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Isolation and Characterization of Cellulolytic Bacteria from Soil around Chicken Coop

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Abstract. Bacteria that utilize cellulose as a substrate and convert it into simpler compounds with the help of cellulase enzymes are known as cellulolytic bacteria. Cellulase enzymes are capable of breaking down cellulose into glucose. Cellulolytic bacteria are bacteria that can produce cellulase enzymes and are typically found in areas containing compost, soil rich in leaf litter, as well as agricultural and livestock soils. The aim of this study is to obtain bacterial isolates from soil around a chicken coop. Bacterial isolates from the soil around the chicken coop were cultured on selective CMC media. The successfully isolated bacteria were then purified to obtain single colonies. The obtained single colonies were characterized to determine the type of cellulolytic bacteria that were successfully isolated. Subsequently, testing was conducted to measure the cellulase enzyme activity produced by the obtained isolates. Four isolates of bacteria successfully grew on the selective CMC media. These four isolates were identified as gram-negative bacteria with a rod-shaped cell morphology. Enzyme activity testing was only performed on isolate LD 2, as it exhibited a clear zone indicating enzyme activity. The highest cellulase enzyme activity value was 0.0062 U/mL, with a 30-minute incubation time of the cell supernatant at 37°C, and a specific enzyme activity of 0.1891 U/mg. Based on the conducted research, only one isolate from the soil around the chicken coop exhibited enzyme activity.

Key words: Cellulolytic Bacteria, Cellulase, Cellulose.

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INTRODUCTION

Cellulolytic microorganisms are microorganisms that are capable of breaking down cellulose with the help of cellulose enzymes produced from bacterial metabolism residues (Sornlake et al., 2017). Cellulose is a compound that is commonly found in nature, along with lignin and other polysaccharides derived from agricultural soil or compost. Cellulose contained in compost or agricultural soils is usually difficult to break down or degrade (Klemm et al., 2006). The accumulation of cellulose in the soil can lead to soil pollution and damage the nutrient content of the soil (Setiawati et al., 2019). Cellulose in the soil also causes the soil structure to become fibrous, requiring enzymatic degradation by cellulolytic bacteria. Cellulolytic bacteria will use cellulose as a substrate, converting it into simpler forms of oligosaccharides, which will then be converted into glucose with the help of cellulase enzymes (Grevitara et al., 2018), (Biswas et al., 2020). Endoglucanase, exoglucanase, and β-D-glucosidase are the three components that make up cellulase (Gupta et al., 2012). Therefore, cellulolytic bacteria will naturally live and thrive in agricultural soil, as well as soil containing compost, such as chicken coops (Ed-Har et al., 2017) [2].

When compared to enzymes produced by animals or plants, the characteristics of enzymes produced by bacteria generally exhibit greater stability across various temperatures and pH levels (Nguyen and Nguyen 2017, Anggriani et al., 2020). In a study conducted by Arifin et al. (2019), 38 bacterial isolates from compost were found to be capable of utilizing cellulose as a substrate or carbon source. Additionally, Ulfa et al. (2014) successfully isolated Bacillus sp., Pseudomonas sp., and Nocardia sp. from peat soil, which exhibited cellulolytic activity. The organic materials present in the soil, primarily composed of cellulose, need to be degraded in order to reduce soil damage, highlighting the crucial role of cellulolytic bacteria in degrading these organic substances (Khotimah et al., 2020). This study aims to determine the abundance of cellulolytic bacteria living in the soil surrounding chicken coops. Therefore, it is necessary to isolate cellulolytic bacteria from the soil around the chicken coop.

