

Isolation and identification of cellulolytic bacteria at fibric, hemic and sapric peat in Teluk Bakung Peatland, Kubu Raya District, Indonesia

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Abstract. Khotimah S, Suharjono, Ardyati T, Nurani Y. 2020. Isolation and identification of cellulolytic bacteria at fibric, hemic, and sapric peat in Teluk Bakung Peatland, Kubu Raya District, Indonesia. *Biodiversitas* 21: 2103-2112. Cellulose degrading bacteria was one of the microbial removers of organic matter contained in the soil into simpler monomers so that it can be utilized by other organisms. The objective of the research was to obtain cellulose-degrading bacteria found on fibric, hemic, and sapric peat in forest and shrubs (oil palm). The bacteria were isolated by pour plate method on 1% CMC media. Selected isolates were assayed quantitatively based on the activity of cellulase enzyme, identified with 16S rDNA. The density of cellulolytic bacteria in the secondary forest peat of fibric, hemic, sapric were 2.1×10^3 cfu/g, 5.9×10^4 cfu/g, and 4.9×10^4 cfu/g whereas, in the area of shrubs/oil palm peat fibric, hemic and sapric 6.9×10^4 cfu/g, 8.4×10^4 cfu/g and 3.4×10^5 cfu/g respectively. There were 19 bacterial isolates that have clear zones around the colony as degradation of cellulose had highest ability to degrade cellulose with clear zones of 5-7 mm. The strain of SB1.1.1 showed highest activity of cellulase enzyme 11.17 U/mL, followed by HH3.1.1 strain and SB2.3 7.83 U/mL. Based on the phylogeny tree, strain SB1.1.1 and HH3.1.1 have the closest kinship relationship with *Bacillus cereus* with a kinship relationship of 100%, while SB2.3 has the closest kinship relationship with *Bacillus stratosphericus* with a relationship of 99.85 %.

Keywords: Activity cellulose bacteria, Borneo, hemic, peat fibric, sapric

INTRODUCTION

The distribution of peatlands in Indonesia varies, around 5.24 million ha is shallow peat, 3.91 million-7 ha was medium peat, 2.76 million ha was deep peat and 2.98 million ha was very deep peat (Maftu'ah & Dedi Nursyamsi 2019). Peat in Teluk Bakung Village, Ambawang Sub-district, Kubu Raya District was deep peat with thickness above 300 m. The area of peat in Kubu Raya District was around 408 369 ha (58%) of West Kalimantan Province, 69.5% was still forest area (Wahyunto et al. 2010), the other is open land (shrub), oil palm plantation land. Tropical peatlands had a low mineral content in comparison to organic matter content (more than 90%), have a high N content and a high C/N ratio, peat soils contain toxic organic acids that cause low pH (Lohila et al. 2011; Ojanen 2013, Linkosalmi et al. 2015) and the content of microelements especially Cu, B, and Zn was very low. In this case, the biological maturation process plays more important role, including aspects of the maturation process and microbial activity and had three levels of peat maturity namely fibric, hemic, and sapric (Kononen et al. 2016). These thick peat deposits contain 11-14% of all C stored in peat soils globally (Page et al. 2012). Decomposition in peat soils was regulated by abiotic environmental factors such as oxygen, humidity, nutrient availability, and pH (Hoyos-Santillan et al. 2015).

The diversity of microbes in peat soils was also

specific, usually dominated by both acidophilic and acidotolerant heterotrophic microbes. Microbial diversity in peatlands played a very important role in the degradation of organic compounds and the biochemical transformation of C, N, P, and S (Elliot et al. 2015; Senga et al. 2015). Microbial density at the top 25 cm of soil surface was highest (Fierer et al. 2003). However, a large number of microbes had been found far below the surface, (Senga et al. 2015; Fritze et al. 2000; Blume et al. 2002). Fierer et al. (2003) reported that around 35% of the total microbial biomass in the top 2 m of terrestrial soil was found at depths > 25 cm. Based on the phylogenetic sequence of 16S rRNA for prokaryotes which were able to hydrolyze cellulose to prokaryotic groups dominated by bacteria (Lynd et al. 2002). Soil bacteria play an important role in the degradation of organic matter, which was largely due to its ability to decompose cellulose-based materials (Soares et al. 2012). Many isolates of cellulolytic bacteria (about 46%) showed the existence of endoglucanase activity, only a small proportion of isolates used exoglucanase (degradation) activity. Endocellulases bind randomly along with cellulose molecules, made several hemispheres, and then separate from the chain, so they quickly reduce the viscosity of CMC (Soares et al. 2012). Progressive endoglucanase, which had so far only been found in bacteria (Alshelmani et al. 2013). Cellulase was the most diverse enzyme that catalyzes a single reaction, namely the hydrolysis of the β -1,4 relationship which joins two

glucose molecules in a cellulose molecule (Top and Wilson 2011). Peat is a pile of decomposed plant residues resulted due to the role of microbial activity, one of which is cellulose bacteria. Therefore, this study focused on the isolation and identification of cellulolytic bacteria isolated from peat soils found in fibric, hemic, and sapric peat in the environment of open forest and peat. Bacterial activity in the composition of organic material (cellulose) determines the level of peat maturity, the higher the level of peat maturity, the more types of decomposing bacteria are found. Peat has three maturities namely fibric, hemic and sapric, inhabited by specific bacteria that negate cellulose in acidic conditions (pH around 3). Bacteria found are expected to be able to increase the fertility of peatlands due to their activity.

