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Isolation and Celulolytic Activity Assay of Actinobacteria Isolated from Palm Oil Wastewater

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Abstract

Cellulose plays an important role in the carbon cycle in nature and is the largest compound. This study aimed to isolate, to characterize and to determine actinobacteria that capable of producing cellulases. The sampling method in this study was carried out by purposive sampling at the outlet point of aeration pond of the wastewater treatment plant (IPAL) from palm oil waste station of PT. Teupin Lada. Isolation of actinobacteria was carried out on Humic Acid Vitamin b Agar (HVA), morphological characterization was carried out on Yeast Malt Agar (YMA), Yeast Starch Agar (YSA), Oatmeal Agar (OA), and microscopic characterization of actinobacteria and measuring the diameter of the clear zone formed on Carboxymethyl Cellulose (CMC) medium using the indicator Congo Red. Eight isolates were obtained from the isolation. Of the 8 isolates obtained, 7 of them were able to produce cellulase enzymes which were measured based on the clear zone formed in the test Congo Red on Carboxymethil Cellulose (CMC), and one isolate did not show any clear zones. The highest value of Cellulolytic Index (IS) was obtained from isolate ATLS-05, namely 8.38 mm.

Keywords: Actinobacteria, Cellulases, Congo red, Liquid waste, Oil palm

Introduction

The high waste load especially palm oil mill effluent (*Elaeis guineensis* Jacq.) or known as Palm Oil Mill Effluent (POME) could cause various problems for the environment and society. POME is wastewater from the palm oil industry, which is one of the most polluting agro-industrial wastes. Palm Oil Mill (POM) liquid waste contained inorganic and organic compounds. Palm oil industrial liquid waste contained high levels of organic substances, causing pollution in the environment around palm oil processing factories [1]. Oil palm liquid waste had a damaging effect on the environment if it was not treated properly. Waste was a potential source of pollution for humans and the environment because it could give off odors [2].

Waste containing organic compounds could generally be broken down by bacteria and could be controlled biologically [1]. One of the bacteria that could remodel these wastes was actinobacteria. Actinobacteria played an important role in the biodegradation of waste, and their wide distribution in soil, compost, water, and other places in the environment was therefore considered important for agricultural and waste industries. Oil palm processing plant liquid waste had the potential to process the hydrolysis of fats and oils into simpler products such as fatty acids and glycerol by microorganisms that grow and develop in them.

Actinobacteria are one of the soil microbes which have the greatest abundance and play an important role in the decomposition process [3]. Cellulases are inducible enzymes that are synthesized by microorganisms during their growth on cellulosic materials [4]. Cellulose is the largest compound and plays an important role in the carbon cycle in nature. The process of breaking down cellulose that occurs enzymatically is carried out by extracellular cellulases produced by several microbes such as actinobacteria [5]. The use of cellulases from bacteria could provide a solution to the problem of pollution, namely reducing the amount of cellulose waste such as leaf piles in the final disposal area, agricultural waste, seaweed on the beach and could be an added value for the use of waste to be processed organic fertilizer [6].

Actinobacteria as enzyme-decomposing bacteria could be seen from several research results including [7], regarding the exploration of actinobacteria from hot springs located in Akoli, Vajreshwari (Mumbai, Maharashtra India) which succeeded in finding cellulase and amylase-producing actinobacteria. Seprianto [8], had also succeeded in screening and isolating cellulolytic bacteria that produce cellulase enzymes from

various types of soil in the environment around Bogor Agricultural Institute (IPB). Research on actinobacteria as a producer of cellulase enzymes from palm oil wastewater was still limited, so it was necessary to conduct further studies to determine the potential of actinobacteria as cellulase degrading from palm oil wastewater.

Seeing that this problem must be solved, research was carried out on the Isolation and Activity Test of Actinobacteria as a producer of cellulase enzymes isolated from the Palm Oil Factory of PT Teupin Lada, East Aceh, which later could produce environmentally friendly technology, namely bioremediation technology or the use of microorganisms. to decompose pollutants in palm oil processing mill effluent. This study aimed to isolate, to characterize, and to see the ability of actinobacteria to produce cellulases.

