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The Effect of Magnetic Field Exposure on Medium to Protease Production by *Bacillus* sp.

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Abstract

This research was purposed to understand the effect of 0.2mT magnetic field exposure treatment for 10 minutes toward medium components to the production of protease in *Bacillus* sp. That magnetic field exposure treatment was given to 8 medium components namely Milk, Yeast, NaCl, KH₂PO₄, MgSO₄, (NH₄)₂SO₄, Agar and Aquadest. Data from Qualitative Proteolytic Activity test on *Bacillus* sp. indicated that in all treatment, the bacteria were able to produce the enzyme. The highest Proteolytic Index (IP) from all those treatments came from the magnetically exposed KH₂PO₄ which was 7.17 at the 10th incubation hour. Treatment of exposure to magnetic fields is also given to the liquid medium. Quantitative data of enzyme activity showed that the best incubation time of protease production by *Bacillus* sp. is the 24th incubation hours with result of 0.031 U/ml. Exposure of 0.2 mT magnetic field for 10 minutes to the NaCl component in Mendel's fluid medium yielded the highest protease activity of 0.067 U/ml.

Keywords: protease, *Bacillus* sp., magnetic field

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1. Introduction

Magnetic field exposure to microorganism affects the growth characteristic and the amount cells at stationary phase. Magnetic field can affect all components of the microorganism cell membrane which then interact with each other so it can stimulate the enzyme activity [1]. 60 minutes exposure of magnetic field with 50 Hz frequency and 10 mT strength affects the morphology of gram-negative *Escherichia coli* and gram-positive *Denitrificans Paracoccus* due to the size increment of those bacteria colony cells. The effect of magnetic field exposure is easier to be observed in the treatment for nutrient agar medium rather than with liquid broth [2].

Treatment of 0.2 mT magnetic field increases the growth rate and affects *Magnetospirillum magneticum* AMB-1 strain in responding stimulus [3]. However, the experiments on the liquid mediums are also done cause the nutrients of medium are the

spread more evenly. In this research, the observation was aimed to know the effect of 0.2 mT magnetic field exposure to culture medium in producing protease *Bacillus* sp.

2. Materials and Methods

Bacillus sp.

Bacillus sp. isolate was obtained from culture collection in Laboratory of Microbiology Faculty of Mathematics and Science, University of Lampung.

Medium

The culture medium used in this research was modified Mendels medium [4], Modified substances of the solid Mendels medium consisted of: 1) Milk, 2) Yeast, 3) NaCl, 4) KH₂PO₄, 5) MgSO₄, 6) (NH₄)₂SO₄, 7) Agar, and h) Aquadest. In this step, 8 different mediums were created and labeled with M₀ until M₈, as listed on Table 1. Every single medium, while being on its liquid state, was exposed by 0.2 mT magnetic field for 10 minutes, and then they were kept till they turned to solid state.

Table 1. Modified Mendels medium substances.

| Treatment Sample | Magnetically Exposed Mendels Medium Substances |
|------------------|---|
| M ₀ | Control |
| M ₁ | Milk |
| M ₂ | Yeast |
| M ₃ | NaCl |
| M ₄ | KH ₂ PO ₄ |
| M ₅ | MgSO ₄ |
| M ₆ | (NH ₄) ₂ SO ₄ |
| M ₇ | Agar |
| M ₈ | Aquadest |

Proteolytic Test

Bacillus sp. isolate was cultured in solid medium and incubated in 37 °C temperature for 10 hours. Proteolytic index was measured by comparing the diameter of Clear Zone area and Bacteria Colony area [5] with this following equation:

$$IP = \frac{B}{A}$$

Note:

- IP : Proteolytic Index
- A : Diameter of Colony Area
- B : Diameter of Clear Zone

[6]

The high IP value (≥ 3) in the isolate indicated that the isolate had higher and maximum potential to be source of Protease [7].

Optimization of Protease Production by *Bacillus* sp.

