

Cellulose degrading bacteria isolated from Palangkaraya, Central Kalimantan, Indonesia as peat fiber decomposer to accelerate peat soil compression

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Abstract. Zulaika E, Solikhah F, Utomo MAP, Endah N, Assavalapsakul W. 2022. Cellulose degrading bacteria isolated from Palangkaraya, Central Kalimantan, Indonesia as peat fiber decomposer to accelerate peat soil compression. Biodiversitas 23: 1648-1654. Peat soil was established from incomplete weathered plants. It delays the process of soil compression. The main components of peat soil are fibers which made of cellulose and lignin that can be enzymatically degraded by cellulolignolytic bacteria. The objective of this study was to isolate indigenous lignocellulolytic bacteria from peat soil that perform peat fiber degradation to accelerate soil compression process. A bacteria screening test was carried out on carboxymethyl cellulose (CMC) media and bacteria identification using 16S rRNA gene markers. Peat fiber decomposition test based on biomass loss, decomposition results were visualized using scanning electron microscopy (SEM) and analyzed using Fourier Transform Infrared (FTIR). The results of screening and identification obtained 5 bacterial species, namely *Pseudomonas taiwanensis* U3 and *Bacillus cereus* D1, D3, U2, U4. After six weeks of incubation, *P. taiwanensis* U3 showed the highest cellulase activity than other isolates with 90% decomposition ability of peat fibers. The results of the decomposition of peat fibers with the addition of isolates visualized smaller pore sizes than control. Stretching occurs in the aromatic group of the C-O ester bond, the C≡C structure of the alkyne group, and the O-H of the alcohol. All obtained bacteria isolates were still viable after six weeks of incubation with 10⁴ CFU g⁻¹ dry weight of fiber. The present investigation deals with isolating indigenous lignocellulolytic bacteria from peat soil that perform peat fiber degradation to accelerate the soil compression process. Our findings are also the first publication that reported *P. taiwanensis* cellulolytic potential.

Keywords: 16S rRNA, decomposition, lignocellulolytic bacteria, peat fiber

INTRODUCTION

Indonesia has about 14.9 million hectares of peatlands, 4.78 million hectares of which is in Kalimantan. Peat from Palangkaraya, Central Kalimantan, Indonesia is hemic type peat. It has a fiber content of 53% which is formed from tropical swamp plants. Peat soil has a very high void ratio and water content, therefore bearing capacity is very low, and the compression time is very long. In geotechnical practice, peat soil does not support building construction foundations. In research of Mochtar et al. (2014), stabilization of peat soil with CaSiO₃ jelly for 20-45 days showed a significant increase in physical properties, after 45 days, the physical properties of peat were decreased, this was due to change in CaSiO₃ jelly which hardened into crystals. In its designation as an infrastructure foundation, efforts to improve soil quality are needed. One of which is by accelerating the decomposition of fibers so as to accelerate the compaction and increase the stability of the peat soil.

In general, the materials that makeup peat soil have not been completely decomposed, mostly consisting of

cellulose, hemicellulose, and lignin (de Souza 2013). The length of the peat soil decomposition process is due to the acidic and anaerobic pH conditions. According to Pichan and O'Kelly (2012), the decomposition process becomes anaerobic with slow microbiological activity in an environment where oxygen is not available. In flooded peatlands, the oxygen content decreases with increasing soil depth, followed by slower microbial activity. Fiber peat content is higher in the deeper peat layer. Naturally, the decomposition process of peat soil continues along with decomposer microorganisms obtaining nutrients. The effects of the decomposition process become genuine when the groundwater level on peatlands decreases, resulting in subsidence of the soil. Therefore the decomposition process must be accelerated and completed before construction. According to Astiani et al. (2016), peatlands are very vulnerable to changes of water regimes.

One alternative that can be developed to accelerate peat soil compression is a biological method, namely bioaugmentation with indigenous cellulolytic bacteria. Cellulolytic bacteria are bacteria that are able to produce enzymes to degrade cellulose so that peat fibers can

decompose faster (Papiyana 2015). This decomposition is environmentally friendly and can be carried out long. Research conducted by Pankratov et al. (2012) found that a cellulolytic bacteria *Telmatobacter bradus* gen. nov., sp. nov can be isolated from the acidic Sphagnum peat soil. Besides, *Bacillus* genera member: *Bacillus cereus* and *Bacillus stratosphericus* isolated from Teluk Bakung Peatland showed high cellulolytic activity (Khotimah et al. 2020). Hence the objective of this research was to figure out the potential of indigenous bacteria from Palangkaraya's peat soil to accelerate decomposition process. Once peat soil compression can be accelerated, it becomes feasible for building construction foundations.