Materials and methods

Isolation and purification of actinobacteria

This study was an exploratory study using spread plate method. Isolation of actinobacteria from palm oil industrial wastewater was carried out by scratching the surface of the HVA media. Samples were taken *by* purposive sampling at the outlet point of the pond. Samples of palm oil liquid waste taken from 7 ponds with different processing. The sample was taken using a sterile sample bottle by removing the bottle cap and then immersed in the waste pool, after being filled it was immediately lifted to the surface and closed again. The sample bottles that were filled with waste were put into the Styrofoam Box and immediately taken to the Laboratory. Some of the criteria for the samples taken were brownish and looked like there was a layer of oil on the surface of the wastewater [1].

Growth of the culture was observed for 7 - 21 days at 37 °C. Furthermore, the isolates were purified on YMA medium by taking a colony that grew apart and showed different morphological characters using quadrant streak method so that the colony activity index cellulolytic could be measured using the formula: Single. Incubation was carried out at 37 °C for 7 - 21 days. The single colony on the Petri dish was then inoculated onto the agar medium for Yeast Starch Agar (YSA) and Oatmeal Agar (OA) using a loop. Incubation was carried out at 37 °C for 7 - 10 days.

Observation of macroscopic and microscopic morphological characteristics of actinobacterial colonies

Macroscopic morphological observations were carried out by observing the colony morphology that was formed including colony shape margins, mycelium color, and dissolved pigments. Actinobacteria that had been examined for different morphological characters would be coded.

Microscopic observation of actinobacteria was to observe the form of hyphae and spores that were formed [9]. Observations were made at the end of the incubation period by growing actinobacterial isolate on YMA medium using a light microscope at a magnification of 100× and 400×.

Characterization of actinobacteria on media *Yeast Starch Agar* (YSA) and *Oatmeal Agar* (OA)

Actinobacterial isolate that had been characterized previously were re-observed on different media, namely Yeast Starch Agar (YSA) and Oatmeal Agar (OA). Actinobacteria isolates were regrown using the 4 quadrant scratchplate method on YSA and OA media, then incubated for 7 - 10 days at 37 °C.

Qualitative cellulolytic activity test Actinobacteria

Isolates to be tested qualitatively were spotted on small Petri containing CMC medium. Incubation was carried out at 37 °C for 7 - 10 days. The cellulolytic activity was tested using Congo Red method. The solution Congo Red (0.1 % w/v) was poured into the culture and left for 15 min. The solution was then discarded and rinsed with 0.1 M NaCl for 15 min 3 times. This washing aimed to dispose of Congo Red which did not bind to polysaccharides. Furthermore, incubation was carried out at 37 °C for 72 h to complete the formation of the clear zone, then observed the clear zone formed. Actinobacterial isolates that were able to break down CMC were shown by the formation of a clear zone around the colony after being tested by Congo Red. The cellulolytic activity index could be determined by measuring the ratio of the clear zone diameter to the colony diameter [6].

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

Data analysis

The data were processed descriptively and displayed in tables and pictures. Data consisted of morphology (shape, edge and elevation, color, and colony pigmentation) and cellulases activity test data.

Results and discussion

Collected Samples

Oil palm liquid waste samples were taken from 7 ponds with different treatments. Sampling in this study was started from pool 1 (anaerobic 1). There were 7 anaerobic pools, namely pool 1 (anaerobic 1) to pool 7 (anaerobic 7). Sampling for anaerobic ponds was only carried out in pond 1 (anaerobic 1). Waste from the 7 anaerobic ponds come from the processing fat pit and cooling pond. The next pool was pool 8 (facultative), which functions as a transitional pool between anaerobic to aerobic pools or was called the bacteria deactivation process. Then pool 9 (aerobic), there was aeration in this pool to provide oxygen with the aimed that oxidation reaction could take place properly. The last sampling was in pond 11 (sedimentation 2) to pond 14 (sedimentation 5) which functions as a sedimentation pond for the final storage of waste before it was discharged into the river.

Isolated samples on Media (HV Agar), and Yeast Malt Agar (YMA)

The oil palm liquid waste sample was isolated on media Humic Acid Vitamin B Agar (HV) using the scatter plate method. The results of the isolation on the HV agar medium showed that as many as 36 colonies which were originally suspected of actinobacteria were able to grow on the medium. The results of the selection of the 36 colonies on the HV medium were carried out by comparing the characteristics of the isolates suspected of being actinobacteria with bacterial colonies in general. Actinobacterial colonies were characterized by the appearance of white spots, round shapes, and forming discs on the surface of the media after 7 - 14 days of incubation.

