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Isolation and Characterization of Thermophilic Bacteria as Cellulolytic Enzyme Producer from the Hot Spring of Ie Seuum Aceh Besar, Indonesia

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Cellulase enzymes can be isolated from thermophilic bacteria obtained from the hot spring Ie Seuum, Aceh Besar. This research aimed to recover and characterize the isolates morphologically and biochemically followed by determination of the thermophile bacterial isolates potential as cellulolytic enzyme producers, The sampling method in this research was conducted by a purposive sampling at temperature of 70°C, 60°C, and 50 °C. Isolation of thermophilic bacteria was carried out on nutrient agar (NA) media. There were four isolates of thermophilic bacteria isolated recovered at 70 °C, five isolates at 60 °C, and seven isolates at 50 °C. Of the 18 isolates obtained, 15 of them were able to produce cellulase enzymes. Cellulase enzyme production can be determined by the presence of clear zones around bacterial colonies on CMC media after addition of 1% congo red drops and wash with 1 M NaCl. The highest five Cellulolytic Index (CI) values were obtained from isolates ISB75; ISB64; ISB52; ISB54; and ISB56 that were 1.23; 2.22; 1.39; 1.59; and 1.10, respectively. Biochemical tests carried out on 5 isolates with the highest cellulolytic index values showed that the bacterial isolate were suspected to be from the genera of *Bacillus* sp.

Key words : cellulase enzymes, hot springs, Ie Seuum, thermophilic bacteri

Enzim selulase dapat diisolasi dari bakteri termofil yang diperoleh dari sumber mata air panas Ie Seuum, Aceh Besar. Penelitian ini bertujuan untuk mendapatkan isolat, karakterisasi morfologi, menentukan isolat bakteri termofil yang mampu menghasilkan enzim selulase, dan mengetahui karakter biokimia isolat terpilih. Metode pengambilan sampel pada penelitian ini dilakukan dengan *purposive sampling* pada suhu 70 °C, 60 °C dan 50 °C. Isolasi bakteri termofil dilakukan pada media *Nutrient Agar* (NA). Hasil isolasi bakteri termofil yang diperoleh adalah empat isolat pada suhu 70°C, lima isolat pada suhu 60 °C, dan tujuh isolat pada suhu 50 °C. Dari 18 isolat yang didapatkan, 15 diantaranya mampu menghasilkan enzim selulase. Produksi enzim selulase dapat diketahui dengan adanya zona bening di sekitar koloni bakteri pada media CMC setelah ditetesi *congo red* 1% dan dicuci NaCl 1M. Nilai Indeks Selulolitik (IS) lima tertinggi diperoleh dari isolat ISB75; ISB64; ISB52; ISB54; dan ISB56 yaitu masing-masing 1,23; 2,22; 1,39; 1,59; dan 1,10. Pengujian biokimia dilakukan terhadap 5 isolat yang memiliki nilai indeks selulolitik tertinggi. Hasil karakterisasi menunjukkan bahwa isolat bakteri tersebut diduga termasuk dalam genus *Bacillus* sp.

Kata kunci : bakteri termofil, enzim selulase, Ie Seuum, sumber air panas

PEnzymes are proteins that are specifically synthesized by living cells to catalyze chemical reactions within cells (Martoharsono 2006). Based on the activity sites, enzymes have two types, namely intracellular enzymes, directly used in cells, and extracellular enzymes, released from cells into the environment to hydrolyze polymer molecules in the environment, such as cellulose, hemicellulose, lignin and others. One of the extracellular enzymes is cellulase (Sholihati *et al.* 2015). According to Pham *et al.* (2010), cellulase is a complex enzyme that is used to break down cellulose into glucose.

Cellulase enzymes can be produced by groups of plants, animals and microorganisms (Said and Likadja 2012). According to Natsir *et al.* (2014), the use of enzymes originating from microorganisms in general is in great demand because it has stability at high temperatures and can be produced in large quantities. Microorganisms that produce cellulase enzymes can be isolated from various sources such as bovine rumen fluid (Setyoko and Budi 2016), mangrove sediments (Setyati and Subagiyo 2012), compost (Baharuddin *et al.* 2010) and hot springs (Mukminin 2014). Tuntun and Huda (2014) states that Hot springs are originated as a result of ground water coming out of the earth's crust after experiencing geothermal heating.

One of the bacteria that can produce cellulase enzymes in hot springs is a group of thermophilic bacteria (Hasanah *et al.* 2015). Thermophile bacteria

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are a group of bacteria that are able to adapt to high-temperature environmental conditions, ranging from 45–80 °C. These thermophilic bacteria are not only tolerant of extreme environmental temperatures, but are also able to survive and reproduce at these temperature conditions (Martharina 2010).

