aligned with ITS sequence references at NCBI database to identify fungal species. The ITS sequences of potential fungal isolate and some reference sequences were aligned to construct a phylogenetic tree. The phylogenetic tree was constructed based on the Neighbor-Joining algorithm and the Tamura-Nei model using MEGA 6 software.

Data analysis

Physical, chemical, fungal abundance, enzyme activity data at pH and temperature optimization were analyzed using one-way ANOVA, followed by Tukey test, using the SPSS 16.0 program. Non-homogeneous data were analyzed using Brown-Forsythe with a p-value of 0.05.

RESULTS AND DISCUSSION

Physiochemical properties of peat soil

Sampling sites for peat soil sampling consists of secondary forest areas and oil palm plantations. Oil palm plantation comprises dry peat as it is located some distance from the Kapuas river. This drainage aims to reduce high water content, create an unsaturated soil for plant root respiration, wash off some organic acids and increase peat decomposition (Tonks et al. 2017). The depth of peat forest areas was >3 m, and the level of peat maturity was determined based on the decomposition level of the plant material (Figure 2). Maturity level consists of three categories of fibric, hemic, and sapric peat.

The chemical characteristics of peat soils in Indonesia are highly diverse. It was determined based on mineral composition, thickness, types of peat composting plants, types of minerals on the substratum (at the bottom of the peat), and the level of peat decomposition. Peatlands in

West Kalimantan, especially in Kubu Raya District, is generally dominated by timber. Therefore, the composition of organic matter is mostly lignin, which typically exceeds 60% of the dry matter, and other components, such as cellulose, hemicellulose, and protein, generally do not exceed 11% (Schropfer et al. 2015).

There was no peat management in peatland in the secondary forest and shrub or peat that the felling feller had just opened. Therefore, the water content was similar. The average water content was 60.6% and classified as moderate. High water content causes the peat to become soft and has a low load-bearing power (Ritung et al. 2011). Bulk density (BD) in the two sampling locations did not differ between fibric, hemic, and sapric peat (about 0.15 g/cm^3) (Table 3). Fibric peat that is generally located in the lower layer has a BD of 0.1 g/cm^3. Palm oil plantation peat and ex-fire peat have a BD of 0.3 g/cm^3, whereas forest peat has a BD of 0.15 g/cm^3 (Nurulita et al. 2016). The total porosity in secondary forest peat and peat in scrub/oil palm ranges from 87 to 93%. Peat has low bearing capacity because of the large pore space, resulting in low soil density and lightweight. Total porosity for fibric/hemic soils is 86%–91% (volume) and 88%–92% for hemic/sapric material, and the average is about 90% by volume (Wahyunto 2015). Intact fiber levels at the two sampling locations differed. Fibric peat has a high intact fiber content. The conductivity at the two sampling locations and the level of maturity of the fibric, hemic, and sapric peat were not significantly different (Table 3).

Figure 2. Vertically varying levels of peat maturity in peat soil profiles: A. Fibric, B. Hemic, C. Sapric

Table 3. Physical properties of peat soils in forests and shrubs in Teluk Bakung Village, Kubu Raya District, West Kalimantan Province, Indonesia

Parameter	Forest			Scrub/oil palm		
	Fibric	Hemic	Sapric	Fibric	Hemic	Sapric
Bulk density (g/cm^3)	0.13 ± 0.03a	0.13 ± 0.02a	0.15 ± 0.03a	0.15 ± 0.01a	0.17 ± 0.03a	0.20 ± 0.05a
Water content pF (% vol)	62.1 ± 7.81a	60.93 ± 9.22a	58.55 ± 6.65a	62.10 ± 5.76a	72.17 ± 1.45a	69.29 ± 1.22a
Particle density (g/cm^3)	1.73 ± 0.01a	1.77 ± 0.03a	1.78 ± 0.04a	1.78 ± 0.03a	1.72 ± 0.04a	1.66 ± 0.01a
Total porosity (%)	92.51 ± 1.54a	92.66 ± 0.87b	91.19 ± 1.72ab	91.58 ± 0.67ab	89.47 ± 1.69ab	87.59 ± 2.75a
Conductivity (cm/h)	3.10 ± 3.33a	9.34 ± 6.55a	16.82 ± 12.4a	17.21 ± 14.33a	2.42 ± 0.77a	7.95 ± 7.57a
Total fiber content (%)	68.67 ± 1.05cd	38.33 ± 2.89ab	28.33 ± 2.89a	71 ± 10.15d	52.96 ± 0.00 bc	25.66 ± 5.13a

Table 4. Chemical properties of peat soils in forests and shrubs in Teluk Bakung Village, Kubu Raya District, West Kalimantan Province, Indonesia

