Cellulase enzyme activity of the bacteria isolated from mangrove ecosystem in Aceh Besar and Banda Aceh

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Abstract. Cellulolytic bacteria is one of the beneficial bacteria that can found in mangrove ecosystem. The purposes of study were to analyse the cellulolytic index, and to analyse the cellulase activity of bacteria isolated from soil mangrove. Qualitatively, assessment of cellulase activity were carried out at the Microbiology laboratory of Fish Quarantine Station, Quality Control and Safety of Fishery Products (SKIPM) Aceh, while quantitatively was observed in microbiology laboratory, Biology Department, IPB. Assessment of qualitative cellulase activity is performed by growing the selected pure isolate on 1% CMC medium then spilled 1% congo red to test its cellulolytic potential. Cellulolytic potential was determined by clear zone performed around the colony after congo red flooded. The quantitative cellulase enzyme activity test carried out by DNS method tested on one selective isolate. There were 21 from 39 isolates showed a clear zone isolated from mangrove soil. The cellulolytic index (CI) obtained ranged from 0.07 to 0.80 classified as low cellulolytic index criteria. The cellulolytic index was higher in bacteria isolated from mangrove rehabilitated than mangrove unrehabilitated. The highest cellulase activity and specific cellulase activity of BTM D32 was at 48 hours with the value were 0.0012 U/ml, 0.077 U/mg, respectively. The result concluded that the bacteria cellulolytic isolated from mangrove soil had low cellulolytic index, low cellulase activity, and low specific cellulase activity.

1. Introduction
Mangrove ecosystem is one of the productive ecosystems in the tropical coastal regions through the litter production [1, 2, 3]. Mangrove litter is a continuous accumulation of vegetative and reproductive components that fall from plants to mangrove forest floor. Litter produced by mangrove vegetations is...
the main source of organic matter available as a food for various types of marine invertebrate organisms and detritivores that inhabit mangrove ecosystem [4, 1]. Mangrove litter degradation and remineralization are factors that contribute to the high nutrient content in mangrove sediments [5]. One of microorganisms that have role to decompose the litter fall in soil is bacteria cellulolytic. Degrading bacteria cellulose has been isolated and characterized from various sources i.e. soil, compost, and rumen ruminants [6, 7, 8]. The litter leaves, rotting wood, and mangrove sediment are rich in organic matter which is a contribution of cellulolytic bacteria [9].

Cellulolytic bacteria are capable of producing enzyme cellulase which functions to break down cellulose into simple sugar [10, 11]. Cellulose as an abundant source of glucose requires accelerating the utilization of cellulolytic bacterial decomposition [12, 9]. Furthermore, the mangrove ecosystem has a diversity of bacteria that can produce extracellular enzymes such as cellulase [13]. Cellulase enzyme can be generated from various sources such as microbes (fungi, bacteria, actinomycetes), plant, animal [14], and invertebrates [15].

Cellulase is a group of enzymes consisting of three major components, endoglucanase, exoglucanase, and β-D-glucosidase [16, 17]. Cellulase enzyme has wide range application in industrial after protease. [18] mentioned that the mangrove ecosystem is potential source of bacteria producing protease, amylase and cellulase. This matter related to protein, carbohydrates and abundant cellulose in mangrove sediment. Moreover, the importance of microbial enzymes and its biotechnology has been reported for cellulase [12], amylase [19, 20], and protease [21, 22]. Some application of cellulase was in textile, paper industry, animal feed, beverage, food industries [23, 16] and pharmaceutical applications [24]. One of the bacteria that can produce cellulase enzymes is Bacillus sp. [25, 26, 11]. Furthermore, [27] reported that genera of Bacillus and Paenibacillus are able to hydrolyse carboxymethylcellulose (CMC). Cellulolytic index (CI) is one of the indices used to determine enzyme activity through the producing of clear zone [28] and each cellulolytic bacteria has difference in the cellulase activity produced. Aceh Province has mangrove ecosystem both mangrove rehabilitated and unrehabilitated along the northern coast of Aceh dominated by Rhizophora sp. and Avicennia sp. are commonly find in this mangrove ecosystem [29]. Several studies on cellulolytic bacteria associated with mangrove habitat have been studied [26, 30, 9, 31], however there is no data and information about the cellulolytic bacteria and their activity in research area. Therefore, the purposes of present study were to analyse the activity of cellulase by hydrolysis capacity testing as qualitatively assay and specific cellulase activity as quantitatively assay.