Optimal determination of *Bacillus* sp. to produce protease enzyme was done by measuring protease activity of cultured *Bacillus* sp. every 6 hours for 30 hours. The incubation time by showing the highest enzyme production is used to determine the optimum time of enzyme production.

Production of Protease Enzymes in Liquid Media by Treatment of Magnetic Field Exposure

In this treatment the enzyme production was performed on four modified Mendels media materials ie a) NaCl, b) MgSO₄, c) KH₂PO₄, d) control. The selection of the four modified Mendels media materials is based on the calculation of the IP values of the three highest treatments. In metallic materials: a) NaCl, b) MgSO₄, c) KH₂PO₄ were each treated with magnetic field exposure of 0.2 mT for 10 minutes in liquid form, then it was given 5 ml of starter *Bacillus* sp. After that the culture of the four treat-

ments incubated in the shaker incubator at a speed of 120 rpm temperature of 40 °C with for 18 hour incubation time adjusting to optimum growth time. The extraction of protease enzymes was performed by centrifugation from finish fermentation at a rate of 3000 rpm for 15 min at 4 °C. this centrifugation will precipitate *Bacillus* sp. while the enzyme will remain in the supernatant to be used as a crude protease [8].

Assay of Protease Activity

Protease activity was assayed by measuring amino acid levels as a hydrolysis product of skim milk by protease enzymes [9]. A total of 0.2 ml of protease was added to the mixture containing 1 ml phosphate buffer with casein substrate pH 7, 0.2 ml standard tyrosine and 0.2 ml of aquadest as blank, incubated at 37 °C for 10 min. After to finish, TCA (0.1 M) 1 ml and protease 0.2 ml were added, incubated at 37 °C for 10 min, and centrifuged at 4000 rpm at 4 °C for 10 min. Then 0.75 ml of supernatant was added and added with 2.5 ml of Na₂CO₃ (0.4 M) solution and 0.5 ml of Folin reagent, incubated at 37 °C for 20 min and measured its absorbance at 578 nm wavelength [10]. The working principle of the Bergmeyer and Grassl method, 1983 ie casein which serves as a substrate will be hydrolyzed by proteases with the help of water into peptides and amino acids. The protease activity was calculated in units of PU (Protease Unit) per ml of the enzyme extract [11].

$$PU = \frac{Asp - Abl}{Ast - Abl} \times \frac{1}{T}$$

Note:

- PU : Protease Activity Unit (Unit / ml)
- Asp: Value of Sample Absorbance
- Ast : Value of Standard Absorbance
- Abl : Value of Blanco Absorbance
- T : Time

3. Results And Discussion

Proteolytic Test on solid media

Observations show that *Bacillus* sp. exhibit proteolytic activity in all treatments (M₀ to M₈). The highest IP value of 4.51 was obtained from treatment of M₂ culture medium and the 10-hour incubation. The IP values of the M₂ treatment (KH₂PO₄) were significantly higher than in the other 7 treatments: M₀, M₁, M₃, M₄, M₅, M₆, M₇, M₈ (Figure 2).