One source of hot springs that can be used as a source of isolation of cellulolytic bacteria found in the Aceh Province is Ie Seuum. Ie Seuum is one of the hot spring bathing areas in the Krueng Raya valley which originates from the crater of the Seulawah volcano. This hot spring area is located in Ie Seuum Village, Mesjid Raya District, Aceh Besar Regency, Aceh Province (Wahyuni 2017). This hot spring has a temperature ranging around 45–80 ºC and a neutral water pH of 7 so that it has the potential to obtain thermophilic bacteria that are able to produce enzymes. Therefore, it is necessary to conduct a research on the Ie Seuum Aceh Besar hot spring to obtain thermophile bacterial isolates that produce cellulase enzymes. The purpose of this research was to isolate thermophilic bacteria, characterize morphologically and biochemically thermophilic bacteria producing cellulase enzymes from the Ie Seuum Aceh Besar hot spring.

MATERIALS AND METHODS

Materials. The materials used in this research were hot water samples, nutrient agar (NA) media, nutrient broth (NB) media, carboxy methyl cellulose (CMC) agar media, $MgSO_4.7H_2O$, K_2HPO_4, $CaCl_2$, $FeSO_4$, KNO_3, yeast extract, glucose, agar, 1% congo red dye, 1M NaCl, violet crystal reagent, safranin, iodine, Kovac's reagent, red methyl, H_2O_2 3%, Simmons citrate agar (SCA), sulfide indol motility (SIM) media, triple sugar iron agar (TSIA) media, methyl red-Vogue Proskauer (MR-VP) media, aquadest, 70% alcohol, blank disc paper, and pipette tips.

Water Sampling. Water sampling was performed using the Ginting method (2009). Hot water samples suspected of containing thermophilic bacteria were taken from the Ie Seuum Aceh Besar hot spring. Thermophile bacterial samples were taken at three different points, namely at 70 ºC, 60 ºC and 50 ºC (Fig 1). Each sample point was repeated three times. Water samples were collected using a long dipper which had been sterilized using 70% alcohol, then put into a hot water flask and tightly closed.

Sample Isolation. One mL of water samples were drawn using a micropipette and dropped into a Petri dish. As much as 30 mL of liquid NA media was poured into a Petri dish which was shaken to keep the suspension flat in the media. After freezing, the Petri dish was incubated at 50 °C for 24 hours. After incubation, bacterial colonies growing on NA media were re-inoculated into sterile Petri dishes containing NA media by means of the quadrant method, then further incubated at 50 °C for 24 hours until single colonies were visible.

Characterization of Thermophilic Bacteria. The characterization of thermophilic bacteria was conducted through macroscopic, microscopic and biochemical observations. Macroscopic observation was the observation of the shape of the colony, edge, elevation, color and surface of the bacterial colony. Microscopic observations were made of the shape of bacterial cells and Gram staining. The isolates which had the highest cellulolytic activity index values were characterized by biochemical properties consisting of catalase tests, indole and motility tests, citrate test, TSIA test, Methyl Red (MR) test, and Voges-Proskauer (VP) test.

Screening of Cellulolytic Bacteria. Pure isolates were grown in NA media, then isolated to NB liquid media for enzymatic assay. One loop of isolate was inoculated into 5 mL NB media and incubated at 50 °C for 24 hours. A 0.5 µL isolates were transferred using micropipette and dropped on blank paper disc on 1% solid CMC media, then incubated at 50 °C for 24 hours. If the isolate had grown on disc paper, then it was flooded using 1% congo red dye for 15 minutes, then rinsed 2-3 times using 1M NaCl and allowed to stand for 15 minutes to detect the clear zone indicating the cellulolytic activity (Rahmadini 2012). The formed diameter of the clear zone was measured using a calipers and the Cellulolytic Index (CI) is calculated (Sari *et al.* 2012) using formula as follows (Kader and Omar 1998):

$$CI = \frac{\text{clear zone diameter - colony diameter}}{\text{colony diameter}}$$

CI = Cellulolytic Index

Data Analysis. Data analysis was carried out descriptively and displayed in the form of tables and images.

RESULTS

Thermophilic Bacteria Isolate from Ie Seuum Hot Spring, Aceh Besar. There were 18 thermophilic bacterial isolates recovered from different temperature comprising of five isolates were from 70 ºC, six isolates

at 60 ºC, and seven isolates at 50 ºC (Fig 2).

Morphological Characteristics of Thermophile Bacterial Isolates. The colony characterization showed that their shapes varied ranging from a circular with spread edge, irregular, wrinkled, circular to circular with a rounded edge. The edge of the colony was branching, choppy, curved, and irregular, whereas the elevation of the colony was flat, like a button, raised and hilly. The whole isolate was creamy with the rough and smooth surface. The results of Gram staining from each isolate showed that all isolates were Gram positive bacteria (Table 1). Based on the Gram staining process all isolates showed the shape of bacilli cells and were purple (maintaining a violet crystalline dye) (Fig 3).