Parameter	Forest			Scrub/oil palm		
	Fibric	Hemic	Sapric	Fibric	Hemic	Sapric
pH	3.05 ± 0.091a	3.16 ± 0.11a	2.95 ± 0.135a	3.0 ± 0.092a	2.96 ± 0.090a	3.00 ± 0.066a
C-Organic (%)	57.17 ± 0.32a	56.71 ± 0.40a	57.00 ± 0.112a	56.77 ± 1.605a	55.91 ± 1.251a	54.72 ± 0.253a
N-Total (%)	1.88 ± 0.02a	1.89 ± 0.012a	1.91 ± 0.023a	1.88 ± 0.01a	1.90 ± 0.011a	1.89 ± 0.006a
P_2O_5	41.28 ± 34.71a	38.71 ± 46.41a	81.95 ± 36.33a	50.75 ± 39.45a	54.93 ± 14.51a	103.17 ± 32.65a
Ca (cmol(+)/kg)	2.69 ± 0.61a	2.65 ± 1.49b	3.55 ± 2.414ab	1.40 ± 0.185ab	1.14 ± 0.410a	1.30 ± 0.513a
Mg (cmol(+)/kg)	0.88 ± 0.45236a	1.13 ± 0.68a	1.3 ± 0.796a	0.58 ± 0.452a	0.65 ± 0.40a	0.94 ± 0.271a
K (cmol(+)/kg)	0.37 ± 0.19a	0.37 ± 0.145a	0.46 ± 0.212a	0.75 ± 0.276a	0.88 ± 0.297a	0.92 ± 0.307a
Na (cmol(+)/kg)	0.61 ± 0.335ab	0.62 ± 0.23ab	0.67 ± 0.427a	1.36 ± 0.469ab	1.49 ± 0.499b	1.58 ± 0.508b
CEC (cmol(+)/kg)	123.23 ± 0.68a	122.22 ± 0.86a	122.67 ± 0.248a	122.35 ± 3.45a	120.48 ± 2.70a	117.93 ± 0.547a

Peat soils in secondary forests and palm oil plantations are highly acidic with pH of about 2–3 (Table 4). The pH of fibric, hemic, and sapric peat soils was not significantly different between the secondary forest and palm oil plantations. The acidity of peat soils is closely related to organic acid content, such as humic acid and fulvic acid (Schropfer et al. 2015). Secondary forest peat and shrubs used for palm oil plantations with different maturity levels have considerably high C-organic content (56%) (Table 4). There was no significant effect on fibric, hemic, and sapric peat at the two sampling locations. The condition of drained peatlands can change because it creates an anaerobic atmosphere. These changes increase microorganisms' activity that degrade soil organic matter (Baldani et al. 2014; Elliott et al. 2015). Secondary forest peat and shrubs used for palm oil plantations have low N-total content (1.88%), and there were no differences in fibric, hemic, and sapric peat (Table 4). This suggested pioneer microorganisms of soil organic matter and N-cycle in the soil cannot work optimally. The activity of microorganisms is strongly influenced by soil pH conditions (Atekan et al. 2014). Secondary forest peat and shrubs used for palm oil plantations have a relatively high P_2O_5 content and no difference in the two sampling locations. The P_2O_5 content in sapric peat was higher than the fibric and hemic peat. It was due to the higher storage and supply of P at sapric peat soils than fibric soil. The phosphorus element (P) in the peat soils was mostly found in the form of P-organic, which undergoes a process of mineralization into P-inorganic by microorganisms (Atekan et al. 2014). CEC on secondary forest peat and shrubs in locations was 121 cmol/kg, and no difference at peat fibric,

hemic, and sapric (Table 4). The peat CEC was high, whereas the base saturation (KB) was considerably low (Table 3). Peat in secondary forests and shrubs that would be planted with oil palms in the sampling area had a considerably low base cation content (Table 4). The peat thickness caused a low base content so that the soil becomes acidic (Kolli et al. 2016). The low content of Ca, Mg, K, and Na might be due to the peat condition, which is always saturated with water and only originated from the accumulation of organic matter, so there is no need to add mineral elements.

