Aktivitas Selulase *Bacillus* sp. yang ditumbuhkan Pada Medium Dengan Penambahan Garam Aluminium dan Paparan Medan Magnet

Cellulase Activity Bacillus sp. Which Was Grown on A Medium with Aluminum Salt and Magnetic Field Exposure

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ABSTRAK

Aluminium adalah jenis kofaktor logam terbaik. Pemaparan medan magnet 0,2mT pada garam aluminium mampu meningkatkan aktivitas enzim selulase pada bakteri *Bacillus* sp. Penelitian ini bertujuan untuk mencari jenis garam aluminium terbaik untuk meningkatkan aktivitas enzim selulase. Aktivtas enzim diuji dengan dua cara yaitu Uji Indeks Selulolitik dan Uji Aktivitas Enzim dengan spektrofotometer. Penelitian ini adalah penelitian deskriptif dengan tiga perlakuan yaitu kontrol, penambahan garam aluminium tanpa paparan medan magnet, dan penambahan garam aluminium yang dipaparkan medan magnet. Jenis garam aluminium yang digunakan adalah $AlCl_3$, $Al_2(PO_4)_3$, dan $Al_2(SO_4)_3$. Konsentrasi garam aluminium yang digunakan adalah 0.01% dan pemaparan medan magnet 0.2 dilakukan selama 10 menit. Hasil penelitian ini membuktikan bahwa aluminium mampu menjadi kofaktor enzim yang efektif. Pemaparan medan magnet pada garam aluminium mampu meningkatkan aktivitas enzim. Jenis garam aluminium terbaik adalah $AlCl_3$ yang dipaparkan medan magnet 0,2mT selama 10 menit. Perlakuan ini mampu meningkatkan aktivitas enzim selulase sebesar 0.69 U/mL. Kemudian $Al_2(PO_4)_3$ mampu meningkatkan aktivitas enzim sebesar 0.41 U/mL dan $Al_2(SO_4)_3$ meningkatkan aktivitas sebesar 0.39 U/mL.

Kata kunci: aktivitas selulolitik, garam aluminium, indeks selulolitik, medan magnet

ABSTRACT

Aluminum is the best metal cofactor enzyme. Exposure to the 0,2mT magnetic field on Aluminium salt is said to be able to increase cellulolytic activity. The purpose of the research is to know if the best aluminum salt can increase cellulolytic activity. This can be tested by Cellulolytic Index Test and Enzyme Activity Test. This experiment is descriptive research with three treatments; control, the addition of aluminum salt without an exposed magnetic field, and added aluminum salt exposed to a magnetic field. Salt aluminum used are $AlCl_3$, $Al_2(PO_4)_3$, and $Al_2(SO_4)_3$. The concentration was 0.01% and exposed to a magnetic field of 0.2mT. The final results tell that aluminum is an effective cofactor. Exposure to a magnetic field on aluminum salts showed an increase in enzyme activity. The best result is $AlCl_3$ exposed to a 0.2mT magnetic field. This was able to increase the cellulolytic activity by 0.69U/mL. Then $Al_2(PO_4)_3$ increased enzyme activity by 0.41U/mL and $Al_2(SO_4)_3$ increased activity by 0.39U/mL.

Keywords: cellulolytic activity, aluminum salt, cellulolytic index, magnetic field

INTRODUCTION

Cellulase enzymes have a significant role in the textile and food industry. Cellulase enzymes are decomposers of organic materials that increase nutrition in animal feed, according to Zilda et al. (2008). The need for cellulase enzymes in the world enzyme industry in Indonesia is almost 50% and is obtained by import. Cellulase enzymes can be

obtained from microorganisms, plants, and animals (Yusak, 2004). Enzymes from microorganisms are widely used because they can reproduce quickly and produce high and stable enzymes (Yusak, 2004).