MATERIALS AND METHODS

Description of research locations

The sampling location was located in Teluk Bakung Village, Ambawang Sub-district, Kubu Raya District, West Kalimantan Province, Indonesia. The total area of the village is 7553,331 ha, with peat depths varying to more than 3 m. The level of peat maturity also varies fibric, hemic, and sapric. This region consists of swamp/peat forests, mixed plantations, and gardens, oil palm plantations, shrubs, and rivers. Administratively, Teluk Bakung Village, Sungai Ambawang Sub-district is located north of Kuala Mandor B Sub-district, south of Sungai Raya Sub-district, west of Pontianak City and east of Sanggau District. Kubu Raya District is located north of Pontianak District, south of the Kayong District, West of Natuna Sea, and East of Landak and Sanggau Regencies. The distance from Kubu Raya city to TelukBakung Village is around 38 km. Geographical location 0°20'43" latitude and 109°15'48" east longitude. The village had an average temperature of 27.1°C, average humidity of 86%, air pressure (mb) 1012.20, wind speed (knots) 4, rainfall (mm³) 223, and sun exposure 55% (Central Statistic Agency Kubu Raya 2016).

Soil sampling

Peat soil samples were taken in Teluk Bakung Village, Kubu Raya District with a purposive random sampling method based on the level of peat maturity. Soil samples were taken at six points in the forest and shrub/oil palm area. At each point, three replications of soil were taken which were composite samples. The soil in peatlands was leveled and cleared from the grass and litter. The peat soil drill (6 mm Stainless steels, 70 mm sample length, stainless steel diameter 3.2 mm pipes) was plugged vertically into the ground then rotated clockwise. The drill was pulled out of the ground and placed above the soil surface perpendicularly, then the soil was removed from the drill and moved into a PVC pipe coated with aluminum foil. The same method was taken to other locations. Land taken was labeled with information and information on depth, date, and location of land acquisition (Agus 2005). Determination of the level of peat maturity was done by 2

methods. Methods for determining the maturity of peat in the field, determination method of peat maturity in the laboratory (Sumawinata et al. 2015).

Soil physicochemical analysis

The characteristics of peat soil analyzed include the level of soil maturity, chemical analysis, and physical analysis to determine the peat soil content. Chemical physics analysis was carried out to determine the total N levels, available P, organic C, soil pH, K, water content, total fiber content, bulk density, and soil temperature.

Isolation and screening of bacterial strains for cellulose degradation

About 25 g of peat soil sample was suspended with 225 mL of sterile saline or 0.85% NaCl (1: 9) in Erlenmeyer flask to obtain a 10⁻¹ dilution series, then homogenized by shaking for one hour to dissolve it so that the microbes attached to the particles the soil can be easily isolated. One milliliter of suspension from the 10⁻¹ dilution series was inserted at nine milliliters of 0.85% NaCl and then diluted to 10⁻⁷. Each peat soil suspension resulting from 10⁻¹-10⁻⁷ dilution was taken 0.1 mL and inoculated aseptically on 1% CMC (Carboxymethylcellulose) media. The composition of CMC media were 1.5 g of CMC (Carboxymethylcellulose) powder in 100 mL of 100% mineral salt solution pH 7. This mixture were then mixed this following minerals, i.e.: (NH₄)₂SO₄ (2.0 g/L), KH₂PO₄ (2.7 g/L), Na₂HPO₄ (5.3 g/L), NaCl (0.2 g/L), MgSO₄ (0.2 g/L), and CaCl₂ (0.05 g/L). This step was conducted by pour plate method. The culture was incubated at 28 ° C for 5 days. CMC media which were overgrown with bacteria were counted by the number of colonies using the Total Plate count/TPC method and purification was carried out on CMC 15 agar media and incubated for 3-5 days (Top and Wilson 2011)

Bacterial potential test for cellulose degradation

Semi-quantitative test of cellulose-degrading bacteria

Pure bacterial culture was inoculated on 1% CMC Broth media, in incubators at 28 °C as long as the culture had a density of 10⁷ cells/ml or calculated Optical density (OD). An inoculum of 100 µL was dropped on a blank disc paper (0.5 cm diameter), placed on a Petri dish which had been filled with Carboxymethylcellulose/CMC to be sterile and incubated at 30 °C for 5 days. After incubation, the media was flooded with 1% Congo Red reagent for 15 minutes, then washed with 1 M NaCl and measured the clear zone around the well using a digital caliper (Behera et al. 2014). Each treatment was repeated three times. The extent of the clear zone of each isolate was analyzed to determine the pure culture of the selected isolate. The highest area of clear zone isolates was chosen as isolates for further treatment.

Bioassay of endoglucanase activity

Pure bacterial culture was inoculated on 1% CMC Broth media, in incubators at 28°C as long as the culture had a density of 10⁷ cells/ml or calculated Optical Density (OD). An inoculum of 5 mL was inoculated in 1% liquid CMC media, incubated at shaking 28°C at a speed of 150

rpm for 5 days. The culture was centrifuged at a speed of 10,000 rpm in cold temperatures of 4°C. The supernatant obtained as a crude extract of cellulase enzymes. Endoglucanase activity was determined by incubating a 1% CMC mixture in 1800 µL 50 mM buffer phosphate (pH 7.0) with 200 µL crude extract of cellulase enzymes at 50°C. After 30 minutes of reaction, 2 mL of dinitrosalicylic acid (DNS) was added and boiled in a water bath for 5 minutes to end the reaction. The sample was cooled to room temperature and its absorbance measured at a wavelength of λ 540 nm. One unit of endoglucanase activity was defined as the number of enzymes that can hydrolyze CMC and release 1 µg glucose per 1-minute reaction at 50 °C (Yun et al. 2010)

Characterization of bacterial colonies and biochemistry

The selected bacteria were observed morphologically; for bacterial colonies including the color and size of the colonies and microscopic observations included the shape of bacterial cells. Selected bacterial was tested for biochemical and physiological tests (Table 1).