The density of cellulolytic degrading fungi at peat soil

The density of cellulolytic fungi in peat soils in secondary forests and shrubs planted with oil palm was significantly different. The population density of cellulolytic fungi in fibric, hemic, and sapric peat of secondary forests was not significantly different that is, were 3.2×10^3, 4.6×10^3, and 5.6×10^3 CFU/g, respectively. The fungi density was evenly distributed at various levels of peat maturity. The fungi density differs in fibric, hemic, and sapric peat of shrubs oil palms of 5.6×10^4, 3.9×10^5, and 4.7×10^4 cfu/g, respectively (Figure 3). In the hemic peat, the fungal density is higher than in fibric and sapric peat, but the density of cellulolytic fungi in fibric and sapric peat is not different. The density of cellulolytic fungi in shrubs/land planted with oil palm was higher than in secondary forests. It is possibly due to the drainage systems that enhance aerobic conditions. These conditions increase the activity of microorganisms to decompose peat. Drainage aims can reduce high water content, create an unsaturated state for breathing plant

roots, wash off some organic acids and increase peat decomposition (Tonks et al. 2017). The majority of microbial decomposition of organic matter such as cellulose are bacteria and fungi. Fungi are the dominant organisms in hydrolyzing cellulose, especially in peat soils. Fungi secrete extracellular enzymes that are capable of decomposing organic matter. The activity of decomposing mold and organic matter determines the maturity level of the peat. The higher the level of maturity of the peat, the more types of decomposing molds are found.

The potency of cellulolytic enzyme activity of fungi isolates

Fifteen fungal species were isolated and selected to determine their ability to degrade cellulose. The SS2.4.1 and SB2.5.1 isolates had the highest clear zone diameter of 24.30 and 23.36 mm, respectively (Figure 4). These fungal isolates were isolated from sapric and fibric peat in shrubs/palm oil (Figure 5). Cellulolytic fungi play important roles in the natural ecosystem to convert and break down cellulose.

Endoglucanase activity of fungi

Ten isolates of cellulose-degrading fungi were assayed for their cellulolytic enzyme activity. Three fungal isolates (HS3.4.1, HS1.5, and SS2.4.1.) had high cellulase activity, at 132, 127.3, and 121 U/mL, respectively (Figure 6). These species were collected from sapric peat in secondary forests and shrubs. High levels of decomposition might be caused by active microorganisms that degrade cellulose. The level of peat maturity was determined by the level of decomposition of the organic matter. The cellulose hydrolyzing microorganisms are dominated by the prokaryotic group in bacteria, and the eukaryotic group in fungi (Lynd et al. 2002). Aerobic filamentous fungi produce extracellular non-composite cellulase systems (Lynd et al. 2002; Wilson 2011). The fungus produces an enzyme endo-1,4-β-D-glucanase decreases β-D-glucose oligomer to glucose and GH61 protein, which encourages cellulose decomposition of oxidative degradation of glucose polymers (Zhang et al. 2014). The β-glucosidase plays an important role in the biological conversion of cellulose to glucose (Doolotkeldieva and Bobusheva 2011).
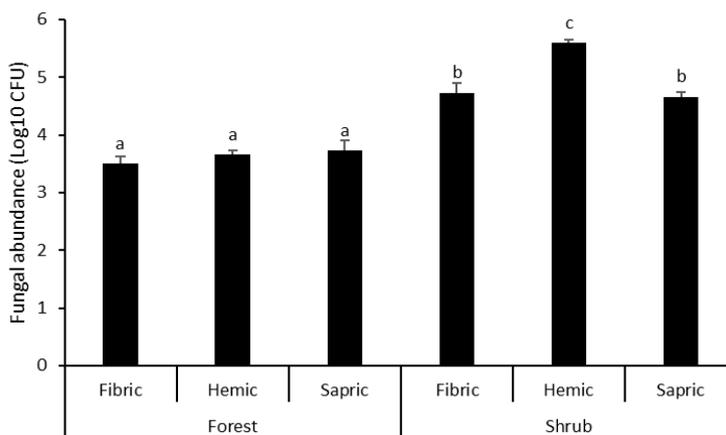