The results of the growth actinobacteria colonies

A total of 36 mixed colonies were then grown on Yeast Malt Agar (YMA) medium for macroscopic and microscopic morphological characterization. The isolation results on YMA medium were obtained as many as 8 actinobacterial isolates that were coded ATLS-01, ATLS-02, ATLS-03, ATLS-04, ATLS-05, ATLS-06, ATLS-07, and ATLS-08 (**Figure 1**). The 8 isolates would also be grown on different media, namely Yeast Starch Agar (YSA) and Oatmeal Agar (OA) to be characterized by macroscopic morphology. The number of isolates obtained from this study was not much, different from previous related studies. The result of a study by Adeline [10] showed that 3 isolates was successfully obtained from empty bunches of oil palm compost and had been identified, namely *Streptomyces* sp., *Nocardiopsis* sp., and *Streptomyces violaceorubidus* which had the potential to produce cellulases. A study by Nurkaya's [11] on the isolation of cellulolytic microbes from several locations in the palm oil industry succeeded in obtaining as many as 10 actinobacterial isolates which were then tested further.

Some of these studies indicated that the results of actinobacteria isolation which could degrade cellulose in palm oil wastewater were not found > 10. This could be caused by measured environmental factors such as pH, temperature, vegetation, humidity, and the growth medium used [12]. Besides, pretreatment of samples before isolation could also affect the results of the isolation obtained [13].

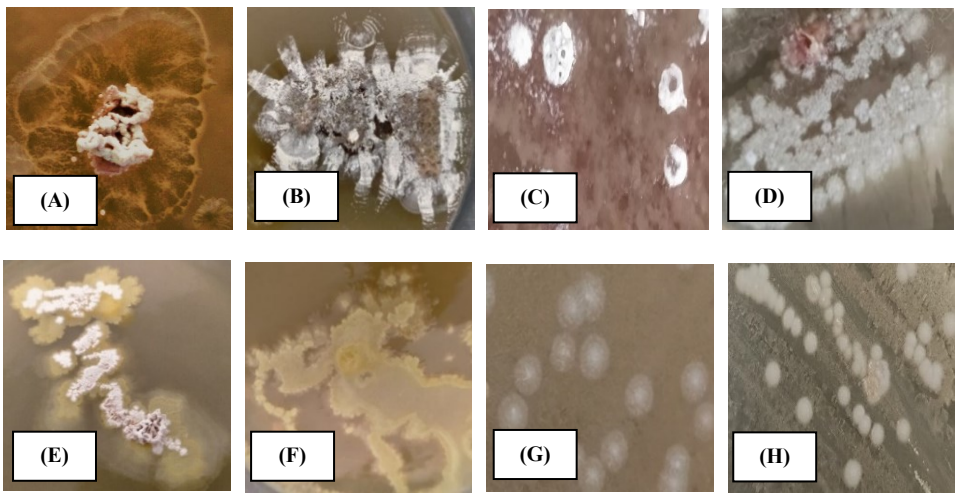


Figure 1 Actinobacteria isolates on *Yeast Malt Agar* (YMA) medium aged 21 days (A) ATLS-01 isolate; (B) ATLS-02; (C) ATLS-03; (D) ATLS-04; (E)ATLS-05; (F) ATLS-06; (G) ATLS-07; and (H) ATLS-08

Actinobacteria colonies growing in *Yeast Malt Agar* (YMA) medium based on the picture above showed different colony shapes and colors. Isolate ATLS-01 and ATLS-05 showed a conical shape, hilly elevation, and white color, isolate ATLS-02, ATLS-04, and ATLS-08 had flat surfaces and rounded irregular and spreading shapes. ALTS-03 isolate had a round colony shape and hilly elevation, while ATLS-06 and ATLS-07 show raised surfaces with a round colony shape.

The characteristics of the 8 isolates obtained showed the same morphology as actinobacteria in general. That the morphological characteristics of actinobacteria, in general, were rough, forming a cone with a dry surface [14]. The form of actinobacteria colonies was round with raised and convex elevations, flat, irregular edges, and powdery, smooth, rough, or wrinkled surfaces [15].