MATERIALS AND METHODS

Cellulolytic bacteria isolation and screening

Cellulolytic isolates were isolated from peat soil in Bereng Bengkel village, Palangkaraya, Central Kalimantan, Indonesia. The screening process was conducted using carboxymethyl cellulose (CMC) agar media with pour plate method. Bacteria culture was then incubated for 72 hours. The composition of the medium is CMC-agar L⁻¹: 10 g CMC, 0.2 g MgSO₄·7H₂O, 0.75 g KNO₃, 0.5 g K₂HPO₄, 0.02 g FeSO₄·7H₂O, 0.04 g CaCl₂, 2 g yeast extract, 1 g D-glucose, and 1.5% agar (Lisdiyanti et al. 2012). The growing colonies were flooded with 0.1% Congo red (15 minutes), then rinsed with 1 M NaCl. Hydrolysis of cellulose was shown by a clear zone around the colony (Lay et al. 2015).

Identification of bacteria using 16S rRNA gene amplification and sequencing

DNA extraction was conducted according to Qiagen®, Germany protocol. DNA was amplified by polymerase chain reaction (PCR) using universal primers 27F (5'AGAGTTTGATCCTGGCTCAG3') and 1492R (5'TACGGTTACCTTGTACGACTT3'). The DNA purity and quantity were measured using ThermoScientific® 2000 nanodrop through 260/280 nm wavelength absorbance. PCR formula consisted of 25 µL PCR MasterMix, 1 µL forward primer, 1 µL reverse primer, 20 µL free-water nuclease, and 3 µL DNA template were mixed into the PCR tube. One-Taq® PCR MasterMix that applied was contained: 20 mM Tris-HCl, 1.8 mM MgCl₂, 22 mM NH₄Cl, 22 mM KCl, 0.2 mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20 and 25 units mL⁻¹ one Taq DNA polymerase. Then the mixture was homogenized using ThermoFischer® homogenizer (Lokhande and Pethe 2016). Amplicons obtained from PCR were then purified using AccuPrep® PCR purification kit. DNA sequences were determined using an ABI 3730XL DNA analyzer (Bioneer, South Korea).

The 16S rRNA gene sequences of cellulolytic bacteria were submitted in the National Center for Biotechnology Information (NCBI) GenBank database; <https://www.ncbi.nlm.nih.gov/>. Phylogenetic analysis was carried out using the Molecular Evolutionary Genetic Analysis 7 (MEGA 7) program (Kumar et al. 2016). The distance between nucleotides was obtained using the

Neighbor Joining algorithm (Saitou and Nei 1987). Bootstrap with 1000 replication was used to obtain statistical support data and a representative phylogenetic tree according to evolutionary history (Fenselstein 1985).

Decomposition test of peat fiber

As much as 2 mL bacterial culture with 0.8 optical density was inoculated into 20 grams of dry peat fiber, then moistened using distilled water and incubated at room temperature for 6 weeks. The content of decomposed peat fiber was calculated according to ASTM (1996): peat fiber was put into an Erlenmeyer, then 100 mL of 5% (NaPO₃)₆ solution was added, then incubated for 3 hours. The mixture was homogenized using stirrer for 10 minutes, then filtered (mesh size 0.15 mm). The fibers were washed with tap water, then soaked in 2% HCl for 2 minutes, then washed again with tap water for 5 minutes. The fibers were placed on Whatman paper no. 4, dried in oven at 105°C for 5 hours. The total mass of peat fiber and Whatman paper minus the mass of Whatman paper is the dry weight of decomposed fiber. Percentage decomposition of peat fiber was calculated based on the ratio of dry weight of fiber (B) to dry weight of initial peat fiber (A):

$$\text{Percentage decomposition of peat fiber} = [(A-B)/A] \times 100\%$$

Where:

A: initial weight of peat fiber (g)

B: final weight of peat fiber (g)

To figure out the results of peat fibers decomposition after the sixth week of incubation, fibers were analyzed using Scanning Electron Microscopy (SEM) (Girardello et al. 2013). Besides, the functional groups of decomposed peat fibers were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with functional group reading on 400 to 4000 cm⁻¹ spectrum range (Romao et al. 2007).