Screening of Thermophile Bacteria that Produce Cellulase Enzymes. The screening stage aimed to determine the cellulolytic activity of the bacterial isolates. This activity is shown by the ability of bacteria to hydrolyze CMC substrate. Colonies that were able to hydrolyze CMC will form a clear zone or a light orange zone around the colony after being flooded with 1% congo red (Fig 4). The clear zone of 18 isolates was measured based on Cellulolytic Index (CI). CI value was calculated by measuring the ratio of clear zone diameter towards colony diameter(Table 3).

Biochemical Test Results of Cellulolytic Bacteria. Biochemical characterization was performed on five thermophilic bacterial isolates showing the highest cellulolytic index values, namely isolates ISB75, ISB64, ISB52, ISB54, and ISB56 (Table 2). Biochemical testing is a way to identify pure cultures of isolated bacteria through their physiological properties.

The bacteria tested showed positive result on catalase test, except for ISB64 which showed negative result. The bacteria tested showed negative results onsimmons citrate test. indole test result of all isolates were negative and motile. The bacteria showed positive results on TSIA media, it was shown by the color change of the media into yellow on the buttocks and slants. The test results on MR media showed positive results only on ISB64 isolate and other isolates showed negative results on VP test media.

Table 1 Results of morphological (macroscopic and microscopic) characterization of thermophilic bacterial isolates from the Ie Seuum hot spring, Aceh Besar

Isolate Code	Macroscopis Character					Microscopic Character	
	Colony Shape	Edge of the Colony	Colony Elevation	Colony Pigment	Colony Surface	Gram	Shape
ISB71	Circular with spread edge	Branching	Flat	Cream	Rough	Positive	Basil
ISB72	Irregular	Irregular	Like a button	Cream	Smooth	Positive	Basil
ISB73	Irregular	Choppy	Convex	Cream	Rough	Positive	Basil
ISB74	Irregular	Curved	Convex	Cream	Smooth	Positive	Basil
ISB75	Irregular	Irregular	Arise	Cream	Rough	Positive	Basil
ISB61	Irregular	Irregular	Convex	Cream	Rough	Positive	Basil
ISB62	Circular with spread edge	Branching	Raised	Cream	Rough	Positive	Basil
ISB63	Irregular	Branching	Hilly	Cream	Rough	Positive	Basil
ISB64	Irregular	Choppy	Raised	Cream	Rough	Positive	Basil
ISB65	Irregular	Curved	Like a button	Cream	Smooth	Positive	Basil
ISB66	Wrinkled	Irregular	Raised	Cream	Rough	Positive	Basil
ISB51	Circular with a rounded edges	Branching	Raised	Cream	Rough	Positive	Basil
ISB52	Irregular	Branching	Raised	Cream	Rough	Positive	Basil
ISB53	Circular	Irregular	Raised	Cream	Rough	Positive	Basil
ISB54	Circular with spread edge	Irregular	Raised	Cream	Rough	Positive	Basil
ISB55	Irregular	Irregular	Flat	Cream	Rough	Positive	Basil
ISB56	Circular with spread edge	Branching	Raised	Cream	Rough	Positive	Basil
ISB57	Irregular	Irregular	Raised	Cream	Rough	Positive	Basil

Description: ISB7: Ie Seuum Bacteria temperature of 70 ˚C
ISB6: Ie Seuum Bacteria temperature of 60 ˚C
ISB5: Ie Seuum Bacteria temperature of 50 ˚C

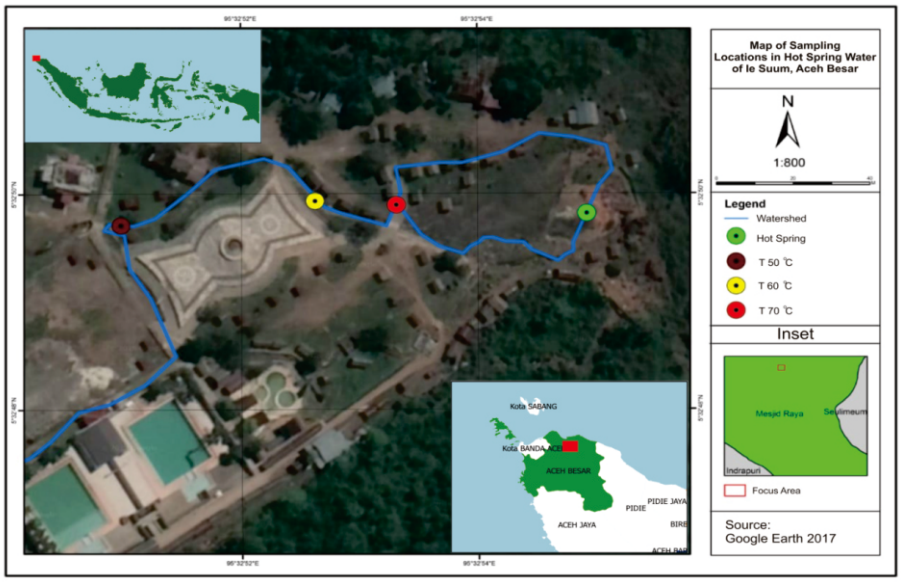

Fig 1 The map of sampling location in Ie Seuum hot springs, Aceh Besar, Aceh, Indonesia.