The characteristics of ATLS-01, ATLS-02, ATLS-03, ATLS-04 and ATLS-05, and ATLS-08 based on the picture above showed that the actinobacteria obtained were thought to belong to the genus *Streptomyces*. Dominance of *Streptomyces* as an isolate was influenced by the isolation medium used [13]. The 6 isolates smelled like soil when the lid was opened and had a powdery consistency. This was consistent with the statement that the genus *Streptomyces* has characteristics that smelled like soil, after the age of 10 - 14 days colonies became dry and granular powder [6].

The characterization of actinobacterial isolates observed included colony morphology using the method referred. The color grouping was carried out by observing the color of the mycelium and pigment dissolved in *Yeast Malt Agar* (YMA) medium. The observed data were then tabulated and presented in (**Table 1**)

Table 1 Morphological characteristics of actinobacteria isolates on YMA media.

Isolate Code	Colony Forms	Edge Colony	Elevation Colony	Characteristics		Pigments Diffused
				Color Colonies		
				Miselium Aerial	Miselium Vegetatif	
ATLS-01	Wrinkle	Grooved	Hilly	White	Dark Brown	Brown Yellowish
ATLS-02	Irregular and spread	Irregular	Flat	White	Brown Blackish	Cream Yellowish
ATLS-03	Round	Siliat	Hilly	White	Brown Raddish	Raddish
ATLS-04	Irregular and spread	Choppy	Embossed	White	Brown Yellowish	Cream
ATLS-05	Wrinkle	Choppy	Hilly	White	Brown Yellowish	No
ATLS-06	Round	Glossy	Embossed	Yellow	Cream	No
ATLS-07	Round	Glossy	Embossed	White	Cream	No
ALTS-08	Round the edge incurred	Siliat	Embossed	White	Cream	No

Table 1 showed that the morphological characteristics of the isolates isolated in this study had different characteristics. Actinobacteria colonies were generally round (50 %), wavy (25 %), silicate (25 %), and smooth (25 %); as well as the mean elevation (50 %). Also, the colony color of the obtained isolates showed a predominantly white color (87.5 %), followed by yellow (12.5 %). Suhartono and Nursanty [17] stated that the appearance of color in actinobacterial colonies could be caused by the pigments produced by 2 types of mycelium, causing color differences in each colony according to the type of actinobacteria. Two types were the mycelium, namely vegetative mycelium (substrate) and aerial mycelium (air) [18]. Amit *et al*. [19] said that the mycelium of actinobacterial substrates varies in shape, size, and thickness, and the colors range from white or almost colorless, yellow, brown, red, pink, orange, green, and black. Whereas aerial mycelium consists of various colors such as white, gray, red, green, blue, and purple series, and various substrate mycelia colors vary from gray, yellow, grayish, orange, medium yellow, pink, pale red, bluish-gray, and white.

Astuty [20] had isolated as many as 20 actinobacterial isolates and grouped them into several colors, namely white, gray, and brown. Husnah *et al*. [21] isolated and characterized 24 actinobacteria from TAHURA Pocut Meurah Intan, Aceh. A total of 10 isolates were also dominated by a white colony.

A total of 4 actinobacterial isolates obtained in this study showed different vegetative mycelium colors, namely dark brown, blackish brown, reddish-brown, and yellowish-brown. While the other 3

isolates were cream-colored. Of the 8 isolates obtained, 5 isolates showed a different color to the vegetative mycelium from the air mycelium. Nonomura [22] stated that based on its ability to produce pigments, the actinobacteria group could be divided into 2, namely groups that produce different colors between vegetative mycelium and air mycelium and groups that do not produce different pigments.

A total of 4 actinobacterial isolates in this study showed diffused pigments in the growing medium. The 4 isolates produced yellowish-brown, reddish-brown, and reddish-brown colors. Anandan *et al*. [23] stated that pigment formation could be influenced by the pH of the medium, aeration, growth temperature, and carbon and nitrogen sources. One of the main features of the pigments produced by the mycelium depends on the composition of the media and the growing conditions. According to Yi Jiang *et al*. [24], pigmentation produced by actinobacteria was divided into 2 types, namely pigments that dissolve in water (seep into the culture media), so that they could change the color of the media. Second, water-insoluble pigments (fat-soluble pigments) give rise to the colony color of actinobacteria. Jiang *et al*. [24] stated that the pigment ability to diffuse from actinobacteria was an important reference in determining new species.