Figure 3. The abundance of cellulolytic fungi at fibric, hemic, and sapric peat in secondary forest and shrub/palm oil plantation
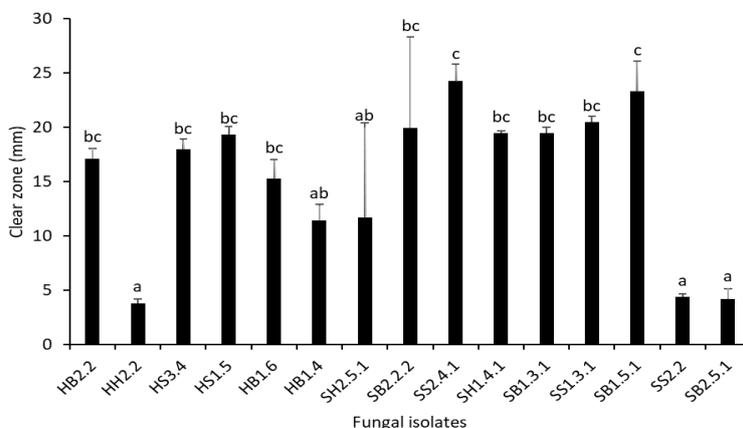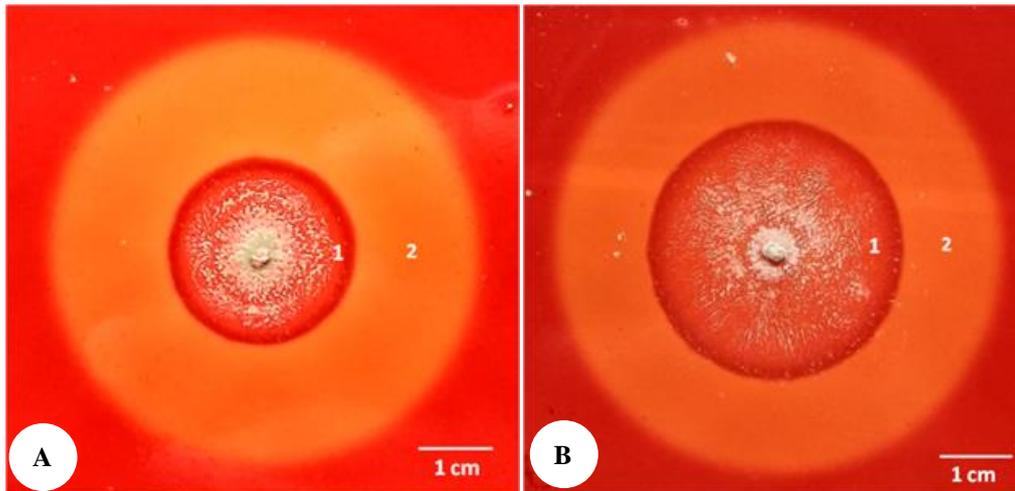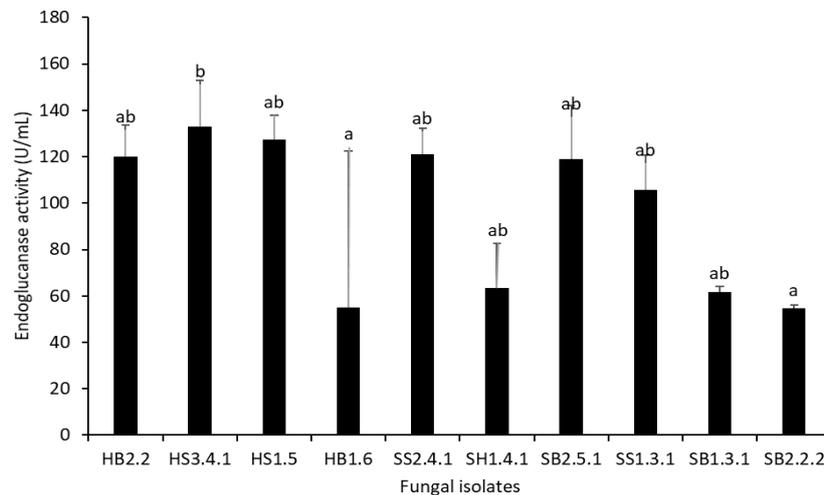

Figure 4. The diameter of the clear zone of the fungal isolates from peat soils indicates cellulolytic activity

Figure 5. The clear zone around the fungal colony on 1 % sodium carboxymethyl cellulose agar medium. A. SB2.5.1, B. SS2.4.1 isolate of fungi
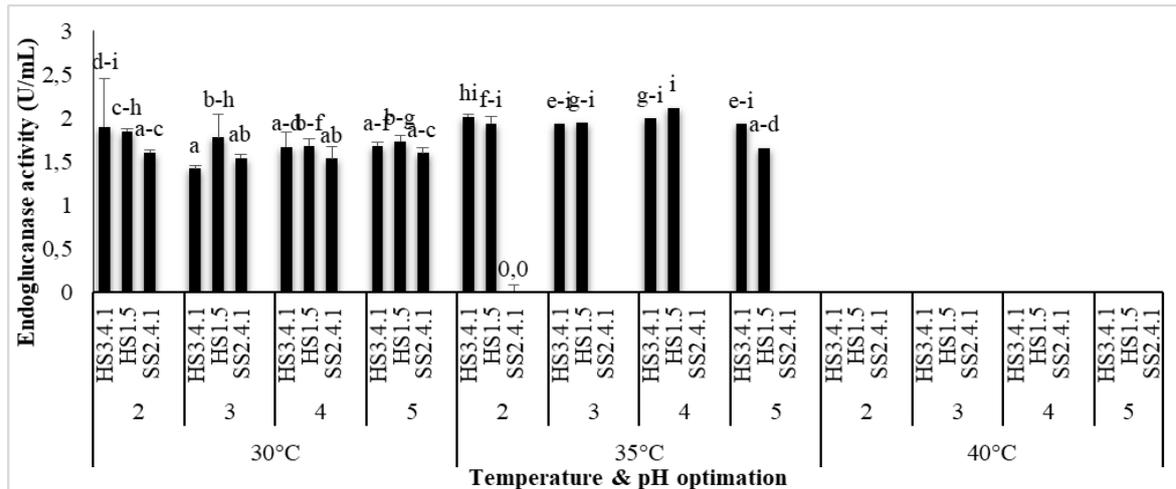