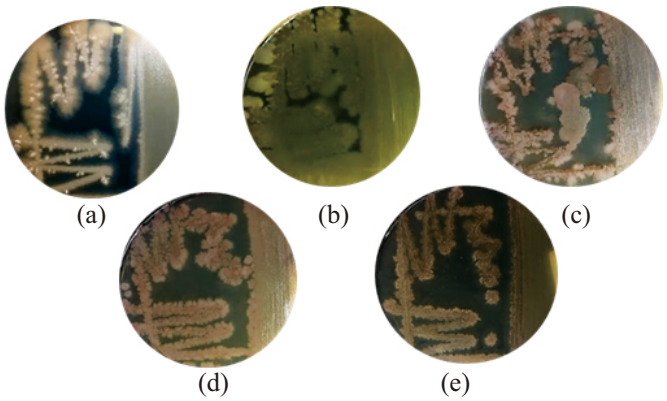

Fig 2 Results of the isolation of thermophilic bacteria, (a) ISB75 isolates, (b) ISB64 isolates, (c) ISB52 isolates, (d) ISB54 isolates, and (e) ISB56 isolates.

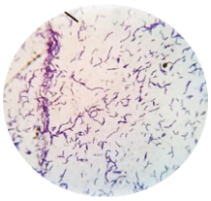

Fig 3 Gram stain results of ISB64 isolates with 1000x magnification.

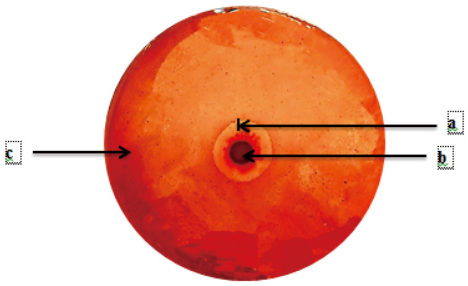

Fig 4 Screening of ISB64 isolate-producing cellulase thermophilic bacteria from the Ie Seuum hot spring, Aceh Besar. (a) Clear zone, (b) Bacterial colony, and (c) CMC media flooded with 1% congo red.

Table 2 Biochemical test results of thermophilic bacterial isolates that produce cellulase enzymes from the hot spring Ie Seuum, Aceh Besar

Biochemical Test	Isolate Code				
	ISB75	ISB64	ISB52	ISB54	ISB56
Catalase	+	-	+	+	+
Simmons citrate	-	-	-	-	-
Indole	-	-	-	-	-
Motility	+	+	+	+	+
TSIA (B/S)	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y
Methyl Red	-	-	-	+	-
Voges Proskauer	-	-	-	-	-
Identification	*Bacillus* sp.1	*Bacillus* sp.2	*Bacillus* sp.1	*Bacillus* sp.3	*Bacillus* sp.1

Description:

+ : Positive reaction - : Negative reaction B : *butt* S : *slant* Y: Yellow

Table 3 Average colony diameter (cm), clear zone diameter (cm), standard deviation values and Cellulolytic Index (CI) values of thermophilic bacteria producing cellulase enzymes from the Ie Seuum hot spring, Aceh Besar

Isolate Code	Colony Diameter (cm)	Clear Zone Diameter (cm)	Cellulolytic Index (CI)
ISB71	0,96 ± 0,19	1,92 ± 0,18	1,00
ISB72	0,52 ± 0,07	1,01 ± 0,12	0,94
ISB73	0	0	0
ISB74	1,03 ± 0,16	1,36 ± 0,14	0,32
ISB75	0,65 ± 0,04	1,45 ± 0,04	1,23
ISB61	0,44 ± 0,07	0,66 ± 0,07	0,50
ISB62	0,75 ± 0,28	0,89 ± 0,19	0,19
ISB63	0,55 ± 0,20	0,82 ± 0,14	0,49
ISB64	0,60 ± 0,02	1,93 ± 0,35	2,22
ISB65	0	0	0
ISB66	0	0	0
ISB51	0,95 ± 0,10	1,80 ± 0,26	0,89
ISB52	0,61 ± 0,15	1,46 ± 0,16	1,39
ISB53	0,61 ± 0,01	1,02 ± 0,02	0,67
ISB54	0,57 ± 0,09	1,48 ± 0,19	1,59
ISB55	0,54 ± 0,04	0,86 ± 0,05	0,59
ISB56	0,79 ± 0,09	1,66 ± 0,21	1,10
ISB57	0,42 ± 0,16	0,85 ± 0,04	1,02