Morphological characterization of actinobacterial isolates on YSA and OA media

After characterizing on YMA medium, the actinobacteria isolates were then grown on Yeast Starch Agar (YSA) and Oatmeal Agar (OA) media as a comparison medium used to characterize the actinobacterial isolates obtained. The morphological characterization of actinobacterial isolates on both media is presented in (**Table 2**).

Table 2 Morphological characteristics of actinobacteria isolates on media Yeast Starch Agar (YSA) and Oatmeal Agar (OA).

Media	Isolates Code	Colony Forms	Edge Colony	Elevation Colony	Color Colonies		P Pigment
					Mycelium Aerial	Mycelium Vegetative	
YSA	ATLS-01	Wrinkle	Irregular	Hilly	Brown	Cream	No
	ATLS-02	Round	Choppy	Flat	White	Cream	No
	ATLS-03	Round	Hilly	Embossed	White	Ruby	Reddish
	ATLS-04	Round	Choppy	Flat	White	Cream	No
	ATLS-05	Round	Glossy	Embossed	White	Cream	No
	ATLS-06	Round	Glossy	Embossed	Cream	Cream	No
	ATLS-07	Round	Glossy	Embossed	White	Cream	No
	ATLS-08	Round	Siliat	Embossed	White	Cream	No
OA	ATLS-01	Irregular	Irregular	Hilly	White and brown in the middle	Cream	Cream Reddish
	ATLS-02	Round	Glossy	Embossed	White bone	White	No
	ATLS-03	Round Irregular	Glossy	Convex	White	White	No
	ATLS-04	Round Irregular and spread	Choppy	Embossed	White	Cream	No
	ATLS-05	Round	Notched	Flat	White	Cream	No
	ATLS-06	Round	Glossy	Embossed	Yellow and cream	Cream	No
	ATLS-07	Round	Siliat	Convex	White bone	Cream	No
	ATLS-08	Round	Glossy	Embossed	White	Cream	No

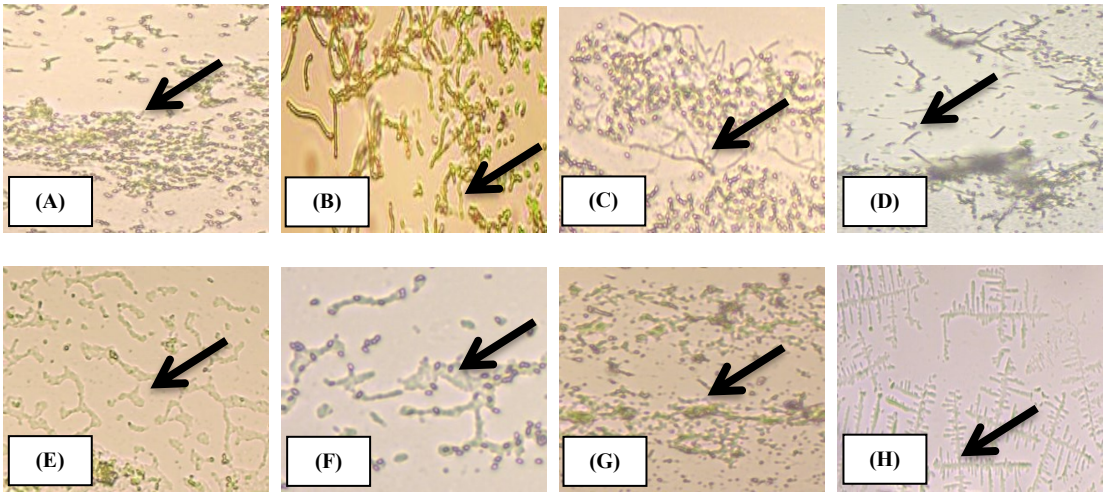
The results of actinobacterial characterization based on **Table 2** showed that there were differences in colony shape, the color of aerial mycelium, vegetative mycelium, and pigment produced from each isolate. The mycelium color on YSA and OA media tended to be different from the mycelium color on YMA media. Colony color in YSA media was dominated by white, and in OA media, cream and white were also dominated. This could be compared with the mycelium color on YMA medium which tends to be brighter and more varied. This characteristic was assumed to be the result of the growth of different

