The Ks and μmax Determination of Proteolytic Lactic Acid Bacteria Isolated from Colostrum of Dairy Cattle with Differences in Energy Source Levels

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ABSTRACT
This study determines the optimal substrate-level used by proteolytic acid lactic bacteria to produce a product expressed in substrate affinity constant (Ks). Proteolytic lactic acid bacteria were isolated from cow colostrum, carried out at the Laboratory of Nutritional Biochemistry, Faculty of Animal Science, Universitas Gadjah Mada. Proteolytic lactic acid bacteria was rejuvenated first in a solid medium, and the amount is multiplied in MRS broth medium to be used as stock. Proteolytic lactic acid bacteria was observed for growth on a defined medium with five variations of glucose substrate levels. The glucose levels were given at 0.15%, 0.1%, 0.05%, 0.01%, and 0% with three replications and one negative control at each level. Proteolytic lactic acid bacteria were incubated at 37°C for 24 hours. Observations were made every two hours. The results of the absorbance observations were used to calculate the Ks value. The Ks value was used to determine the substrate level in the next liquid fermentation. The Ks and μmax values obtained in the proteolytic lactic acid bacteria were 0.0569 gram/100 ml and 0.0125 per hour, respectively.

Keywords*: Lactic acid bacteria, Ks (substrate affinity constant), Define medium*

1. INTRODUCTION

Lactic acid bacteria produce lactic acid as the main product and also protease enzymes. Therefore, it can be utilized as a probiotic candidate that benefits host livestock. Besides, lactic acid bacteria are also used as a feed fermenter for feed preservative agents and improve feed quality.

The substrate's utilization by lactic acid bacteria will show a different response, characterized by cell biomass, end products, or derivatives of the bacteria. The efficiency of the substrate used by bacteria can be expressed in the form of ks. Ks is a constant that states the level of enzyme affinity for the substrate. The smaller Ks value means that less level substrate is used to achieve a half maximum enzymatic reaction speed. Therefore, the substrate requirement by microbes can be optimized by calculating the ks value of a microbe.

One of the enzymatic reaction parameters is hydrolysis reaction speed (V), catalyzed by the enzyme. The V increases with increasing substrate concentration [S] until a state is reached where the addition of substrate concentration [S] no longer increases the initial rate of the reaction and if all enzymes are saturated with the substrate [ES]. The condition in which V cannot increase again with increasing [S] is called the maximum speed (Vmax) [1].

In general, bacteria have several growth phases: lag phase, logarithmic phase, stationary phase, and the death phase. Lactic acid bacteria provide a maximum product when the bacteria are at the end of the logarithmic phase. Yuliana [2] states that making a bacterial growth curve is an essential part of a study because it can describe the characteristics of bacterial colonization.

2. MATERIALS AND METHOD

2.1. Material

The materials used in this study were proteolytic lactic acid bacteria, MRS medium, agar, define medium, glucose substrate, 70% alcohol, cotton pads, and distilled water. The tools used in this research were Petri dish, measuring cylinder, beaker glass, Erlenmeyer glass, hangetube, test tube, osse ring, bunsen, laminar airflow, magnetic and stirrer, autoclave, spectrophotometer, incubator, micropipette, blue tip, yellow tip, white tip, pH meter, Wattman filter paper number 1, funnel, and centrifuge.

2.2. Rejuvenation of Proteolytic Lactic Acid Bacteria

Media was made by dissolving 2.61 grams of MRS and 1 gram of agar in 50 ml of distilled water in Erlenmeyer. The media is heated and homogenized using a hotplate and stirrer until it boils. Tools and materials are sterilized by autoclaving at a temperature of 121°C with one psi pressure for approximately 30 minutes. Laminar is sterilized with alcohol and UV light for 30 minutes. Media was poured into the Petri dish as much as 15 ml. After solid, the bacteria were inoculated on the media using a ring. The inoculum was incubated in an incubator at 37°C for 24 hours.

