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Study of Kinetic Fermentation of Proteolytic Lactic Acid Bacteria Isolated from Colostrum of Dairy Cattle with Inulin Substrate

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ABSTRACT
This research aimed to determine the effect of the additional inulin level as a carbohydrates source for the fermentation kinetics of the proteolytic lactic acid bacteria (LAB Pro 4). The fermentation kinetics were observed using growth charts of lactic acid bacteria (LAB) or LAB Pro 4 with inulin substrate and derived to the equation Michaelis–Menten. Inulin is used as a source of carbohydrates because it contains high carbohydrate chemical elements. The inulin concentrations used to determine the Ks and μmax values of proteolytic four LAB were 0, 0.01, 0.05, 0.1, and 0.15%. Observations were made every hour for 24 hours of incubation until they reached the stationary phase. The results obtained were the Ks value of 0.02 gram/100 mL and the Vmax value of 0.0168 per hour.

Keywords: *Fermentation kinetics, Inulin, Lactobacillus*

1. INTRODUCTION

Fermentation is an effort to produce economic value sourced from raw materials rich in fiber or protein with processing assisted by microbes. Fermentation produced a product that was simpler and easier to digest than the original material. Through fermentation technology can degrade complex nutrients into simple ones so as to increase digestibility [3]

Fermentation uses the help of microbes that are often used, namely lactic acid bacteria (LAB). The LAB will produce lactic acid that resulted in a lower the pH during fermentation process. The LAB use substrates such as carbohydrates to produce lactic acid [10]. The characteristics of lactic acid bacteria, in general, are that their cells react positively to the Gram strain. Glucose fermentation will produce lactic acid. The type of fermentation of lactic acid bacteria includes heterofermentative, namely those whose fermentation results in addition to lactic acid there are other organic acids such as acetate, CO_2 gas, and ethanol.

Lactic acid bacteria are obtained from several isolations such as milk, colostrum, or humans or animals' digestive tract [7]. LAB are often used from the genus *Lactobacillus* and *Bifidobacterium*. Probiotics from LAB are often used in disease management and preservation technologies [10]

Synbiotics are a combination of probiotics and prebiotics to carry out functions for fermentation. Synbiotics are a combination of probiotics and prebiotics, which have a synergistic effect because prebiotics can increase probiotic bacteria's growth. Synbiotics have begun to be developed as health support for the digestive tract [1]

Prebiotics is food ingredients containing oligosaccharides that cannot be digested by the host but have a beneficial effect on the host by stimulating the host's growth microflora of the digestive tract [11]. The

growth of lactic acid bacteria requires a substrate that is commonly known as a prebiotic. Prebiotics can be carbohydrates that are difficult to digest in the digestive tract of animals, so they need to be hydrolyzed first.

Prebiotics are used for microbiota viability. Inulin is a source of prebiotics that are widely used as processed food products such as fermented milk. Inulin is obtained from several plants. Inulin is a food component that is difficult to hydrolyze by stomach acid but can stimulate the growth of probiotic bacteria. Inulin functions as a dietary fiber that is a carbohydrate group that cannot be hydrolyzed by human body enzymes but is fermented by the intestinal microflora to affect intestinal function [8] Inulin is a natural polymer of a carbohydrate group with a monomer, namely fructose, whose number on one polymer strand varies. The inulin fermentation product by Lactobacilli produces short-chain fatty acids (SCFA), which contain acetate, butyrate, and propionate [1].

Fermentation kinetics is an enzymatic reaction for the formation of products by fermentation bacteria in the fermentation process. Fermentation kinetics study is useful for understanding the fermentation process that can take place. Fermentation kinetics elements could run if they can estimate the substrate's amount needed to the optimal [5]. The critical element in the Michaelis - Menten equation is Ks, which is specific for a particular enzyme with a specific substrate at certain pH and temperature conditions. The Ks value can estimate the amount of substrate needed so that fermentation by LAB can run optimally.

2. MATERIALS AND METHOD

2.1. Material

The equipment used are incubator or oven, water bath, hot pan, and magnetic stirrer, pH meter, funnel, filter paper, thermometers, refrigerators, centrifuges, tube centrifuges, autoclave, analytical balance, vortex, laminar airflow, test tubes, spectrophotometers, micropipette, tip, Erlenmeyer tube, hangetube, tube rack, stirrer, beaker, bunsen, and blender.

The materials used included proteolytic LAB Pro 4. Extraction materials for substrate sources were inulin and aquadest. The chemicals and growth media for bacteria are deMann Rogosa Sharp (MRS) broth, Nutrient Broth (NB), aquades, and yeast extract. The ingredients for the determination of total carbohydrates are Anthrone and H_2SO_4 reagents. The ingredients for the determination of lactic acid levels are 10% trichloroacetic acid (TCA), 20% $CuSO_4$, 4% $CuSO_4$, H_2SO_4, p-hydroxybiphenyl solution, and aquades.

2.2. Methods

2.3.1. *Growth Lactic Acid Bacteria*

2.3.1.1. Enrichment of Lactic Acid Bacteria

The enrichment of LAB Pro 4 isolates were conducted by planting in sterile MRS broth. The number of isolates planted was 10% of the total MRS planting medium. After planting, they were incubated in an oven at 37°C for 24 hours.