actinobacteria according to the media used, namely YMA, YSA, and OA. This could occur due to differences in the composition of the YMA, YSA, and OA media used. Astuty [20] also found differences in mycelium color between YMA, YSA, and OA media. The most varied mycelium colors were found in YMA medium. According to Astuty [20], the grouping of actinobacteria in different media was seen based on the color of the colony. The International Streptomyces Project (ISP) had recommended the color of air mycelium (aerial) in different media to be used as a taxonomic character [25].

The formation of aerial mycelium and vegetative mycelium tended to be faster on YSA and OA media on the 4th day of incubation. This could be due to differences in the media used which contained different compositions. Research from Utarti *et al.* [26], showed that the media of Oatmeal Agar (OA) and Yeast Starch Agar (YSA) would accelerate the formation of air mycelium which subsequently produced spores because it was known to contain more complex carbon sources, namely oatmeal and starch. The developing spores could change the color of the medium (diffused pigment) when it was nutritious, humidity, temperature, and other conditions to fulfill a sufficient life. Yeast Starch Agar (YSA) or ISP 4 media is a medium that is poor in nutrients, containing lots of minerals and starch as a source of carbon. Starch in ISP 4 medium must first be degraded into shorter-chain compounds by producing an amylase enzyme that aims to break down starch into more available carbon [27].

Microscopic characterization

Microscopic characterization was carried out to see the differences in the hyphae form of the actinobacterial isolates. The results of microscopic characterization of actinobacterial isolates could be seen in (**Figure 2**).



Note: →Actinobacteria hyphae

Figure 2 Microscopic observation of actinobacterial isolates with 10×40 magnification (A) ATLS-01; (B) ATLS-02; (C) ATLS-03; (D) ATLS-04; (E) ATLS-05; (F) ATLS-06; (G) ATLS-07; and (H) ATLS-0.

The results of microscopic characterization based on **Figure 2** above showed that isolates ATLS-01, ATLS-02, ATLS-03, and ATLS-04 had the form of hyphae flexuous *(*rectiflexibilis*)* and ATLS-05 and ATLS-06 have hyphae in the form of open spirals, whereas ATLS-07 and ATLS-08 have the hyphae from Rectus. Several previous studies, namely from Armaida and Khotimah [10], had characterized the actinobacteria of the genus *Streptomyces* with the form of hyphae flexuous. Another study, Astuty [20], characterized 20 indigenous actinobacteria from peat soils as many as 20 isolates of the genus actinobacteria *Streptomyces* with the categories hyphae spirals, rectiflexibiles, and retinaculiaperti. Another research, obtained isolates *Streptomyces* based on microscopic observations, namely in the form of mycelium which is like a root and is irregular, forming a twisted and elongated chain [28].

Based on this, isolates ATLS-01, ATLS-02, ATLS-03, ATLS-04, and ATLS-07, and ATLS-08 were thought to be of the genus *Streptomyces*, and isolates ATLS-05 and ATLS-06 were thought to be of the genus *non-Streptomyces*. The genus *Streptomyces* had the form of hyphae straight *(*rectis*),* flexuous *(*rectiflexibilis*),* curved (looped/retinaculiperti), and helicoidal (spirals). Air hyphae (aerial) grown prominently on the surface of the medium in a long spiral, short, or twisted like a chain of spores [29]. The function of air hyphae was as a means of reproduction. Substrate hyphae (vegetative) was

hyphae that was formed at the bottom or bottom of actinobacterial cultures which function as a means of absorbing nutrients, while dissolved pigments was the colors secreted by the culture into the growing medium [30].

Environmental Characteristics Factors Environmental

Characteristic factors measured from palm oil wastewater samples are temperature, pH, COD, and BOD content, total nitrogen (N-Total), total suspended solids, and oil and fat. The results of these measurements could be seen in (**Table 3**).

Table 3 Measurement of the environmental characteristics of PT. Teupin Lada.