2. Methods Implementation
2.1 Research Location and Time
The research conducted in mangroves areas along the northern coast of Aceh Province, it was started from September to October 2020, then it was continued on August to September 2021. Soil sampel were collected at 6 stations divided into 3 stations in mangrove rehabilitation (station 1, 2, and 3) and 3 stations in unrehabilitated (station 4, 5, and 6), and each station has 3 repetitions. Stations 1, 2 until 6 were located in Lambadeuk, Dayah Teungoh, and Gampong Pande, Ruyung, Lamreh, and Gampong Lampanah, respectively. Observation of cellulase activities were carried out at the Microbiology laboratory of Fish Quarantine Station, Quality Control and Safety of Fishery Products (SKIPM) Aceh, while cellulase activity quantitatively was observed in microbiology laboratory, Biology Department, IPB.

2.2 Materials
The isolate of bacteria used in this observation were pure bacteria isolated from soil of mangrove ecosystem, pure isolate of BTMD32, carboxy methyl cellulose (CMC) agar media, MgSO4.7H2O, K2HPO4, CaCl2, FeSO4, KNO3, yeast extract, glucose, agar, 1% congo red, 1M NaCl, aquadest, 70% alcohol, and pipette tips, autoclave, and incubator.
2.3 Hydrolysis capacity testing

Isolate bacteria observed was from soil samples in mangrove ecosystems that have clear zones. Samples observation were carried out at the Microbiology Laboratory, Fish Quarantine Station, Quality Control and Safety of Fishery Products (SKIPM) Aceh. The ability of bacteria to produce cellulase enzymes quantitatively was tested by hydrolysis capacity. One loop of isolate was inoculated into petri dish containing 1% CMC media, and it was incubated at 70°C for 48 hours. Cellulase activity was carried out using the 1% congo red. The isolate media was flooded using 1% congo red and dye for 15 minutes in order to detect the clear zone, then rinsed 2-3 times using 1M NaCl. Bacteria isolates that were able to hydrolysis CMC were indicated by the formation of a clear zone around the colony after they was flooded by congo red, and diameter of clear zone formed can be measured. The appearance of a hydrolysis zone around the colonies indicates synthesis of extracellular cellulase [28]. The clear zone data would be used to determine the cellulolytic index [32]. The higher of the cellulolytic index (CI) explain the higher the cellulase enzyme produced by bacteria. The category of cellulolytic index (CI) was low if the CI ≤ 1CI, medium if the CI value = 1 to 2, and high category if CI ≥ 2 [33]. The cellulolytic index was calculated using the following equation [32]:

\[
\text{Cellulolytic index} = \frac{\text{clear zone diameter (cm)} - \text{colony diameter (cm)}}{\text{colony diameter (cm)}}
\]  

(1)

2.4 Quantitative cellulase activity testing

Quantitative testing of cellulase activity was carried out using the 3.5-\text{dinitrosalicylic acid} (DNS) method [34]. A total of one loop of bacteria isolate was grown in 1% CMC liquid media and incubated at room temperature for 24 hours. Hereinafter, 1 ml of bacteria culture was taken and inoculated into 100 ml of 1% CMC liquid media, and incubated for 24-30 hours at room temperature above a shaking incubator. Every 6 hours duration, a bacterial culture was taken. The cellulase enzyme production was harvesting at 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours. As much as 3 ml of culture was taken and put into an Eppendorf tube, then centrifuged for 10 minutes at 10,000 rpm. The centrifuged supernatant was separated from the pellet and the supernatant was used as crude extract enzyme (EEK) to measure its activity using DNS reagent. The substrate used in the measurement of cellulase activity was 1% CMC dissolved in 0.1 M phosphate buffer with pH 7.0 and the incubation time was 30 minutes. The standard sugar used as control was glucose. The reducing sugar produced from the reaction was measured by a
spectrophotometer with a wavelength (\(\lambda\)) of 540 nm. Cellulase enzyme activity was calculated using the following formula [34].

