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# Isolation and characterization of soil actinobacteria as cellulolytic enzyme producer from Aceh Besar, Indonesia

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**Abstract.** *Fitri L, Bessania MA, Septi N, Suhartono S. 2021. Isolation and characterization of soil actinobacteria as cellulolytic enzyme producer from Aceh Besar, Indonesia. Biodiversitas 22: 5169-5180.* Cellulolytic actinobacteria are cellulase-producing bacteria capable of degrading cellulose. This study aimed to isolate, characterize, evaluate the cellulolytic ability, and to determine physiological characterization of soil cellulolytic actinobacteria isolated from the Ujung Pancu area, Aceh Besar. Isolation of actinobacteria from soil samples was performed using serial dilution method on Yeast Malt Agar (YMA) medium. Morphological characterization was carried out by growing isolates on YMA, Oatmeal Agar (OA), and Yeast Starch Agar (YSA) media. Cellulolytic ability was determined by calculating the cellulolytic index (IS) on 1% carboxymethyl cellulose (CMC) medium after adding 0.1% congo red solution. Physiological characterization of cellulolytic actinobacteria tested in this study was salinity, pH, and carbon source in liquid Yeast Malt (liquid YM), and the growth was measured at a wavelength of 581nm. The results showed that a total of nine isolates of actinobacteria were isolated, which belonged to the genus *Streptomyces*. Cellulolytic test results showed that eight isolates had the ability to degrade cellulose. Isolates AUP-04, AUP-03, and AUP-01 had the highest cellulolytic index value. Physiological characterization results revealed that three isolates had different tolerances for salinity levels, pH, and types of carbon sources. AUP-03 isolate grew well at 10% salinity with an OD value of 0.88, isolate AUP-01 grew at 5% salinity with an OD value of 0.49, whereas isolate AUP-04 grew well on media that did not contain salinity. All three isolates grew well at pH 6 with OD values of 0.93, 1.12, and 1.27. AUP-03 and AUP-01 isolates grew well on media containing dextrose as carbon source with OD values of 0.154 and 0.17, respectively, while isolate AUP-04 grew well on glucose-containing media with an OD value of 0.22.

**Keywords:** Actinobacteria, cellulolytic, characterization, isolation, morphology, physiology, soil

## INTRODUCTION

Cellulose is an organic compound as the main form of glucose storage and is produced by photosynthesis in plants. Cellulose is the most important component of all elements in plants and plays a role in the carbon cycle in nature. Therefore, cellulose is the most abundant organic material in nature (Devi et al. 2013). Cellulose is very difficult to degrade so it cannot be used and stored in nature as waste. However, its enormous potential as a renewable energy source was only discovered after the discovery of cellulose-degrading enzymes or cellulases (Rajagopal and Kanna 2017). Cellulase is a bioactive compound produced by microorganisms during the growth of materials containing cellulose. As knowledge increases about how cellulase works, microorganisms have been used in the enzymatic hydrolysis of cellulose (Behera et al. 2017).

Cellulase has potential in biotechnology that is useful in various industries, including food industry, animal feed, textile and laundry, paper and pulp, agriculture, research and development, biofuels, pharmaceuticals, and waste management. Currently, cellulases together with hemicellulases and pectinases account for about 20% of the world enzyme market (Behera et al. 2017). Although a large number of microorganisms are capable of degrading cellulose, only a few of them are capable of hydrolyzing cellulose by producing cellulase. Various studies have reported on the degradation of cellulosic materials, but few studies have reported which microorganisms meet industrial requirements (Bai et al. 2012).

Actinobacteria are one of the largest groups of microorganisms that have been reported to play a major role in biotechnology discoveries, because they are able to produce various kinds of secondary metabolites. Actinobacteria are a group of Gram-positive bacteria that are generally widespread in soil and litter, so they are better known as soil bacteria. Apart from soil, actinobacteria are also found in inland and marine sediments (Putri et al. 2018). Actinobacteria are also referred to as filamentous bacteria because their morphological characteristics are more like filamentous fungi by forming spores and mycelium, but the cell structure and cell wall composition of actinobacteria resemble that of bacteria (Das et al. 2008). In nature, actinobacteria play a role in the decomposition process. One of the roles of actinobacteria in the decomposition process is that they can degrade cellulose by producing cellulase (Nurkanto 2008).

The growth of actinobacteria is strongly influenced by environmental conditions. Soil actinobacteria grow in pH range of 6 to 9 and optimum growth occurs at a pH close to neutral (Barka et al. 2016). Actinobacteria grow at a

salinity concentration of no more than 3%. The carbon source used by actinobacteria comes from several types of carbohydrates, such as sucrose, glucose, and fructose (Nurkanto and Agusta 2016).

Aceh is one of the regions in Indonesia with very fertile soil conditions so that it has the potential to have the presence of actinobacteria that have cellulolytic capabilities. Unfortunately, there are very few research publications on the isolation of soil actinobacteria from Aceh. Only three studies of isolation of soil actinobacteria from Aceh have been successfully reported by Husnah et al. (2012) from Pocut Meurah Intan Forest Park, Aceh Besar and Suhartono and Nursanty (2012) from Darussalam, Banda Aceh.

Currently, there is no research on actinobacteria that have the potential to degrade cellulose from Aceh soil. Ujung Pancu is one of the areas located in Peukan Bada Sub-district, Aceh Besar District, is a fertile coastal and forest ecosystem on the hills. Environmental factors such as salinity concentration, pH, and differences in carbon sources are known to have an influence on the growth of actinobacteria. This is because bacteria can only grow well in certain environmental conditions (Singh et al. 2013).

Therefore, this study aimed to isolate soil actinobacteria from Ujung Pancu area in Lampageu Village, Peukan Bada District, Aceh Besar and test their ability to degrade cellulose. In addition, physiological characterization was also performed to obtain information on isolates of cellulolytic actinobacteria that grow under the influence of certain carbon sources, pH, and salinity.

## MATERIALS AND METHODS

### Sample collection

The soil samples were collected from the Ujung Pancu area, Lampageu Village, Peukan Bada District, Aceh Besar, Aceh. Soil samples were taken from five points with a random method. Each point was 50 m apart and 10 cm in depth (Figure 1). The samples were then put into a sterile plastic clip and were taken to the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia.

### Isolation and purification of actinobacteria

Isolation of actinobacteria from soil samples was carried out using serial dilution method (Husnah et al. 2012). The Yeast Malt Agar (YMA) medium was used for isolation. The composition of YMA media was: 4 grams of Yeast extract, 10 grams of Malt extract, 4 grams of dextrose, 20 grams of agar, and 1000 mL of sterile distilled water.