2.3. Enrichment of Proteolytic Lactic Acid Bacteria

Media was prepared, heated, and homogenized as the previous step. Also, tools and materials are sterilized by the same method. Furthermore, osse rings are sterilized by burning on a bunsen. Bacterial colonies that grow on a solid medium are taken using the osse tip. The bacterial colony at the end of the osse is immediately inoculated into a new liquid medium. The bacteria contained in the liquid medium were incubated in an oven at 37°C for 24 hours.

2.4. Optical Density Observation

2.4.1. Media Production

A total of 0.3 grams of glucose was dissolved in 20 ml of distilled water to make the main solution with a concentration of 1.5%. The main solution was diluted to obtain a glucose solution with a concentration of 1%, 0.5%, and 0.1%. Define medium is prepared according to the recipe and then dissolved with distilled water in an Erlenmeyer glass. Define medium is heated and homogenized using a hotplate and stirrer until boiling.

2.4.2. Sterilization of Tools and Materials

Laminar is cleaned using tissue paper moistened with alcohol. After that, sterilize it using a UV light for about 30 minutes. The media in the hangetube is sterilized in an autoclave and a solution of sugar and blue tip at a temperature of 121°C, one psi pressure for approximately 30 minutes.

2.4.3. Growth of Proteolytic Lactic Acid Bacteria

The addition of the glucose substrate-level was adjusted to the treatment, with each level having a control tube. The substrate level of 0% was added with 1 ml of distilled water in each tube. The substrate level of 1.5% was added 1 ml into each tube until the substrate level was 0.15%. 1% substrate level is added 1 ml in each tube until the substrate level becomes 0.1%. The substrate level of 0.5% was added as much as 1 ml into each tube until the substrate level was 0.05%. The substrate level of 0.1% was added as much as 1 ml into each tube until the substrate level was 0.01%. Each substrate addition level consisted of 3 replication tubes and one negative control tube. 1ml of proteolytic lactic acid bacteria was added to the hangetube. The proteolytic lactic acid bacteria were incubated in an incubator at 37°C.

2.4.4 Optical Density Observation

Observation of microbial growth was carried out using the optical density method. Microbial growth was observed every two hours for 24 hours. Absorbance observations were made with a wavelength of 600 nm. Blanks were taken from tubes containing only media at each substrate treatment level.

2.4.5 Determination of Ks and μmax Values

The absorbance of each replication at each substrate-level was graphed between the absorbance value and time. The graph produces an equation $y = ax + b$. The value of a is the slope. Value of a is made a graph between 1/a and 1/substrate. The graph produces a new regression equation, $y = cx + d$. The value of μmax is 1/d while the value of ks is $\frac{1}{d}c$.

3. RESULT AND DISCUSSION

3.1 Determination of Ks and μmax Values

The value of the specific growth rate of proteolytic lactic acid bacteria was obtained from the equation's slope value in the proteolytic lactic acid bacteria's logarithmic phase. In general, the logarithmic phase of lactic acid bacteria was found at 3 to 12 hours of incubation at each substrate-level treatment. The absorbance measurement at the beginning of the incubation period resulted in a low absorbance, where the dead lactic acid bacteria might have been added. Death from bacteria can be caused by bacteria not being able to adapt well to the new media. During reinoculation, bacteria were grown on an MRS broth medium, while a defined medium was used when observing microbial

growth. Setyati [3] stated that differences influence microbes' adaptability in the media used during reinoculation and growth.

Table 1. The relationship of increasing the substrate level [S] with the specific growth rate (μ)

Substrat level [S] (%)	Slope/μ (per hour)
0.01	0.0019
0.05	0.0034
0.1	0.0127
0.5	0.0187

The absorbance value in the logarithmic phase is made a linear regression equation where each treatment level's slope or specific growth rate is obtained. The relationship of the increase in substrate-level with the specific growth rate is presented in Table 1.

The increase in substrate level is directly proportional to the specific growth value of LAB Pro. The greater the added substrate level, the greater the slope value or the specific growth rate. The higher the added substrate level, the greater the microbial growth. Microbes use the carbon source as an energy source [4]. In general, one of the benefits of microorganisms using substrates is for the growth of cell biomass [2]. Therefore, increasing the substrate level impacts increasing the growth of cell biomass from lactic acid bacteria.