\[
\text{Enzyme activity (Unit/ml)} = \frac{(\text{Cons. sample sugar} - \text{cons. control sugar}) \times (\text{diluent Factor} \times 1000)}{\text{weight of glucose} \times \text{incubation time}}
\]  \(2\)

The total protein content of the enzyme can be determined by testing using the Bradford method. The standard used was Bovine Serum Albumin (BSA). Total protein content was calculated by looking at the OD or absorbance data when reacting the sample with Bradford's reagent. The specific activity of the enzyme was calculated by the following formula [35].

\[
\text{Specific activity (U/mg)} = \frac{\text{Activity of Enzyme (U/mL)}}{\text{total protein (mg/mL)}}
\]  \(3\)

3. Result and Discussion
3.1 Hydrolysis capacity testing

Hydrolysis capacity testing aimed to determine the cellulase activity by showing the clear zone produced by each isolates. There were 21 isolates that had clear zones from 39 isolates obtained from mangrove soil at northern coast of Aceh. The highest number of isolates producing cellulase enzymes were found at station 1 as many as 6 isolates, followed by station 5, station 6, 2, 3, and 4 where the isolate numbers were 4, 4, 3, 2, and 2 isolates, respectively. Figure 2 showed the clear zone/halo formed after flooded by 1% congo red. [28] explained that the halo around the colonies indicates production of extracellular cellulase by bacteria. The colony of bacteria are positively produce extracellular enzyme could be show a clear zone around the colony with a red background [36]. Furthermore, 1% congo red solution given in CMC media to detect halo zone that can be hydrolyses by the cellulase [37].

![Figure 2. One of bacteria isolate (A), cellulolytic bacteria that produce cellulase enzyme (B); clear zone (1), bacteria colony (2) and (3) CMC media was flooded with 1% Congo red.](image)

Table 1 described the cellulolytic index (CI) obtained from each isolate that shows the halo zone. The CI obtained was ranged from 0.07 to 0.80. The highest CI was Isolate BTME33 (0.80), and followed by isolate BTMF22 (0.68) and BTMD32 (0.57). Those isolates was isolated from mangrove unrehabilitated. Station 1, 2, and 3 were located in mangrove rehabilitated which is a mangrove area planted after the 2004 tsunami, whereas station 4, 5, and 6 were located in mangrove unrehabilitated (no human intervention for planted). Both of them have different number of vegetation, species and abundance, soil texture, and soil organic carbon. The highest CI value was at station 5 (BTME33), it was assumed because the average of soil organic carbon higher in this station compare with the other
Soil organic carbon in the station 5 was 1.35%. Station 5 located in mangrove unrehabilitated contained more litter production than mangrove rehabilitated. [29] informed that the mangrove litter production in Northern coast of Aceh was 97.37 gm$^{2}$day$^{-1}$ indicated high litter production in mangrove unrehabilitated, and litter was the highest contribution of organic carbon in the soil. Moreover, [38] mentioned that enzymatic activity is associated with habitat characteristics i.e. temperature, moisture, and substrate condition. Furthermore, previous research reported that soil enzyme activities were significantly affected by input of Carbon and Nitrate in the soil [39; 40]. Figure 3 showed that the average of cellulolytic index each station and soil organic carbon (SOC).