Figure 6. Endoglucanase activity of selected fungi isolates.

## Optimum pH and temperature for fungal cellulase activity

Three fungal isolates (HS3.4.1, HS1.5, and SS2.4) which had high cellulase activity, were cultured at various pH and temperatures. The results showed that isolates HS3.4.1 and HS1.5 were able to grow at pH 2, 3, 4, and 5, and able to degrade cellulose (Figure 7). The isolates HS3.4.1 and HS1.5 were also able to grow at 35°C and capable of degrading cellulose. These isolates were collected from sapric peat. In sapric peat, almost all the remaining plant or cellulose had been degraded to into simple compounds.

Isolates HS3.4.1, HS1.5 and SS2.4.1 were able to degrade cellulose at 30°C ranged 1.4-2.1 U / mL (Figure 7). Isolates HS3.4.1 and HS1.5 were potential to degrade cellulose at 35°C. At 40°C the three fungal isolates were unable to grow. The results showed that three isolates could grow and degrade cellulose at different pHs, at 30ºC

(Figure 7). The ability to grow at a wide pH range may be caused by one enzyme, active at several pH values, or several enzymes with different optimal pH (Kunitake and Kobayashi 2017). *Trichoderma* and *Penicillium* have cellulolytic enzyme activity of 141.4 U / mL and 108.0 U / mL at optimum pH and temperature of 4.0 and 30°C (Zhang et al. 2014). Most fungal endoglucanase activity has optimum at pH 4 - 6. The relatively wide optimum pH of these three fungi showed that they could degrade cellulose in different pH environments (Figure 6). *Penicillium* possible grow in a wider range of soil pH (3–8) and may be influenced by other factors such as other cellulolytic fungi as competitor (Kornillowicz-Kowalska et al. 2003). *P. citrinum* grows optimally at 37°C; other fungal species have a maximum growth temperature of 30°C (Houbraken et al. 2010). Most of the fungal endoglucanases have optimum activity at pH 4 - 6 (Fernandez et al. 2017).

Figure 7. Cellulase enzyme activity (Endoglucanase) of selected fungi isolates cultured on 1% CMC broth media at various pH and temperatures
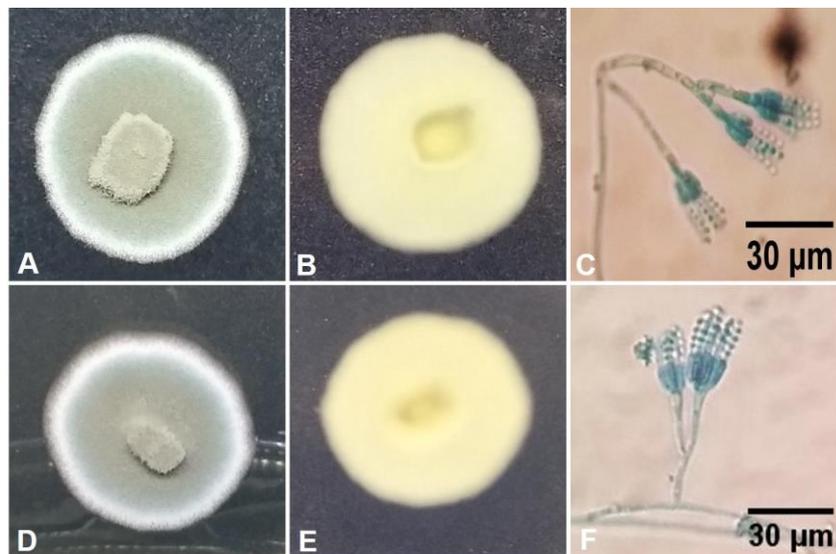

Figure 8. Morphological characters and microscopic structure (magnification (400 X) of HS3.4.1 and HS1.5 isolates. HS3.4.1 strain a) colonies viewed from above, b) colonies viewed from below c) hyphae structures, conidia; strain HS1.5 d) colonies viewed from above, e) colonies viewed from below f) hyphal and conidial structures

Phylogenetic tree of selected fungi isolate

Two isolates (HS3.4.1 and HS1.5) have morphological characters as follows: colony size (27–28 mm), filamentous colony shape, flat colony surface, having concentric lines, white pigmentation, inherent dry texture, optical features greyish green, white edges, and the edge of the colony thoroughly (Figure 8).