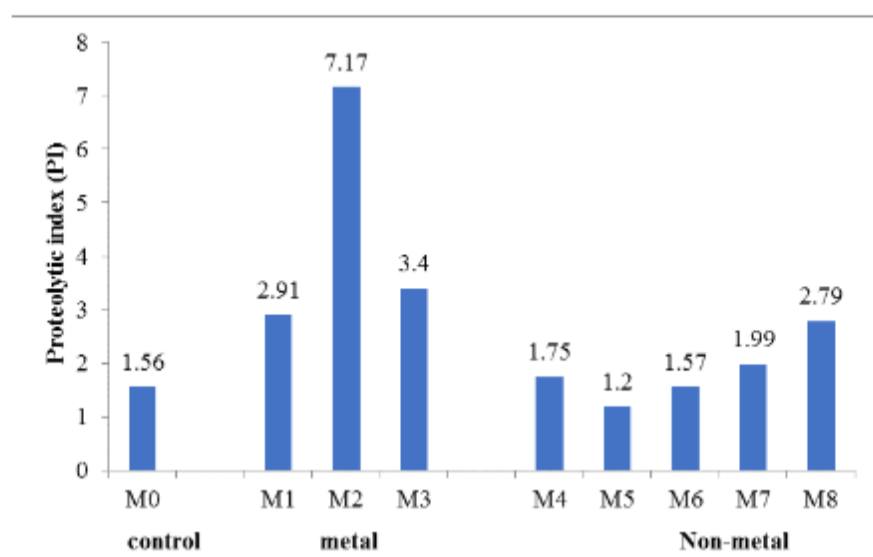


Figure 1. The average value of PI at 10 hours incubation time

Note: Proteolytic Index Value (PI) *Bacillus* sp. in the solid medium without the magnetic field treatment (M0), the magnetic field treatment of 0.2 mT on the components i.e. NaCl (M1), KH_2PO_4 (M2), MgSO_4 (M3), milk (M4), yeast (M5), $(\text{NH}_4)_2\text{SO}_4$ (M6), agar (M7), aquadest (M8)

KH_2PO_4 in bacterial culture media serves as a buffer and it is a major mineral important for cell growth [12]. Based on the results of his research, [13] states that KH_2PO_4 was useful for energy metabolism as cell membrane stabilizer and in amino acid synthesis. The results of [14] stated that the addition of KH_2PO_4 in the appropriate amount will increase the growth of *Bacillus* sp. Metallic ions are elements that it can conduct electricity and heat well [15], while nonmetallic ions are a composite of some non-metallic elements.

The IP value of the component exposed to the magnetic field is a larger metal ion compared with the nonmetallic. Exposure of 0.2 mT magnetic field for 10 minutes on Mendels media component gives a different effect. The presence of a magnetic field in the cell and medium environments can store magnetic fields to penetrate through cell membranes and extracellular media. Thus, the production of proteases is done on media whose metal content is exposed to a magnetic field.

Optimization of Protease Production by *Bacillus* sp.

Optimization of protease production was used to know the optimum time of *Bacillus* sp. in producing protease enzymes. Determination of the duration of production is done by means, measuring the value of enzyme activity every 6 hours for 30 hours. The results showed that *Bacillus* sp gave the highest protease activity (0.031 U / ml) occurring at the 24th hour incubation (Figure 2).

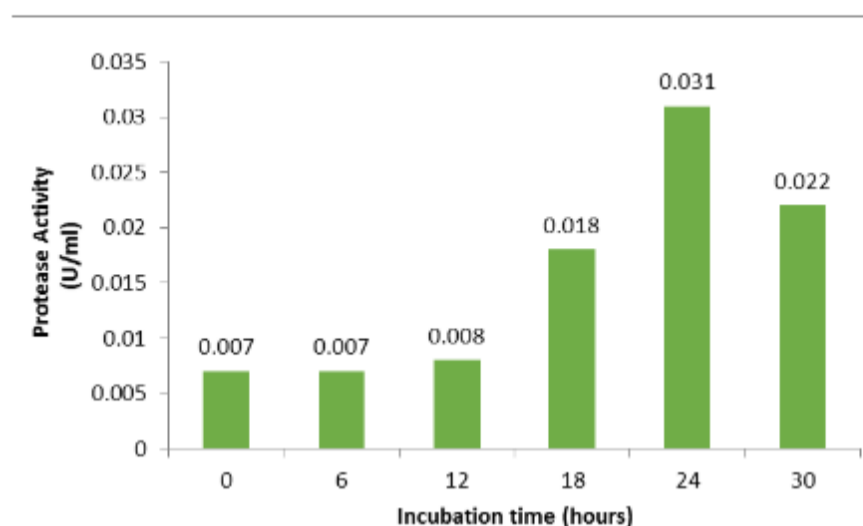


Figure 2. Effect of incubation time on protease production by *Bacillus* sp.