Viability test

After six weeks of incubation on peat fiber, the viability of the isolate was measured by its growth ability on nutrient agar medium using the pour plate method, then followed by incubation for 24 hours (Harley and Prescott 2002).

RESULTS AND DISCUSSION

Cellulolytic bacteria

The cellulolytic isolates that formed clear zone around the colony were D1, D3, U2, U3 and U4, the visible zone was stained with congo red as qualitative indicator of extracellular cellulase production (Figure 1). Congo red interaction with β-(1,4)-D-glucan bounds in CMC produced a red color on CMC agar medium. CMC was degraded into smaller oligosaccharides fragments that cannot congo red. Most of the hydrolysis zones were produced by the activity of endoglucanase which is one of the components of cellulase (Saini and Tewari 2012).

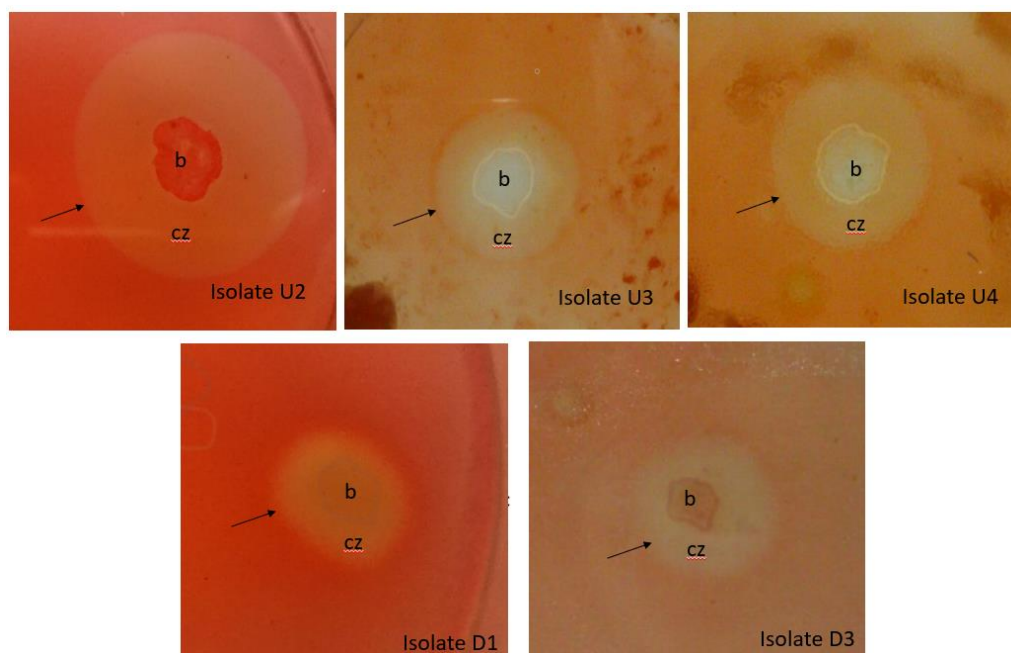


Figure 1. Cellulolytic bacteria isolates on CMC agar plates. The arrows around the colonies showed cellulolytic activity zone of cellulase. Note: b: bacterial zone; cz: clear zone

Bacteria species identification using 16S rRNA marker

Cellulolytic bacteria from peat soil were identified by analyzing its 16S rRNA gene sequences. The obtained sequences are then submitted and compared with nucleotide sequences reserved in NCBI GenBank database. Isolates D1 (MT3735311.1), D3 (MT373532.1), U2 (MT373533.1) and U4 (MT373535.1) showed 99% similarity and 100% recovery with the bacterial species *B. cereus*. U3 isolate (MT373534.1) showed 99% similarity and 100% recovery with the bacterial species *Pseudomonas taiwanensis*.