Bacteria identification based on 16S rDNA Sequences

Bacterial isolates that can produce superior EPS are identified molecularly based on the 16S rDNA sequence. Isolation of chromosomal DNA was carried out using the Zymo-Spin™ Kit. Pure DNA obtained, agarose gel electrophoresis (0.8%) was performed to ensure that the DNA was extracted successfully. DNA was amplified using universal 16S rDNA primers namely 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-GGT TAC CTT CTT ACG ACT T-3') (Chen et al. 2015). Amplifiers were sent for sequencing at the 1st BASE DNA Sequencing Service, Malaysia. After that, sequences were analyzed using sequence scanner software and trimming if necessary. Sequence results from both primers were combined with the contig feature using the Bioedit software. The final sequence result was used to identify bacteria using BLAST from NCBI. The isolate sequences obtained were compared with reference sequences (ingroups and outgroups) from the GenBank database to create phylogeny trees using MEGA 6 software for Windows-based on the Neighbor-Joining algorithm and Tamura-Nei models.

Data analysis

Data on physical and chemical properties, the abundance of cellulose-degrading bacteria, assay potential and enzyme activity were analyzed by ANOVA followed by the Tukey test with the help of the SPSS 16 program to obtain a comparison of peat forest profiles and the level of maturity at different locations, and for screening cellulose bacteria.

RESULTS AND DISCUSSION

Sampling and determining the level of peat maturity

The peat soil sampling area was located in Teluk Bakung Village, Ambawang Sub-district, Kuburaya district, consisted of secondary forest areas and shrub areas

planted with oil palm which were secondary forest peat areas where deforestation occurred during 1, 2, and more than 3 years ago, which have been made drainage channels. The area was dry peat because it was located some distance from the Kapuas river. Sampling from one point to another within 100 m starts with this drainage which was used for the sampling point. Three points were taken from each area with the maturity level of fibric, hemic, and sapric peat. In soil sampling in the forest and bush area the peat depth was greater than 3 meters, and determining the level of peat maturity or weathering level of peat was differentiated based on the level of decomposition of the original plant material or fiber (Figure 1).

Table 1. Characteristics of cellulose-degrading isolates isolated from fibric, hemic and sapric peat in secondary forest and shrubs (palm oil) in Teluk Bakung, Ambawang Sub-district, Kubu Raya District, West Kalimantan, Indonesia

Characteristic	Isolate		
	SB2.3	HH3.1.1	SB1.1.1
Color of the colony at TSA media	Cream	Cream	Cream
Colony diameter (mm)	3,56	4, 23	4, 23
Gram reaction	+	+	+
Cell shape	Rod	Rod	Rod
Catalase	+	+	+
Test TSIA	As/As,G-H2S-	As/As,G-H2S+	As/As,G-H2S+
Spores	+	+	+
Oxidase	+	-	-
Motility	-	-	-
Nitrate	+	+	+
Lysin	-	+	+
Ornithine	-	-	-
H ₂ S	-	+	+
Glucose	-	+	+
Mannitol	-	-	-
Xylose	-	-	-
ONPG	+	+	+
Indole	-	-	-
Urease	-	+	+
V-P	+	+	+
Citrate	-	+	+
TDA	-	-	-
Gelatin	-	+	+
Malonate	-	-	-
Inositol	+	-	-
Sorbitol	-	-	-
Rhamnose	-	-	-
Sucrose	-	-	-
Lactose	-	+	+
Arabinose	-	-	-
Adonitol	-	-	-
Raffinose	-	-	-
Salicin	-	-	-
Arginine	-	-	-
Coagulase	-	-	-
Hemolisa	Beta	Beta	Beta
Test sensitive Novobiocin	No	No	No
Starch hydrolysis	-	+	+
Casein hydrolysis	+	+	+

Note: + : present,-: not present, TD: not yet to be identified, Alk/As: lactose or sucrose fermentation, As/As: glucose and lactose or sucrose fermentation, Alk/Alk: unfermented sugar

Physicochemical analysis of soil fibric, hemic and sapric peat

Secondary forest peat and shrubs that were used as oil palm plantations at different levels of maturity have a very high C-organic content (56%). There was no significant effect on fibric, hemic, and sapric peat at the two sampling locations. The thickness of the sapric peat, hemic in these two locations was small so that there is no difference in its C level. Secondary forest peat and shrubs used as oil palm plantations have low N-total content (1.88%) and there was no difference in fibric, hemic and sapric peat. Secondary forest peat and shrubs that were used as oil palm plantations have a fairly high P₂O₅ content. There was no difference in the two sampling locations, but the P₂O₅ content value in fibric, hemic and sapric peat, P₂O₅ sapric peat content was greater than the fibric and hemic peat. This was due to the higher storage and supply of P in sapric peat soils than fibric (Figure 2) (Zhang et al. 2014).