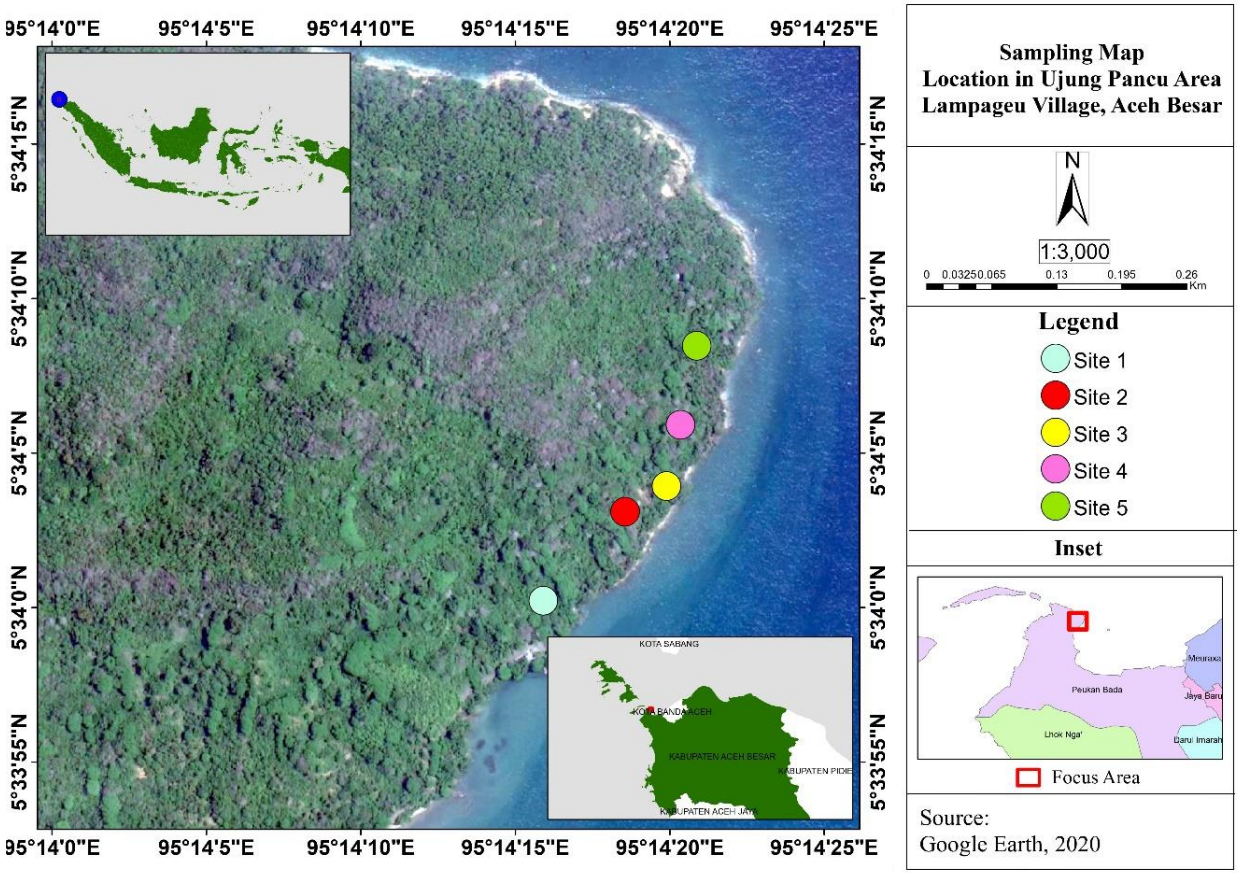



**Figure 1**. Map of sampling locations in the Ujung Pancu area, Lampageu, Aceh Besar, Indonesia

A total of 10 grams of sample was mixed into 90 mL of sterile distilled water. Then 1 mL of sample was taken and suspended in 9 mL of sterile distilled water to obtain desired serial dilutions of $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$. 0.1 mL samples was taken from the dilutions of $10^{-2}$, $10^{-3}$ and $10^{-4}$, then spread on YMA medium using streak-plate method, and incubated at 30°C for 14 days until the colonies grew. Purification of actinobacterial isolates was done on YMA medium. The colonies that grew on each YMA medium were further purified on YMA using streak-plate method and incubated at 30°C for 14 days until the colonies grew.

**Morphological characterization**

Actinobacterial isolates grown on YMA medium were characterized macroscopically and microscopically. Macroscopic characterization included colony morphology in the form of elevation, aerial mycelium color, substrate mycelium color, and pigmentation. Microscopic characterization was performed by Gram staining. Microscopic characterization aimed to observe the shape of the mycelium using a light microscope with 1000x magnification. Actinobacteria isolates were grown on different media, such as Yeast Starch Agar (YSA) and Oatmeal Agar (OA), to observe differences in their cultural characteristics.

**Screening of cellulolytic enzyme-producing actinobacteria**

The potential test of cellulose-degrading isolates was carried out using a method by Murtiyaningsih and Hazmi (2017). The isolates were grown on 1% carboxymethyl cellulose (CMC) medium then incubated for 7 days at 30°C temperature. The grown isolates were doused in 0.1% congo red solution and then left for 15 minutes. The isolates were then rinsed thrice with 1% NaCl solution to remove congo red solution. The isolates were re-incubated at 30°C for 72 hours for the formation of clear zone. Actinobacterial isolates that were able to decompose CMC were characterized by the formation of a clear zone around the colony after testing with congo red solution. Cellulolytic activity index was determined by measuring the ratio of diameter of clear zone to the diameter of colony. The formula for determining the cellulolytic activity index was:

$$\text{Cellulolytic Index} = \frac{[\text{clear zone diameter (mm)} - \text{colony diamater (mm)}]}{\text{colony diameter (mm)}}$$

**Culturing cellulolytic actinobacteria**

Actinobacterial isolates that had the highest cellulolytic index were re-cultured on YMA and incubated at 30°C for 14 days. Isolates grown on YMA medium were taken using sterile straws and toothpicks, then inoculated into liquid YM medium. The composition of the liquid YM media was as follows: 4 grams of yeast extract, 10 grams of malt extract, 4 grams of dextrose, and 1000 mL of sterile distilled water. Isolates were incubated at 30°C for 10 days in an orbital shaker at 150 rpm. Isolates that grew well were further used for physiological characterization (salinity, pH, and carbon source).

**Effect of salinity on cellulolytic actinobacteria**

A 0.1 mL suspension of actinobacterial isolates was taken using a micropipette and inoculated into each test tube that already contained YM broth medium with various concentrations of NaCl. The concentrations of NaCl used were 0% (without the addition of NaCl), 2.5% (2.5 grams of NaCl), 5% (5 grams of NaCl), 7.5% (7.5 grams of NaCl), and 10% (10 grams of NaCl).

The test tubes were closed with sterile cotton and sealed, then incubated for 10 days at 30°C using an orbital shaker at 150 rpm. The experiment was repeated twice for each concentration of NaCl (test tube). The growth of the isolates was then observed by measuring the optical density using a spectrophotometer at a wavelength of 581 nm.

**Effect of pH on cellulolytic actinobacteria**

A 0.1 mL suspension of actinobacterial isolates was taken from 10 days old YM broth using a micropipette and inoculated into YM broth test tubes containing various pH concentrations (pH 2, 4, 6, 8, and 10). Test media with various pH concentrations were made by adding 1% HCl or NaOH into YM broth solution with a pH of 2, 4, 6, 8, and 10. The test tubes were covered with sterile cotton and sealed using parafilm and incubated for 10 days at 30°C. The experiment was repeated twice for each pH concentration. The growth of the isolates was observed by measuring the optical density at a wavelength of 581 nm.

**Effect of carbon source on cellulolytic actinobacteria**

A total of 0.1 mL of suspension of actinobacterial isolates aged 10 days from YM broth medium was taken using micropipette. Then the suspension was inoculated into each test tube containing test medium for the effect of carbon sources, namely YM broth medium added with various carbon sources consisting of. A 0.1 mL suspension of actinobacterial isolates was taken from 10 days old YM broth using a micropipette, then inoculated into YM broth test tubes containing various carbon sources i.e. 0.2 grams of dextrose, 0.2 grams of glucose, 0.2 grams of fructose, and 0.2 grams of sucrose. Then test tubes were covered with sterile cotton and sealed using parafilm and incubated for 10 days at 30°C. The experiment was repeated twice for each type of carbon source. The growth of isolates was then observed at a wavelength of 581 nm.

## RESULTS AND DISCUSSION

**Result of actinobacterial isolates**

A total of nine actinobacteria isolates namely, AUP-01, AUP-02, AUP-03, AUP-04, AUP-05, AUP-06, AUP-07, AUP-08, and AUP-09 were obtained from the soil of Ujung Pancu, Aceh Besar. Based on the location points of the soil samples taken, isolates AUP-01, AUP 05, and AUP-09 were isolated from site 1; isolate AUP-06 isolated from site 2; isolate AUP-08 isolated from site 3; and isolates AUP-03, AUP-04, AUP-07 isolated from site 5. Based on the dilution factor used in the serial dilution method, isolate AUP-07 obtained from the dilution factor $10^{-2}$, isolates AUP-01, AUP-02, AUP-03, AUP-04, AUP-05, AUP-06,

and AUP-09 were obtained from a dilution factor of $10^{-3}$; and isolate AUP-08 obtained from the dilution factor of $10^{-4}$.

The depth of soil sample for actinobacteria isolation in this study was 10 cm. According to Bhattarai (2015), actinobacteria could be isolated from the soil with a depth of 10 to 15 cm. Algafari (2014) recommended that soil samples should be taken at a depth of 10 cm to isolate actinobacteria. At this depth, actinobacteria are found in large numbers because they have excellent nutrients and oxygen for bacterial growth. Isolation results showed that the colonies were generally round, rough in shape and held tightly to the media. In addition, actinobacteria isolates had an earthy odor. According to Asquith et al. (2013) this soil-like odor comes from an actinobacterial secondary metabolite compound called Geosmin. The number of soil actinobacteria isolates obtained from Ujung Pancu area was different from previous studies. Husnah et al. (2012) succeeded in obtaining 24 isolates of soil actinobacteria from the Pocut Meurah Intan Forest Park, Aceh Besar. Sukmawaty et al. (2020) succeeded in isolating 15 isolates of actinobacteria from the soil of the Malindo pine forest, South Sulawesi. Kanti (2005) reported 31 isolates from the Jambi National Forest Park. The environmental conditions of the soil, such as degree of acidity (pH), temperature and soil moisture were also measured in the present study. The results showed that the average soil pH, average temperature and soil moisture were 5.4, 26.9°C and 10-75%, respectively. According to Wink et al. (2017), actinobacteria grow well at temperature of 25 to 30 °C. Barka et al. (2016) stated that actinobacteria isolated from soil grow well in the pH range of 6 to 9 and also grow very well at near neutral pH. Farida (2008) noted that the growth of actinobacteria is decreased at pH 5, making actinobacteria intolerant to acid. Kumalasari (2012) reported that actinobacteria need low humidity, which should be below 80%. Therefore, the soil conditions in the Ujung Pancu area had a temperature and humidity suitable for the growth of actinobacteria. But, the acidity of soil in Ujung Pancu was not suitable for the growth of actinobacteria.