Isolates D1, D3, U2 and U4 in the phylogenetic tree (Figure 2), belong to the same cluster as other members of *B. cereus* sensu-stricto and *B. cereus* sensu-lato. *Bacillus cereus* sensu-lato is a group of endospore-producing Gram-positive bacteria with a broad ecological spectrum but very close phylogenetic relationships (Jensen et al. 2003; Fayad et al. 2019). Members of *B. cereus* sensu-lato are *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, *B. mycoides*, *B. pseudomycoides*, *B. toyonensis*, and several new members such as *B. gaemokensis*, *B. manliponensis*, *B. bingmayoensis*, and *B. wiedmannii*. The ability of *B. cereus* D1, D2, U2 and U4 from peat soil to produce cellulase was supported by Vilain et al. (2006), that the soil is one of the habitats of *B. cereus* in the saprophytic phase. Saprophytic microorganisms such as *B. cereus* has been known to produce hydrolytic enzyme such as cellulase (Chantarasiri et al. 2015; Sari et al. 2017). *Bacillus cereus* is also found in acidic soils (Chau et al. 2015). In this acidic environment, *B. cereus* possesses several mechanisms to withstand its cell, such as modification of cell architecture and composition, change of metabolisms that produced alkaline substance, and internal pH homeostasis (Duport et al. 2016). Its ability to produce alkaline substance through urease and arginine

deiminase action showed upstanding potential to be developed as a neutralizing agent for acidic soil (Duport et al. 2016; Mahdavi et al. 2017).

Isolate U3 belongs to *Pseudomonas* genus. This genus shows common characteristics such as rod-shaped Gram-negative bacteria, does not form spores, and obligate aerobic (Palleroni 2010). The obtained *P. taiwanensis* U3 isolate from peat soil was supported by research by Situmorang et al. (2015) and Gusmawartati et al. (2017), who successfully isolated *Pseudomonas* from the peat soil habitats. Besides Sethi et al. (2013), Khatiwada et al. (2016), Maharsiwi et al. (2020) reported that *Pseudomonas* is one of the cellulolytic bacteria which showed cellulose hydrolysis activity. This study is the first report that unravels *P. taiwanensis* U3 ability to degrade cellulose in an acidic environment.

Peat fibers decomposition

All bacteria isolate was able to decompose peat fibers faster than control (without isolate). The decomposition ability of each isolate is shown in Table 1.

Peat fiber decomposition is relatively increased until the final incubation week. *Pseudomonas taiwanensis* U3 showed higher decomposition rate than the other isolates, which was almost 90%. This is in accordance with cellulase activity screening which *P. taiwanensis* U3 showed the most spacious cellulolytic clear zone (Figure 1). In the control chamber, there is also decomposition activity of around 60%, it is possible that there are other indigenous bacteria in the peat fiber besides tested isolates. The high level of decomposition is determined by the type of peat soil, in this study the Palangkaraya peat soil was classified as dry and fibrous. According to van der Linden and van Geel (2006), the decomposition rate of dry peat soil is relatively higher than wet peat. All of the peat fiber

decomposition has positive correlation between incubation time (2.4, 6 weeks) and % decomposition ($r: 0.8, p < 5\%$). It signifies that the longer the incubation time, the higher percentage decomposed peat fiber.

The surface morphology of the control peat fiber (without isolate addition) which visualized with SEM-

500x, showed a loose arrangement and large pores. Meanwhile, the peat fibers decomposed by the isolates showed a denser arrangement and smaller pores (Figure 3), this indicates the activity of the decomposition of peat fibers by cellulolytic bacteria.