Peat in secondary forests and shrubs planted with oil palms in the sampling area had very low base cation content (Figure 2). At the sampling location, it had a very low value, the depth of the peat was more than 3 m. There was no significant difference in water content in the two sampling locations and in the maturity level of fibric, hemic, and sapric peat. The water content had an average of 417%, the water content was not included as high which was classified as moderate, because the peat soil water content ranges from 100-1.300% of its dry weight, meaning that peat can absorb water up to 13 times its weight. High water content caused BD to be low, peat to be soft and low load-bearing power (Osono et al, 2009). There was no difference in Bulk density (BD) in the two sampling locations either in fibric, hemic and sapric peat had an average of 0.15 g/cm³ (Table 1). BD of peat soil depended on the level of decomposition, BD varies between 0.1 to 0.2 g/cm³ (Figure 2).

Abundance of cellulose-degrading bacteria

There were 19 cellulolytic bacteria at fibric, hemic and sapric peat in Teluk Bakung Peat Area, Ambawang Sub-

district, Kubu Raya District. The abundance of cellulolytic degrading bacteria in peat soils in the secondary forest area and shrubs to be planted with oil palm is significantly different, in secondary forests the abundance is smaller than the abundance in shrubs. The abundance of cellulolytic degrading bacteria in sapric peat was higher in abundance compared to fibric and hemic peat. Abundance of cellulolytic bacteria in secondary forests on fibric, hemic, and sapric peat respectively 2.1x10³, 5.9x10⁴ and 4.9x10⁴ cfu/g. In shrubs peat abundance of cellulolytic bacteria in fibric, hemic, and sapric peat respectively 6.9x10⁴, 8.4 x10⁴ and 3.4x10⁵ cfu/g (Figure 3).

Cellulolytic potential of bacteria isolated from secondary forest peat and shrub peat

Pure cellulolytic bacterial strains were tested for cellulose degradation by measuring the clear zone in bacterial culture by 1% CMC agar. The clear zone formed around the bacterial colony showed the activity of the cellulose enzyme that is secreted. Nineteen bacteria had been isolated and selected to determine their ability to degrade cellulose. Bacterial strains that had high cellulose degradation ability as indicated by the extent of the clear zone formed. There was one strain of bacterial HH3.1.1 having a clear zone size of 6.7 mm and there are 6 other bacterial isolates that had a clear zone size of between 5 to 6 mm (Figure 4). This bacterial strain was isolated from fibric, hemic, and sapric peat in shrubs and secondary forests (Figure 5).

Test cellulase enzyme activity

Bacteria that can degrade cellulose were tested for their cellulose enzymes. There were seven strains of bacteria tested for their cellulose enzymes. Bacteria had a high clear zone capable of expressing enzymes to degrade cellulose to glucose. Bacteria that had a high clear zone may not necessarily cellulase enzymes degrade high cellulose as well. Seven of the bacteria tested for SB1.1.1 isolate had the highest enzyme activity (11.17 U/mL), followed by HH3.1 and SB2.3 7.83 U/mL (Figure 6).

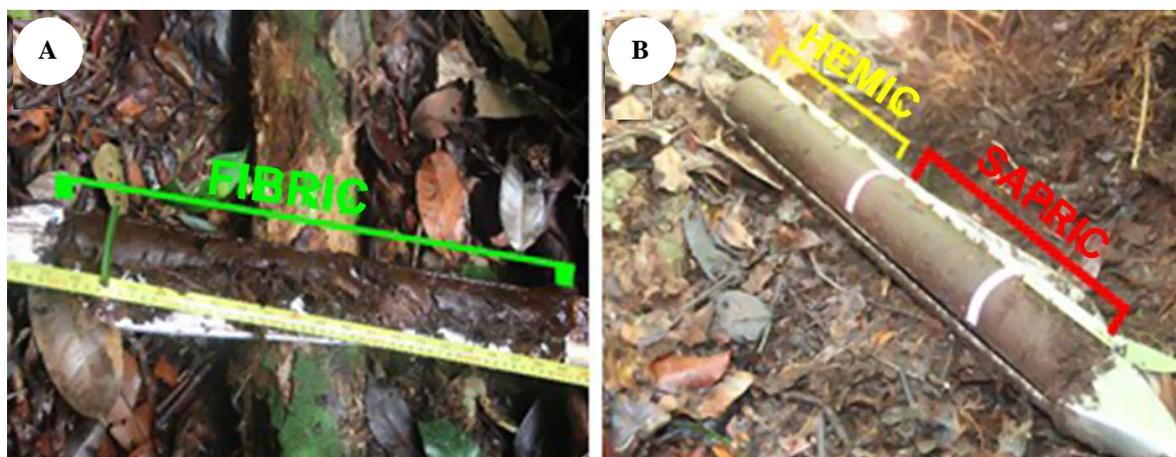


Figure 1. Variation of vertical level of peat maturity in peat soil profiles: A. Fibric peat, B. Hemic peat and sapric peat