Table 1. Colony diameter, clear zone diameter, and cellulolytic index of bacteria cellulolytic

| No. | Isolate code | Diameter colony (cm) | Clear zone diameter (cm) | Cellulolytic index (IS) |
|-----|--------------|----------------------|--------------------------|------------------------|
| 1   | BTMA11       | 0.85 ± 0.11          | 1.10 ± 0.07              | 0.29                   |
| 2   | BTMA13       | 0.64 ± 0.29          | 0.87 ± 0.36              | 0.36                   |
| 3   | BTMA21       | 0.82 ± 0.69          | 1.11 ± 0.71              | 0.35                   |
| 4   | BTMA22       | 0.69 ± 0.16          | 0.87 ± 0.15              | 0.26                   |
| 5   | BTMA23       | 0.72 ± 0.28          | 1.00 ± 0.41              | 0.39                   |
| 6   | BTMA33       | 0.81 ± 0.62          | 1.15 ± 0.86              | 0.42                   |
| 7   | BTMB11       | 0.39 ± 0.01          | 0.54 ± 0.04              | 0.39                   |
| 8   | BTMB12       | 0.79 ± 0.49          | 1.00 ± 0.65              | 0.27                   |
| 9   | BTMB21       | 0.58 ± 0.16          | 0.77 ± 0.18              | 0.32                   |
| 10  | BTMC21       | 0.73 ± 0.02          | 0.89 ± 0.15              | 0.22                   |
| 11  | BTMC31       | 0.40 ± 0.01          | 0.55 ± 0.01              | 0.38                   |
| 12  | BTMD31       | 0.33 ± 0.01          | 0.41 ± 0.03              | 0.26                   |
| 13  | BTMD32       | 0.14 ± 0.20          | 0.22 ± 0.31              | 0.57                   |
| 14  | BTME11       | 0.52 ± 0.15          | 0.80 ± 0.11              | 0.52                   |
| 15  | BTME12       | 2.13 ± 0.25          | 2.27 ± 0.29              | 0.07                   |
| 16  | BTME14       | 0.67 ± 0.22          | 0.85 ± 0.17              | 0.28                   |
| 17  | BTME33       | 0.18 ± 0.11          | 0.32 ± 0.11              | 0.80                   |
| 18  | BTMF11       | 1.99 ± 0.01          | 2.14 ± 0.02              | 0.07                   |
| 19  | BTMF21       | 0.41 ± 0.03          | 0.58 ± 0.01              | 0.40                   |
| 20  | BTMF22       | 0.43 ± 0.06          | 0.72 ± 0.11              | 0.68                   |
| 21  | BTMF32       | 0.37 ± 0.01          | 0.47 ± 0.03              | 0.28                   |

The CI value of present study was ranged from 0.07 to 0.80 and this value was categorized as low. According to [33], CI value would be categorized as low if ≤ 1, medium if 1-2, and high if ≥ 2. The CI value was lower than the previous result done by Behera et al (2014) where the CI was ranged from 1.18 to 2.5. Research done by [41] found that the genus Pseudomonas has the highest CI value (1.3). Isolate KFY-40 has the highest cellulolytic isolated from soil mangrove Malaysia with the value of 3.40 [30]. The difference in cellulolytic index may be caused by different types of isolates that have the ability to produce cellulase enzymes. According to [42], the difference in cellulolytic index values was due to each isolate having different abilities in hydrolysing CMC media so that it affected the size of the clear zone formed. Cellulolytic bacteria are often isolated from soil containing leaf litter because the soil contains relatively rich organic matter and leaf litter with relatively complex polysaccharide content. These conditions cause soil and leaf litter to become good habitats for various microorganisms [43]. Each cellulolytic bacteria produce a cellulase enzyme complex is varied, depending on the genes possessed and carbon source used [44; 45]. The diameter of the clear zone is generally larger than the diameter of the colony, because the cellulase enzyme is secreted into the surrounding environment by cellulose-degrading bacteria. Bacteria can’t enter cellulose molecules because the size of cellulose is larger than the size of bacterial cells [46].
Figure 3. The average of cellulolytic index and soil organic carbon (SOC) in study area

3.2 Quantitative Testing of Cellulase Enzyme Activity

Quantitatively, measurement of cellulase enzyme activity was tested on BTMD32 which had high cellulolytic index for 2.5 days of incubation (54 hours). The isolates (BTME33 and BTMF22) that also had high cellulolytic index already mentioned in other scientific article. Quantitative testing of cellulase activity aimed to obtain the optimum time of producing cellulase enzyme. Measurements were carried out 9 times, namely at 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours (hr). Based on figure 4 showed that the highest cellulase activity was 0.0012 U/ml at the 48 hours and decreased until 54 hour of observation. There were 3 times of exponential phase that was 18 and 30 hours of incubation time. The figure 4 explained that the cellulase enzyme start to generate at 6 hours which the value of 0.0002 U/ml. Enzyme activity that goes up and down is caused by different cell growth phases hourly. [47] reported the highest cellulase activity was at 24 hours (0.08 U/ml).