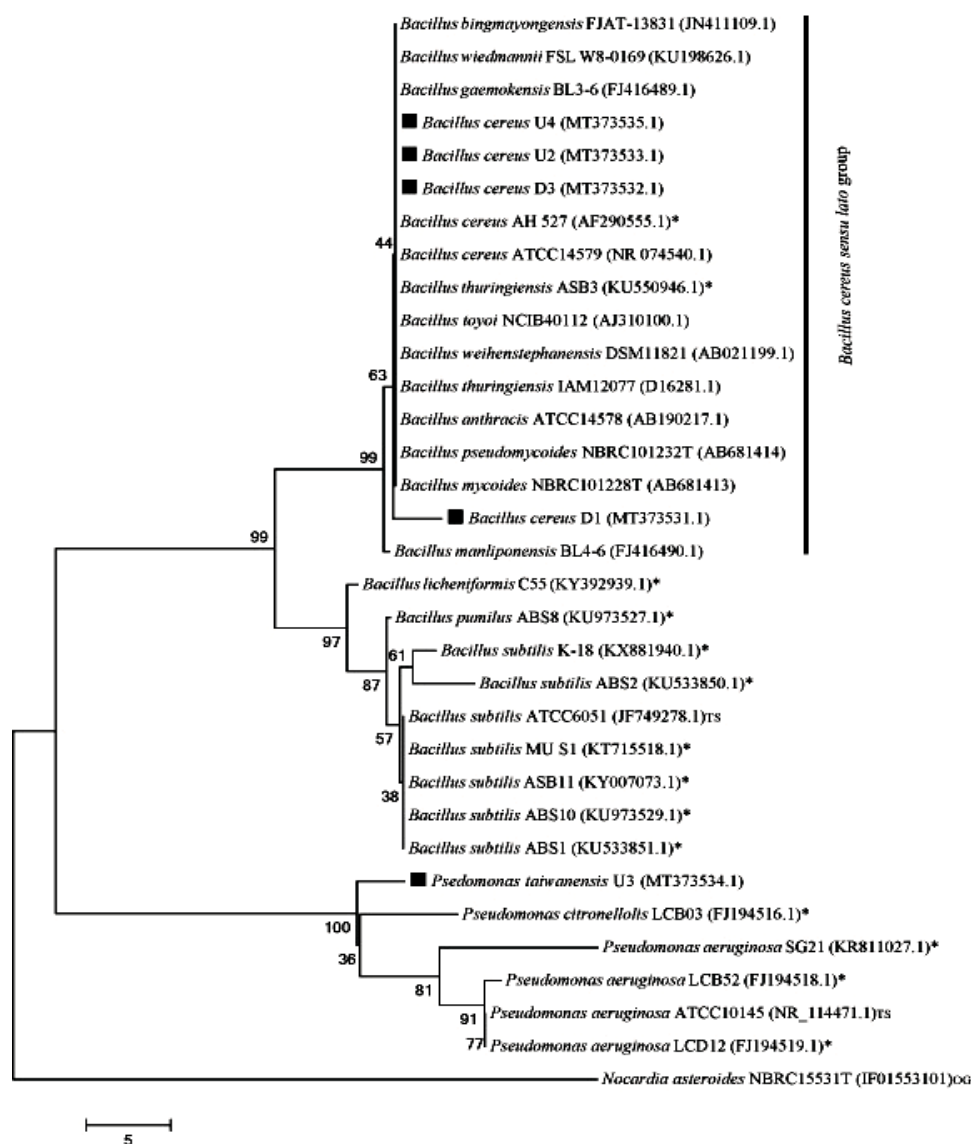


Figure 2. Reconstruction of phylogenetic tree of cellulolytic bacteria based on 16S gene rRNA sequence using neighbor-joining method (bootstrap value = 1000)

Table 1. Peat fiber decomposition (%)

Isolate	Peat fiber		
	Week 2	Week 4	Week 6
Control	60.37 ± 1.32	64.63 ± 0.84	65.53 ± 1.28
<i>Bacillus cereus</i> D1	78.28 ± 0.73	82.57 ± 1.88	84.47 ± 0.82
<i>Bacillus cereus</i> D3	81.46 ± 0.70	85.18 ± 1.25	87.35 ± 0.61
<i>Bacillus cereus</i> U2	78.04 ± 1.28	82.24 ± 1.29	83.27 ± 1.25
<i>Bacillus cereus</i> U4	81.14 ± 0.87	83.13 ± 1.49	86.40 ± 0.55
<i>Pseudomonas taiwanensis</i>	86.53 ± 1.30	88.51 ± 0.93	89.03 ± 0.81

In the research of Pichan and O'Kelly (2012), peat fiber degradation causes modification in the structure, number and size of peat fibers. The rate of decomposition affects the porosity of the peat, affects the particle size and structure of the peat fiber. As the decomposition increases, the particle size of the peat fiber become smaller, while the composition of the peat fiber that has not been decomposed tends to be more tenuous than the decomposed fiber.

The decomposition of peat fiber also causes stretching and changes in the chemical structure of its functional groups. Based on FTIR analysis, changes in functional groups are visualized by the appearance of peaks at several wavelengths. Chemical stretching occurs in the alcohol functional group (OH) at a wavelength of $3680.3\text{--}3937.58\text{ cm}^{-1}$, the aromatic group and the $\text{C}\equiv\text{C}$ structure of the alkyne group at $1811.63\text{--}2291.63\text{ cm}^{-1}$, and the C-O bond of the ester group at $1169\text{--}1500\text{ cm}^{-1}$ (Figure 4).