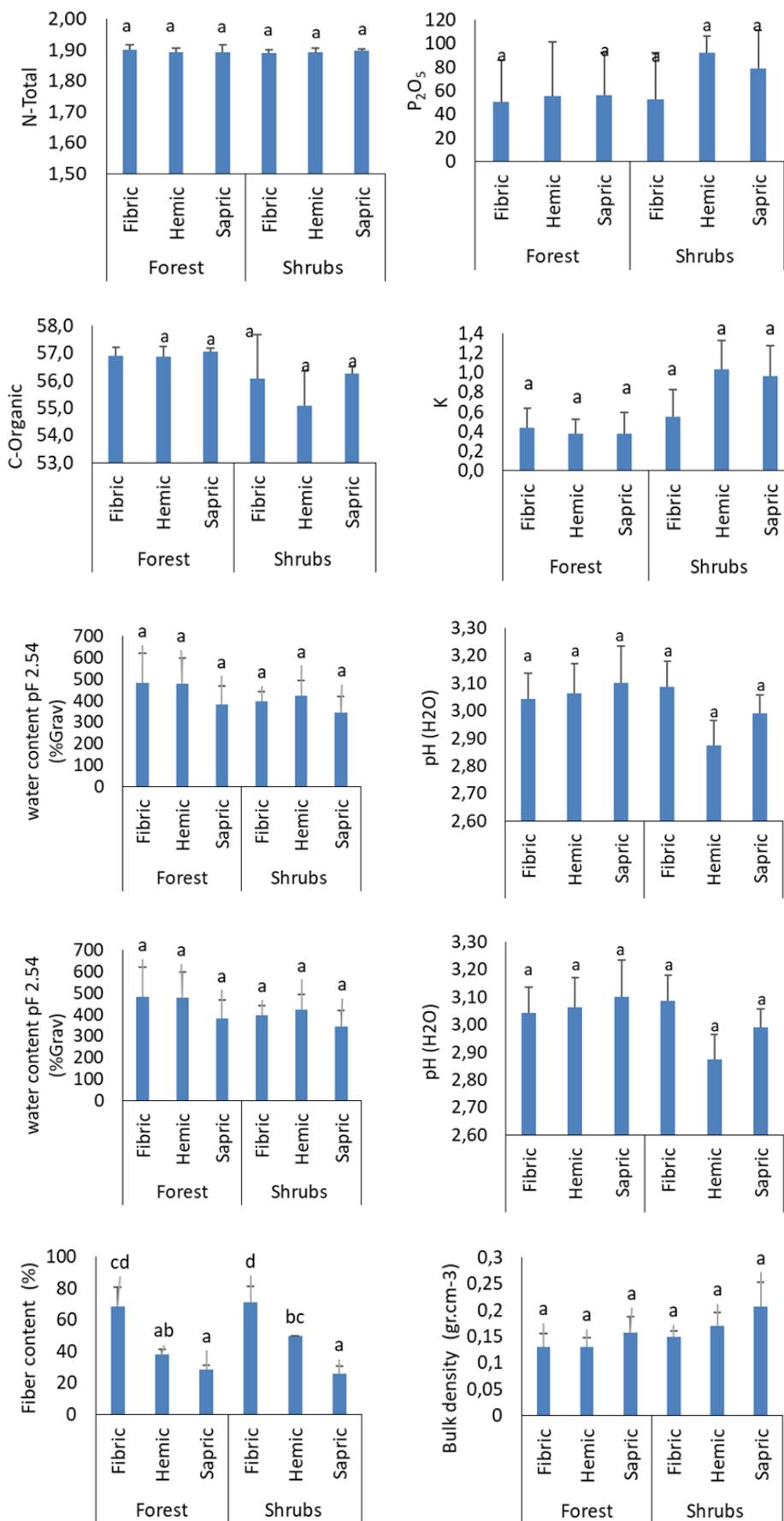


Figure 2. Physicochemical properties of secondary, fibric, hemic and sapric peatland forests and shrubs (oil palm)

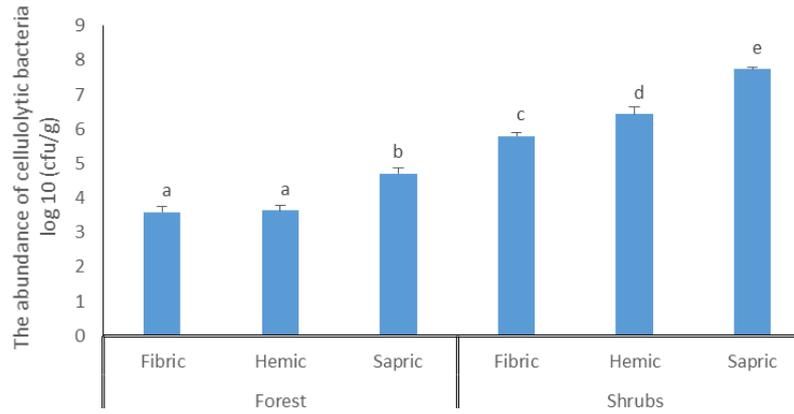


Figure 3. Abundance of cellulolytic bacteria in secondary forest peat and shrub peat on fibric, hemic and sapric peat