Figure 4. Cellulase activity of isolate BTMD32 isolated from soil mangrove

When the enzyme activity increases, it is possible that the cell will also experience an exponential phase, while when there is a decrease in activity, the cell has reached a stationary phase which is then followed by a death phase which causes a decrease in the activation of the enzymes produced [48]. Furthermore, [49] mentioned that the microorganism no only produce cellulase but also protease, protease could be damage the activity of the cellulase enzyme the stationary phase. At the 6 hours until 12 hours of observation time was the lag phase where the bacteria are still adapting to the new environment. Bacteria growth at 18, 30, and 48 hours of incubation time could be called as log and
stationary phases where bacteria begin to replicate DNA and then cells begin to grow [50]. The decreasing of cellulase activity experienced a death phase, where the number of dead cells is more than living cells [47], [44] found that the optimal incubation time for cellulase enzyme production was 48 - 72 hours, but 72 hours incubation time is not a stationary phase because cellulolytic activity has not yet decline. Furthermore, increasing of enzyme production was associated with increasing of cell growth indicated that cellulose was actively used by cellulolytic bacteria [51].

![Figure 5. Specific cellulase activity of isolate BTMD32 isolated from soil mangrove](image)

Figure 5 explained the specific cellulase activity which was included protein concentration (mg/ml). The highest specific cellulase activity was at 48 hours with the value was 0.097 U/mg. The specific cellulase activity explained that at certain time they were no specific activity at 24, 36 and 54 hours (0.000 mg/ml), and at 18 and 30 hours, the specific activity were 0.030 U/mg and 0.097 U/mg, respectively. The specific cellulase activity of isolate BTMD32 was lower than previous research done by [52]. They obtained that the specific activity of cellulase enzymes gradually increased up to 48 hr (0.44 U/mg) and significantly decreased at 72 hr. The protein concentration was ranged from 0.010 to 0.014 mg/ml (Table 2). The protein concentrations was fluctuated and was assumed would not affect the specific cellulase activity.

| Time (hours) | Protein concentration (mg/ml) | Specific cellulase activity (U/mg) |
|--------------|-----------------------------|----------------------------------|
| 6            | 0.011                       | 0.022                            |
| 12           | 0.010                       | 0.012                            |
| 18           | 0.014                       | 0.030                            |
| 24           | 0.012                       | 0.000                            |
| 30           | 0.010                       | 0.046                            |
| 36           | 0.013                       | 0.000                            |
| 42           | 0.012                       | 0.030                            |
| 48           | 0.012                       | 0.097                            |
| 54           | 0.013                       | 0.000                            |

4. Conclusion
Bacteria isolated from mangrove soil in the northern coast of Aceh have the ability to produce the cellulase classified as extracellular enzymes. There were 21 isolates of cellulolytic bacteria that had cellulolytic index by producing a clear zone around the bacteria colonies. The larger the clear zone, the greater the ability of bacteria to produce cellulase. The cellulase and specific cellulase activity of isolate BTMD32 produced the highest activity at the 48 hours of observation time. Isolates of cellulolytic bacteria isolated from soil in both mangrove rehabilitated and unrehabilitated sites had low ability to
produce cellulase enzymes qualitatively and quantitatively. This situation may cause the litter decomposition, and soil organic carbon content become low in study area.