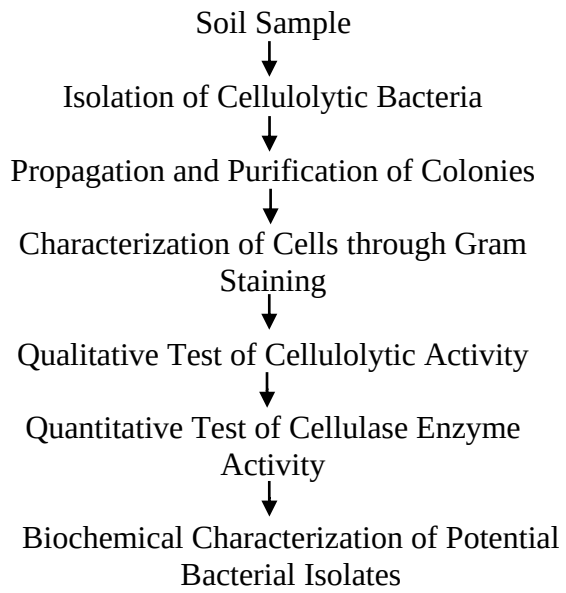
MATERIALS AND METHODS

Work Procedure

This research was conducted from February 12th to March 8th, 2018, at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural

Sciences, Bogor Agricultural University. The research framework is as follows [3].

The equipment used includes a UV-Vis spectrophotometer, magnetic stirrer, centrifuge, incubator, vortex, autoclave, microscope, spatula, analytical balance, dropper pipette, spreader rod, Bunsen burner, and common glassware used in the laboratory. The main material used in the experiment is soil samples obtained from the soil around the Babakan Raya community chicken coop, Dramaga District, Bogor, West Java.



Sample Collection

Soil sampling was conducted by taking soil samples around the chicken coop at a depth of approximately 5-10 cm, totaling 5 grams. The samples were wrapped in aluminum foil and placed in sample plastic bags. Subsequently, the samples were taken to the laboratory.

Isolation and Selection of Cellulolytic Bacteria

The method used for isolating cellulolytic bacteria was serial dilution. One gram of soil sample was dissolved in 9 mL of physiological saline solution (0.85% NaCl) and homogenized using a vortex. Then, serial dilutions were performed until a concentration of 10-6 was obtained. 0.1 mL from the last two dilution series (10-5 and 10-6 suspensions) was inoculated onto Petri dishes containing CMC media to achieve dilutions of 10-6 and 10-7. The suspensions in the media were spread evenly using a spreader rod. Afterward, they were incubated at 37°C for 48-72 hours (Lamid et al., 2011), (Top and Wilson, 2011), (Benard et al., 2015). Following the incubation period, bacterial colonies that grew on the media were observed. Colony morphology was examined by observing their shape, edge, elevation, and color (Howard et al., 2003).

Cellulolytic Bacteria Purification

Bacterial colonies that grew on the media after incubation were further purified using the zig-zag streak technique on CMC media. They were then incubated for 48 hours at room temperature. Single colonies formed

during this process were used as isolate stocks and stored for subsequent testing (Howard et al., 2003).

Cellulolytic Bacteria Characterization

Observation of cell morphology was carried out using the gram staining technique. Pure bacterial isolates grown on CMC media were collected, and 1-2 loopfuls of bacterial colonies were spread onto a glass slide with a drop of distilled water, dried, and fixed using a Bunsen burner. The fixed bacterial smear was flooded with crystal violet for 1 minute, rinsed with distilled water, dried, and then flooded with iodine for 2 minutes. After rinsing with distilled water and drying, the smear was flooded with 45% alcohol for 30 seconds, rinsed with distilled water, and then counterstained with safranin for 30 seconds. Finally, it was rinsed with distilled water, and any excess water was blotted with tissue. Bacterial staining was observed using a light microscope at magnifications of 400× and 1000×.

Measurement of Clear Zone Cellulolytic Activity

Pure isolates were also tested for cellulolytic activity by determining their cellulolytic index. Isolates were incubated in CMC media for 48 hours at room temperature. After the incubation period, the cellulolytic activity of the isolates was tested using 0.1% congo red indicator reagent and 0.2 M NaCl solution as the rinsing solution. The isolates were treated with congo red reagent until fully saturated, left undisturbed for 15 minutes, and then rinsed with 0.2 M NaCl. The final step involved measuring the cellulolytic index produced by the bacterial isolates (Khalila et al., 2020).