The ability of a bacterium to produce enzymes is directly proportional to the amount of bacterial growth. Suitable growing media will increase the production of bacteria. Another environmental factor that is now widely studied is the magnetic field. Sumardi et al. (2018) stated that exposure to a magnetic field of Al and Fe Metal added to the modified Mandels medium increased the protease enzyme's activity. Exposure to a magnetic field of 0.2mT for 10 minutes to paramagnetic Al metal ions is thought to cause a positive interaction between Al metal ions and proteins on the bacterial membrane of *Bacillus* sp., thereby causing an increase in the length of the bacterial cell size. It is directly proportional to the increase in its proteolytic activity (Sumardi et al., 2018).

So, research was conducted to determine the activity of the bacterial cellulase enzyme *Bacillus* sp., which was grown in a medium added with Aluminum salt and exposed to a magnetic field of 0.2mT.

MATERIALS AND METHODS

Test Bacteria and Cellulase Selective Medium

Bacillus sp. culture taken from the collection in the Laboratory of Microbiology, Faculty of Mathematics and Natural Sciences, University of Lampung. The cellulase enzyme-producing bacteria were isolated from the intestines of native chickens in 2019.

The experimental medium used was Mandels Medium with a composition of 0.5% CMC, 0.2% NaCl, 0.35% yeast extract, 0.245% KH_2PO_4, 0.035% $MgSO_4$, 0.17% $(NH_4)_2SO_4$, 0.5% skim milk and 1.5% agar dissolved in one liter of distilled water with a normal pH setting of 7-8. Agar is used as a compacting medium for qualitative enzyme tests on bacteria. The aluminum salt used is 0.01% $Al_2(PO_4)_3$, 0.01% $AlCl_3$, or 0.01% $Al_2(SO_4)_3$. Exposure to the magnetic field used was 0.2mT with an exposure time of 10 minutes after the aluminum salt was dissolved in water and sterilized by autoclave.

The research treatments:

1. Control : Medium without adding aluminum salts and without exposure to magnetic fields.
2. P 1 : Addition of $AlCl_3$ without exposure to a magnetic field.
3. P 2 : Addition of $AlCl_3$ exposed to a 0.2mT magnetic field.
4. P 3 : Addition of $Al_2(PO_4)_3$ without exposure to a magnetic field.
5. P 4 : Addition of $Al_2(PO_4)_3$ exposed to a 0.2mT magnetic field.
6. P 5 : $Al_2(SO_4)_3$ without exposure to a magnetic field.
7. P 6 : Addition of $Al_2(SO_4)_3$ exposed to a 0.2mT magnetic field.

Cellulolytic Index Determination

Cellulolytic Index (CI) is a numerical value indicating the ratio between the diameter of the clear zone and the diameter of the colony (Durham et al., 1987). The CI value of the isolate ≥ 3 indicates that the isolate has a good potential and is optimal for producing extracellular cellulase enzymes (Said and Likadja, 2012).

Figure 1. Mandels medium with CMC

The Cellulolytic Index can be calculated using the following equation:

$$CI = \frac{B}{A} \quad (1)$$

Information:
CI: Cellulolytic Index
A: Colony bacteria diameter
B: Clear zone diameter / Colony diameter
(Febriyanto *et all.*, 2015)

Cellulase Enzyme Production in Liquid Media

Production of cellulase enzymes using Mandels liquid medium modified by adding aluminum salts $Al_2(PO_4)_3$, $AlCl_3$, or $Al_2(SO_4)_3$. Based on cellulolytic test results, the best bacterial isolates were inoculated in 25 mL Mandels C MC liquid medium, then incubated in a shaking incubator with an agitation speed of 120 rpm at room temperature for 24 hours. This incubated bacterial suspension is a starter for enzyme production. Cellulase enzyme production was carried out by inoculating 5 mL of starter *Bacillus* sp. in 45 mL Mandels liquid medium modified by magnetic and metal treatment. The culture was incubated in the Shaker Incubator at 120 rpm at room temperature in all treatments. For 24 hours (Sumardi et al., 2018).