No	Parameters	Unit	Yield	Method *
1.	pH in the Laboratory		8.46	4500-H^+-B
2.	Total Nitrogen (N-Total)	mg/L	20.60	4500-N org
3.	Total Suspended Solids	mg/L	210	2540 D
4.	Oil and Fat	mg/L	6.0	5520 B
5.	COD with $K_2C_2O_7$	mg/L	214.58	5220 C
6.	BOD 5 days 20 °C	mg/L	98.6	5210 B

Source: PT. PKS Teupin Lada, East Aceh

Based on the data in **Table 3**, the environmental condition factor was the temperature in each pool which is 20 °C. This could be assumed as one of the factors causing the number of actinobacterial isolates found in this study not too much because the temperature was not suitable for the growth of actinobacteria. Rao [31] stated that the environment temperature suitable for the growth of actinobacteria is in the range of 25 - 35 °C. The temperature of palm oil liquid waste was 80 - 90 ℃. However, according to Anandan *et al*. [23], the types of actinobacteria that could grow at 20 ℃ were mesophilic actinobacteria, which could grow at optimal temperatures from 20 to 42 °C, among which there were thermotolerant species, which could survive at 50 °C. So it could be assumed that the environmental temperature factor of the sampling site was an important factor for the growth of actinobacteria. Meanwhile, the suitable incubation temperature for the growth of actinobacteria ranges from 28 - 37 ℃ [31]. Anandan *et al*. [23] added that incubation temperatures of 28, 37 and 45 °C were the optimal temperatures for the isolation of moderate soil mesophilic, thermotolerant, and thermophilic actinobacteria.

Another environmental factor that was measured was pH 8.46. Actinobacteria could be divided into 3 groups based on their tolerance to pH. The acidophilic group (pH range 3.5 - 6.5) with an optimum pH of 4.5 - 5.5. Neutrophilic group (pH range 5.0 - 9.0) with an optimum pH of 6.8 - 7.0. Alkalophilic group (pH range 9.0 - 11). So that the pH conditions of the oil palm liquid waste samples in this study were 8.46 which allows actinobacteria to survive in this environment [32].

In addition to environmental temperature and pH factors, Total Nitrogen (N-Total), total suspended solids, oil and grease, COD with K2C2O7, and also BOD are environmental factors of palm oil liquid waste that were also measured. These factors could affect the life of microorganisms in it, including actinobacteria. Mahida [33] stated that the ratio of carbon and nitrogen in palm oil waste could have an impact on microbial activity. Generally, the breakdown rate would be fast at a ratio of 10 - 30. Mahfut [34] in his research stated that a high COD value means that the value of the organic matter in the waters was also high. Mahida [33] stated that, the higher the BOD value of water, the heavier the degree of organic pollutants. This was due to the decomposition process requires high dissolved oxygen. The BOD number depends on the amount, type of nutrient, chemical substance, temperature, pH, and the number and type of microbes.

Cellular enzyme activity test

Measurement of actinobacterial isolates capable of producing cellulases was indicated by the formation of a clear zone around the colony on the culture media. The results of actinobacteria screening that were capable of producing cellulase enzymes could be seen in (**Figure 3**).

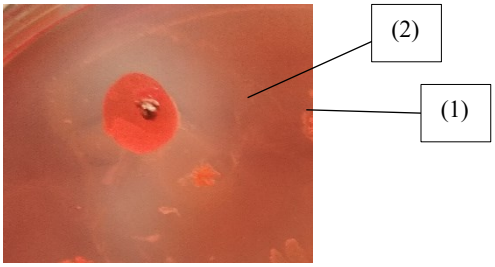


Figure 3 Test results for the activity of cellulase-producing actinobacteria on CMC aged 10 days; (1) Colony diameter (mm); (2) Clear zone diameter (mm)

Observation of actinobacterial isolates that were capable of producing cellulase enzymes was carried out on the 10th day of incubation. Based on the image above, 7 isolates were obtained (ATLS-01, ATLS-02, ATLS-03, ATLS-05, ATLS-06, ATLS-07, and ATLS-08) which were able to produce cellulase enzymes and 1 isolate that was not zoned clear namely ATLS-04. According to Pesrita *et al*. [35], the clear zone formed around the colony in each test isolate was caused by the fact that the isolate produced cellulase enzymes that were able to hydrolyze cellulose to glucose. The larger the clear zone, indicating the high the cellulase enzyme produced [3]. Microorganisms were allowed to grow in media containing cellulose as the only carbon source, they must produce cellulase enzymes to utilize cellulose in their growth and metabolic needs [36]. said that glucose was used as a source of carbon and a source of nutrition for cellulolytic bacteria [37].