A linear regression equation between 1/[s] and 1/μ is made based on the slope's value. The equation obtained is Y = 4.608x + 80.99. The graph of this equation can be seen in Figure 1.

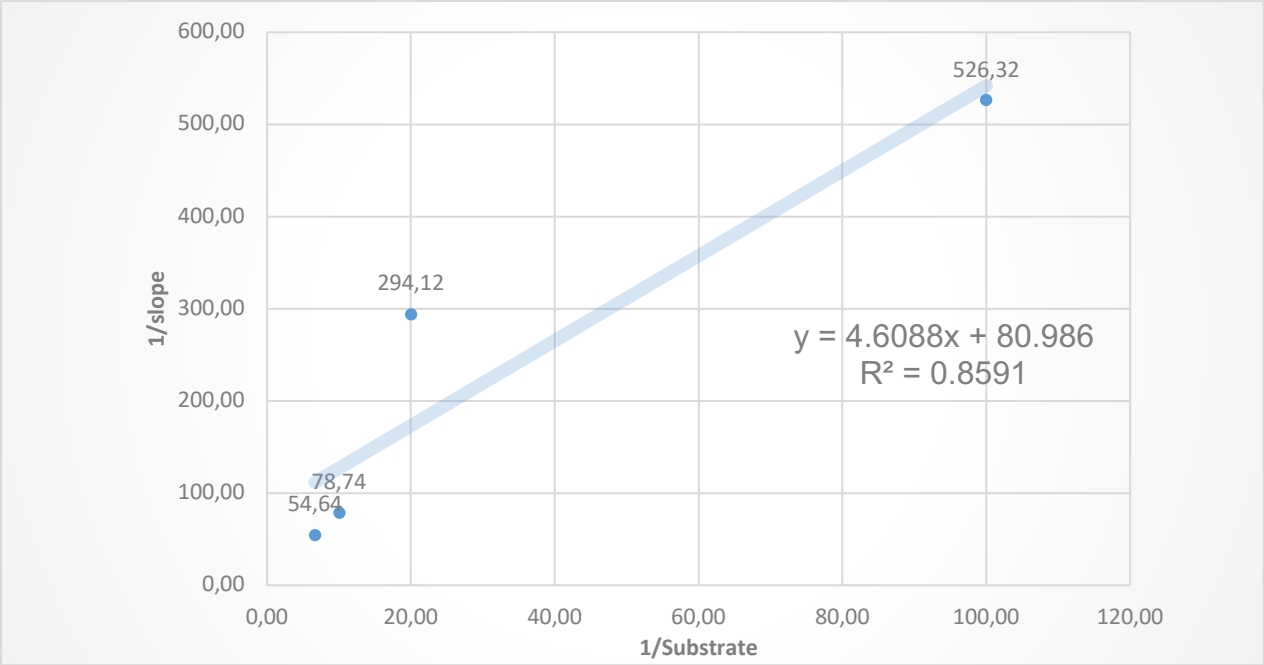

Figure 1. Curves for determining the values of Ks and μmaks

Based on the value of the curve equation, the value of Ks and μmaks can be determined. The Ks value obtained based on this equation is 0.0125 per hour with a Ks value of 0.0569 gram/100 ml. The Ks and μmax values of Lactobacillus plantarum FNCC 250 were 0.04 g/L and 0.17 per hour, respectively, whereas in Lactobacillus sp [4]. FNCC 401 has Ks and μmax values of 0.06 g/L and 0.26 per hour, respectively. Different values of the Ks and μmax values were possible in different isolates. Besides that, the different types of substrates will also produce different Ks and μmax values. It is possible because each enzyme produced by microbes works specifically on a substrate. The difference in Ks value is caused by the bacterial isolate characteristics, where Ks shows the relationship between affinity value and cell growth rate [5].

4. CONCLUSION

Proteolytic lactic acid bacteria grown in a defined medium with substrate-level variations of 0.01, 0.05, 0.1, and 0.5 have different growth responses at each substrate level, where there is an increase in the specific growth rate for each level. The Ks and μmax values obtained in the proteolytic lactic acid bacteria were 0.0569 gram/100 ml and 0.0125 per hour, respectively.

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