**Morphological characterization of actinobacteria**

*Macroscopy characteristics*

Actinobacteria isolate colonies grown on YMA media can be seen in Figure 2.

Actinobacterial colonies on YMA medium were rough, starchy and irregularly shaped, with relatively distinct colony colors. Isolates AUP-01, AUP-03, AUP-06, and AUP-08 had white colonies. According to Husnah et al. (2012) the colony characteristics of actinobacteria are divided into two groups. The first group has a velvety and colored colony surface, and is capable of producing aerial mycelium. The second group has a smooth and colorless colony surface, and did not produce aerial mycelium.

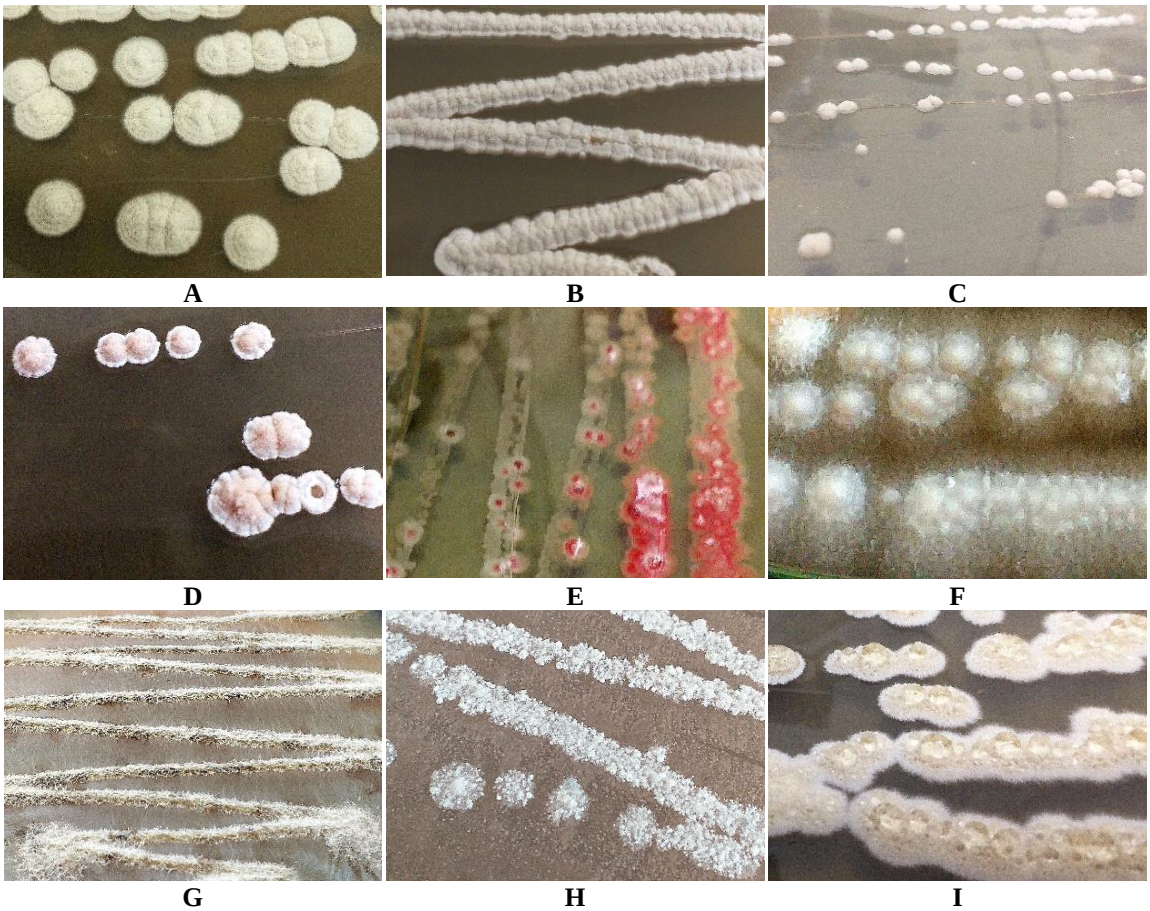



**Figure 2**. Growth of actinobacteria isolates on Yeast Malt Agar media: A. AUP-01, B. AUP-02, C. AUP-03, D. AUP-04, E. AUP-05, F. AUP-06, G. AUP-07, H. AUP-08, I. AUP-09

Isolates that were macroscopically characterized on YMA medium then grown on Oatmeal Agar (OA) and Yeast Starch Agar (YSA) media. Actinobacteria isolates grown on OA medium had brownish white color (AUP-03 and AUP-09 isolates), brown (AUP-01 and AUP-07 isolates), pink (AUP-05 isolates), black (AUP-02 isolate), blackish green (AUP-08 isolate), white (AUP-06), and grey (AUP-04 isolate) of aerial mycelium. Actinobacterial isolate colonies grown on YSA medium had different aerial mycelium colors from those of OA medium. Aerial mycelium color of isolates grown on YSA medium was white (AUP-01, AUP-03, AUP-06, AUP-08 isolates), brownish-white (AUP-02 and AUP-07 isolates), black (AUP-04 isolate), and red (AUP-05 and AUP-09 isolates). Although the same actinobacterial isolates had different colony colors on OA and YSA media, these isolates had rough, starchy, and irregular colonies (Figures 3 and 4).

OA medium was also included in the International Streptomyces Project category 3 or also called ISP 3 media. wet al. (2020) stated that OA and YSA media contain more complex carbon sources in the form of oatmeal and starch to accelerate the growth of aerial mycelium and spores. The starch contained in YSA medium must be broken down first with the help of amylase so that it becomes a carbon source available for the growth of actinobacteria (Wulandari and Nanik 2016).

Isolates grown on YMA, OA, and YSA media, were then subjected to macroscopic morphological characterization. The results showed that colony color, mycelium color, and pigment production were not always the same on different mediums. Nurkanto and Agusta (2015) reported that the morphological characterization of actinobacteria was influenced by the composition of the media. The morphological characterization of isolates on YMA, YSA, and OA media can be seen in Table 1.

Based on Table 1, actinobacteria isolates grown on YMA, YSA, and OA and YSA media showed different characteristics. This may be due to the differences in composition between YMA, YSA, and OA media, so the same isolate produces different characteristics. The diversity of morphological characteristics of actinobacterial isolates on various media could be used as information in grouping actinobacteria (Astuty 2017). Therefore, the results of the characteristics of actinobacterial isolates grown on various media were used as a basis for identifying the type of actinobacteria.

One of characteristics in the grouping of actinobacteria according to the recommendations of the International Streptomyces Project is the color of the aerial mycelium (Oskay 2009). Barka et al. (2016) added that the morphological characterization of actinobacteria is used as a reference in classifying actinobacteria, but it is not used to determine the type of actinobacteria.