The HS3.4.1 and HS1.5 isolates were identified phylogenetically. The results showed that the two isolates belong to *Penicillium citrinum* based on the ITS sequence (Figure 9). *Penicillium citrinum* has a vertical terminal metulae with small conidia (2–3 μm), sharing symmetrical biverticillate conidiophores with occasional additional branches. Sporulation on PDAs with bluish-gray conidia and reversing yellow. Conidiophores arising from aerial hyphae, predominantly irregularly biverticillate. *Penicillium citrinum* is a filamentous fungus common in nature (tropical), and it is found in various soil substrates, tropical cereals, spices, and indoor environments (Samson et al. 2004). *P. citrinum* produced citrinin mycotoxins consistently (Syrchin et al. 2015), and several other electrolytes, such as tanzowaic A acid, quinolactacins, quinocitrinine, asteric acids, and compactins (Syrchin et al. 2015; Mahishi et al. 2014). *P. citrinum* also interacts with plant roots. Therefore, it is very beneficial because the fungus produced gibberellins, to increase stem growth (Fernandez et al. 2017). The most common fungi genera were *Aspergillus*, *Penicillium*, *Chaetomium*, and *Trichophyton*, most of which can degrade cellulose (Arenas-Castro et al. 2016).
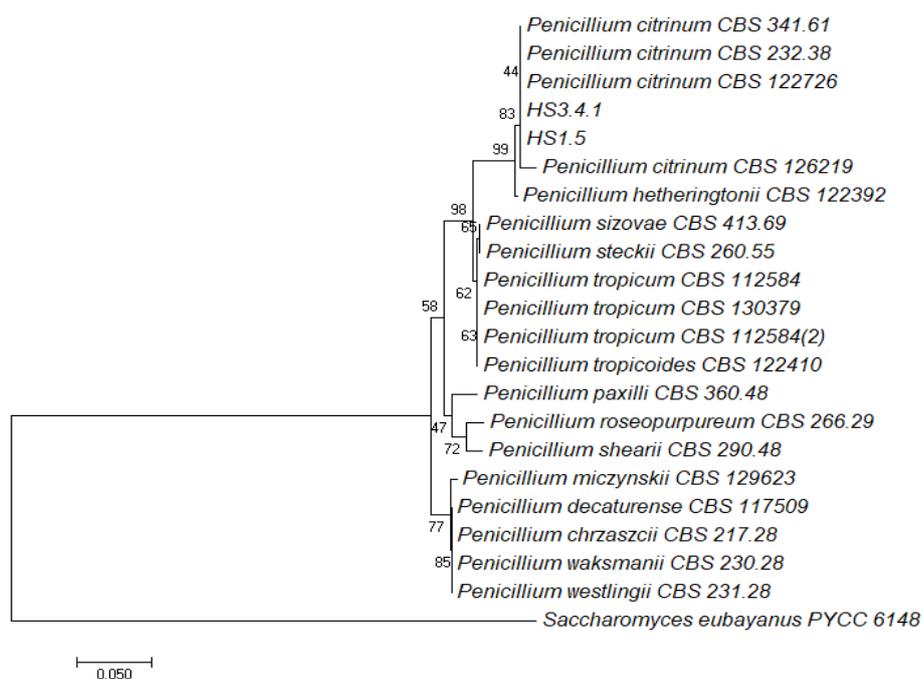
Figure 9. Neighbor-joining phylogenetic tree of HS3.4.1, HS1.5 and reference strain types based on ITS region of DNA sequences, with, Tamura-Nei algorithms of 1000 bootstrap replicates

In conclusion, the density of cellulolytic fungi in secondary forest at the fibric, hemic, and sapric peat were 3.2×10^3, 4.6×10^3, and 5.6×10^3 cfu/g, respectively. The cellulolytic fungi density in the shrubs/peat and palm oil area were 5.6×10^4, 3.9×10^5, and 4.7×10^4 cfu/g, respectively. Fifteen fungal isolates could degrade cellulose. The isolates HS_1, $HS_3.4.1$, $SB_2.2$, $SS_2.2$, and $SH_1.4$ had high cellulase activity that indicated by clear zone diameter of 20–25 mm. The isolates HS3.4.1, HS1.5, and SS2.4.1 cellulase enzyme activity at 132.16, 127.3, and 121 U/mL, respectively. The HS3.4.1 and HS1.5 isolates were able to grow and degrade cellulose at pH 2, 3, 4, and 5 and at temperatures 30°C and 35°C. Based on the ITS sequence similarity, HS3.4.1 and HS1.5 closely related to *Penicillium citrinum*. Indigenous fungi of peat soil of various levels of maturity can degrade high cellulose, capable of decomposing or reducing organic C. The fungus can grow at an acidic pH of about 3-4, a high temperature of 35°C. This fungus can be used as a biofertilizer candidate on peat soils, especially fibric peat with high fiber content and low in nutrients. The cellulases secreted by these fungi can be developed commercially with further research.