2.3.2. *Liquid Fermentation in Defined Medium*

The growth of lactic acid bacteria was defined as a medium with an inulin substrate. LAB Pro 4 that had been grown on a defined medium were added with inulin as the substrate. The inulin concentrations used were 0; 0.01; 0.05; 0.10 and 0.15%. Then observed the growth of bacteria using a spectrophotometer with a wavelength of 600 nm every hour until the stationary phase. The results obtained are recorded.

The resulting value at incubation was changed using logarithmic calculations to obtain growth results with the directional coefficient's value. The bacterial growth rate is measured by connecting 1/S and 1/μ so that the new equation y = bx + a is obtained. This equation can be used to calculate the value of Ks and μmax (Robinson dan Tiedje, 1983):

$$\frac{1}{\mu} = y \text{ dan } \frac{1}{s} = x,\ a = \frac{1}{\mu_{max}} \text{ dan } b = \frac{K_s}{\mu_{max}}$$

where μ is bacteria substrate coefficient, s is the substrate concentration, μ_{max} is the maximum growth rate of bacteria, and K_s is the substrate concentration of μ (half of μ_{max}).

3. RESULT AND DISCUSSION

3.1. Enzymatic Kinetic Reaction

Inulin is a probiotic substrate that contains Fructooligosaccharides (FOS). The FOS is a carbohydrate that cannot be digested in the digestive tract, so lactic acid bacteria must degrade it. FOS can be used as a probiotic [8].. The determination of fermentation kinetics used data from the growth of LAB Pro 4 with inulin as a substrate and on a limited medium.

The inulin concentration at the time of observation used 0%; 0.01%; 0.05%; 0.10%; and 0.15% and with a LAB Pro 4 concentration of 1% in each tube. Observations were carried out for 24 hours using a spectrophotometer with a wavelength of 600 nm. The relationship between the optical density (OD) absorbance value and the incubation time can be seen in Figure 1.

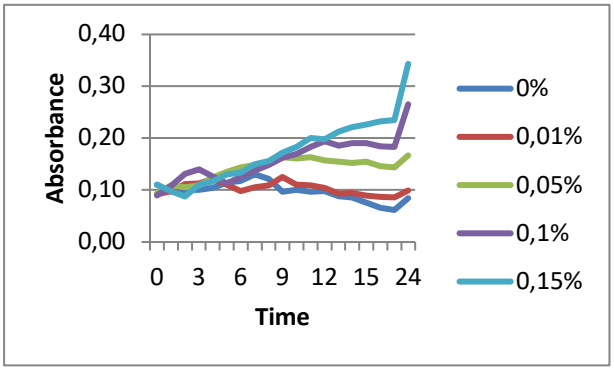

Figure 1. Relationship between optical density (absorbance) and incubation time

Figure 1 shows the growth of LAB Pro 4 with different inulin concentrations. It is because lactic acid bacteria will grow faster with higher levels of the substrate given. It was found that 0.15% of inulin levels had faster growth, followed by 0.10% inulin levels. Higher the inulin levels in fermentation by *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*, the faster the growth rate. The determination of the reaction rate (v) obtained is the initial velocity associated with the enzyme's substrate hydrolysis. The product formed is related to the hydrolysis time in the enzyme log phase [8]. The graph between 1/slope and 1/level is presented in Figure 2.

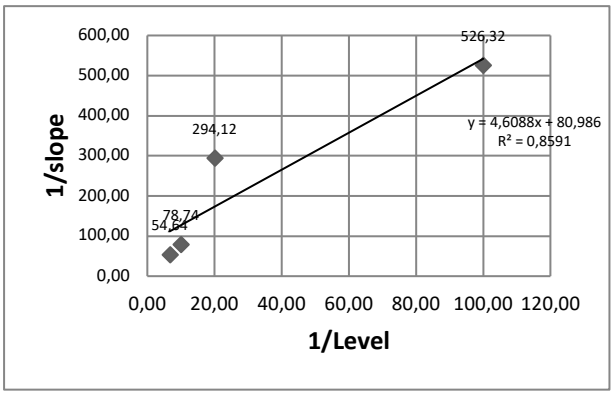

Figure 2. The relationship between the slope of the growth to the concentration of inulin levels.

Figure 2 shows the relationship between 1/S and 1/q. The regression equation obtained in the graph is Y = 1.3735X + 59.465. Based on the regression equation obtained, the Ks value for the growth of LAB Pro 4 with inulin as a source of carbohydrates or prebiotics is 0.2 grams/100mL. In the other experience, Ks value on the inulin A1-KG substrate with mesophilic bacteria dahlia tuber rhizosphere is 0.0312 g/100mL. The smaller the Ks value obtained, the less substrate needs to reach the maximum point of growth. Also, the resulting qmax value of 5.8×10^{-3}/hour [2]. The specific growth rate (μ) value at 12 to 30 hours was 0.0081 hours^{-1}. The experiment was obtained from inulin dahlia tuber flour with yeast isolate DUCC Y-015, one of the inulinase-producing microbes [4].

4. CONCLUSION

Based on research conducted using inulin as a fermentation substrate of LAB Pro 4, the Ks value was 0.2 gram/100mL, and qmax was 2.8×10^{-3}/hour. It is necessary to develop the Ks value obtained in various feed ingredients as a fermentation method.

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