Description:

ISB7: Ie Seuum Bacteria temperature of 70 °C

ISB6: Ie Seuum Bacteria temperature of 60 °C

ISB5: Ie Seuum Bacteria temperature of 50 °C

DISCUSSION

Thermophilic Bacteria Isolate from Ie Seuum Hot Spring, Aceh Besar. The results obtained are different from other research, where Hastuti *et al.* (2012), succeeded in isolating 44 thermophilic bacterial isolates from the Semerup hot spring, Kerinci, Jambi. Moreover, Ardani *et al.* (2012), isolating four chitinolytic thermophile bacterial isolates from the Ie Seuum hot spring, Aceh Besar. Lutfi (2012), succeeded isolating 6 themophilic bacteria isolates that produced cellulase from Panggo hot spring, Sinjai Regency, South Sulawesi and Hasanah (2015) succeeded isolating 5 isolates of cellulase-producing thermo-alkalifilic bacteria in Pariangan hot spring, West Sumatra.

The difference in the number of isolates successfully isolated from each point can be influenced by environmental conditions that support-bacterial life. The environmental conditions comprises biotic and abiotic factors, including the presence of dry leaves, grass and moss, as well as temperature and pH (Pitri *et al.* 2015). Environmental conditions contained in the sampling location at Ie Seuum were deciduous leaves, grasses, moss, inorganic waste and other organic

sources which were a source of energy for microorganisms. Abiotic environmental conditions that occured in sampling location were temperature and pH. The temperature range at the hot water sampling location was 45–80ºC with pH of 7.

Morphological Characteristics of Thermophile Bacterial Isolates. The results obtained in the study were aligned with other study (Wahyuni 2017) signifying that morphological features of the thermophilic bacteria were irregular and circular colony forms with the color of the cream colony and arise and flat colony elevation, and rough and smooth shiny colonies. The result of Runtuboi *et al.* (2018) showed that the bacteria had of regular, rhizoid, and irregular rounded colony form, the elevation of colonies were convex, like a button and flat, the colony margin were flat, bumpy, curved, and jagged, the colony pigment were white and yellow, and the texture of colony surface were shiny, rough and wrinkled.

Observations on the morphological characteristics of bacterial colonies need to be made in order to facilitate the process of identifying the types of bacteria. According to Lay (1994), based on the morphological characteristics of bacterial colonies, the process of identification of types of microorganisms can be carried out, but to obtain perfect identification results it must proceed with biochemical tests.

The results of Gram staining from each isolate showed that all isolates were Gram positive bacteria. Based on Gram staining, all isolates were bacilli and had purple color (maintaining a violet crystalline dye). This is in accordance with Wahyuni (2017); Mukminin (2014); and Sianturi (2008) studies, the result of the studies showed that Gram staining of hot water bacteria were Gram positive bacilli bacteria. According to Lay (1994), Gram staining is used to determine the morphology of bacterial cells as well as to distinguish between Gram positive and Gram negative bacteria. Gram-positive bacteria in Gram staining will be purple while Gram-negative bacteria will be red. Gram-positive bacteria in Gram staining are purple due to the violet-iodine crystalline complex which is retained even if given a pale alcohol solution, while Gram-negative bacteria are red because the complex is soluble when giving 96% alcohol solution so that it absorbs the red color from safranin.

Screening of Thermophile Bacteria that Produce Cellulase Enzymes. The formation of clear zone around the colony on the CMC media is caused by the process of cellulose degradation by cellulolytic bacteria. Hydrolyzed cellulose in the media so that if inundated by congo red 1% will produce a clear zone because congo red cannot bind to the media without β-1,4-glycosidic bonds contained in cellulose polymers due to the presence of cellulase enzymes so that the binding hydrolyzed cellulose polymers (Jo *et al.* 2011). The clear zone formed could be seen by adding congo red dye then rinsed with 1 M NaCl. The addition of congo red dye was to clarify the clear zone formed so that it could be easily observed (Astriani 2017).

Congo red is sodium salt from benzidinediazo-bis-1-naphthylamine-4 sulfonic acid ($C_{32}H_{22}N_6Na_2O_6S_2$) so that this dye will dissolve and be washed by other sodium salts, such as NaCl (Hartanti 2010). Washing with NaCl will fade congo red especially in the area around the colony that contains hydrolyzed cellulase derivatives such as cyclodextrin, cellobiose and glucose because congo red is not tightly bound so that a clear zone appears (Seprianto 2017).

