Fermentation Kinetics of Isolate Lactic Acid Bacteria Probiotic BR 12 and BR 17 were Isolated from Broiler Chicken Meat

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Abstract. The purpose of this study was to determine the Lineweaver-Burk equation, maximum speed (μ_{max}) and maximum specific / per hour growth rate Ks (constant saturation) of probiotic lactic acid isolates BR 12 and BR 17 with limited sucrose. The material in this study was probiotic isolates of Lactic Acid Bacteria (LAB) BR 12 and BR 17 which were isolated from broiler chicken meat, determined media, and sucrose. The method used was growed probiotic LAB isolates in the media determined with a sukora concentration that wasdifferent from 0-1%. The results showed that the Lineweaver-Burk equation of the LAB probiotic BR 12 was y = 3.503x + 10.10. the maximum speed (μ_{max}) LAB probiotic BR 12 was0.0075 hour-1 and the constant Michaelis-Menten (KS) was 0.264g / 100 ml. Lineweaver-Burk equation of LAB probiotic BR 17 was y = 2.809x + 11.40. The maximum speed (μmaks) of LAB probiotics BR 17 was 0.088 / hour and the Michaelis-Menten constant (Km) = 0.2464 g / 100ml. The conclusion of this study was that the Lineweaver-Burk equation BR 12 was different from BR 17. The value of μ_{max} and Ks BR 17 was lower than BR 12.

Keywords: Fermentation Kinetics, Lactobacillus Fermentum BR 17, Broiler Chicken Meat.

1. Introduction

Lactic Acid Bacteria (LAB) were Gram positive, non-spore, negative catalytic, anaerobic, microaerophilic or aero-tolerant and they produce lactic acid as the main product of fermentation [1]. Lactic acid bacteria can grow in plants [2], fish [3] and animals [4]. Lactic Acid Bacteria (LAB) wasone organism that ferments food ingredients through carbohydrate fermentation and generally produces large amounts of lactic acid. These bacteria contribute significantly to the increase in taste and texture of fermented products [5]. Isolates of BR 12 and BR 17 were probiotic LAB isolated from broiler chicken meat. Isolates isolated from meat can be used as starters in meat fermentation [6]. Fermented meat, which wasa fermented sausage, usually uses sucrose as an energy source for LAB [7]. One thing that wasneeded so that the enzymatic reaction can run efficiently wasto estimate the amount of substrate needed [8]. So, it wasimportant to know the need for sucrose as an energy source for the growth of probiotic LAB isolates BR 12 and BR 17.

The success of the fermentation process wasstrongly influenced by the success in optimizing the desired microbial growth factors. These factors will provide different conditions for each microbe according to their respective environment so that it affects the

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fermentation kinetics [9]. Fermentation kinetics can provide information about the speed of cell biomass production and environmental influences on growth rates [10]. Fermentation kinetics in used substrate can be measured used the Michealis-Menten equation [11].

$$v = \frac{d[P]}{d} \quad (1)$$

The kinetics of bacterial growth can also be calculated used a monod equation which was an analogy of the Micheais-Menten equation used enzyme kinetics [12]. The Monod equation was often used to describe the relationship between growth and substrate concentration. The monod equation model is:

$$\mu = \mu max \frac{S}{Ks + S} \quad (2)$$

The value of μ shows the specific value of the growth rate, μ_{max} was the maximum speed, S was the concentration of the substrate given. The value of K_s was the level of S equal to μ half μmak, the value of K_s in the monod equation was an analogy of K_m in the Michealis-Menten equation model. The K_s value reflects the proportional relationship that bacteria make in used the substrate for growth [13].

The study of the growth kinetics of microbial culture can be used to estimate the efficiency of production costs in large scale [9]. V_{max} lipase enzyme with coconut oil substrate of 2.11 × 10-3 mmol / minute, palm oil substrate 2.30 × 10-3 mmol / minute on and olive oil substrate 1.60 × 10-3 mmol / minute on [8]. The pancreatic lipase K_m value obtained was 1.21 × 104 ppm in coconut oil; 2.29 × 104 ppm on palm oil; and 1.60 × 104 ppm in olive oil. The cellulase kinetics of Actinomycetes acp-7 isolates in solid rice straw media obtained μ_{max} by 31.25 g per K at 74.34 g [14]. The lactic acid bacterial fermentation of t5 isolates derived from tempoyak obtained μ_{max} by 0.0598 (hour-1) while the K_s value was 1.2236g / g [9].

The purpose of this study was to find out μ_{max} (maximum specific growth rate / per hour) and isolate K_s (constant saturation) LAB probiotic BR 12 and BR 17 with limited sucrose.