The cellulase enzymes produced by isolates from each treatment were extracted by centrifuging 5 mL of culture at 6000 rpm for 10 minutes. Centrifugation of liquid media will precipitate *Bacillus* sp. cells due to gravity. The supernatant formed contains enzymes excreted by *Bacillus* sp. will be used as a test sample for cellulase enzyme activity (Yusufa et al., 2013).

Cellulase Enzyme Activity Test

Measurement of cellulase enzymes was carried out by measuring reducing sugar levels which were carried out in 7 test tube groups consisting of controls and samples (6 treatments). As a control treatment, 1 mL of 1% CMC solution was added to 1 mL of Crude Enzyme Extract for control treatment then incubated at 25-35°C for 30 minutes in an incubator shaker. After that, 2 mL of DNS was added to the sample, incubated at 100°C for 10 minutes, and cooled.

In the treatment sample, 1 mL of crude extract enzyme for each treatment was added with 1 mL of 1% CMC then vortexed and incubated at 25-35°C for 30 minutes in an incubator shaker. After that, 2 mL of DNS was added to the sample, incubated at 100°C for 10 minutes, and cooled. After all the treatments were ready, absorbance was measured in the seven treatment tubes using a spectrophotometer with a wavelength of 540 nm. The results of the absorbance value will be analyzed for the value of cellulase enzyme activity with Equation 2.

$$\text{CA (U/mL)} = \frac{(X\ sample - X\ COntrol)\ .DF\ .10^3}{Incubation\ time\ x\ Glucose\ MW} \quad (2)$$

Information:
CA: Cellulase Activity (U/mL)
DF: Dilution Factor: 1000
Incubation Time: 30 minutes
Glucose MW: Molecular Weight: 180.18 mg/mL

RESULT AND DISCUSSION

Cellulolytic Index Test is a test conducted to determine cellulolytic activity qualitatively. Meanwhile, cellulase enzyme activity is a test performed to quantitatively observe cellulase enzyme activity by looking for the value of glucose levels resulting from the degradation of CMC (Carboxyl Methyl Cellulose). This research uses these two data as a reference. The data from calculating the cellulolytic index and cellulase enzyme activity can be seen in Figures 2 and 3.