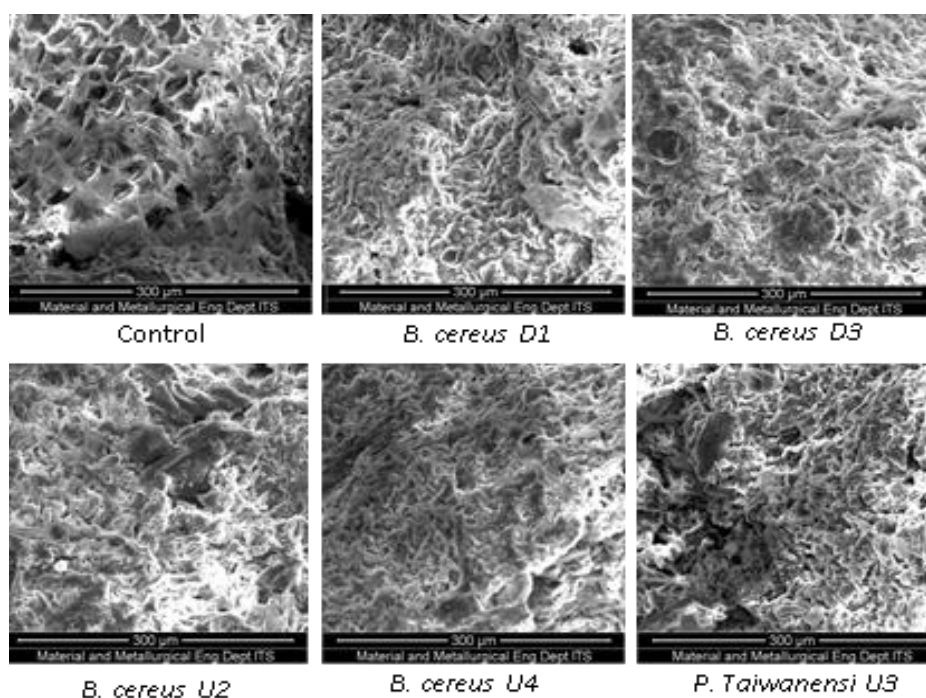


Figure 3. Peat fiber from the decomposition of isolates visualized by SEM (500x). Control figure is fiber decomposition without the addition of cellulolytic bacterial isolates

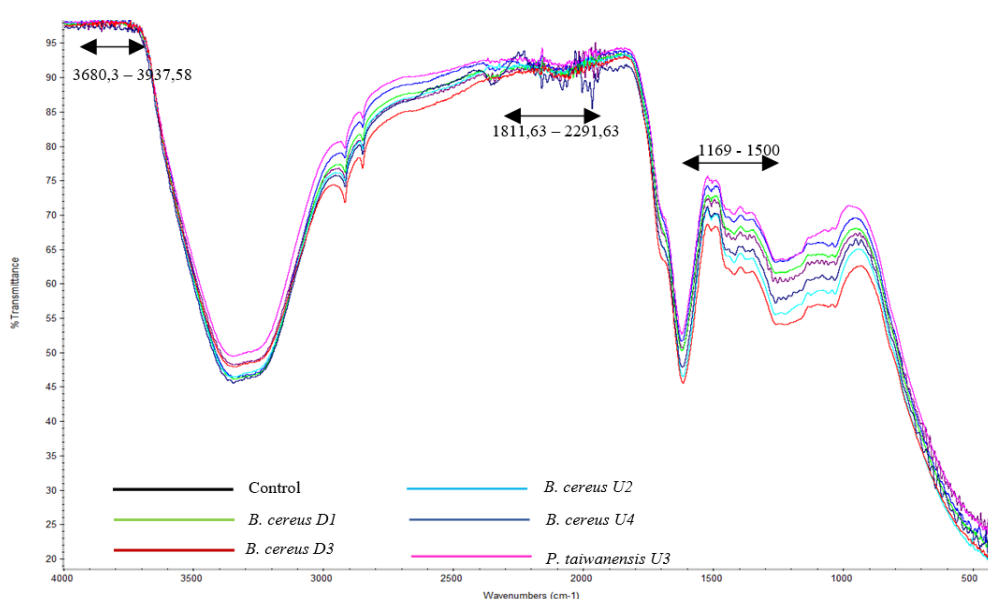


Figure 4. FTIR of decomposed peat fiber. The wavelength of $1169\text{--}1500\text{ cm}^{-1}$ shows the stretching of the C-O bond from the ester group, $1811.63\text{--}2291.63\text{ cm}^{-1}$ is the stretching of the aromatic group and the C-C structure of the alkyne group, $3680.3\text{--}3937.58\text{ cm}^{-1}$ is the stretching of the O-H functional group in the alcohol