2. Materials and Methods

Liquid fermentation isolates from BR 12 and BR 17 used limited sucrose medium. LAB isolates that have been grown with medium defined in hungate tubes [15]. The difined medium consists of

1. Mineral solution I :
   KH_2HPO_4 = 0.101
2. Mineral solution II :
   KH_2HPO_4 = 0.101
   $(NH_4)_2SO_4$ = 0.1542
   $MgSO_4.7H_20$ = 0.031611
   $CaCl_2$ = 0.0204315
   NaCl = 0.1542

The composition of a liquid medium defined:

1. 25.7 ml mineral solution I
2. 25.7 ml mineral solution II
3. 0.34 g yeast extract
4. 119.17 ml of aqudest

5. sucrose (according to the level used)

The number of bacteria used in planting in this medium was as much as 10% of the new planting medium. Sucrose as a source of energy for the growth ofisolates LAB probiotic BR 12 and BR 17 with different concentrations of 0.0; 0.1; 0.2; 0.4; 0.6; 0.8; 1%. The bacteria were then incubated at 37ºC. Each treatment was repeated 3 times. The isolates of LAB probiotic BR 12 and BR 17 were observed for growth by measured optic density (OD) on a spectrophotometer with a wavelength of 600nm. Observation was carried out every hour until it reaches the stationary phase.

Determination of the maximum rate (μ_{max}) and the Michaelis-Menten constant (Km) was done used the Lineweaver-Burk curve by graphing the relationship between (1/v) as the Y axis against (1 / S) as the X axis. The data obtained was made regression linear so that the linear line equation was obtained. The slope of linear regression was included in the Lineweaver-Burk equation, namely y = ax + b to get the maximum rate (μ_{max}) and the Michaelis-Menten (km) constant correctly. μ_{max} was obtained from 1/b while K_m was obtained from $\mu_{max} \times$ ax.

3. Results and Discussion

a. Growth curve

Liquid fermentation kinetics of LAB probiotics BR 12 and BR 17 used limited sucrose media were carried out to determine growth kinetics by determined the values of Ks and μmax. The growth curve of probiotic BR 12 and BR 17 LAB isolates with sucrose energy sources was shown in Figure 1. and Figure 2. LAB growth was influenced by nutrition. The higher the nutrients available, the higher the growth of BAL isolates, and the longer the stationary phase. Lactic acid bacteria use carbohydrates for their growth and produce the main products of lactic acid [16]. an increase in lactic acid during fermentation was associated with an increase in the value of optical density from LAB growth. Carbohydrate fermentation was caused by enzymatic hydrolysis of bacteria [17].

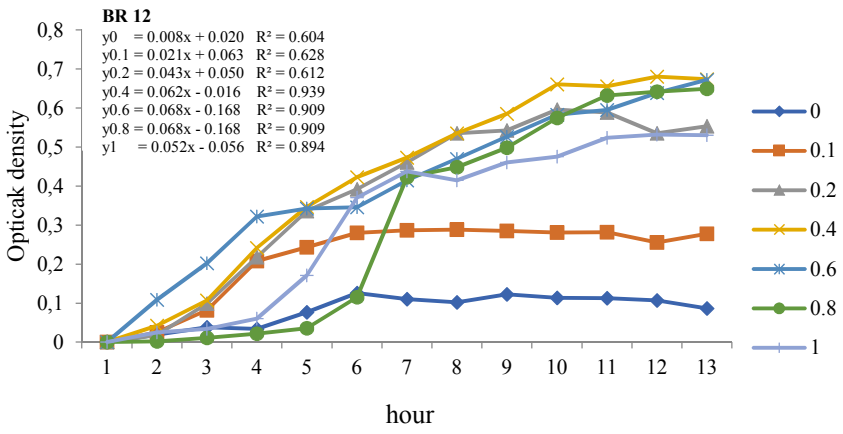

Fig. 1. Graph of growth of BR 12 LAB isolates in liquid form at limited sucrose concentrations (0; 0.1; 0.2; 0.4; 0.6; 0.8; 1).

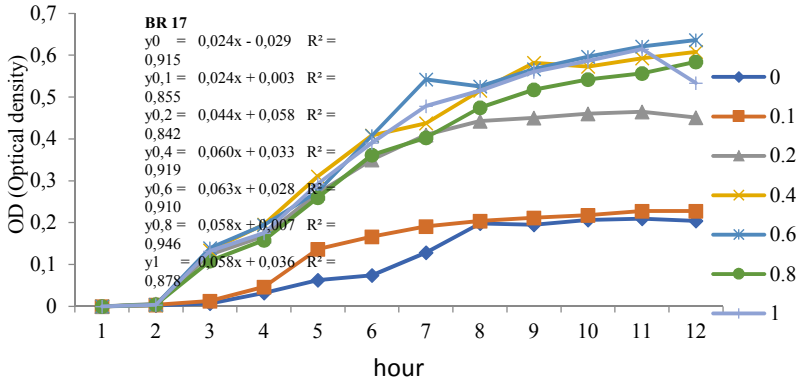

Fig. 2. Graph of growth of BR 17 LAB isolates in liquid form at limited sucrose concentrations (0; 0.1; 0.2; 0.4; 0.6; 0.8; 1).

b. The value of μ_{max} and Ks

The kinetic growth of LAB BR 12 and BR 17 was used to measure the reaction rate (Table 1). in table 1 shows that the higher the substrate level, the higher the velocity μ in the BR 1 2 and BR 17 LAB, because high energy reserves will accelerate growth. monod equation can be obtained Ks and μmax values. The values of Ks and μmax obtained can show the efficiency of media use by LAB probiotics BR 12 and BR 17.