The degradability of cellulose was classified based on the cellulolytic index value with a low category if the IS value was ≤ 1 mm, moderate category if the IS value was 1 - 2 mm, and high if the IS value was ≥ 2 mm [43]. The Cellulolytic Index (IS) value of the actinobacterial isolates obtained in this study could be seen in **Table 4**.

Table 4 Cellulolytic Index (IC) value of actinobacteria isolates.

No.	Isolate Code	Diameter (mm)		CI	Information
		Colony	Clear Zone		
1	ATLS-01	5.0	19.5	2.9	High
2	ATLS-02	5.0	17.3	2.46	High
3	ATLS-03	5.0	12.2	1.44	Moderate
4	ATLS-04	5.0	-	-	-
5	ATLS-05	5.0	46.9	8.38	High
6	ATLS-06	5.0	8.0	0.6	Low
7	ATLS-07	5.0	18 4	2.68	High
8	ATLS-08	5.0	18.6	2.72	High

Based on the results of the calculation of the Cellulolytic Index (IC), isolates that had a high cellulolytic index were Isolate ATLS-01 which was 2.9 mm, ATLS-02 was 2.46 mm, ATLS-05 was 8.38 mm, ATLS-07 and ATLS-08, namely 2.68 mm and 2.72 mm, isolates with the medium category was ATLS-03 isolates at 1.44 mm, and isolates with low categories was isolates ATLS-06 with 0.6 mm. Research on cellulase-producing actinobacteria had been conducted by several researchers. One of them was Nurkanto [3] who isolated actinobacteria from waste soil as phosphate and a cellulolytic laxative on CMC media. Based on his research, 37 actinobacterial isolates of the genus *Streptomyces* had an average cellulolytic clear zone ratio of ≤ 3.5. Research by Saini *et al*. [38] succeeded in selecting 20 actinobacterial isolates from different samples in India, and 17 of them showed hydrolysis zone from the production of extracellular cellulase from actinobacteria.

The actinobacterial isolates showed the diameter of the hydrolysis zone as well as the cellulolytic index values (ranging from 2.5 to 9.0) which were observed for various different isolates. The Cellulolytic Index (CI) of the isolates suspected of being *Streptomyces* (Isolates ATLS-01, ATLS-02, ATLS-03, ATLS-05, and ATLS-08) in this study showed that the ability of these isolates to degrade high cellulose and showed zone differences. According to Pesrita *et al.* [35], the difference in clear zones produced was thought to be caused by different microorganisms that affect internal factors (genes) and external factors

(nutrition, temperature, and incubation time). Research from Nurkanto [3] on the forest after the Bangkirai hill fire in East Kalimantan and its potential as cellulose-degrading obtained as many as 78 isolates *Streptomyces,* 20 of which showed cellulolytic enzyme activity with an average of 1.5 mm including in the moderate category.

Many previous studies had found that the genus *Streptomyces* had a high cellulose hydrolysis ability. Das *et al*. [39] have also succeeded in isolating actinobacteria which could degrade cellulose, namely *Streptomyces griseochromogenes Streptomyces rochei, Streptomyces plicatus* and *Streptomyces enissocaesilis* from diverse habitats. According to Nurkanto [3], the genus *Streptomyces* was the most efficient genus in degrading cellulose because of its growth rate and high activity compared to other genera.

Conclusions

The conclusions that could be drawn based on the research were the number of actinobacterial isolates from palm oil wastewater was 8 isolates. Based on the results of microscopic observations, as many as 6 isolates obtained were thought to belong to the genus *Streptomyces,* and 2 other isolates were *non-Streptomyces*. Isolates capable of producing cellulase enzymes were divided into several categories, namely low ATLS-06 with an IS 0.6 mm. The medium category was ATLS-03 isolate with an CI of 1.44 mm, and the high category with the highest Cellulolytic Index (CI) value was found in ATLS-05 isolates with an IS of 8.38 mm. It is hoped that further research can be carried out regarding the identification of actinobacterial species molecularly and purification of cellulose enzymes so that they are known more specifically.

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