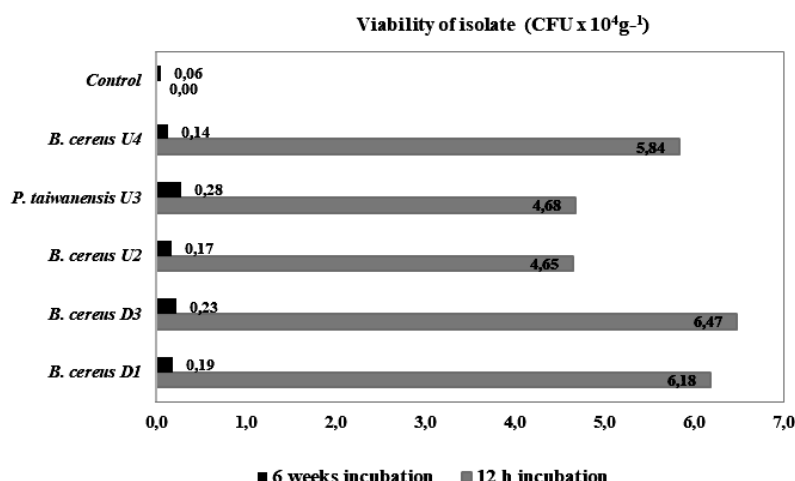


Figure 5. Viability of isolates at initial incubation time (12 hours) and after 6 weeks. *Pseudomonas taiwanensis* U3 has the highest viability and density of 0.28×10^4 CFU g⁻¹

The decreasing peak with low transmittance value was observed in the wavelength spectrum of $3000\text{--}3600\text{ cm}^{-1}$, this indicated that there are still O-H groups in the cellulose-lignin material. Peaks with low transmittance values are also found in the wavelength spectrum $1592\text{--}1632\text{ cm}^{-1}$ which indicates high levels of aromatic groups with benzene rings in peat fibers. Based on the results of the FTIR above, it can be marked that there are changes in the functional group of the cellulose-lignin material. Although peat fibers have not completely degraded yet, the stretching of some functional group indicates the activity of enzymes that play important role in chemical bonds spilling in cellulose-lignin material of peat fiber.

The results of Kruer-Zerhusen et al. (2017) research showed the degradation of cellulose components by cellulase has not given complete degradation results. This may caused by the unbalanced proportion of cellulases activity, while endoglucanase works extra than other cellulase components, therefore FTIR results still manifest the presence of complex chemical bond structure and slight change in the bond in the aromatic ring.

Cellulolytic bacteria viability

At the sixth week after incubation, all isolates were still viable in the peat fiber, although the bacterial density was greatly decreased compared to the initial inoculation number. The viability of each bacteria are available in Figure 5.

Colony number of peat fiber with cellulolytic bacteria application was higher than control, i.e. $0.14 - 0.28 \times 10^4$ CFU g⁻¹. At the end of incubation, the highest CFU was found in peat fiber added with *P. taiwanensis* U3 which was 0.28×10^4 CFU g⁻¹. The low viability and relatively small CFU may caused by the nutrients available in the peat fiber are slowly decomposed. In a similar study, the average number of colony on decomposed Sphagnum peat was 10^5 CFU g⁻¹ dry mass after 10 days of incubation (Pichan and O'Kelly 2012), while on Icelandic peat it was 2.6×10^5 CFU g⁻¹ dry peat (Hunter et al. 2006).

Based on investigation above, there were 5 isolates of cellulolytic bacteria that could be isolated from peat soil in Palangkaraya. Four bacterial isolates were belong to *B. cereus* species, namely isolates D1, D3, U2 and U4, while U3 was identified as *P. taiwanensis*. All bacterial isolates were able to minimize the pores in the peat fiber soil. The highest percentage of peat fiber decomposition was *P. taiwanensis* U3 with a value of 89.03 ± 0.81 on 6th week of incubation period.

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The authors declare that there is no competing interest.

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