$$Clear\ Zone\ Index\ (\text{mm}) = \frac{Clear\ Zone\ Diameter\ (\text{mm}) - Colony\ Diameter\ (\text{mm})}{Colony\ Diameter\ (\text{mm})}$$

Cellulase Enzyme Activity

Quantitative testing of extracellular cellulase enzyme activity was conducted using the method developed by Miller (1959). Two loopfuls of bacterial culture were inoculated into 17 mL of 1% liquid CMC and incubated in a shaking incubator at 121 rpm and 37°C for 24 hours. After 24 hours, the 24-hour pure bacterial culture was centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant, which contains crude enzyme, was collected and analyzed for enzyme activity. Additionally, 3 mL of the bacterial culture was taken and placed in a tube to measure its absorbance using a spectrophotometer at a wavelength of 600 nm. The sterile medium was used as the blank.

The cellulase enzyme activity test involved three treatments: the sample, blank, and control. The sample tube was filled with

1 mL of 1% CMC solution in 0.05 M phosphate buffer, to which 1 mL of crude enzyme was added and homogenized. The blank and control treatments each used 1 mL of 1% CMC solution in 0.05 M phosphate buffer without the addition of crude enzyme. All treatments were incubated at 27°C for 5 minutes, 10 minutes, 20 minutes, 30 minutes, and 40 minutes. Then, 2 mL of dinitrosalicylic acid (DNS) reagent was added to each treatment. After adding the DNS reagent, 1 mL of water was added to the blank treatment, while 1 mL of crude enzyme was added to the control treatment. The mixtures were then heated in boiling water at 100°C for 5 minutes. After cooling down, the cellulase enzyme activity was measured using a spectrophotometer at a wavelength of 550 nm for the sample, blank, and control treatments.

Table 1. Concentration of Glucose Standard Solutions

Glucose Concentration	Glucose Stock (mL)	Distilled Water (mL)
0	0	5
0,1	0,1	4,9
0,2	0,2	4,8
0,3	0,3	4,7
0,4	0,4	4,6
0,5	0,5	4,5

The glucose standard solutions were prepared using the Bernfeld method (1955). 10 mg of glucose was dissolved in 10 mL of distilled water to obtain a glucose stock solution with a concentration of 1 mg/mL (1000 ppm). The glucose standard solutions were prepared by mixing the glucose stock solution with distilled water in various concentrations (Table 1). Then, 2 mL of DNS reagent was added to each mixture. The mixtures were heated in boiling water at 100°C for 5 minutes and cooled for 10 minutes. The glucose absorbance was measured at a wavelength of 550 nm.

Protein Content Determination using the Bradford Method

0.1 mL of crude enzyme extract (CEE) was added to 5 mL of the Bradford reagent and homogenized using a vortex. The mixture was then incubated at room temperature for 5 minutes, and its absorbance was measured at a wavelength of 595 nm using a spectrophotometer. The standard protein curve was prepared by mixing 0.1 mL of Bovine Serum Albumin (BSA) with 5 mL of the Bradford reagent (containing 50 mg Coomassie Brilliant Blue, 25 mL 95% ethanol, and 50 mL orthophosphoric acid 85%). The absorbance measurements of the standard curve were entered into Microsoft

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Excel to obtain the equation for measuring the sample protein concentration (y = ax + b). In the equation, y represents the sample absorbance, and x represents the protein concentration (Bradford, 1976).

RESULTS AND DISCUSSION

The isolation of cellulolytic bacteria from soil samples around the chicken coop was carried out using serial dilution. The purpose of serial dilution is to reduce the number of suspended microorganisms in the liquid. A 1:9 ratio was used for the sample and the first dilution, and subsequently, each dilution contained 1/10 of the microorganism cells from the previous dilution.

After incubating the 10-6 and 10-7 dilutions using the pour plate method for 48-72 hours, bacterial colonies grew on the CMC media as shown in Figure 1.

