Production of starter culture to produce biomass of probiotic indigenous lactic acid bacteria

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Abstract. The purpose of this study was to compare the mixture of three strains probiotic lactic acid bacteria (LAB) that would be produced into strarter cultures. The bacteria consisted of: *Lactobacillus murinus* Ar-3, *Streptococcus thermophilus* Kp-2, and *Pediococcus acidilactici* Kd-6. The study was conducted by observing the growth of LAB in the glucose yeast peptone medium for 24 hours. The variables observed were the generation time of three strains of LAB. Data from the research result analyzed using regression test to see the relationship between incubation time and optical density value of each bacterium. The result showed that the generation time of each strain was *Lactobacillus. murinus* Ar-3 was 3.25 ± 0.006 hours, *Streptococcus thermophilus* Kp-2 3.68 ± 0.1 hours, and *Pediococcus. acidilactici* Kd6 3.74 ± 0.04 hours. The results of the regression analysis showed that the comparison of *L. murinus* Ar-3, *S. thermophilus* Kp-2, *P. acidilactici* Kd6 in starter culture production was found to be 1,00: 1,18: 1,16 v / v of each strain probiotics. It could be concluded that the generation time of three strains of probiotic bacteria is different.

1. Introduction

Probiotics are living microorganisms, which upon ingestion in certain numbers exert various health benefits beyond the inherent basic nutrition [1; 2; 3]. It is recommended that probiotic products contain at least 10^7 cfu/g living microorganisms [4; 5]. Probiotics products usually incorporate intestinal species of *Lactobacillus* because of their long tradition of safe use in the dairy industry as well as the fact that some strains of lactic acid bacteria (LAB) are capable of exerting their beneficial effects by balancing the intestinal flora and eventually competing with pathogens for gut colonization [6].

The nutritional requirements for *Lactobacilli* to grow include carbohydrates, peptides, fatty acid esters, salts and nucleic acid derivatives. It has limited biosynthetic abilities and therefore vitamins and amino acids are often added to the medium [7; 8). Nevertheless, the possibility of obtaining high biomass from a low cost medium is very challenging due to the production of lactic acid concomitant with the cell growth [9; 10, 11; 12]. For industrial applications, Lactobacillus bacteria are not simple to grow in large scale bioreactors, therefore high cell density cultivations are more important as their biomass is becoming very valuable (13). This research was aimed to know the generation time of three strain LAB probiotic to produce biomass of starter culture.

2. Material and Methods

2.1. *Material.*

2.2. Three indigenous lactic acid bacteria namely *Lactobacillus murinus* Ar-3, *Streptococcus termophilus* Kp-2, and *Pediococcus acidilactici* Kd-6.

2.3. Methods

2.3.1. General. Inoculums preparation. The preparation of inoculums started with transferring the stock culture into a liquid Glucose Yeast Peptone (GYP) media. After the growth of culture, the microorganisms were transferred to a plate of solid GYP medium. The plate was incubated at 37°C for 48 h in order to allow sufficient growth of colonies.

The grown colonies were either used to initiate a fermentation process or were stored back at 4°C as stock culture which can be prepared by culturing the colonies in slant agar followed by adding 30% of sterilized glycerol. The LAB inoculums were prepared by inoculating a single colony of them into 10 mL broth media which was then incubated at 37°C for 24 h. One milliliter of inoculums was transferred into bijou bottle containing 9 mL media. Cultures were incubated for 10 h at 37°C before being transferred into flask.

Sample in flasks were taken by using aseptic technique for every 30 min by flaming the cap swabbed with 70% ethanol. Twelve milliliter of sample was transferred into a bijou bottle, which was then being divided for measuring optical density (OD, A660 nm). The flasks then were transferred back to the incubator to continue the fermentation process.

2.3.2. Statistic. To observe the generation time, a regression analysis was made which showed the relationship between the optical density (OD) and the time of fermentation and carried out the calculation of each parameter of fermentation kinetics [14].

3. Results and Discussion

The observation of optical density (OD) and growth curves showed that *Lactobacillus murinus* Ar-3, *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 experienced an adaptation phase in GYP media for 2 hours, namely at the 0 hour incubation time to 2 hour. Microbes that were inoculated into a medium, will first experience adaptation to adapt in new environmental conditions [15]. The phase lag occurs briefly because the bacteria has previously undergone refreshment on the same media as those used for growth so the process of adaptation to the media is rapid. *Lactobacillus fermentum* and *Lactobacillus plantarum* undergo a 2-hour adaptation phase on GYP media [16].

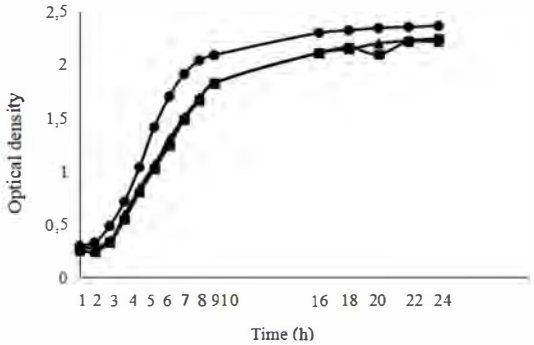

Figure 1. The growth of lactic acid probiotic (●) *Lactobacillus murinus* Ar-3, (■) *Streptococcus thermophilus* Kp-2 and (▲) *Pediococcus acidilactici* Kd-6 in *glucose yeast peptone broth* (GYP) medium.