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References
[1] Bouillon S, Koedam N, Raman A V and Dehairs 2002 Primary Producers Sustaining Macro-Invertebrate Communities in Intertidal Mangrove Forests Oecologia. 130 441-448
[2] Coronado–Molina C, Alvarez–Guillen H, Day Jr J W, Reyes E, Perez B C, Vera–Herrera F and Twilley R 2012 Litterfall Dynamics in Carbonate and Deltaic Mangrove Ecosystems in the Gulf of Mexico Wetlands Ecology and Management. 20 123-136
[3] Wang’ondu V W, Bosire J O, Kairo J G and Koedam N 2014 Litter Fall Dynamics of Restored Mangroves (Rhizophora mucronata Lam. and Sonneratia alba Sm.) in Kenya Restoration Ecology. 22(6) 824-831
[4] Betoulle J L, Fromard F, Fabre A and Puig H 2001 Characterization of Litter and Its Contributions to Soil Nutriment in a Mangrove of French Guiana French Canadian Journal of Botany. 79 238–249
[5] Bosire J O, Guebas F D, Kairo J G and Koedam N 2005 Litter Degradation and CN Dynamics in Reforested Mangrove Plantations at Gazi Bay Kenya Biological Conservation. 126(2)
[6] Murtiyantingsih H and Hazmi M 2017 Isolasi dan Uji Aktivitas Enzim Selulase pada Bakteri Selulolitik Asal Tanah Sampah Jurnal Ilmu-Ilmu Pertanian. 15(2) 17-23
[7] Arifin Z, Gunam I B W, Antara N S and Settyo Y 2019 Isolasi Bakteri Selulolitik Pendegradasi Selulosa dari Kompos Jurnal Rekayasa dan Manajemen Agroindustri 7(1) 30-37
[8] Yogyaswari S A, Rukmi M I and Raharjo B 2016 Ekplorasi Bakteri Selulolitik dari Cairan Rumen Sapi Peranakan Fries Holland (PFH) dan Limousine Peranakan Ongole (Limo) Jurnal Akademika Biologi, 5(4) 70-80
[9] Kurniawan A, Sari S P, Asriani E, Kurniawan A, Sambah A B, Triswiyana I and Prihanto A A 2019 Kapasitas Hidrolisis Bakteri Pendegradasi Selulosa dari Ekosistem Mangrove Journal of Tropical Marine Science. 2(2) 76-82
[10] Júnior F L S, Dias A C F, Fasanella C C, Taketani R G, Oliveira de Souza Lima A, Soares Melo I and Andreote F D 2013 Endo- and Exoglucanase Activities in Bacteria from Mangrove Sediment Brazilian Journal of Microbiology. 44(3) 969-976
[11] Padilha I Q M, Carvalho L C T, Dias P V S, Grisi T C S L, Honorato da Silva F L, Santos S F M and Araújo D A M 2015 Production and Characterization of Thermophilic Carboxymethyl Cellulase Synthesized by Bacillus sp. Growing on Sugarcane Bagasse in Submerged Fermentation Brazilian Journal of Chemical Engineering. 32(01) 35-42
[12] Behera B C, Sethib B K, Mishrab R R, Duttac S K and Thatoi H N 2017 Microbial Cellulases–Diversity and Biotechnology with Reference to Mangrove Environment: A review Journal of Genetic Engineering and Biotechnology
[13] Dias A C F, Andreote F D, Taketani R G, Tsai S M, Azevedo J L and Melo I S 2011 Archaeal Communities in the Sediments of Three Contrasting Mangroves J. Soils Sediments 11 1466–1476
[14] Acharaya S and Chaudhary A 2012 Bioprospecting Thermophiles for Cellulase Production: a Review Brazilian Journal Microbiology. 43(3) 844–856
[15] Gautam S P, Bundela P S, Pandey A K, Khan J, Awasthi M K and Sarsaiya S 2011 Optimization for the Production of Cellulase Enzyme from Municipal Solid Waste Residue by Two Novel Cellulolytic Fungi Biotechnology Research International. 810425 1-8
[16] Anoop Kumar V A, Chandra Kurup R S, Snishamol and Prabhu G N 2019 Role of Cellulases in Food, Feed, and Beverage Industries Department of Microbiology Springer Nature Singapore Pte Ltd. 323-343

[17] Gupta P, Samant K and Sahu A 2012 Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential International Journal of Microbiology. 578925 1-5