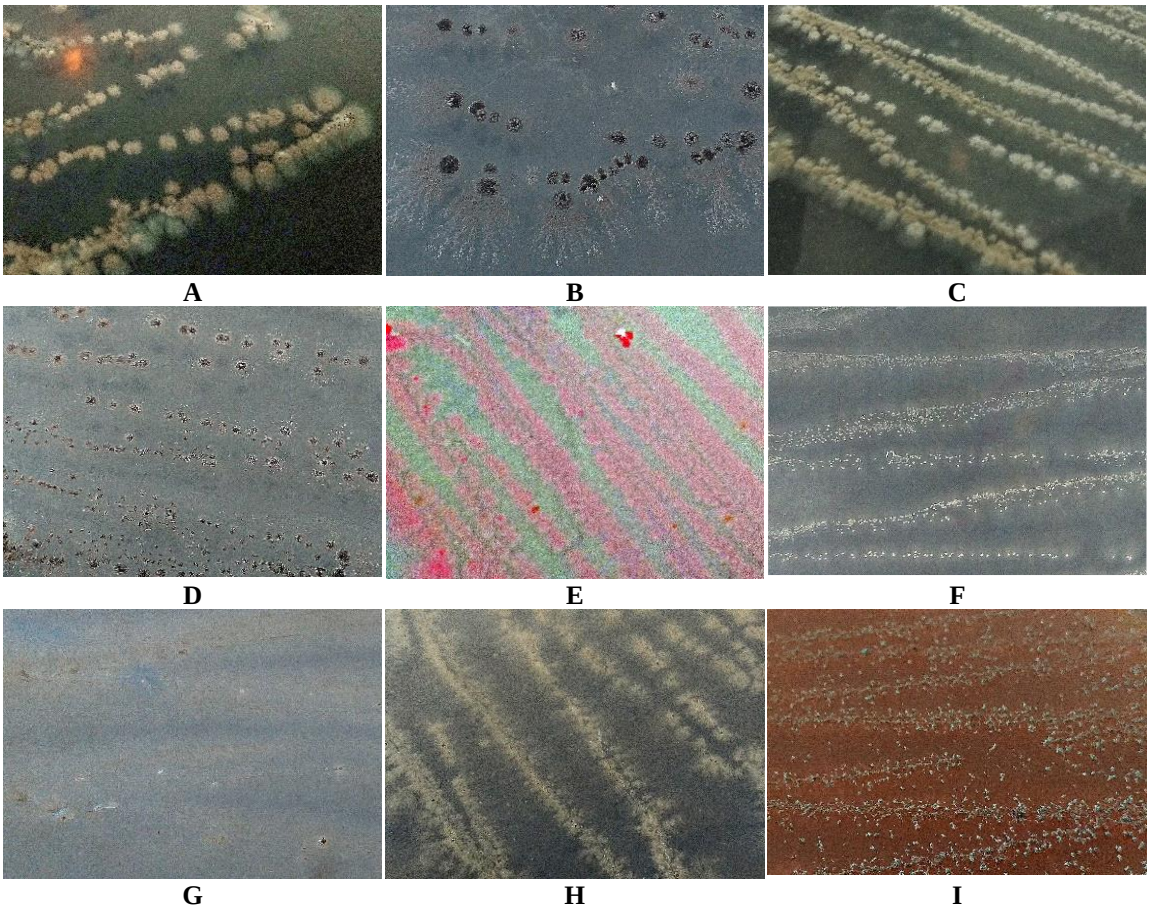



**Figure 3**. Growth of actinobacteria isolates on Oatmeal Agar media: A. AUP-01, B. AUP-02, C. AUP-03, D. AUP-04, E. AUP-05, F. AUP-06, G. AUP-07, H. AUP-08, I. AUP-09

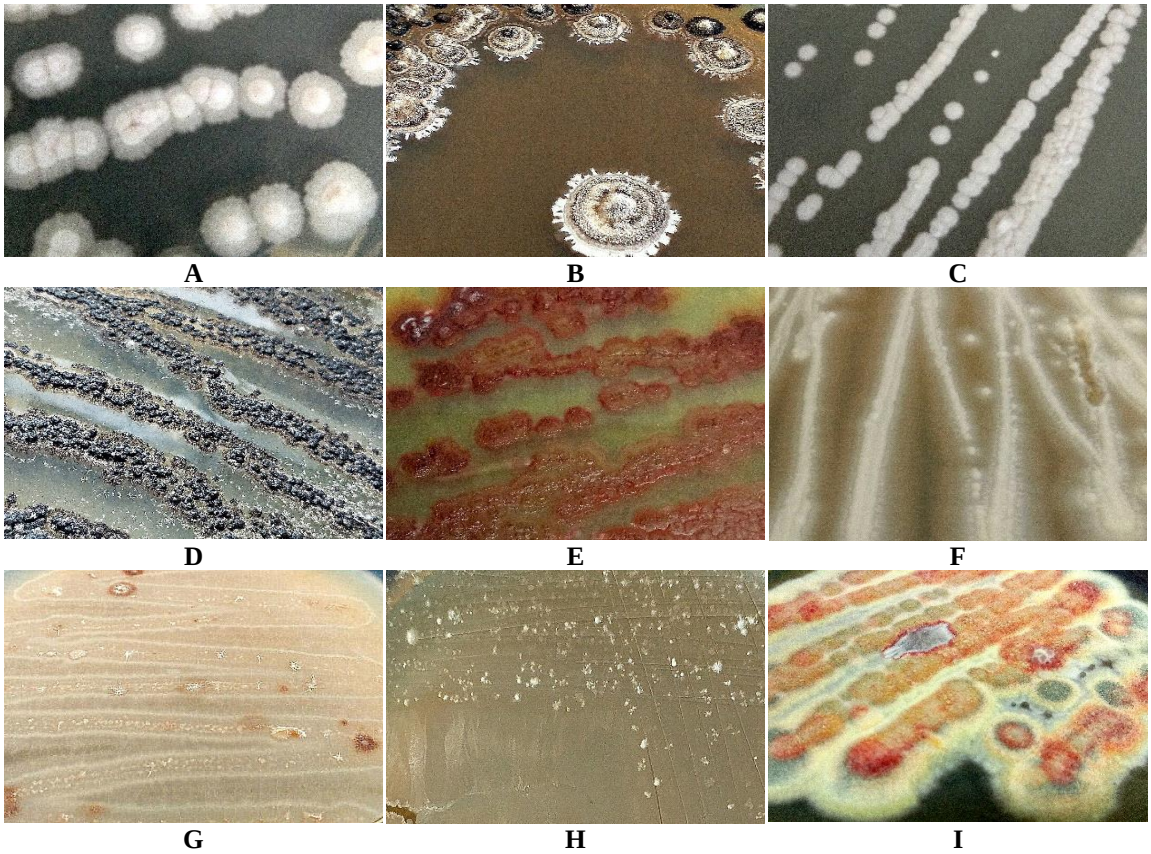



**Figure 4**. Growth of actinobacteria isolates on Yeast Starch Agar media: A. AUP-01, B. AUP-02, C. AUP-03, D. AUP-04, E. AUP-05, F. AUP-06, G. AUP-07, H. AUP-08, I. AUP-09

**Table 1.** Morphological characteristics of actinobacterial isolates on Yeast Malt Agar (YMA) Oatmeal Agar (OA), and Yeast Starch Agar (YSA) media

| Media | Isolates | Mycelium color | | Pigmentation | Elevation |
|---|---|---|---|---|---|
| | | Substrate | Aerial | | |
| YMA | AUP-01 | Tawny | White | None | Convex |
| | AUP-02 | Tawny | Grey white | None | Crateriform |
| | AUP-03 | Tawny | White | None | Convex |
| | AUP-04 | Brown | Tawny | None | Crateriform |
| | AUP-05 | Red | Red | None | Flat |
| | AUP-06 | White | White | None | Flat |
| | AUP-07 | Brown | Tawny | None | Flat |
| | AUP-08 | Tawny | White | Brown | Flat |
| | AUP-09 | Yellow | Cream | None | Pulvinate |
| OA | AUP-01 | Grey | Brown | None | Convex |
| | AUP-02 | Grey | Black | None | Flat |
| | AUP-03 | Cream | Brownish white | None | Convex |
| | AUP-04 | Grey | Grey | None | Flat |
| | AUP-05 | Pink | Pink | None | Flat |
| | AUP-06 | White | White | None | Flat |
| | AUP-07 | Brownish white | Brown | None | Flat |
| | AUP-08 | Cream | Blackish green | Cream | Raised |
| | AUP-09 | Black | Brownish white | Red | Flat |
| YSA | AUP-01 | Cream | White | White | Convex |
| | AUP-02 | Brown | Brownish white | Dark brown | Convex |
| | AUP-03 | Cream | Brownish white | None | Convex |
| | AUP-04 | Grey | Black | None | Raised |
| | AUP-05 | Yellowish red | Yellowish red | None | Flat |
| | AUP-06 | White | White | None | Flat |
| | AUP-07 | Yellow | Brownish white | None | Flat |
| | AUP-08 | Brown | White | None | Flat |
| | AUP-09 | Yellowish red | Bluish red | None | Crateriform |

The presence of substrate mycelium in this study was observed on the reverse side of the Petri dish. According to Dhanasekaran and Yi (2016), substrate mycelium or vegetative mycelium grows inside or on the surface of the media. Substrate mycelium has various shapes, sizes, and thicknesses. The color of the substrate mycelium was white, yellow, brown, red, orange, grey, black, to almost no color. The results of the study of thermophilic actinobacteria conducted by Fitri et al. (2019) showed that actinobacteria isolates grown on YMA medium had brown, yellowish-white, and orange substrate mycelium, isolates grown on OA medium had white substrate mycelium, and isolates grown on YSA medium had yellow and white substrate mycelium.