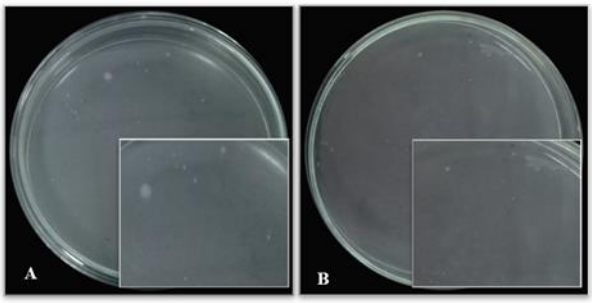

Figure 1. Growth of Cellulolytic Bacteria from Soil Samples around the Chicken Coop. (A) 10-6 Dilution and (B) 10-7 Dilution.

The isolation results revealed four bacterial colonies that exhibited distinct characteristics when grown on CMC media (Table 2). After isolating the bacteria, a purification process was carried out to obtain pure single colonies. Each growing colony was directly purified because it represented a type of cellulolytic bacteria. CMC media is a specific medium that contains a carbon source used for the growth of cellulolytic bacteria, making it specific for the growth of cellulolytic bacteria (Begum et al., 2013).

Table 2. Characteristics of Cellulolytic Bacterial Colony Morphology

No	Isolate Code	Shape	Size	Surface	Color	Optical Characterization	Elevation	Margin
1	LD 1	Irregular	Large	Smooth	Cream	Transclucent	Flat	Undulate
2	LD 2	Circular	Medium	Rough	Cream	Transclucent	Umbonate	Entire
3	LD 3	Irregular	Small	Rough	Cream	Opaque	Umbonate	Undulate
4	AD 4	Circular	Small	Smooth	White	Opaque	Flat	Entire

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Purification of cellulolytic bacteria was performed using CMC media and the zig-zag streak technique to obtain single bacterial colonies. These single colonies can be used as isolates for further testing. The results of the zig-zag streak technique and the obtained isolates can be seen in Figure 2.

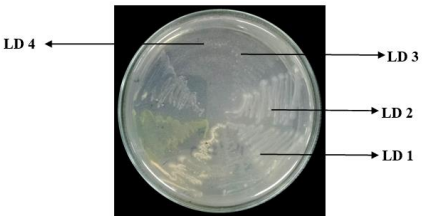

Figure 2. Zig-Zag Streak Technique

Gram staining is a differential staining method used to classify bacteria into two groups: Gram-positive and Gram-negative bacteria, as well as to determine their shape, either bacillus or coccus.

Based on the gram staining results, all four bacterial isolates obtained exhibited Gram-negative characteristics and had a bacillus shape. Differences in cell wall structure affect the staining response, where Gram-positive bacteria with a single layer cell wall composed of peptidoglycan and teichoic acid have a stronger affinity for absorbing the purple color. This is due to the narrow pores of the cell wall that retain the purple color after alcohol decolorization. Gram-negative bacteria, with a cell wall composed of three layers including lipopolysaccharides, lipoproteins, and lipids, are more likely to be washed away by alcohol. Hence, they appear red when stained with safranin (Pelczar & Chan, 2005). Kotimah et al., 2020 reported that cellulolytic bacterial isolates obtained from Teluk Bakung Petland, Kubu Raya, Indonesia, had a bacillus cell shape. Other studies have also reported that 71.6% of the isolated bacteria had a bacillus shape, while 28.3% had a cocci shape (Haldar & Nazareth, 2018).

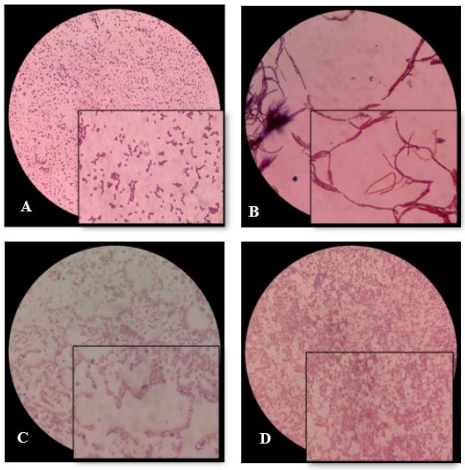

Figure 3. Gram Staining Results Observation: (A) LD 1 Negative Bacillus, (B) LD 2 Negative Bacillus, (C) LD 3 Negative Bacillus, (D) LD 4 Negative Bacillus, with a magnification of 1000x.