ACKNOWLEDGEMENTS

REFERENCES

1st BASE. 2015. Universal Primers Offered. http//base-asia.com/dna-sequencing-services/universal-primers-offered. Accessed May 8, 2017.

Agus F, Hairiah K, Mulyani A. 2011. Pengukuran Cadangan Karbon Tanah Gambut [Peat Soil Carbon Reserve Measurement]. Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian, Bogor. [Indonesian]

Arenas-Castro H, Muñoz-Gomez SA, Uribe-Acosta M, Castaño-Castaño L, Lizarazo-Medina, P.X. 2016. Richness, cellulolytic activity, and fungicide susceptibility of fungi from a bird biological collection. Acta Biol Colomb 21 (1): 167-173. DOI: 10.15446/abc.v21n1.49240

Atekan A, Nuraini Y, Handayanto E, Syekhfani S. 2014. The potential of phosphate solubilizing bacteria isolated from sugarcane wastes for solubilizing phosphate. J Degraded Mining Lands Manag 1 (4): 175-182. DOI: 10.15243/jdmlm.2014.014.175

Bahrami A, Emadolin I, Atashi MR, Bork HR. 2010. Land-use change and soil degradation: A case study, North of Iran. Agriculture and Biol J North Am 1 (4): 600-605.

Baldani JI, Reis VM, Videira SS, Boddey LH, Baldani VLD. 2014. The Art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologist. Plant Soil 384 (1-2): 413-431. DOI: 10.1007/s11104-014-2186-6

Baldrian P, Vorıskova J, Dobiasova P, Merhautova V, Lisa L, Valaskova V. 2011. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. Plant Soil 338 (1-2): 111-125. DOI: 10.1007/s11104-010-0324-3

Behera BC, Parida S, Dutta SK, Thatoi HN. 2014. Isolation and identification of cellulose degrading bacteria from mangrove soil of Mahanadi river delta and their cellulase production ability. American J Microbiol Res 2 (1): 41-46. DOI: 10.12691/ajmr-2-1-6

Doolotkeldieva TD, Bobusheva ST. 2011. Screening of wild-type fungal isolates for cellulolytic activity. Microbiol Insights 4: MBI.26418. DOI: 10.4137/mbi.s6418

Elliott DR, Caporn SJM, Nwaishi F, Nilsson RH, Sen R. 2015. Bacterial and fungal communities in degraded ombrotrophic peatland undergoing natural and managed re-vegetation. PLoS ONE 10 (5): eo124726. DOI: 10.1371/journal.pone.0124726

Fernandez RD, Bulacio N, Alvarez A, Pajot H, Aragon R. 2017. Fungal decomposers of leaf litter from an invaded and native mountain forest of NW Argentina. Antonie van Leeuwenhoek 110 (9): 1207-1218. DOI: 10.1007/s10482-017-0893-8

Houbraken JAMP, Frisvad JC, Samson RA. 2010. Taxonomy of Penicillium citrinum and related species. Fungal Divers 44 (1): 117-133. DOI: 10.1007/s13225-010-0047-z

Kjoller A, Struwe S. 1982. Microfungi in ecosystems: Fungal occurrence and activity in litter and soil. Oikos 39 (3): 391-422. DOI: 10.2307/3544690

Kolli R, Asi E, Tonutare T, Astover A, Szajdak L, Tamm I. 2016. Fabric and properties of mineral soils underlying a shallow peat mantle in Estonia. Quat Int 418: 84-93. DOI: 10.1016/j.quaint.2015.08.045

Kornillowicz-Kowalska T, Iglik H, Wojdylo B. 2003. Correlation between the abundance of cellulolitic fungi and selected soil properties. Acta Mycol 38 (1-2): 161-172. DOI: 10.5586/am.2003.017

Kunitake E, Kobayashi T. 2017. Conservation and diversity of the regulators of cellulolytic enzyme genes in Ascomycete fungi. Curr Genet 63 (6): 951-958. DOI: 10.1007/s00294-017-0695-6