The presence of aerial mycelium in the study was observed on the front side of the dish. Aerial mycelium is a set of hyphae that develop from the substrate mycelium and grow into the air (Dhanasekaran and Yi 2016). Aerial mycelium has a variety of colors, such as white, grey, red, green, blue, and purple. Fitri et al. (2017), reported that isolates of endophytic actinobacteria grown on YMA medium produced grey, white, greenish grey, bluish grey, and turquoise aerial mycelium, while isolates grown on OA medium produced green, grey, white, and bluish green aerial mycelium.

Most of the actinobacteria isolate in this study did not produce pigmentation. Only isolates AUP-08 and AUP-09 produced yellowish white and red pigments. Pigment causes differences in media color, colony color, and mycelium color (substrate and aerial) based on water solubility. Pigment production depends on the media content, condition, and age of the isolate (Dastager et al. 2006). The pigments that cause the color change of the medium are water-soluble pigments, while the pigments that give rise to colony color are fat-soluble pigments (Yi et al. 2016). Most actinobacteria produce a pigment called melanin. These pigments are generally black and brown in color and are used in pharmaceutical and cosmetic products (Dastager et al. 2006).

*Microscopy characteristics*

The result of Gram staining revealed that all isolates showing purple color were classified as Gram-positive bacteria. According to Dhanasekaran and Yi (2016), actinobacteria are grouped into Gram-positive bacteria because they have a peptidoglycan cell wall with a thickness of 20-80 mm. Isolates were observed at 1000x magnification and microscopic observations can be seen in Figure 5. Actinobacteria isolates observed under the microscope had different aerial mycelium shapes. Aerial mycelium can produce spores whose structure is also called spore beading. The characteristics of the aerial mycelium were based on Dhanasekaran and Yi (2016). Isolate AUP-02 had an elastic or flexuous shape, isolate AUP-03, AUP-04, AUP-06, AUP-07 and AUP-08 had a straight or straight shape, isolate AUP-01 had a curved or spiral shape, isolate AUP-05 had a biverticillate form. According to Vinothini et al. (2018) structure of spore beading in aerial mycelium of actinobacteria is divided into simple and verticillate forms. The simple form is divided into recture (straight), flexous (flexible), retinaculum-apartum (open loop), and spiral. The verticillate form consists of monoverticillus, monoverticillus-spiral, biverticillus, and biverticillus-spira.

Astuty (2017) reported that 20 isolates of peat soil actinobacteria from the genus *Streptomyces* had spiral, straight, and open loop-shaped mycelium. Armaida and Khotimah (2016) reported that *Streptomyces* isolates had a flexuous shape of mycelium. This is reinforced by the statement of Maheswari and Chandra (2000) that genus *Streptomyces* has aerial mycelium in the form of flexuous, spiral, straight, and open loops. On the basis of macroscopic and microscopic characteristics, all nine isolates belonged to the genus *Streptomyces*. *Streptomyces* is the largest genus of actinobacteria that grow faster than other genera, making it the most abundant genus found in all types of ecosystems (Nurkanto 2008). Taxonomically, *Streptomyces* belongs to the family Streptomycetaceae, order Actinomycetales, and class Actinobacteria. These bacteria belong to Gram-positive bacteria and are not acid-fast (non-acid-fast), are aerobic and chemoorganotrophic, form aerial mycelium and branched substrates, and produce pigments. *Streptomyces* spores are non-motile. Colonies of *Streptomyces* often initially have a smooth surface and then form aerial mycelium which may appear floccose, granular, powdery, or velvety (Rosenberg et al. 2014).

**Screening of cellulolytic enzyme-producing actinobacteria**

Actinobacterial isolates that contain cellulolytic enzyme were characterized by the formation of a clear zone around the colonies on carboxymethyl cellulose (CMC) medium after being stained with congo red. CMC medium contains cellulose as a carbon source and a substrate source in enzymatic reactions (Nurkanto 2007). Alam et al. (2004) stated that CMC was referred to as the best substrate to induce the formation of extracellular cellulolytic enzymes. The results of cellulose-degrading actinobacterial isolates can be seen in Figure 6.

Observation of actinobacteria isolates capable of degrading cellulose was carried out on the 10th day of incubation. Of the nine isolates, only 8 isolates i.e. AUP-01, AUP-02, AUP-03, AUP-04, AUP-05, AUP-06, AUP-07, AUP-08 was used in the cellulolytic test. AUP-09 isolate was not tested because it was contaminated. The ability of actinobacteria to produce cellulase enzymes was indicated by the formation of a clear zone around the colony. Isolates that produce cellulase are able to hydrolyze cellulose into glucose (Pesrita et al. 2017). The large clear zone formed indicated the high cellulase produced.

The diameter of the isolate colonies growing on CMC medium was in the range of 9.7 - 27.2 mm. The diameter of the clear zone was in the range of 12.9 - 42.5 mm. The diameter of clear zone formed on CMC medium was larger than the diameter of the colony. Choi et al. (2005) grouped the cellulolytic index based on three categories, low ($\leq$ 1), moderate (1-2), and high ($\geq$ 2). The results of cellulolytic index values of actinobacterial isolates can be seen in Table 2.

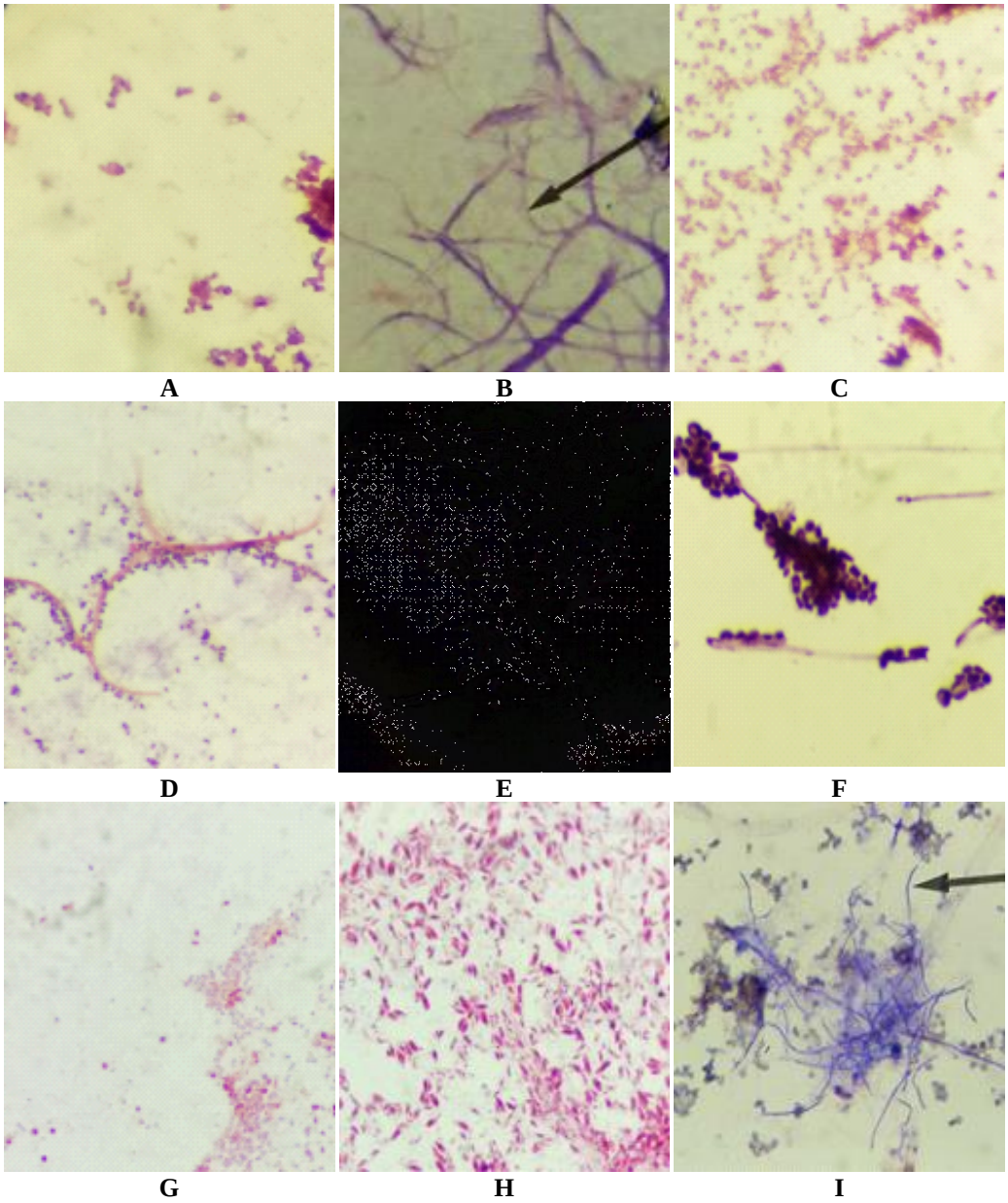



**Figure 5.** Microscopic observation of actinobacterial isolates by Gram staining at 1000x magnification: A. AUP-01, B. AUP-02, C. AUP-03, D. AUP-04, E. AUP-05, F. AUP- 06, G. AUP-07, H. AUP-08, I. AUP-09