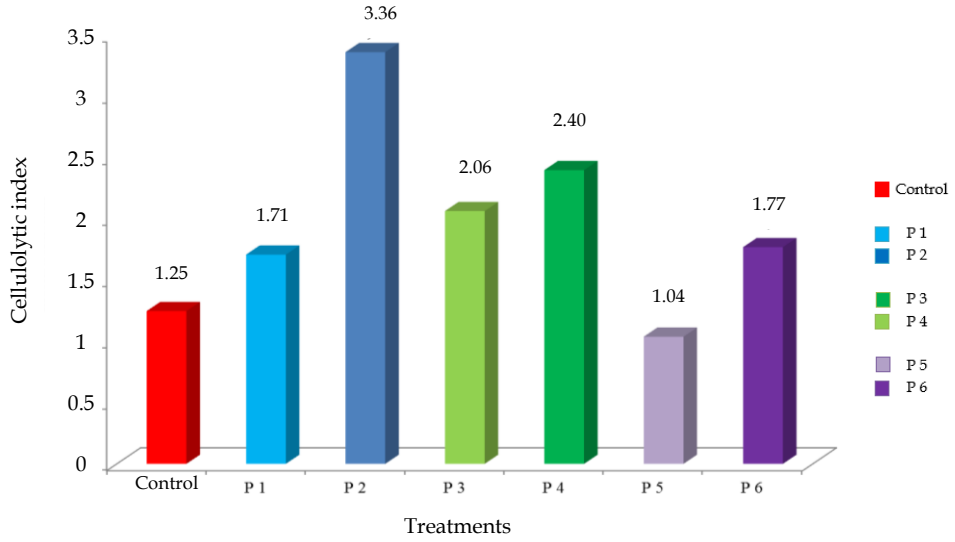

Figure 2. The average value of *Bacillus* sp. cellulolytic index

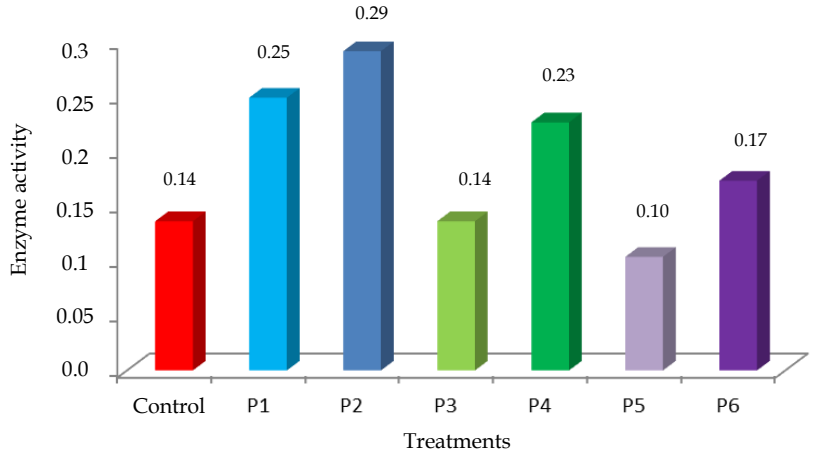

Figure 3. The average value of *Bacillus* sp. enzyme activities

Description:

Control	: Without the addition of aluminum salts and exposure to the field magnets
P 1	: Addition of AlCl3 {Aluminum chloride}}.
P 2	: Addition of aluminum salt Al2Cl3 {Aluminum chloride} exposed to a 0.2mt magnetic field for 10 minutes.
P 3	: Addition of $Al_2(PO_4)_3$ {Aluminum (III) Phosphate}.
P 4	: Addition of salt $Al_2(PO_4)_3$ {Aluminum (III) Phosphate} exposed to 0.2mT magnetic field for 10 minutes.
P 5	: Addition of $Al_2(SO_4)_3$ {Aluminum (III) Sulfate} salt.
P 6	: Addition of $Al_2(SO_4)_3$ {Aluminum (III) Sulfate} exposed to 0.2mT magnetic field for 10 minutes.

Cellulolytic Index

The Cellulululolytic Index of *Bacillus* sp. on a solid medium can be seen in forming a clear zone around the growing colony. The selection medium used in this research contained CMC (carboxymethyl cellulose). Cellulose hydrolyzed by extracellular enzymes in the form of glucose cellulases will

not have ®-D-glucan bonds so that the congo red dye will not bind to the medium and form a clear zone after being rinsed with 1 M NaCl (Zhang et al., 2006).

The wider the clear zone formed indicates that the bacteria can produce cellulase enzymes to degrade CMC as a substrate contained in the bacterial growth medium (Vijayaraghavan et al., 2013).

Cellulase enzyme is a type of enzyme that can degrade cellulose by breaking the ®-1,4 glycoside bond, which is an oligosaccharide derived from cellulose and glucose. This enzyme is classified into three groups depending on how it hydrolyzes cellulose in the growth medium (Lynd et al., 2022). This research obtained data on cellulolytic index values with three treatment repetitions. The results of the cellulolytic index values can be seen in Figure 2.

Cellulase Enzyme Activity

The cellulase activity test measured the sugar content, a product of CMC hydrolysis in Mandels medium. The addition of aluminum salts in the medium can increase the activity of enzymes. The rate of cellulase enzyme activity also differs depending on the type of aluminum salt used. The addition of $AlCl_3$ salt was able to increase the value of the cellulolytic index by 0.35 U/mL. Then followed by $Al_2(PO_4)_3$ of 0.34 U/mL and $Al_2(SO_4)_3$ of 0.19 U/mL. Exposure to a magnetic field also increased the enzyme activity than without exposure to a 0.2mT magnetic field. The highest increase in activity occurred in treatment 2. The addition of $AlCl_3$ salt exposed to a 0.2mT magnetic field for 10 minutes increased cellulolytic activity up to 0.69 U/mL. Then, in treatment 4, it was 0.41 U/mL. This research shows that exposure to a 0.2mT magnetic field for 10 minutes on aluminum salts can affect the enzyme activity of *Bacillus* sp. bacteria (Figure 3).