[18] Subagiyo, Djarod M S R and Setyati W A 2017 Potensi Ekosistem Mangrove Sebagai Sumber Bakteri Untuk Produksi Protease, Amilase Dan Selulase Jurnal Kelatun Tropis. 20(2) 106–111

[19] De Souza P M and Magalhães P De O E 2010 Application Of Microbial βAmylase In Industry: A Review Brazilian J. Microbiol. 41 850-861

[20] Dar G H, Kamili A N, Nazir R, Bandh S A and Malik T A 2014 Biotechnological Production Of A-Amylases For Industrial Purposes : Do Fungi Have Potential To Produce A- Amylases, International J. Biotechnol. Molec. Biol. Res. 5(4) 35-40

[21] Mienda B S, Yahya A, Galadima I A and Shamsir M S 2014 Res. J. Pharma. Biol. Chem. Sci. 5(1) 388- 396

[22] Hamza T A 2017 Bacterial Protease Enzyme: Safe And Good Alternative For Industrial And Commercial Use Int. J. Chem. Biomol. Sci. 3(1) 1-10

[23] Kuhad R C, Gupta R and Singh A 2011 Review article: Microbial Cellulases and Their Industrial Applications Enzyme. 280696-10

[24] Abubakar F A and Oloyede O B 2013 Production and Activity of Cellulase from Aspergillus niger using Rice Bran and Orange Peel as Substrates International Journal of scientific research and management. 1(5) 285-291

[25] Sadhu S, Saha P, Sen S K, Mayilraj S and Miti T 2013 Production, Purification and Characterization of a Novel Thermotolerant Endoglucanase (CMCase) from Bacillus Strain Isolated from Cow Dung Spingerplus Jornal. 2 1-10

[26] Chantarasariri A 2015 Aquatic Bacillus cereus JD0404 Isolated From the Muddy Sediments of Mangrove Swamps in Thailand and Characterization of Its Cellulolytic Activity Egypt J Aquat Res. 41(3) 257–64

[27] Afzal S, Saleem M, Yasmin R, Naz M and Imran M 2010 Pre and Post Cloning Characterization of a ‘1,4’ Endoglucanase from Bacillus sp. Molecular Biology Reports. 37 1717-1723

[28] Saini A, Aggarwal Ne K and Yadav A 2017 Isolation and Screening of Cellulose Hydrolysing Bacteria from Different Ecological Niches Bioengineering and Bioscience. 5(1) 7-13

[29] Dewiyanti I, Nurfadillah N, Setiawati S and Elrahimi A S 2019 Litter Production and Decomposition of Mangrove in the Northern Coast of Aceh Besar district, Aceh province IOP Conference Series Materials Science and Engineering. 567 012025

[30] Naresh S, Balakrishnan K, Ahmad A N G and Yi Peng 2019 Isolation and Partial Characterization of Thermophilic Cellulolytic Bacteria from North Malaysian Tropical Mangrove Soil Tropical Life Sciences Research. 30(1) 123-147

[31] Biswas S, Al Saber Md, Ara Tripty I, Adnan Karim Md, Aminul Islam Md, Shazid Hasan Md, Rubayet Ul Alam A S M, Kabir Jahid Md, Nazmul Hasan Md 2020 Molecular characterization of cellulolytic (endo- and exoglucanase) bacteria from the largest mangrove forest (Sundarbans), Bangladesh Annals of Microbiology. 70(68) 1-11

[32] Bradner J R, Gillings M, Nevalainen K M H 1999 Qualitative Assessment of Hydrolytic Activities in Antarctic Microfungi Grown at Different Temperatures on Solid Media World Journal of Microbiology and Biotechnology. 15(1) 131–132

[33] Choi Y W, Hodgkiss I J and Hyde K D 2005 Enzyme Production by Endophytes of Brucea javanica Journal Agricultural Technology. 29 55-66

[34] Miller G L 1959 Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar Analytical Chemistry. 31(3) 426-428