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66 (4): 506-577. DOI: 10.1128/mmbr.66.4.739.2002

Mahishi PK, Tiwari KL, Jadhav SK. 2014. Physiochemical and microbial studies of paper mill effluent, Raipur (Chhattisgarh), India. Banat's J Biotechnol V (9): 57-62. DOI: 10.7904/2068-4738-v(09)-57

Nurulita Y, Adetutu EM, Kadali KK, Shahsavari E, Zul D, Taha M, Ball AS. 2016. Assessment of the influence of oil palm and rubber plantations in tropical peat swamp soils using microbial diversity and activity analysis. J Agric Chem Environ 5 (2): 53-65. DOI: 10.4236/jacen.2016.52006

Osono T. 2005. Colonization and succession of fungi during decomposition of Swida controversa leaf litter. Mycologia 97 (3): 589-597. DOI: 10.1080/15572536.2006.11832789

Osono T, Ishii Y, Takeda H, Seramethakun T, Khamyong S, To-Anun C, Hirose D, Tokumasu S, Kakishima M. 2009. Fungal succession and lignin decomposition on Shorea obtusa leaves in a tropical seasonal forest in northern Thailand. Fungal Divers 36: 101-119. DOI: 10.1.1.465.2471

Ritung S, Wahyunto W, Nugroho K, Sukarman S, Hikmatullah H, Suparto, Tafakresnanto C. 2011. Peta Lahan Gambut Indonesia Skala 1:250.000 [Map of Indonesian Peatlands Scale 1: 250,000]. BBSDLP, Bogor, Indonesia. [Indonesian]

Samson RA, Frisvad JC. 2004. Penicillium subgenus Penicillium: New taxonomic schemes and mycotoxins and other extrolites. Stud Mycol 49 (49): 1-260.

Saragih SD. 2009. Types of fungi at some level of peat maturity. [Thesis]. University of North Sumatra, Medan. [Indonesian]

Schropfer SB, Bottene MK, Blanchin L, Robinson LC, de Lima V, Jahno VD, da Silva Barud H Robeiro SJL. 2015. Biodegradation evaluation of bacterial cellulose, vegetable cellulose and poly (3-hydroxybutyrate) in soil. Polimeros 25 (2): 154-160. DOI: 10.1590/0104-1428.1712

Sumawinata B, Djajakirana G, Handayani L. 2015. Assessment of physical, chemical and biological properties of peat soils. IPN Toolbox, Jakarta. [Indonesian]

Syrchin SO, Kharkevych OS, Pavlychenko AK, Yurieva OM, Nakonechna LT, Nekleva YS, Kurchenko IM. 2015. Extracellular cellulolytic complexes production by microscopic fungi. Biotechnol Acta 8 (5): 78-85. DOI: 10.15407/biotech8.05.078

Tonks AJ, Aplin P, Beriro DJ, Cooper H, Evers S, Vane CH, Sjogersten S. 2017. Impacts of conversion of tropical peat swamp forest to oil palm plantation on peat organic chemistry, physical properties and carbon stock. Geoderma 289 (1): 36-45. DOI: 10.1016/j.geoderma.2016.11.018

Voriskova J, Baldrian P. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME J 7 (3): 477-486. DOI: 10.1038/ismej.2012.116

Wahyunto W. 2015. Peatlands in Indonesia. Term/definition, classification, extent, distribution, updating of spatial data on peatlands. IPN Toolbox, Jakarta. [Indonesian]

Wahyunto W, Supriatna W and Agus F. 2010. Land use change and recommendation for sustainable development of peatland for agriculture: Case study at Kubu raya and Pontianak Districts, West Kalimantan, Indonesia. Indones J Agric Sc 11 (1): 32-40. DOI: 10.21082/ijas.v11n1.2010.32-40

Wilson DB. 2011. Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol 14 (3): 259-263. DOI: 10.1016/j.mib.2011.04.004

Yin LJ, Lin HH, Xiao ZR. 2010. Purification and characterization of a cellulose from Bacillus subtilis YJI. J Mar Sci Technol 18 (3): 466-471.

Zhang P, Tian X, He X, Song F, Ren L, Jiang P. 2008. Effect of litter quality on its decomposition in broadleaf and coniferous forest. Eur J Soil Biol 44 (4): 392-399. DOI: 10.1016/j.ejsobi.2008.04.005

Zhang Z, Liu JL, Lan JY, Duan CJ, Ma QS, Feng JX. 2014. Predominance of Trichoderma and Penicillium in cellulolytic aerobic filamentous fungi from subtropical and tropical forests in China, and their use in finding highly efficient β-glucosidase. Biotechnol Biofuels 7 (1): 107. DOI: 10.1186/1754-6834-7-107