The cellulolytic ability of the bacteria isolated from the soil around the chicken

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coop can be observed through the clear zone formed by the bacterial colonies (Figure 4).

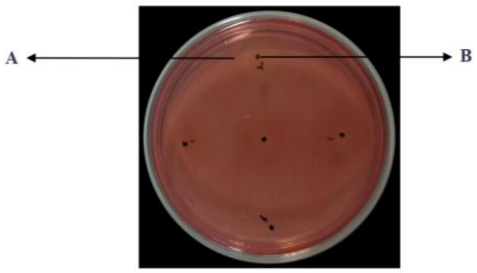

Figure 4. Clear Zones formed on CMC Media after Congo Red Solution Application: (A) Diameter of Clear Zones, (B) Diameter of Colonies.

Based on the cellulase enzyme activity test results, only one isolate, LD 2, produced a clear zone, while the other three isolates (LD 1, LD 3, and LD 4) did not produce a clear zone. Congo red dye interacts strongly with polysaccharides containing β-(1,4) and β-(1,3) glycosidic bonds, forming a color-glucan complex. The formation of a clear zone indicates the absence of a color-glucan complex due to the hydrolysis of the CMC glycosidic bonds. On the other hand, the red-colored areas indicate the presence of a color-glucan complex, indicating no substrate (CMC) hydrolysis (Jo et al., 2011), (Liang et al., 2014). The cellulolytic index produced by the bacterial colony of isolate LD 2 was 1.4193 cm. Therefore, isolate LD 2 was selected as a potential isolate for further testing.

The enzyme activity test began with the preparation of a glucose standard curve, and the equation obtained was y = 2.281x - 0.051. The R2 value obtained was 0.993. With the obtained equation, the cellulase enzyme activity can be calculated as the x-value from the absorbance measurement y-value. The equation y = ax - b is used to determine the x-values for the sample and control, which are then inserted into the enzyme activity equation: 1 unit/mL for 40 minutes.

Table 3. Cellulase Enzyme Activity Measurement Results

Incubation Time (Minutes)	LD 2	Control	X LD 2	X Control	Unit Activity (U/mL)	Spesific Activity (U/mg)
5	0,1220	0,1320	0,0758	0,0802	-0,0049	-0,1474
10	0,2190	0,2010	0,1184	0,1105	0,0044	0,1326
20	0,1290	0,1060	0,0789	0,0688	0,0028	0,0847
30	0,2820	0,2050	0,1460	0,1122	0,0062	0,1891
40	0,2480	0,2010	0,1311	0,1105	0,0029	0,0866

Based on the enzyme activity test results during the 40-minute incubation period, as shown in Table 3, no enzyme activity was observed at the 5-minute incubation time. At the 10-minute incubation, the enzyme activity started to appear at a value of 0.0044. However, at the 20-minute incubation, the enzyme activity decreased to 0.0028, and at the 30-minute incubation, it increased again. The highest enzyme activity was observed at the 30-minute incubation with a value of 0.0062, which then decreased to 0.0029 at the 40-minute mark. These results indicate fluctuating values, likely due to the instability of the enzyme activity. Nevertheless, there is still enzyme activity present, albeit unstable. The enzyme activity testing was performed on the supernatant harvested from bacteria incubated for 24 hours at 37°C. At this time, the bacterial growth enters the exponential phase, which is the phase of the highest metabolic activity (Warly et al., 2019). The highest reported enzyme activity produced by cellulolytic bacteria isolated by Sonia and Kusnadi (2014) was 0.08 U/mL from isolates incubated for 24 hours. The testing results from the supernatant produced by the cellulolytic bacterial isolate still do not show stability as the enzyme tested has not been purified and is still in the form of supernatant (Murtiyaningsih & Hazmi, 2017). There are several factors that influence enzyme activity, including temperature, pH, organic matter, and incubation time (Orsi et al., 2018) (Yang et al., 2018).