Aluminum salts dissolved in water will release their ionic bonds so that the ions will move freely with a stable dipole moment. Positively charged ions will bond with negative ions and vice versa. Metal ions can be activated in several ways. (a) Becomes part of the enzyme's active site so that it supports the substrate to bind to the enzyme. (b) Metal salts will release their bonds with each other and become active ions so they can bind with H ions to enter the cell. So that it can support the transport of substances in cell metabolism. (c) Metal ions will bind to inhibitor ions that want to attach to the enzyme's active site. (d) Metal ions will become replacement ions on the active site of the enzyme or substrate. However, in general, the magnetic field will affect changes in cell structure and be able to change the growth rate of bacteria. The magnetic field can also change the flow of ion movement in the membrane to accelerate cell regeneration and reproduction (Sudarti et al., 2014).

Enzymes have characteristics that can work with the help of cofactors. Cofactors are groups of protein ions in enzymes that affect the catalytic ability of an enzyme. Metal ions are one type of cofactor in the form of a prosthetic group that can enhance catalytic enzymes (Sumardjo, 2006). Aluminum metal ions are a type of heavy metal which some will be able to enter the cell through the membrane, and some other ions will be retained and unable to pass through the cell membrane. Al ions that accumulate will affect the cell's pH and ion balance. It can help the process of cell metabolism be faster and more stable so that the bacteria experience cell elongation. (Sumardi, 2020). Fatmawati and Ummi (2018) stated that *Bacillus* sp is a type of bacteria capable of biosorption and degrading heavy metals in the membrane. It can happen because the bacterial membrane comprises Teichoic Acid and Peptidoglycan. Lipid and protein content in peptidoglycan can bind metal ions with carboxylic, hydroxyl, amino, and phosphate groups. Al^{3+} metal ion, the positively charged, will bind to the surface of the bacterial cell membrane, which is negatively charged because it is composed of various anions. Metal ions also easily bind to the sulfhydryl groups of proteins and the hydroxyl groups of phospholipids and can increase the permeability of cell membranes (Satya and Larasati, 2012).

In this research, aluminum metal ions in various types of aluminum salts increased the bacterial cellulase enzyme *Bacillus* sp activity. Exposure to a 0.2mT magnetic field for 10 minutes in aluminum salt solution has a more significant positive impact on the growth and

rate of cellulase activity of *Bacillus* sp. The magnetic field is said to increase the ionic strength of heavy metal ions. Aluminum is a type of ferromagnetic metal that has a small electromagnetic field. However, Aluminum can attract stable magnetic lines of force. Exposure to a 0.2mT magnetic field is known to affect the ionization of an element. The addition of aluminum salts to the medium can increase enzyme activity.

Chang (2005) states that the ionization energy of an ion or element is directly proportional to its electronegativity. The higher the electronegativity, the more difficult it is to remove an electron. When aluminum is exposed to an external magnetic field, it creates a parallel or stable dipole moment. Metal ions will carry this magnetic property and will only be damaged at high curie temperatures (Smith, 1993). Thus, when in the cell. The heavy metal Al will continue to carry magnetism from magnetic field exposure. This condition will help the salt ions attract water molecules from the protein. Aluminum salts in the medium can stabilize enzymes by reducing the excess electrostatic charge on the enzyme molecules (Sumardi, 2018).

Exposure to magnetic fields on the medium's components can also increase the metabolic rate of bacterial cells. Hernawati et al. (2016) proved that exposure to a magnetic field in the growth medium positively responded to celluloid activity because elements exposed to a magnetic field are thought to have ionization energies and high electron affinities, thereby increasing enzyme activity.

The addition of $AlCl_3$ showed the highest activity value even before being exposed to a magnetic field. $AlCl_3$ is composed of ions (Al^{3+}) and (Cl^-). Aluminum chloride is also one of the largest electrolytes in the cytoplasm. This electrolyte plays a role in controlling the osmotic pressure in the cell. The Al metal ion in this compound easily binds to protein and hydroxyl sulfhydryl groups in phospholipids (Devi and Prasad, 1991).