This is similar to [16] which suggest that an increase in protease enzyme activity resulting from APP-4 isolates from 18 hours to 24 hours from 0.118 U/ml to 0.524 U/ml. After reaching optimum production time, protease production activity of *Bacillus* sp. decreased at the 30th hour of 0.022 U / ml. Decreased activity of *Bacillus* sp. occurred after incubation of 24 hours. This can occur because of the reduced amount of substrate that inhibits the formation of substrate enzyme complexes. In addition, the availability of bacterial nutrition has begun to diminish and bacterial cells begin lysis that goes with the death phase [17].

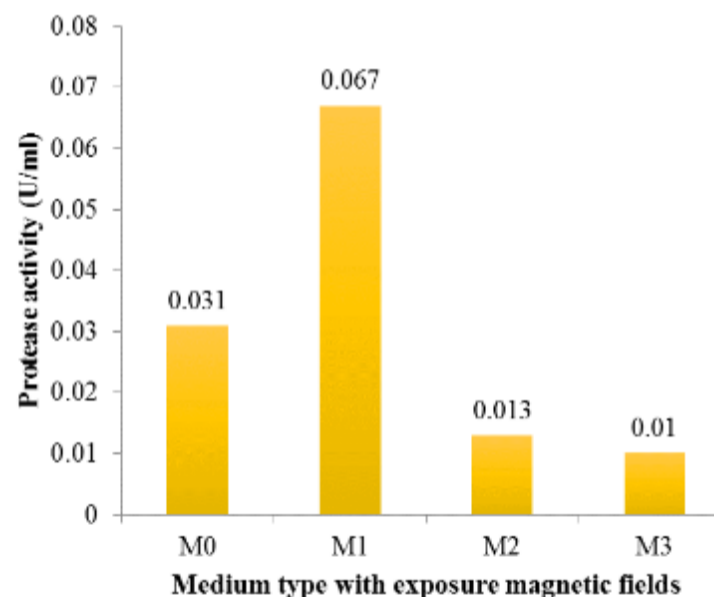


Figure 3. The effect of 0.2 mT magnetic field exposure of metal ion components on *Bacillus* sp. protease production in liquid medium

Note : M0 : control (no magnetic field exposure)
 M1 : NaCl exposed magnetic field
 M2 : KH_2PO_4 exposed magnetic field
 M3 : MgSO_4 exposed magnetic field

Optimization of protease production was used to know the effect of 0.2 mT magnetic field exposure to component of liquid medium in producing protease *Bacillus* sp. If the value of the highest proteolytic index on solid media is from the exposure of the magnetic field to KH_2PO_4 , dif-

ferent results in the liquid medium. In the liquid medium the component having the highest protease activity (0.067 U/ml) is present in the NaCl compound exposed to the magnetic field (Figure 3).

It is assumed that NaCl ions in liquid media are more able to move freely when crossing cell plasma membranes than in solid media, thus balancing electrons inside and outside the cell membrane. As already known, the weight of the Sodium atom is the smallest among other metal treatments. The weight of the sodium atom is 23, the weight of magnesium atom is 24, and the weight of the potassium atom is 39.

Increased of enzyme activity may occur cause the addition of certain metals is required as part of the active side of the enzyme. The mechanism of the metal ion in enzyme enzyme activity may occur in several ways, namely (a) being an integral part of the active site, (b) changing the electrical charge, (c) removing ion inhibitors, (d) switching less effective ions on the enzyme's active site or substrate. The metal ion inhibition of protease activity at a particular concentration relates to ionic strength, where the ionic strength affects the conformation or the three-dimensional structure of the enzyme protein or substrate protein [18].

4. Conclusions

Based on the results of this study concluded the following, namely;

1. Exposure of magnetic field of 0.2 mT for 10 min to KH_2PO_4 component on solid medium Mendels produced the highest proteolytic index value by 7.17 after 10 hours incubation.
2. The best incubation time of protease production by *Bacillus* sp. i.e. at 24 hours 0.031 U / ml.
3. Exposure of magnetic field of 0.2 mT for 10 minutes to the NaCl component in liquid medium Mendels produced enzyme activity by 0.067 U/ml.

5. Acknowledgement

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