Monod equations describe an increase in μ when substrate concentration increases and slows down to the maximum specific growth value (μmax). When the substrate concentration in the condition μ was equal to half μ_{max}, then the saturated concentration of half saturation can be known. Ks value was an important element in the biodegradation process because Ks shows the relationship between affinity value and bacterial cell growth rate [18]. The relationship between 1 / [S] and 1 / [μ] LAB probiotics BR 12 and BR 17 isolates used limited sucrose was illustrated by the Lineweaver-Burk curve, this can be seen in Figure 3.

Table 1. Reaction rates of isolates BR 12 and BR 17

Time (hours)	Sucrose	Isolate LAB BR 12			Isolate LAB BR 17		
		μ	1/[S]	1/ μ (hours^{-1})	μ	1/[S]	1/ μ (hours^{-1})
13	0,0	0.008			0,023		43,47
13	0,1	0.021	10,00	47.61905	0,024	10,00	41,67
13	0,2	0.043	5,00	23.25581	0,047	5,00	21,27
13	0,4	0.062	2.50	16.12903	0,061	2.50	16,39
13	0,6	0.068	1.67	14.70588	0,064	1.67	15,62
13	0,8	0.068	1.25	14.70588	0,059	1.25	16,95
13	1,0	0.052	1,00	19.23077	0,060	1,00	16,67

Based on the graph in Figure 3, the BR 12 equation, $y = 3.503x + 10.10$, $R^2 = 0.918$ and BR 17 were $y = 2.809 x + 11.40$, $R^2 = 0.928$, the maximum value of LAB isolates from BR 12 probiotic isolates was 0.099/ hour and BR 17 was 0.088 / hour. Specific speed of probiotics LAB BR 17 was higher than probiotics LAB BR 12. This speed difference was caused by genetic variation. Constant Michaelis-Menten (Ks) BR 12 was 0.34 g / 100ml and

BR 17 was 0.246 g / 100 ml. The Ks value in this study was still below Ks LAB. The Ks value of LAB ranges from 0.3 - 4.5 g / 100 ml [19]. The Ks value indicates that BR 12 requires sucrose of 0.099 g in every 100 ml of media to produce optimal bacterial growth rates, whereas BR 17 requires sucrose 0.246 g. BR 17 isolates require fewer sucrose substrates compared to BR 12, this was due to genetic variation. The results of this study indicate that the growth rates of BR 12 and BR 17 on the sucrose substrate were low. this was because sucrose was disaccharide so its use must be broken down into monosaccharide [20].

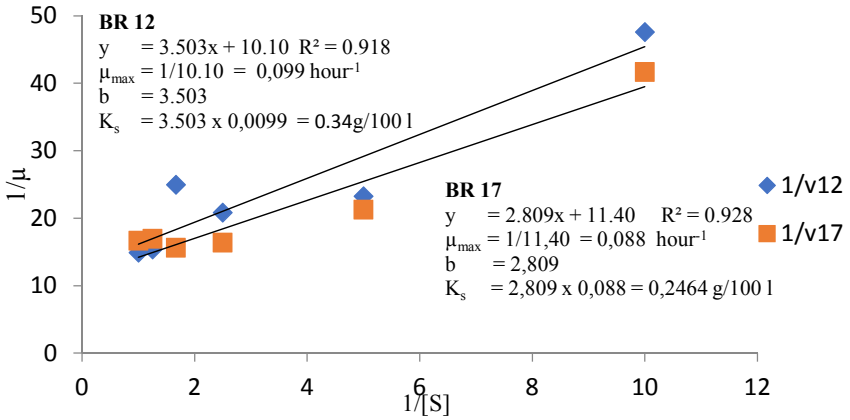

Fig. 3. Lineweaver-Burk curve between 1 / [S] and 1 / [μ] LAB probioticBR 12 and BR 17 used limited sucrose.

4. Conclution and Suggestion

a. Conclusion

The conclusion of this study were that the LAB BR 12 and BR 17 fermentation kinetics isolated from chicken showed Lineweaver-Burk equation BR 12 different BR 17. The value of μ_{max} BR 17 was lower than BR 12, and Ks BR 17 was lower than BR 12.

b. Suggestion

More research was needed to determine the specific characteristics of isolates BR 12 and BR 17 which were seen in terms of growth kinetics.

5. Conclusion

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