The following $AlCl_3$ constituent ion is chloride. *Chloride* is the main ion that helps cells to control intracellular activity and secretion of transepithelial electrolyte fluids. Chloride (Cl^-) is the largest isoelectric, facilitating the transport of substances in the intracellular and extracellular systems. The presence of chloride in the cell makes it easier for substances to enter the cell. Cl^- will bind O_3 and O_4, then turn it into O_2. The oxygen will become an electron transport ion, making metabolism faster. Metabolism that takes place quickly causes an increase in enzyme production by bacteria. Al ions also make bacterial cells elongate, facilitating the movement of ions within the cell, which also facilitates the pathways of cell metabolism to increase cell growth and cellulolytic activity.

The next aluminum salt compound is $AlPO_4$. This aluminum salt comprises $2Al^{3+}$ and $(PO_4)^{3-}$ ions. Phosphates that are degraded by bacteria can reduce the pH value of bacteria so that they are acidic. Bacteria *Bacillus* sp. can live in an acidic environment, and it is also in line with research results which show an increase in the value of cellulase activity produced by bacteria. However, in some conditions, bacteria will secrete Pi from the hydrolysis of phosphates, which can precipitate heavy metal ions on the cell wall. Pi secretion can also increase the proton gradient on the transmembrane so that the bacterial cell can transport metal ions into the cell. Metal ions in bacterial cells do not affect the metabolism or growth of bacteria.

This research also used aluminum salts in the form of $Al_2(SO_4)_3$. This type of aluminum salt is often used in the water treatment industry. This type of aluminum is the best antibacterial because it can inhibit the growth of bacteria. $Al_2(SO_4)_3$ is composed of compounds $2Al^{3+}$ and $3(SO_4)^{2-}$. Sulfate in cells requires many steps to be used by cells in cell chemical reactions. Sulfate ions that enter the cell need to be converted into new sulfites that bacteria can assimilate into the sulfur cycle. Sulfur assimilation to become ADP (adenosine-3,5--diphosphate) requires a long step, and it is related to the lower cellulase activity results compared to other types of aluminum salts. However, this research has yet to be discovered whether the length of the incubation period and temperature can have a positive effect.

Aluminum salts exposed to a magnetic field undergo biomineralization with the cell walls so that Al^{3+} ions do not poison the bacterial cells. Ions carried by salt compounds

can also react inside or outside the cell. When inside the cell, ions in aluminum salt compounds will be reduced or oxidized with other ions in the cell and collaborate to increase the rate of cell metabolism so that bacteria can produce more cellulase enzymes than under normal conditions. Meanwhile, Al ions which cannot pass through the cell wall, will affect extracellular enzymes in the form of cellulase enzymes produced by bacteria. Al ions will become enzyme cofactors.

Al metal type cofactor will affect the conformation of the cellulase enzyme by attaching to the active side of the enzyme or the other side. The cellulase enzyme produced by the bacterium *Bacillus* sp. will bind Al ions on the allosteric active site so that the enzyme's active site will increase and also cause enzyme activity to increase (Wijayanti and Salirawati, 2018). Al metal ions also bind to many ligands to attract and bind substrates to enzymes quickly and powerfully. Al metal ions can stabilize enzymes and induce protein conformation on the active sites of enzymes so that the degradation process by enzymes dashes (Baehaki, Rinto, and Budiman., 2011).

CONCLUSION

This research proved that aluminum salts can affect the activity of the bacterial cellulase enzyme *Bacillus* sp. exposure to a 0.2mT magnetic field for 10 minutes changed the cellulolytic enzyme activity value of *Bacillus* sp. The best aluminum salt is aluminum chloride salt, with an enzyme activity value of 0.35 U/mL. Exposure to a magnetic field of 0.2mT doubled the enzyme's activity by 0.69 U/mL.

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