[35] Lowry O H, Rosbrough N J, Farr A L and Randall R J 1951 Protein Measurement with the Folin Phenol Reagent J. Biol. Chem. 193 265–275
[36] Yoo J S, Jung Y J, Chung S Y, Lee Y C and Choi Y L 2004 Molecular Cloning and Characterization of CMCase Gene (celC) from Salmonella yphimurium UR J. Microbiol 42(3) 205-210

[37] Zhang Y Y, Dong J D, Yang B, Ling J, Wang Y S and Zhang S 2009 Bacterial Community Structure of Mangrove Sediments in Relation to Environmental Variables Accessed by 16S rRNA Gene-Denaturing Gradient Gel Electrophoresis Fingerprinting Sci Mar. 73 487–498

[38] Sinsabaugh R L 1994 Enzymic Analysis of Microbial Pattern and Process Biol Fertil. 17 69-74

[39] Allison S D, Czimczik C I and Treseder K K 2008 Microbial Activity and Soil Respiration Under Nitrogen Addition in Alaskan Boreal Forest Glob. Change Biol. 14 1156–1168

[40] Jian S, Jianwei L, Chen J, Gangsheng W, Melanie A M, Kudjo E D, Dafeng H and Yiqi L 2016 Soil Extracellular Enzyme Activities, Soil Carbon and Nitrogen Storage Under Nitrogen Fertilization: A Meta-Analysis Soil Biol. Biochem. 101 32-43

[41] Astriani M 2017 Skrining Bakteri Selulolitik Asal Tanah Kebun Pisang (Musa paradisiaca) Jurnal Biota. 1(3) 6-10

[42] Pitri R E, Agustien A and Febria F A 2015 Isolation and Characterization of Amylothermophylic Bacteria from Medang River Hot Springs J Bio UA. 4(2) 119-122

[43] William R and Govind N S 2003 Identification of carbohydrate degrading bacteria in subtropical regions Rev. Biol. Trop. 51(4)

[44] Meryandini A, Wahyu W, Besty M, Titi C S, Nisa R and Hasrul S 2009 Isolation of Cellulolytic Bacteria and Their Enzyme Characteristics Makara Sains. 13 (1) 33-38

[45] Sunarti T C, Meryandini A, Sofiyanto M E and Richana N 2010 Saccharification of corncob using cellulolytic bacteria for bioethanol production Biotropia. 17(2) 105–114

[46] Zverlova V V, Holl W and Schwarz H 2003 Enzymes for Digestion of Cellulose and other Polysaccharides in the Gut of Longhorn Beetle Larvae, Rhagium inquisitor L. (Col. Cerambycidae). International Biodeterioration & Biodegradation. 51(3) 175-179

[47] Sonia N M O and Joni K 2015 Isolation and Partial Characterization of Cellulase Enzyme from Isolate OS-16 Cellulolytic Bacteria Origin Bromo-Tengger Desert JPA. 3(4) 11-19

[48] Abalos J M F, Arribas A R, Garda A L and Santamaria R I 1997 Effect of Carbon Source on the Expression of celA1, a Cellulase-Encoding from Streptomyces halstedii JM8 FEMS Microbiol Letters. 153 97-103

[49] Martina A, Yuli N and Sutisna M 2002 Optimasi Beberapa Faktor Fisik tehadap Laju Degradasi Selulosa Kayu Albasia Pariserianthes falcatairia (L) Nielsen dan Karboksimetilselulosa (CMC) secara Enzimatik oleh Jamur, Jurnal Natur Indonesia 4(2) 156-163

[50] Thiel T 1999 Science in the Real World : Microbes in Action University of Missouri Saint Louis. Missouri

[51] Seo J K, Park T S, Kwon I H, Piao M Y, Lee C H and Ha J K 2013 Characterization of Cellulolytic and Xylanolytic Enzymes of Bacillus licheniformis JK7 Isolated from the Rumen of a Native Korean Goat AJAS, 26(1) 50–58

[52] Iqbalysah T M, Uli A, Rika S U, Frida O and Febriani 2019 Concomitant Cellulase and Amylase Production by a Thermophilic Bacterial Isolate in a Solid-State Fermentation Using Rice Husks Nat Resour 53 327–333.