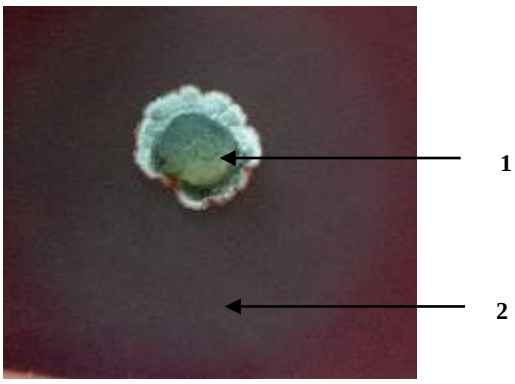



**Figure 6**. Cellulose degrading potential of actinobacterial isolates on carboxymethyl cellulose (CMC) medium after 10 days of incubation: (1) colony (2) clear zone

**Table 2.** Cellulolytic activity index value of actinobacterial isolates

| Isolates | Diameter (mm) | | Cellulolytic Index | Category |
|---|---|---|---|---|
| | Colony | Clear zone | | |
| AUP-01 | 10.2 | 39.3 | 2.85 | High |
| AUP-02 | 10.4 | 37.6 | 2.61 | High |
| AUP-03 | 10.1 | 39.7 | 2.93 | High |
| AUP-04 | 9.7 | 35.3 | 2.63 | High |
| AUP-05 | 22.2 | 27.6 | 0.24 | Low |
| AUP-06 | 21.5 | 26.3 | 0.25 | Low |
| AUP-07 | 10.6 | 12.9 | 0.18 | Low |
| AUP-08 | 27.2 | 42.5 | 0.,56 | Low |
| AUP-09 | - | - | - | - |

Table 2 showed that isolates AUP-01, AUP-02, AUP-03 and AUP-04 had a high cellulolytic index, while isolates AUP-05, AUP-06, AUP-07, and AUP-08 had a low cellulolytic index. Isolates that had a high category of cellulolytic index have better cellulolytic activity than isolates that had a low category of cellulolytic index.

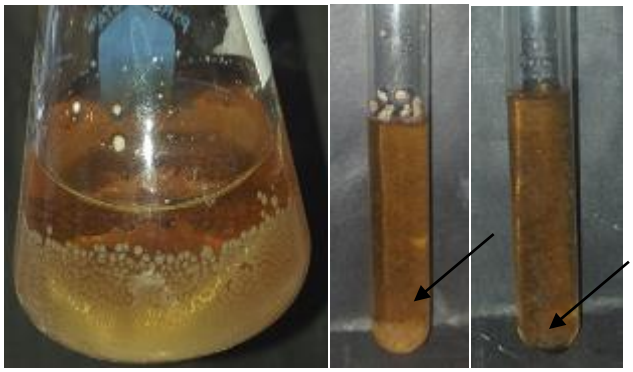
The measurement of cellulase enzyme activity on actinobacteria grown on CMC medium has been published by several researchers. Nurkanto (2007) succeeded in testing the cellulolytic activity of 36 isolates from fire forests in East Kalimantan consisting of the genera *Streptomyces, Nocardia, Miconomspora*, and *Actinoplanes* with an average clear zone ratio of not more than 1.75. Furthermore, Nurkanto (2008) measured cellulolytic activity in isolates from the Waigeo Islands, Papua, which included the genera *Streptomyces*, *Micromonospora*, *Streptosporangium*, *Actinoplanes*, *Nocardia*, and *Pseudonocardia* with an average clear zone ratio of above 2, some even reached 7. Pesrita et al. (2017) also reported 13 actinobacterial isolates from bagasse had cellulolytic properties. Yurnita (2020) also obtained 8 actinobacterial isolates from palm oil waste that showed cellulase activity.

It can be concluded that actinobacterial isolates from forest soils in the Ujung Pancu area, Aceh Besar, were able to degrade cellulose by producing cellulase as an extracellular enzyme. Putri and Setiawan (2019) reported that isolates that have the potential to produce cellulase extracellular enzymes belong to the genera *Streptomyces*, *Kitasatospora*, *Nonomurae*, *Asanoa*, *Streptosporangium*, and *Dactylosporangium*. Several other studies also showed that *Streptomyces* hydrolyzes cellulose with cellulase. *Streptomyces viridobrunnes* species produce cellulase in the form of endoglucanase (Da Vinha et al. 2011). Cellulase enzymes in the form of carboxymethyl cellulase are also produced by actinobacteria from the *Streptomyces longispororuber* species (Prasad et al. 2013).

**Culturing cellulolytic actinobacteria on liquid media**

Based on the results of the cellulolytic test, it was observed that isolates AUP-03, AUP-01, and AUP-04 had the highest cellulolytic index values. All three isolates were grown on liquid YM medium for physiological characterization. It was observed that actinobacteria grown on liquid medium showed the formation of a precipitate at the bottom of the medium (Figure 7.A and 7.B). According to Luti and Yonis (2014), actinobacteria that grow in liquid media showed the presence of mycelium strands that assemble like pellets at the bottom of the media.

The results showed that the presence of pellets that were only at the bottom of the media meant that the liquid media used as actinobacterial growth media did not have the same turbidity (homogeneous). The bottom of liquid medium looked more cloudy than the top because of the mycelium structure (Figure 7.B). The breakdown of the mycelium structure was performed by adding sterile glass beads into a test tube in which the test isolates were to be homogenized (Figure 7.C).



**A** **B** **C**

**Figure 7**. Results of actinobacterial growth on liquid YM medium. A. Erlenmeyer flask, B. test tube with pellet structure, C. test tube after homogenization

Several studies were reported on the growth of actinobacteria in liquid media. Yakoob and Pradeep (2019) reported that the growth of several strains of *Bifidobacterium thermoacidophilum* in liquid media forms homogeneous sediment, but it is spread evenly by shaking. Species of *Scardovia inopinata* grown in liquid media have homogeneous sediments in liquid culture, disperse easily, and never form lumps (Teixeira et al. 2020). In addition, Hand et al. (2018) also reported that *Acidothermus* genus grown in liquid media had moderate turbidity and cells tended to agglomerate and settle after 3 days.

**Physiological characteristics of cellulolytic actinobacteria**

*Salinity*

The results showed that AUP-03 isolate had a good tolerance to high salinity concentration (10%) with an optical density (OD) value of 0.88, followed by AUP-01 isolate with a value of 0.88. The highest (0.49) OD was observed in AUP-01 followed by AUP-03 isolate (0.18) OD value at 5% salinity concentration. Isolate AUP-04 showed the lowest OD value at all salinity concentrations. It was observed that highest OD value of AUP-04 isolate (media without the addition of NaCl) was 0.11. The growth of AUP-03, AUP-01, and AUP-04 isolates in media with different salinity concentrations can be seen in Figure 8.

Based on the diagram, it can be seen that AUP-03 and AUP-01 isolates grew well at 10% and 5% salinity concentrations. This may be due to the adaptation made by actinobacteria to salt levels in their environment. According to Roeßler and Müller (2002) bacteria that grow in habitats with high salinity concentrations (2%-30%) had the ability to accumulate compatible solutions. According to Lee and Fan (2020), compatible solutions are dissolved organic molecules that are neutral.

AUP-03 and AUP-01 isolates were isolated from an environment close to the coast with high salinity conditions. Octavina et al. (2021) reported that Ujung Pancu beach had high salinity levels ranging from 24-35 ppm in its environment. AUP-04 isolate grew well on media that did not contain NaCl. Based on the results of the present study, it could be seen that actinobacteria had a wide tolerance to various concentrations of salinity. Hamid et al. (2015) noted that actinobacteria are widely distributed in natural ecosystems, especially in soil, and have good

tolerance to salinity. Isolate AUP-03 grown in media with salinity concentration (10%) grew best as compared to the other two isolates (AUP-01 and AUP-04). This is in accordance with the statement of Patil et al. (2011) that actinobacteria isolates are more dominantly found in dry alkaline soils. In addition, actinobacteria are found in habitats with high salinity concentrations due to their ability to tolerate high salt concentrations (Shivakumar 2000).

*pH*

The results showed that AUP-03, AUP-01, and AUP-04 isolates were grown on media with different pH i.e. 2, 4, 6, 8, and 10 concentrations. The optical density (OD) values of AUP-03, AUP-01, and AUP-04 isolates were 0.93, 1.12, and 1.27, respectively. The growth of AUP-03, AUP-01, and AUP-04 isolates in media with different pH concentrations can be seen in Figure 9.