The presence of clear zones caused by cellulase enzymes released into the media is colorless metabolites. Bacteria produce extracellular enzymes to hydrolyze food sources that contain cellulose contained in the media. Cellulose molecules cannot enter into bacterial cells because of their large size, therefore cellulose molecules are hydrolyzed in advance by cellulase enzymes to become simpler cellobiose molecules. These molecules will then be transported into bacterial cells and used as a carbon source for their growth activities (Lutfi 2012).

The clear zone of 18 isolates was measured based on Cellulolytic Index (CI) (Table 3). Only 15 isolates of 18 isolates obtained had the ability to produce cellulase enzymes. Four isolates were recovered from 70 ºC, four isolates at 60 ºC, and at seven isolates at 50 ºC with a range of cellulolytic index of 0.19–2.22. Isolate ISB75; ISB64; ISB52; ISB54; and ISB56 had the highest cellulolytic index values of 1.23; 2.22; 1.39; 1.59; and 1.10, respectively. Isolate ISB62 had the lowest cellulolytic index value of 0.19. According to Choi *et al*. (2005), if the cellulolytic index value ≤ 1 is in the low category, between 1–2 is in the medium category, and ≥ 2 is in the high category. Cellulolytic Index (CI) values obtained in this study were moderate to high category.

Similar result was shown in the study by Behera's (2014), the result showed that bacteria obtained from mangrove soil in Mahanadi river which had cellulolytic index value of 1.18 to 2.5 cm. A different result was obtained on the study by Sari *et al.* (2012) that succeeded isolating cellulolytic bacteria from Medang River hot springs with the highest cellulolytic index value of 5.40, meanwhile a study by Sonia and Kusnadi

(2015) obtained cellulolytic bacteria isolated from Tengger-Bromo desert that had cellulolytic index values of 1.1 to 11. A study by Nababan *et al.* (2019) showed that cellulolytic bacteria isolated from several forest soils in Bali and compost had cellulolytic index value of 4.50 to 7.98. According to Pitri *et al.* (2015), the difference in cellulolytic index values is due to the ability of each isolate to hydrolyze CMC substrate differently so that it influences the size of the formed clear zone. According to Arji *et al*. (2018), the size of clear zone formed depends on the bacteria ability to degrade complex molecules into simple molecules.

Based on the results of the enzyme testing, it can be seen that the highest number of cellulase-producing isolates were found at 50 ºC, that is, as many as seven isolates and isolates with the highest cellulolytic index values were found at 60 ºC. This is consistent with the research of Sari *et al*. (2012) demonstrating that eleven isolates producing cellulase enzymes were recovered from 50 ºC of the Medang River hot springs, but the highest cellulolytic index values were shown by the isolates recovered from 78 ºC. Ibrahim and El-diwany (2007) signified that if the selection results only get a few isolates that produce clear zones, this shows that bacteria that produce less cellulase caused by the low content of organic matter in the media that can be used as a carbon source by cellulolytic bacteria.

Biochemical Test Results of Cellulolytic Bacteria. Bacteria isolates that showed positive catalase means that they are able to convert hydrogen peroxide into water and oxygen, allowing these bacteria classified into aerobic or facultative aerobic bacteria (Sonia and Kusnadi 2015). The negative result of the simmons test showed that all of these bacteria did not use citrate as their carbon source. The negative result of indole showed that the bacteria did not have the ability to break down amino acid tryptophan into indole compounds (Arfah *et al.* 2014). Bacteria that are motile indicated that the bacteria had flagellum which allowed the movement of bacteria. TSIA test showed that all bacteria were able to ferment lactose and sucrose. ISB64 isolate showed MR positive which means mixed acid fermentation on media was occurred. All bacteria isolates were unable to use butanadiol fermentation through VP test because it showed negative results (Natsir *et al.* 2014).

Based on these data (Table 2), bacterial identification can be done. Identification in this study was carried out based on "*Cowan and Steel's Manual for the Identification of Medical Bacteria Third Edition*" by looking at the data from microscopic, macroscopic and biochemical characterization of thermophilic isolates, it was concluded that the five thermophilic bacterial isolates were thought to belong to the *Bacillus* genera. According to Corbin (2004), the colony of *Bacillus* sp. has the general characteristics of having a whitish beige color and the shape of a round and irregular colony. *Bacillus* sp. has a colony edge shape that is flat and uneven, the surface is rough and not slimy, and even tends to dry and powder. This is consistent with the morphological characterization results of bacterial isolates obtained. In general, the *Bacillus* sp. has a positive stem and Gram cell shape. *Bacillus* sp. is motile, including positive Gram in the form of short bacilli to single bacillus with a single arrangement, producing spores that are usually resistant to heat, aerobic, positive catalase and some species produce negative catalase. Each species differs in the use of sugar as well as fermentative activity (Hatmanti 2000; Puspita *et al.* 2017).