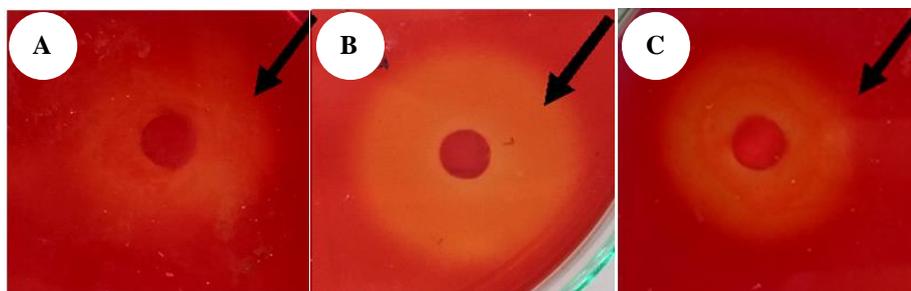


Figure 4. Clear zones isolate. A. SB1.1.1, B. HH3.1.1, C. SB2.3

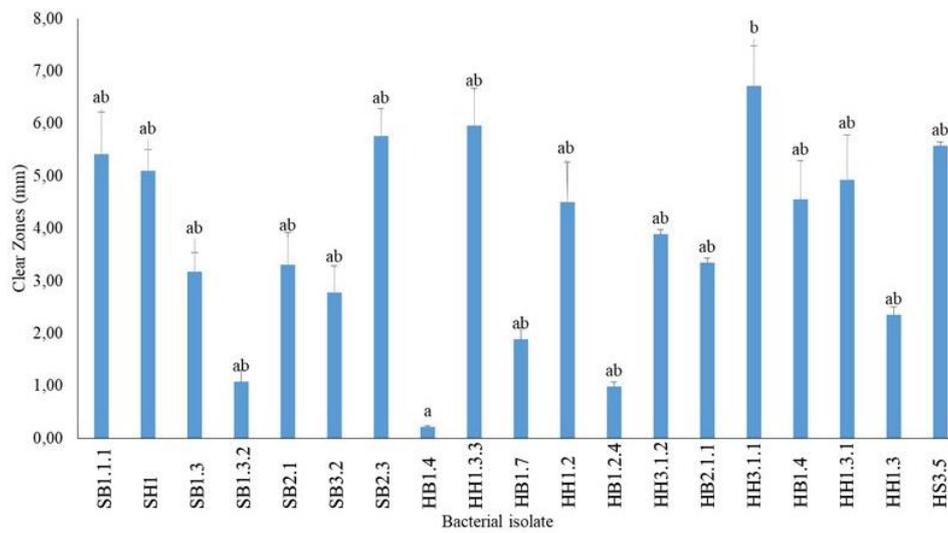


Figure 5. Clear zone of cellulolytic bacteria

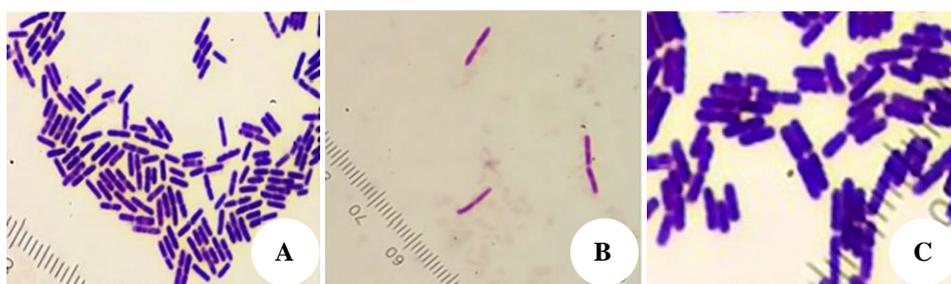


Figure 7. The bacterial cell forms: A. SB1.1.1, B. HH3.1.1, C. SB2.3 gram are positive, bacilli

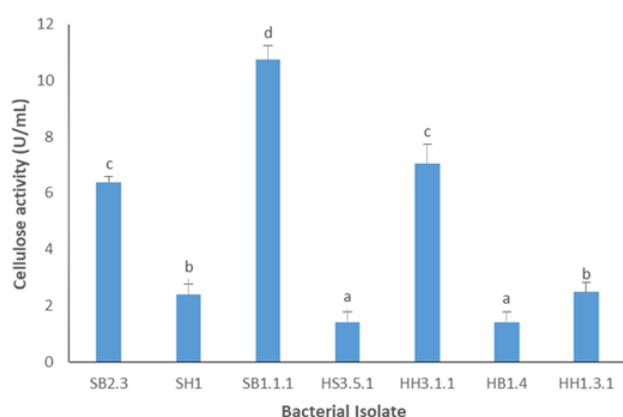


Figure 6. Test the activity of cellulase enzymes in selected cellulose strains

Bacteria identification based on 16S rDNA sequences and characterization of bacterial colonies and biochemistry

Based on the results of cellulase activity test there were 7 bacterial strains that had been able to degrade cellulose, there was three bacterial strain that had the highest cellulase enzyme activity compared to the others, namely, strain SB1.1 followed by HH3.1.1 and SB2.3. These three bacterial strains were identified molecularly using 16S rDNA. Based on the phylogeny tree (Figure 8) strains SB1.1.1 and HH3.1.1 had the closest kinship with *Bacillus cereus* with a kinship of 100% and SB2.3 had the closest severity relationship with *Bacillus stratosphericus* with kinship 99.5%. SB1.1.1 isolate was cellulose-degrading bacteria isolated from shrub peat (open land for <1 year to be planted with oil palm with 9a drainage system, while SB2.3 was cellulose-degrading bacteria isolated from shrub peat (open land for > 1 year) which had been planted with oil palm, while isolate HH3.1.1 was cellulose-degrading bacteria isolated from secondary forest peat with dense vegetation and no drainage system. All three bacteria had the form of stem cells and were gram-positive (Figure 7) and had biochemical and physiological characteristics (Table 1). Based on the characters matched with the Bergey's identification book Manual of Determinative Bacteriology, the isolates SB1.1.1 and HH3.1.1 were classified as *Bacillus cereus*, while SB2.3 was classified as *Bacillus stratosphericus*.