The growth and survival of actinobacteria may be strongly influenced by the pH of environment. The results showed that the best growth of cellulolytic actinobacteria isolates (AUP-03, AUP-01, and AUP-04) was observed in medium containing pH 6. This is in accordance with the statement by Akond et al. (2016) that actinobacteria, classified as neutrophilic bacteria, showed optimal growth in the pH range of 6 to 8.

The ability of actinobacteria to tolerate various pH concentrations for their growth media was in accordance with Bull (2010) that actinobacteria had flexible metabolic physiology that allowed them to survive under unfavorable environmental conditions. However, actinobacteria that grew under optimum environmental conditions had a greater potential to produce industrially valuable enzymes, including cellulase enzymes. Tseng et al. (2007) stated that actinobacteria at optimum growth conditions have the ability to produce cellulase enzymes that functioned in recycling nutrients back to the soil which increased soil productivity.

*Carbon source*

The results showed that AUP-03 and AUP-01 isolates had good tolerance to media enriched with carbon sources in the form of dextrose. The optical density (OD) values of AUP-03 and AUP-01 isolates were 0.154 and 0.17, respectively, while AUP-04 isolate showed good (0.22) tolerance to media with added carbon source in the form of glucose. The growth of AUP-03, AUP-01, and AUP-04 isolates in media with different carbon sources can be seen in Figure 10.

This was in accordance with the statement of Pandey et al. (2005), that dextrose was the most suitable carbon source for the growth of actinobacteria compared to sucrose, fructose, and glucose. In addition, Manivasagan et al. (2010) also stated that sucrose and starch are poor carbon sources because they did not support the growth of actinobacteria. The AUP-04 isolate showed good growth in the medium where a carbon source was added in the form of glucose. This is in accordance with the findings of Raytapadar and Paul (2001) that glucose is the best carbon source that can be utilized by actinobacteria in their growth as compared to starch, lactose, sucrose, and mannitol.

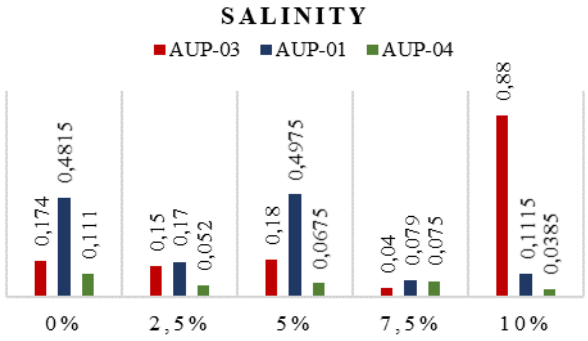



**Figure 8**. Growth pattern of isolates in liquid media with different salinity concentrations

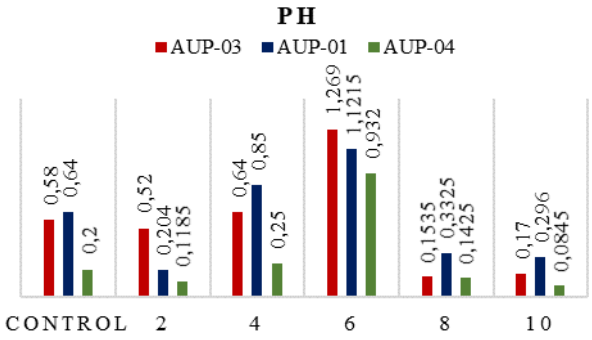



**Figure 9**. Growth pattern of isolates in liquid media with different pH concentrations

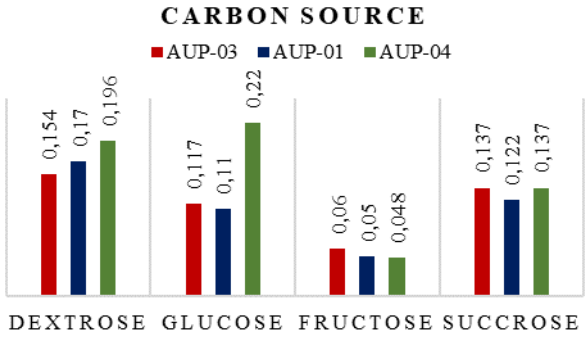



**Figure 10**. Growth pattern of isolates in liquid media with different carbon sources

It is concluded that from nine actinobacterial isolates that had different morphological characteristics, three isolates had the highest cellulolytic index. All three isolates also had different physiological characteristics based on salinity, pH, and carbon source.

## REFERENCES


Akond MA, Jahan MN, Sultana N, Rahman F. 2016. Effect of temperature, pH and NaCl on the isolates of Actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh. Am J Microbiol Immunol 1: 10-15.

Alam MS, Sarjono PR, Aminin ALN. 2004. Isolation, purification, characterization of cellulolytic enzymes produced by *Streptomyces omiyaensin.* J Biol Sci 10: 1647-1653. DOI: 10.3923/pjbs.2004.1647.1653

Algafari RN. 2014. Assessment of profile depth, site of sampling, type of media and methods used for the isolation of Actinomycetes. Intl J Microb 6: 553-558.

Armaida E, Khotimah S. 2016. Characterization of porifera-associated Actinomycetes (*Axinella* spp.) from Lekumutan Island, West Kalimantan. Protobiont 1: 69-70.[Indonesian].

Asquith EA, Evans CA, Geary PM, Dunstan RH, Cole B. 2013. The role of Actinobacteria in taste and odour episodes involving geosmin and 2-methylisoborneol in aquatic environments. J Water Suppl Res Technol AQUA 62: 452-467. DOI: 10.2166/aqua.2013.055

Astuty E. 2017. Isolation and morphological characterization of indigenous Actinomycetes from peat. J Natur Envir Sci 16: 7-15. [Indonesian].

Bai S, Kumar MR, Kumar DM, Balashanmugam P, Kumaran MB, Kalaichelvan PT. 2012. Cellulase production by *Bacillus subtilis* isolated from cow dung. Arch Appl Sci Res 4: 269-279.

Barka EA, Vasta P, Sanchez L, Gaveau-Vallant N, Jacquard C, Klenk HP, Clement C, Ouhdouch Y, van Wezel GP. 2016. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80: 1-43. DOI:10.1128/MMBR.00019-15

Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN. 2017. Microbial cellulases-Diversity & biotechnology with reference to mangrove environment: A review. J Gen Eng Biotech 15: 197-210. DOI: 10.1016/j.jgeb.2016.12.001

Bhattarai B. 2015. Variation of soil microbial population in different soil horizons. J Microbiol Experi 2: 75-78. DOI: 10.15406/jmen.2015.02.00044

Bull AT. 2010. Actinobacteria of The Extremobiosphere in Extremophiles. Springer-Verlag, Berlin.

Choi YW, Hodgkiss IJ, Hyde KD. 2005. Enzyme production by endophytes of *Brucea javanica*. J Agri Technol 1: 55-66.

Da Vinha FN, Gravina-Oliveira MP, Franco MN, Macrae A, da Silva Bon EP, Nascimento R P, Coelho RRR. 2011. Cellulase production by *Streptomyces viridobrunneus* SCPE-09 using lignocellulosic biomass as inducer substrate. Appl Biochem Biotech 164: 256-267. DOI: 10.1007/s12010-010-9132-8

Das S, Lyla PS, Khan SA. 2008. Distribution and genetic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal. Chi J Ocean Limnol 26: 166-177. DOI: 10.1007/s00343-008-0166-5

Dastager S, Li WJ, Dayanand A, Tang SK, Tiang SP, Zhi XY, Xu LH, Jiang CL. 2006. Separation, identification, and analysis of pigment (melanin) production in *Streptomyces*. Afr J Biotech 5: 1131-1134.

Devi YG, Kumar BL, Lokeswari N, Arjun P, Harasriramulu S. 2013. Isolation of the marine actinomycetes from the Bay of Bengal sediments. Intl Res J Pharm Appl Sci 3: 121-123.

Dhanasekaran D, Yi J. 2016. Actinobacteria: Basics and Biotechnological Applications. IntechOpen, London.

Farida Y. 2008. Combined Method of Screening Marine Actinomycetes Producing Compounds with Anticancer Potential. [Dissertation]. Universitas Gadjah Mada, Yogyakarta. [Indonesian].