The results of this study are in accordance with Huwae and Aditiawati (2020), who found that cellulase-producing bacteria obtained from sila hot spring had similarity to the Genus Bacillus. However, different results were obtained by Arji *et al.* (2018), where the bacteria isolated from Lainea hot spring, Southeast Sulawesi was likely belong to the Genus Listeria.

REFERENCES

Ardani F, Yasmin Y, Fitri L. 2012. Potential of chitinolytic hot springs bacteria as biological control of *Aedes aegypti* larvae. J Bio Ed. 4(2): 77-81.

Arfah, RA, Patong AR, Ahmad A, Djide MN. 2014. Isolation and identification of amylase-producing thermophile bacteria from hot springs in Lejja, South Sulawesi. Al-Kimia. 2(2): 36-46. doi: 10.24252/al-kimia.v2i2.1644.

Arji M, Rezky W, Nurhaliza WOS, Yanti NA. 2018. Isolation, characterization, and bacteria potential test of producing termostabil enzyme from Lainea hot water sources, Southeast Sulawesi. Biowallacea. 5(1): 726-734. doi: 10.33772/biowallacea.v5i1.4592.

Astiani M. 2017. Screening for cellulolytic bacteria from banana garden *(Musa paradisiaca)*. Biota Journal. 3(1): 6-10. doi: 10.19109/Biota.v3i1.871.

Baharuddin AS, Razak MNA, Hock LS, Ahmad MN, Aziz SA, Rahman NAA, Shah UKM. 2010. Isolation and characterization of themophilic cellulase-producing bacteria from empty fruit bunches-palm oil mill effluent compost. Am J Applied Sci. 7(1): 56-62. doi: 10.3844/ajassp.2010.56.62.

Behera BC, Parida S, Dutta SK., Thatoi HN. 2014. Isolation

and identification of cellulose degrading bacteria from mangrove soil Mahanadi river delta and their cellulose production ability. AJMR. 2(1): 41–46. doi: 10.12691/ajmr-2-1-6.

Choi YW, Hodgkis IJ, Hyde KD. 2005. Enzyme production by endophytes of *Brucea javanica.* J Agricultural Technol. 29: 55-66.

Corbin BD. 2004. Identification and characterization *Bacillus thuringiensis.* J Bacteriol. 186: 7736-7744.

Ginting Y. 2009. Bacterial isolation and thermophile amylase activity test from the spirit of hot springs of North Sumatra mountain. [dissertation]. Medan (ID): Universitas Sumatera Utara.

Hartanti. 2010. Isolation and selection of thermophilic cellulolytic bacteria from Gunung Pancar Hot Crater, Bogor. [dissertatiton]. Bogor (ID): Institut Pertanian Bogor.

Hasanah A, Megahati RRP, Wati M. 2015. Thermo-alkalifilic bacteria producing cellulases in Pariangan hot springs, Tanah Datar district, West Sumatra. Journals. Padang (ID): Teacher Training and Education College (STKIP) PGRI West Sumatra. 1-5.

Hastuti W, Agustien A, Nurmiati. 2012. Screening and characterization amilo-thermophilic bacteria from Semurup hot springs, Kerinci, Jambi. J Bio UA. 1(2): 150-155. doi: 10.25077/jbioua.1.2.%25p.2012.

Hatmanti A. 2000. Introduction of *Bacillus* spp. Oseana. 25(1): 31-41.

Huwae LMC, Aditiawati P. 2020. Isolation and identification of cellulase-producing thermophilic bacteria from Sila hot springs. ACROPORA. 3(1): 32-35. doi: 10.31957/acr.v3i1.1214.

Ibrahim ASS, El-diwany AI. 2007. Isolation and identification of new cellulases producing thermophilic bacteria from an egyptian hot spring and some properties of the crude enzyme. Aust J Basic Applied Sci. 1(4): 473-478.

Jo WS, Park HN, Cho DH, Yoo YB, Park SC. 2011. Optimal media conditions for the detection of extracellular cellulase activity in *Ganoderma neo-japonicum*. Mycobiology. 39(2): 129-132. doi: 10.4489/MYCO. 2011.39.2.129.

Kader AJ, Omar O. 1998. Isolation of cellulolytic fungi from sayap Kinabalu Park, Sabah. Serawak. ARBEC. 1–6.

Lay BW. 1994. Microbial analysis in the laboratory. Jakarta: PT Raja Grafindo Persada.

Lutfi Z. 2012. Screening of thermophilic bacteria producing cellulase enzymes from Panggo hot springs, Sinjai Regency, South Sulawesi. [dissertation]. Makassar (ID): UIN Alauddin Makassar.