Protein content measurement was performed using the Bradford method. The principle of this method involves the binding of coomassie brilliant blue G-250 (CBBG) dye to proteins containing amino acid residues. The CBBG reagent in an acidic environment exists as an anion that binds to proteins, forming a blue color (Bradford, 1976). In this experiment, the protein binding the coomassie brilliant blue dye is the cellulase enzyme in the form of crude enzyme. Bovine Serum Albumin (BSA) was used as an accurate standard solution.

The protein content measurement using the Bradford method started with the construction of a BSA standard curve, and the equation obtained was $y = 0.636x + 0.015$. The R2 value obtained was 0.984. The absorbance read using a spectrophotometer was 0.036. This value represents the y-value in the equation $y = ax + b$. By using this formula, the soluble protein content of the tested bacterial culture was determined to be 0.033 mg/mL. Iqbalsyah et al. (2019) reported the highest protein concentration produced by cellulolytic bacterial isolates from soil in the Pulau Weh area to be 0.44 U/mg.

CONCLUSION

There were four isolates of cellulolytic bacteria that were successfully isolated from the soil around chicken coops using special media for the growth of cellulolytic bacteria. Of the four isolates, the isolate with the LD 2 code had the highest cellulolytic activity value. LD 2 isolates had the highest value of specific enzyme activity at 30 minutes of incubation at room temperature, namely 0.1891 U/mg.

REFERENCES

Anggriani, L., Mubarik, NR., & Budiarti, S. (2020). Lipase-producing ability of bacteria from inasua (fish fermented product) from Central Moluccas, Indonesia. Biodiversitas, 21(2), 622-628.

Arifin Z., Gunam IBW., Antara NS., & Setiyo Y. (2019). Isolasi Bakteri Selulolitik Pendegradasi Selulosa dari Kompos. Jurnal Rekayasa dan Manajemen Agroindustri, 7(1), 30-37.

Begum, S., Meignanalaksmi, & Dhevi, P. (2013). Isolation and Characterization of Cellulase Producting Paracoccus Pantotrophus FMR19 (JX012237) from Goat Rumen Fluid and its Effects on pH, Temperature and Carbon Sources. International Journal of Advanced Biotechnology and Research, 4(3), 384-390.

Benard, L.D., Tuah, P.M., Suadin, E.G., & Jamian, N. (2015). Isolation and characterization of surface and subsurface bacteria in seawater of Mantanani Island, Kota Belud, Sabah by direct and enrichment techniques. IOP Conference Series Materials Science and Engineering, 78(01), 20-33.

Biswas, S., Al Saber, M.D., Tripty, I.A., Karim, Islam, Hasan, Rubayet, Jahid, & Hasan. (2020). Molecular characterization of cellulolytic (endo-andexoglucanase) bacteria from the largest mangrove forest (Sundarbans), Bangladesh. Ann Microbiol, 70 (68), 1-11.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing principles of protein-dye binding. Analyze Biochemistry, 72(2), 248-254.

Ed-Har, A.A., Widyastuti, R., & Djajakirana, G. (2017). Isolasi dan Identifikasi Mikroba Tanah Pendegradasi Selulosa dan Pektin dari Rhizosfer Aquilaria Malaccensis. Buletin Tanah dan Lahat, 1(1), 58-64.

Grevitara, Y., Rahma, B., Septirangga, H., Dahlia, I., & Suarsini, E. (2018). Isolation and Identification of Cellulose Degrading Bacteria from Banana Peel Compost. Jurnal Biologi El-Hayah, 7(1), 6-11.

Gupta, P., Samant, K., & Avinash, S. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Intl J Microbiol, 2012, 1-5.

Haldar, S., & Nazareth, S.W. (2018). Taxonomic diversity of bacteria from mangrove sediments of Goa: metagenomic and functional analysis. 3 Biotech, 8(436), 1-10.

Howard, R.L., Masoko, P. & Abotsi, E. (2003). Enzyme activity of a Phanerochaete chryso-sporium cellobiohydrolase (CBHI. 1) expressed as a heterologous protein from Escherichia coli. African Journal of Biotechnology, 2(9), 296-300.