Discussion

The maturity level consists of three categories. Fibric peat was characterized when the peat was squeezed with the palm of the hand in a wet state, the fiber content left in the palm after squeezing was three quarters or more ($>3/4$), brown in color). Hemic (half-cooked) peat, which was peat which had a moderate level of weathering, some material had undergone weathering and partly in the form of fiber, when squeezed with your palms in a wet state, peat passes through the fingers between the fingers and the fiber content left in the palm after squeezing was between less than three quarters to a quarter or more ($1/4$ and $<3/4$), brown. Sapric peat (mature), i.e. peat whose weathering level was

advanced (ripe) when squeezed, it was very easy for peat to pass between the fingers and the fibers left in the palm of the hand was less than a quarter ($<1/4$), dark brown to black (Wahyunto 2015).

There was no significant effect on fibric, hemic, and sapric peat at the two sampling locations. The thickness of the sapric peat, hemic in these two locations was small so that there is no difference in their C level. The condition of drained peatlands could change the condition of anaerobic peat to aerobic. This resulted in an increase in the activity of microorganisms remodel soil organic matter (Doolotkeldieva et al. 2011; Zhang et al. 2014). Pioneering microorganisms of soil organic matter and N-fixing have not been able to work optimally. The activity of microorganisms was strongly influenced by soil pH conditions (Kunitake 2017). In soils that had acidic pH, microorganism activity was very low. The availability of N for plants on peat soils was generally low, although the analysis of total N was generally relatively high because it comes from N-organic. Comparison of C and N content of peat soils was relatively high, generally ranging from 20-45 and increasing with increasing depth (Wahyunto et al. 2010). This was due to the higher storage and supply of P in sapric peat soils than fibric (Zhang et al. 2014). Peat in secondary forests and shrubs planted with oil palms in the sampling area had very low base cation content. Oligotrophic peat, like many found in Kalimantan, had a very low base cation like K, especially in thick peat. The thicker the peat, the lower bases it contains and the reaction of the soil becomes more acidic (Baldani et al. 2014). The low K content was thought to be due to the condition of the peat which was always saturated with water and only comes from the accumulation of organic matter so that there was no addition of mineral elements. Fibric peat which was generally located in the lower layer had BD 0.1 g/cm³, but coastal peat and peat in the river flow can have BD > 0.2 g/cm³ due to the influence of mineral soil. Oil palm plantation peat and ex-fire peat had BD 0.3 g/cm³, while forest peat had BD 0.15 g/cm³ (Nurulita et al. 2016). The levels of intact fibric peat fibers were higher than hemic and sapric peat. Sapric peat had a smaller intact fiber content, almost no residual plant structure remains visible and amorphous material was formed (Kalisz et al. 2010).

The abundance of cellulolytic bacteria in shrubs/land planted with oil palm is higher than in secondary forests, this was due to the shrub area which was also a secondary forest that has been felled for 1, 2, and more than 3 years ago, and drainage systems have been made, so as to enable aerobic conditions. These conditions increase the activity of microorganisms in decomposing peat (Lynd et al. 2002; Laiho 2006). The depth of the peat soil in the sampling area was above 3 m, which was deep peat. The abundance of cellulose bacteria in the area was relatively low, according to Elliot et al. (2015) the number of bacteria in peat usually ranges from 10⁸-10⁹ cfu/g. Fierer et al. (2003) state that the density of microorganisms was highest at the surface above 25 cm, microbiological studies focus on surface soil above 25 cm, whereas deep soils were rarely studied (Senga et al. 2015). The density of cellulose bacteria in sapric peat soil was higher compared to hemic and fibric peat. The depth of

the sapric peat was thinner and its location is above the ground surface an average of 25 cm. The depth of the deepest fibric peat was above 3 m, so the density of cellulose bacteria was smaller. In addition to the density of

cellulose bacteria influenced by the depth of peat soil was also influenced by aerobic conditions and nutrition where it grows.