The logarithmic phase of *Lactobacillus murinus* Ar-3 was reached at the second to ninth hour. Whereas *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 reached log phase peaks at 8 hour. Cell counts at 8 hour Ar-3 7.99 x 10^7 cfu / ml, Kp-2 7.98 x 10^7 cfu / ml, 7.93 x 10^7 cfu / ml.

The logarithmic phase is the cell experiencing division until the maximum number of cells is reached. The logarithmic phase is characterized by the line of exponents on the growth curve [17].

At 12h to 24 h, a stationary phase is marked by a fixed growth curve line. In the stationary phase the number of cell populations remains because the number of cells dividing is equal to the number of cells that die. The stationary phase describes the accumulation of metabolites resulting from cell metabolic activity and the nutrient content begins to run out, consequently there is nutrient competition so that some cells die and others grow, so the number of bacteria is relatively constant.

The generation time was determined using the growth value in the logarithmic phase [18]. With OD660 logarithmic phase data, linear regression was made. The x-axis shows the incubation time at 37 °C (hour) and the y-axis shows the number of bacteria (OD660 value). Based on the regression calculation in the logarithmic phase, different incubation times were obtained between the three probiotic strains of LAB. Linear regression analysis of *Lactobacillus murinus* Ar-3 isolates, *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd6 obtained successive regression formulas y = 0.2455x + 0.0699; y = 0.21179x-0.0271 and y = 0.2173x-0.0832 (Figure 2).

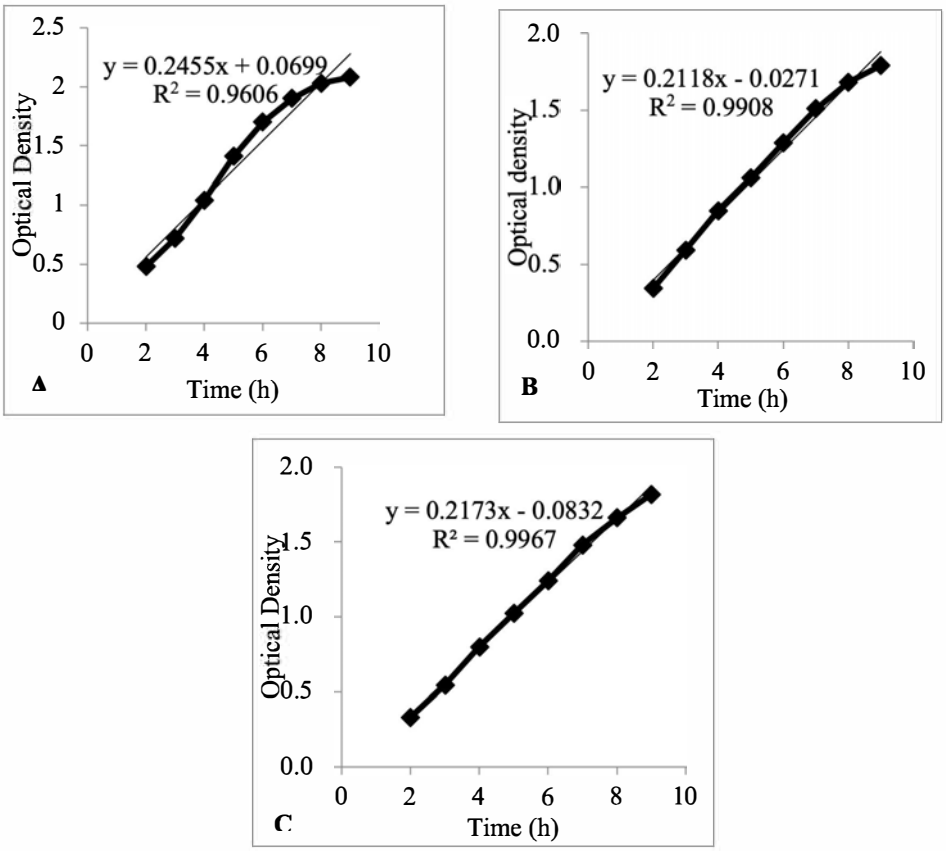

Figure 2. Regression of the logarithmic phase of probiotic bacteria: A. *Lactobacillus murinus* Ar-3. B. *Streptococcus thermophilus* Kp-2 and C. *Pediococcus acidilactici* Kd-6

Lactobacillus murinus Ar-3 has a generation time of 3.25 ± 0.006 hours, *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 of 3.68 ± 0.1, and 3.74 ± 0.04 hours. The generation time of *Lactobacillus murinus* Ar-3 was faster than that of *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 ($P < 0.05$). Based on the generation time of *Lactobacillus murinus* Ar-3, *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 the ratio of the composition of the mixture is 1: 1.18: 1.6 v/v of each strain probiotics. The Comparative value is used to mix three bacterial strains when

producing starter culture biomass. Once calculated the time of generation is obtained as presented in Table 1.

Table 1. Generation time of probiotic lactic acid bacteria in PGY medium

	L. murinus Ar-3	*P. acidilactici* Kd-6	S. *thermopillus* Kp-2
Generation time	3,25	3,56	3,72
	3,26	3,77	3,78
	3,25	3,71	3,71
Average	3,25±0,006[b]	3,68±0,1[a]	3,74±0,04[a]

4. Conclusion

Based on the results of the study it can be concluded that the generation time of three strains of probiotic bacteria is different. So that the exact ratio in mixing *Lactobacillus murinus* Ar-3, *Streptococcus thermophilus* Kp-2, *Pediococcus acidilactici* Kd-6 for the production of starter cultures was 1: 1.18: 1.16 in v/v of each probiotics culture.

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