Iqbalsyah, T.M., Amnam U., Utami, R.S., Oesman, F., & Febriani. (2019). Concomitant cellulase and amylase production by a thermophilic bacterial isolate in a solid-state fermentation using rice husks. Agriculture and Natural Resources, 53(4), 327-333.

Jo, W.S., Park, H.N., Cho, D.H., Yoo, Y.B., & Park, S.C. (2011). Optimal media conditions for the detection of extracellular cellulase activity in Ganoderma neo-japonicum. Mycobiology, 39(2), 129-132.

Khalila, R., Fitri, L., & Suhartono. (2020). Isolation and characterization of thermophilic bacteria as cellulolytic enzyme producer from the hot spring of Ie Seuum Aceh Besar, Indonesia. Microbiology Indonesia, 14(1), 25-33.

Khotimah S., Suharjono, Artyati, 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(05), 2103-2112.

Klemm D., Schuman D., Kramer F., Hebler N., Hornung M., Schmauder HP, & Marsch S. (2006). Nanocelluloses as Innovative Polymers in Research and Aplication. Adv Polym Sci. Springer Berlin Heidelberg, 205, 49-96.

Lamid, M., Nugroho, T.P., Chusniati, S. & Rochiman, K. (2011). Eksplorasi bakteri selulolitik asal cairan rumen sapi potong sebagai bahan inokulum limbah pertanian. Jurnal Ilmiah Kedokteran Hewan, 4(1), 37-42.

Liang, Y.L., Zhang, Z., Wu, M., Wu, Y., & Feng, J.X. (2014). Isolation, screening, and identification of cellulolytic bacteria from natural reserves in the subtropical region of china and optimization of

cellulase production by Paenibacillus terrae ME27-1. BioMed Research International, 2014, 1-13.

Miller, G.L. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar. Analyze Chemmistry, 31 (3), 426-428.

Murtiyaningsih, H., & Hazmi, H. (2017). Isolation and cellulase enzyme activities assays in cellulolytic bacteria origin from soil waste. Agritrop, 15 (2), 293 - 308.

Nguyen, T.H., & Nguyen, V.D. (2017). Characterization and applications of marine microbia enzyme in biotechnology and probiotics for animal health. Adv Food Nutr Res, 80, 37-74.

Orsi, W.D., Richards, T.A., & Francis, W.R. 2018. Predicted microbial secretomes and their target substrates in marine sediment. Nat Microbiol 3 (1): 32-37.

Pelczar, M.J. & Chan E.C.S. (2005). Dasar-dasar mikrobiologi. UI Press Jakarta.

Setiawati, M.R., Ufah, N., Hindersah, R., & Suryatmana, P. (2019). Peran Mikroba Dekomposer Selulolitik dari Sarang Rayap dalam Menurunkan Kandungan Selulosa Limbah Pertanian Berselulosa Tinggi. Soilrens, 17(2), 1-8.

Sonia, N.M.O. & Kusnadi, J. (2015). Isolation and partial characterization of cellulase enzyme from isolate OS-16 cellulolytic bacteria origin Bromo-Tengger desert. Jurnal Pangan dan Agroindustri, 3 (4), 11-19.

Sornlake, W., Rattanaphanjak, P., Champreda, V., Eurwilaichitr, L., Kittisenachai, S., Roytrakul, S., Fujii, T., & Inoue, H. (2017). Characterization of cellulolytic enzyme system of Schizophyllum communemutant and evaluation of its efficiency on biomass hydrolysis. Bioscience, Biotechnology, and Biochemistry, 81 (7), 1289-1299.

Top, E., & Wilson, D. (2011). Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol, 14, 1-5.

Ulfa A., Khotimah S., & Linda R. (2014). Kemampuan Degradasi Selulosa oleh Bakteri Selulolitik yang Diisolasi dari Tanah Gambut. Protobiont, 3(2), 259-267.

Warly, L., Marli, Y., & Riyanto, I. (2019). The activity of cellulose enzyme from indigenous bacteria “Bacillus sp. YLB1" as bioactivator. Jurnal Peternakan Integratif , 7 (2), 10-18.