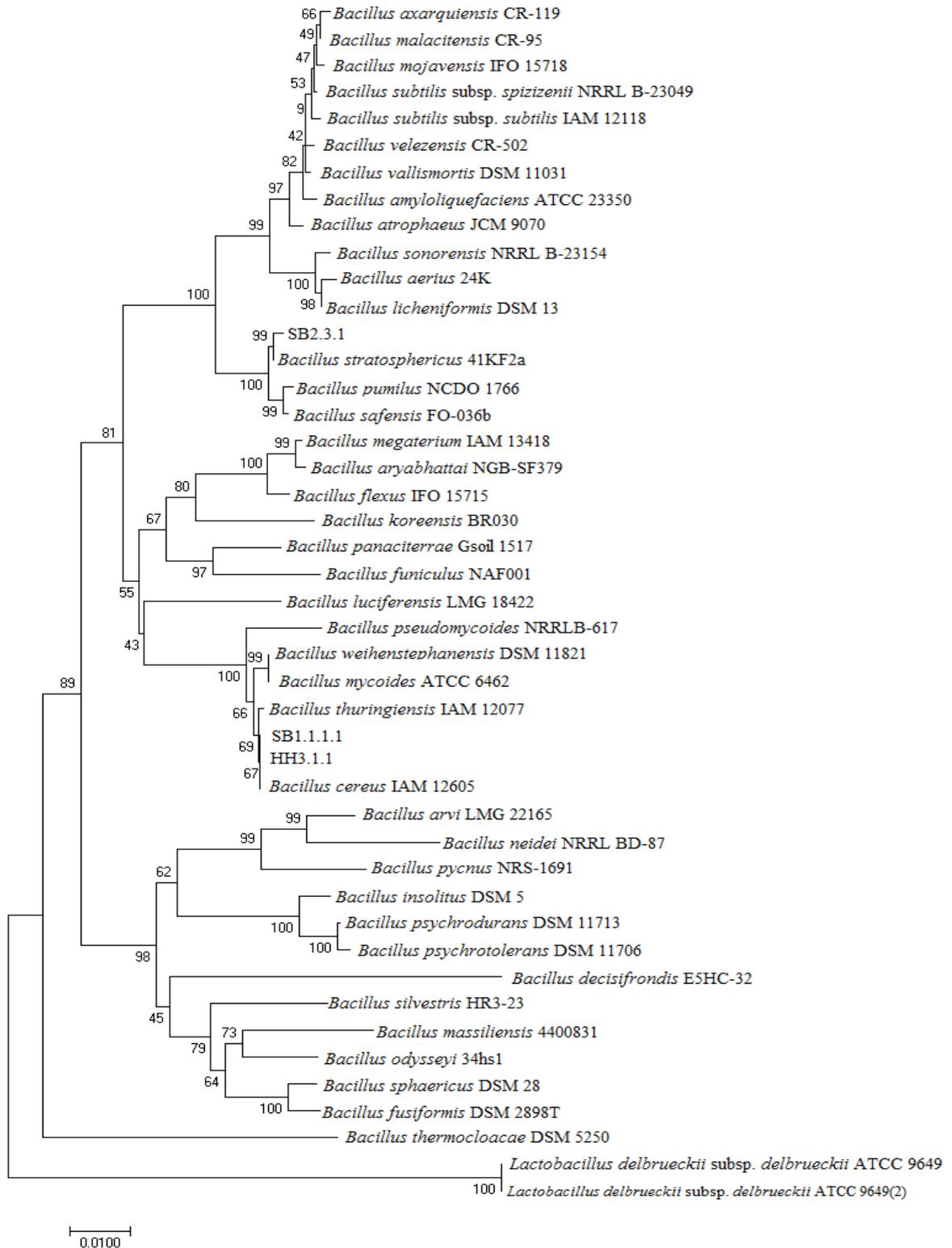


Figure 8. SB1.1.1, HH3.1.1 and SB2.3 isolate phylogeny tree with reference strain type based on 16S rDNA sequences with Neighbor-Joining algorithm, Tamura-Nei with bootstrap 1000 times

This bacterial strain was isolated from fibric, hemic, and sapric peat in shrubs and secondary forests. Among prokaryotes, bacteria represent the ability to degrade cellulose. According to Behera et al. (2014), cellulose-degrading bacteria include *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp., *Brucella* spp. had a clear zone ranging from 1.18 to 2.5 mm which indicates its ability to degrade cellulose and the resulting enzyme activity ranges from 2.471-98.253 U/mL/min. According to Behera et al. (2014) cellulose-degrading bacteria such as *Bacillus* spp., had the activity of the enzymes produced ranging from 2.471-98.253 U/mL/min. This bacterial strain was isolated from the shrub fibric peat (open land to be planted and already planted with oil palm aged 1-2 years). The open land peat has been drained to allow a high level of decomposition. Microbes capable of hydrolyzing cellulose for prokaryotic groups were dominated by bacteria, with aerobic conditions, the bacteria produce an enzyme endo-1,4- β -D-glucanase which decreases oligomer β -D-glucose to glucose which encourages cellulose decomposition by oxidative degradation of glucose polymers (Top & Wilson 2011; Zhang et al. 2014; Harris et al. 2010). Fibric peat was formed from piles of plant remains that are still easily identified, almost all of them have not been degraded or little has been degraded. The isolate was obtained and has the ability to degrade cellulose derived from fibric and hemic peat. In fibric peat bacteria actively degrade cellulose and in hemic peat, some have decayed so that many isolates actively degrade cellulose.

The 16S rDNA sequences in these three isolates were amplified using universal primers 27f. The 16S rDNA sequences of the three isolates were compared with those of the entire *Bacillus* genus in the Gen-Bank database using BLAST on the NCBI website <http://www.ncbi.nlm.nih.gov/BLAST>. The similarity matrix and phylogeny tree were constructed using mega-7. Based on the Maximum-likelihood method and the Tamura Nei algorithm with bootstrap 1,000 times (Fig 8), the isolate SB1.1.1 was shown. HH3.1.1 had 100% similarity of 16S rDNA sequence with *Bacillus cereus* IAM 12605 and isolate SB.2.3 has 99.85% similarity with *Bacillus stratosphericus* 41Kf2a. Ventorino et al. (2015) reported that based on phylogenetic analysis, the 16S rRNA gene sequence of forty cellulolytic bacteria identified 20% included the *Bacillus* group. *Bacillus cereus* was a bacterium that shows the highest cellulase activity among all cellulolytic bacterial isolates found (Yang et al. 2014).

In conclusion, there were 19 cellulolytic bacterias at fibric, hemic and sapric peat in Teluk Bakung Peat Area, Ambawang Sub-district, Kubu Raya District. Based on the 16S rDNA sequences, the HH3.1.1 and SB1.1.1 identified as *Bacillus cereus*, where as SB2.3 identified as *Bacillus stratosphericus*. These HH3.1.1, SB1.1.1 and SB2.3 strains had the highest enzyme activity, 11.17 U/mL, 7.83 U/mL, and 7.83 U/mL, respectively.

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