Martharina D. 2010. Characterization of thermophilic lactic acid bacteria from Gunung Pancar White Crater, Bogor. [dissertation]. Bogor (ID): Institut Pertanian Bogor.

Martoharsono S. 2006. Biochemistry I. Yogyakarta: Gadjah Mada Press.

Mukminin A. 2014. Isolation of thermophilic cellulolytic bacteria from Pacet Mojokerto hot spring and testing cellulase enzyme activity. [dissertation]. Malang(ID): UIN Maliki Malang.

Nababan M, Gunam IBW, Wijaya IMM. 2019. Production of crude cellulase enzyme from cellulolytic bacteria. J RMA. 7(2): 190-199. doi: 10.24843/JRMA.2019.v07. i02.p03.

Natsir NAN, Natsir H, Dali S. 2014. Exploration and characterization of thermophile bacteria producing amylase enzymes from Panggo Hot Spring, South Sulawesi. National Seminar on Biochemistry. Jakarta (ID): UIN Syarif Hidayatullah. 72-81.

Pelczar M J, Chan ECS. 2005. Fundamentals of microbiology 1. Translated by Hadioetomo RS, Imas T, Tjitrosomo SS. Jakarta: Figures Universitas Indonesia Press.

Pham TH, Quyen DT, Nghiem NM. 2010. Optimization of endoglucanase by *Aspergillus niger* VTCC-F021. Aust J Basic Apllied Sci. 4(9): 4151-4157.

Pitri RE, Agustien A, Febria FA. 2015. Isolation and characterization of amylothermophylic bacteria from Medang River hot springs. J Bio UA. 4(2): 119-122. doi: 10.25077/jbioua.4.2.%25p.2015.

Puspita F, Ali M, Pratama R. 2017. Isolation and characterization of morphology and physiology of *Bacillus* sp. endophytic of Palm Oil (*Elaeis guineensis* Jacq.). J Agrotek Trop. 6(2): 44-49.

Rahmadini I. 2012. Purification and characterization of cellulase enzymes from bacteria isolated from seaweed waste. [dissertation]. Bogor (ID): Bogor Agricultural University Postgraduate School.

Runtuboi DYP, Gunaedi T, Simonapendi M, Pakpahan NNL. 2018. Isolation and identification of thermophilic bacteria from hot springs in Moso, Muara Tami District, Jayapura City, Papua Province. Papua Bio J. 10(2): 68-73. doi: 10.31957/jpb.474.

Said MI, Likadja JC. 2012. Isolation and identification of bacteria that potential to produce protease enzymes in the tannery industry in PT. Adi Satria Abadi (ASA) Yogyakarta. JITP. 2(2): 121-128.

Sari UM, Agustien A, Nurmiati. 2012. Screening and characterization of thermophilic cellulolytic bacteria in Medang River hot springs, Kerinci, Jambi. J Bio UA. 1(2):166-171. doi: 10.25077/jbioua.1.2.%25p.2012.

Seprianto. 2017. Isolation and screening of cellulolytic bacteria from various types of soil as a producer of cellulase enzymes. IJOBB. 1(2): 64-70.

Setyati WA, Subagiyo. 2012. Isolation and selection of bacteria that produce extracellular enzymes (proteolytic, amylolytic, lipolytic and cellulolytic) derived from manggrove area sediments. IJMS. 17(3):164-169. doi: 10.14710/ik.ijms.17.3.164-169.

Setyoko H, Utami B. 2016. Isolation and characterization of cow rumen liquid cellulase enzyme for biomass hydrolysis. Proceeding Biology Education Conference. 13(1):863-867.

Sholihati AM, Baharuddin M, Santi. 2015. Isolation and test of cellulase enzyme activity from *Bacilllus subtilis* bacteria. Al-Chemistry. 3(2):78-90. doi: 10.24252/al-kimia.v3i2.1672.

Sianturi, DC. 2008. Bacterial isolation and thermophilic rough amylase activity test from the Panen Sibirubiru hot spring. [dissertation]. Medan(ID): Universitas Sumatera Utara.

Sonia NMO, Kusnadi J. 2015. Isolation and partial characterization of cellulase enzyme from isolat OS-16 cellulolytic bacteria origin Bromo-Tengger desert. JPA. 3(4): 11-19.

Tuntun M, Huda M. 2014. Isolation and identification of thermophilic bacteria from the Natar Lampung South geothermal hot springs. Journal of Health Analyst. 3(1):297-304. doi: 10.26630/jak.v3i1.439.

Wahyuni D. 2017. Morphological characteristics and activity tests of thermophilic bacteria in the Ie Seuum Tourism Area, Mesjid Raya District as a support for microbiology practicum. [dissertation]. Banda Aceh (ID): UIN Ar-Raniry.