Fitri L, Meryandini A, Iswantini D, Lestari Y. 2017. Characterization of ethanolic extract of *Streptomyces* sp. as a pancreatic lipase inhibitor produced by endophytic *Streptomyces* sp. AEBg12. Biosaintifika 9: 177-184. DOI: 10.15294/biosaintifika.v9i2.8907

Fitri L, Putri KA, Ismail YS. 2019. Isolation and characterization of thermophilic actinobacteria as proteolytic enzyme producer from Ie Seuum Hot Spring, Aceh Besar, Indonesia. Biodiversitas 20: 2802-2808. DOI: 10.13057/biodiv/d201004

Hamid AA, Ariffin S, Mohamad, S. A. S. 2015. Identification and optimal growth conditions of actinomycetes isolated from mangrove environment. J Analy Sci 19: 904-910.

Hand TH, Das A, Roth MO, Smith CL, Jean-Baptiste UL, Li H. 2018. Phosphate lock residues of *Acidothermus cellulolyticus* Cas9 are critical to its substrate specificity. J Sust Chem Eng 7: 2908-2917. DOI: 10.1021/acssynbio.8b00455

Husnah M, Suhartono, Yulvizar C. 2012. Isolation of soil Actinomycetes from Forest Park of Pocut Merah Intan as potential producer of antimicrobial compounds. In: Proceeding of The 2nd Annual International Conference Syiah Kuala University 2012 & The 8th IMT-GT Uninet Bioscience Conference. AIC Universitas Syiah Kuala, Banda Aceh.

Kanti A. 2005. Cellulolytic Actinomycetes isolated from soil in Bukit Dua Belas National Park, Jambi. Biodiversitas 6: 85-89. DOI: DOI:10.13057/biodiv/d060203

Kumalasari AM. 2012. The potential of actinomycetes as a source of antibiotic bioactive compounds from the Bantimurung karst area, South Sulawesi. Pelita-Jurnal Penelitian Mahasiswa. UNY 59-72. [Indonesian].

Lee Y. Fan C. (2020). Mechanistic exploration of the catalytic modification by co-dissolved organic molecules for micropollutant degradation during fenton process. Chemosphere 258: 127338. DOI: 10.1016/j.chemosphere.2020.127338

Luti KJK, Yonis RW. 2014. An induction of undecylprodigiosin production from *Streptomyces coelicolor* by elicitation with microbial cells using solid-state fermentation. Iraq J Sci 55: 1553-1562.

Maheswari MU, Chandra TS. 2000. Production and potential applications of a xylanase from a new strain of *Streptomyces cuspidosporus*. W J Microbiol Biotechnol 16: 257-263. DOI: 10.1023/A:1008945931108

Manivasagan P, Venkatesan J, Sivakumar K, Kim SK. 2010. Pharmaceutically active secondary metabolites of marine actinobacteria. J Microbiol Res 169: 262-278. DOI: 10.1016/j.micres.2013.07.014

Murtiyaningsih H, Hazmi M. 2017. Isolation and test of cellulase enzyme activity on cellulolytic bacteria from waste soil. J Agritop 15: 294-297. [Indonesian]

Nurkanto A. 2007. Identification of forest soil actinomycetes after Bangkirai hill fire in east Kalimantan and the potential as cellulose degradation and phosphate solvent. Biodiversitas 8: 314-319. DOI: 10.13057/biodiv/d080414

Nurkanto A. 2008. The diversity of Actinomycetes in the Waigeo Islands, Raja Ampat District, Papua and its potential as cellulose degraders and phosphate solvents. Berita Biologi 9: 9-18. [Indonesian]

Nurkanto A, Agusta A. 2015. Molecular identification and morpho-physiological characterization of Actinomycetes producing antimicrobial compounds. J Biol Ind 2: 193-205. [Indonesian].

Octavina C, Agustina S, Sarong MA, Sari PH, Sahidin A, Razi NM, Agustiar M, Sakinah R, Fazillah MR. Length-weight relationship of Lingula sp. in Aceh Southern Shore. IOP Conf Ser Earth Environ Sci 674: 012021. DOI: 10.1088/1755-1315/674/1/012021

Oskay M. 2009. Antifungal and antibacterial compounds from *Streptomyces* strains. Afr J Biotech 8: 3007-3017.

Pandey A, Shukla A, Majumdar SK. 2005. Utilization of carbon and nitrogen sources by *Streptomyces kanamyceticus* M 27 for the production of an anti bacterial antibiotic. Afr J Biotech 4: 1-3

Patil RC, Mule AD, Mali GV, Tamboli RR, Khobragade RM, Gaikwad SK, Katchi VI, Patil D. 2011. Isolation of marine actinomycetes from the mangrove swamps for biotechnological exploration. J Life Sci 5: 1030-1036.

Pesrita A, Linda TM, Devi S. 2017. Selection and activity of local Riau Actinomycetes cellulase enzymes on sugarcane bagasse lignocellulosic media. Jurnal Riau Biologia 2. [Indonesian].

Prasad P, Singh T, Bedi S. 2013. Characterization of the cellulolytic enzyme produced by *Streptomyces griseorubens* (Accession No. AB184139) isolated from Indian soil. J King Saud Univ Sci 25: 245-250. DOI: 10.1016/j.jksus.2013.03.003

Putri AL, Setiawan R. 2019. Isolation and screening of Actinomycetes producing cellulose and xylanase from Mamasa Soil, West Sulawesi. IOP Conf Ser Earth Environ Sci 674: 012021. DOI: 10.1088/1755-1315/308/1/012035

Putri AL, Lisdiyanti P, Kusmiati M. 2018. Identification of Mamasa freshwater sediment Actinomycetes, West Sulawesi and their activities as antibacterial and phosphate solvents. J Biotek Bios Indon 5: 139-148. DOI: 10.29122/jbbi.v5i2.2953

Rajagopal G, Kannan S. 2017. Systematic characterization of potential cellulolytic marine actinobacteria *Actinoalloteichus* sp. MHA15. Biotech Rep 13: 30-36. DOI: 10.1016/j.btre.2016.12.003

Raytapadar S, Paul AK. 2001. Production of an antifungal antibiotic by *Streptomyces aburaviensis* IDA-28. Microbiol Res 155: 315-323. DOI: 10.1016/S0944-5013(01)80010-0

Roeßler M, Müller, V. 2002. Chloride, a new environmental signal molecule involved in gene regulation in a moderately halophilic bacterium, *Halobacillus halophilus*. J Bacteriol 184: 6207-6215. DOI: 10.1128/JB.184.22.6207-6215.2002

Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F. 2014. The Prokaryotes: Actinobacteria. Springer, Berlin.

Shivakumar DK. 2000. Actinomycetes. Annamalai University Press, India.

Singh OV, Gabani P, Copeland E, Chandel AK. 2013. Ultraviolet radiation-resistant isolates revealed the cellulose-degrading species *Cellulosimicrobium cellulans* (UVP1) and *Bacillus pumilus* (UVP4). Biotech Biochem Appl 59: 395-404. DOI: 10.1002/bab.1038

Suhartono S, Nursanty R. 2012. Bioprospecting soil Actinomycetes: isolation and antibacterial assay. Jurnal Biologi Edukasi 4: 1-6.

Sukmawaty E, Sari SR, Masri M. 2020. Characterization of soil Actinomycetes from Malino pine forest rhizosphere of South Sulawesi. Elkawnie J Islam Sci Tech 6: 315-328. DOI: 10.22373/ekw.v6i2.5383

Teixeira N, Parolo CCF, Malz M, Devine DA. 2020. Gene expression profile of *Scardovia* spp. in the metatranscriptome of root caries. Brazil Or Res 1: 34-47. DOI: 10.1590/1807-3107bor-2020.vol34.0042

Tseng M, Hoang KC, Yang MK, Yang SF, Chu WS. 2007. Polyester-degrading thermophilic Actinomycetes isolated from different environments in Taiwan. Biodegradation 18: 579-583. DOI: 10.1007/s10532-006-9089-z

Vinothini G, Kavitha R, Latha S, Arulmozhi M, Dhanasekaran D. 2018. Cell aggregating temperament and biopotency of cultivable indigenous actinobacterial community profile in chicken (*Gallus gallus domesticus*) gut system. Arab J Sci Eng 43: 3429-3442. DOI: 10.1007/s13369-018-3083-8

Wink J, Mohammadipanah F, Hamedi J. 2017. Biology and Biotechnology of Actinobacteria. Springer International Publishing, Switzerland.

Wulandari S, Sulistyani N. 2016. Effect of media on growth of Actinomycetes isolate code Al35 and optimization of antibacterial metabolite production based on fermentation time and pH. Media Farmasi Jurnal Ilmu Farmasi 13: 186-198. DOI: 10.12928/mf.v13i2.7770 [Indonesian].

Yakoob R, Pradeep BV. 2019. *Bifidobacterium* sp as probiotic agent-roles and applications. J Pur Appli Microbiol 13: 1407-1417. DOI: 10.22207/JPAM.13.3.11