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The Growth Curve and Total Colonies Number of Lactobacillus spp on the Pink Guava (Psidium guajava Linnaeus) Juice Medium

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***Abstract*—Probiotics are bacteria that are beneficial to human health. Probiotic drinks from cow milk medium use carbohydrates, vitamins, and minerals for its fermentation process. Pink guava fruit has a higher content of fructose, glucose, sucrose, vitamins (B1-B3) and phosphorus and is non-cholesterol than cow milk. This study aims to find out the total number of colonies and an overview of the growth curve of Lactobacillus spp cultured in the medium of pink guava juice (Psidium guajava Linnaeus) in vitro and then compared with the Indonesian national standard for probiotic drinks (107 CFU/ml). The growth of Lactobacillus spp in pink guava medium observations every 12 hours for 60 hours. The method used for calculating the number of colonies is the TPC (Total Plate Count) method then described in the form of a growth curve. The results showed the growth curve Lactobacillus spp in the media of pink guava juice from the log phase until the death phase starting at the 12th hour, 24, 36, 48 to 60 with TPC amounts of 21.5x1010CFU/ml, 49x1010CFU/ml, 49.5x1010CFU/ml, 25.5 x1010CFU/ml and 13x1010 CFU/ml respectively. The stationary phase on the curve begins at the 24th to the 36th hour. The conclusion of this research is that the pink guava juice media can be an excellent growth medium for Lactobacillus spp the conclusion of this research is that the pink guava juice media can be an excellent growth medium for Lactobacillus spp.**

Keywords—colony number, growth curve, lactobacillus spp, pink guava juice

I. INTRODUCTION

People usually use cow's milk as a growth medium for probiotic bacteria [1]. One type of probiotic bacteria is. Lactobacillus spp. These bacteria use lactose-type carbohydrates in cow's milk as the main source of nutrients for metabolism. Lactose is a disaccharide so it must be broken down into monosaccharides (glucose and galactose) assisted by vitamins and minerals as cofactors and coenzymes in order to be used in the glycolysis process [2]. Lack of cow's milk media in addition to the content of vitamins and minerals in small amounts, also fat content in the form of very high cholesterol [3]. So, to overcome the shortage in the medium of cow's milk, it is necessary to look for other alternative media for growth media Lactobacillus spp.

Guava fruit is a fruit that has a content of carbohydrates, fibre, minerals (phosphorus) and vitamins (B1-B3) that exceed cow's milk. In addition, guava fruit is non-cholesterol and non-lactose compared to cow's milk. Guava also contains a lot of vitamin C and tannins that are useful to facilitate the digestive system and blood circulation. Guava is easy to get because it bears fruit all year round in the tropics and the price is much cheaper compared to cow's milk. Carbohydrates contained in guava are monosaccharides in the form of fructose and glucose, as well as disaccharides in the form of sucrose [4]. Based on the carbohydrate content, the guava can act as an alternative medium of growth Lactobacillus spp.

II. METHODS

This research is a descriptive research conducted in the laboratory in vitro against Lactobacillus spp using the medium of pink guava juice (Psidium guajava L.). The research was conducted in the Laboratory of Microbiology Faculty of Medicine UNJANI. This study aims to create a growth curve and calculate the total number of colonies of Lactobacillus spp on the medium of pink guava juice (Psidium guajava L.). Observations are made every 12 hours for 60 hours, the number of colonies is calculated then compared to the SNI standard, which is a minimum of 10^7 CFU/ml [5].

A. *Lactobacillus Spp Suspension Manufacturing and Culture Procedure*

Colonies of bacteria grown in MRSA media are incubated for 48 hours at 37ºC. Bacteria are then mixed with a 0.9% sterile NaCl solution until turbidity is obtained in accordance with McFarland standard 0.5 (10^8 CFU/ml bacteria) [6]. Transfer Lactobacillus spp bacterial inoculum in TSB media into 5 test tubes containing 10 ml each, then incubation with a temperature of 37ºC [6]. The appropriate observation time of 12, 24, 36, 48 and 60 hours.

B. *Procedure for Making Pink Guava Juice Media*

The old, fresh, and cooked pink guava is washed thoroughly. Guava cut into pieces, added water with water ratio: guava = 3:1. Stir until evenly then filtered with a filter cloth [7]. The filtering result is inserted into 5 test tubes of 10 ml each and then inserted into the autoclave for sterilization at a temperature of 121 ºC 15 minutes for 2 hours.

C. *Growth Curve*

The method used in the creation of the growth curve is the plate count. Data retrieval is done every 12 hours for 60 hours. The calculation of colonies was carried out by making a dilution series in NaCl of 0.9%. dilution 10^{-1}-10^{-11}. One ml of each dilution is put in a sterile petri dish then poured 10 ml of MRSA media [6].

Bacterial culture is done duplo, then incubated at a temperature of 37ºC for 24 hours. Growing colonies are counted. The data included in the calculation are colonies numbering 30-300 colonies. After the chart is created, the X axis declares the time and the Y axis declares the log number of cells. The time when bacteria achieve the highest activity in the medium of pink guava juice can be determined from this chart [6,8].

III. Results and Discussion

A. *Total Plate Count (TPC) Results*

The calculation of the number of bacterial colonies in this study was conducted using dilution techniques and then calculated by TPC method [6]. The results of counting the number of Lactobacillus spp colonies grown in the medium of pink guava juice (Psidium guajava Linnaeus) can be seen in Table 2.

TABLE I. TPC Lactobacillus Spp on the Medium of Pink Guava Juice (Psidium Guajava Linnaeus)

Hour to	TPC (CFU/ml)						
12	99,5 x10^5	87 x10^6	71 x10^7	63,5 x10^8	41 x10^9	21,5 x10^{10}	16 x10^{11}
24	148,5 x10^5	128 x10^6	109 x10^7	93,5 x10^8	69,5 x10^9	49 x10^{10}	29 x10^{11}
36	149 x10^5	128,5 x10^6	109 x10^7	94,5 x10^8	69,5 x10^9	49,5 x10^{10}	29 x10^{11}
48	129 x10^5	99,5 x10^6	81 x10^7	60 x10^8	47 x10^9	25,5 x10^{10}	9,5 x10^{11}
60	87 x10^5	68,5 x10^6	50 x10^7	45 x10^8	21 x10^9	13 x10^{10}	3,5 x10^{11}

Based on the observations in Table 2, Lactobacillus spp bacteria can grow on the medium of pink guava juice (Psidium guajava Linnaeus). Guava has a substrate that can be used for the growth of Lactobacillus spp bacteria, namely simple carbohydrates monosaccharides (glucose and fructose) and types of disaccharides (sucrose) and complex carbohydrates in the form of fibre for their energy source [9]. In addition, guava has a lot of vitamin B1-B3 content as well as phosphorus needed to help the metabolic process of Lactobacillus spp which acts as a cofactor of complex enzymes that catalyse the formation of pyruvate acid, lactic acid, and energy [10,11].

B. *Lactobacillus spp Growth Curve in Pink Guava Sari Media (Psidium guajava Linnaeus)*

Based on the calculation of TPC Lactobacillus spp that grows on the medium of pink guava juice (Psidium guajava Linnaeus), obtained growth curve as in Figure 1.

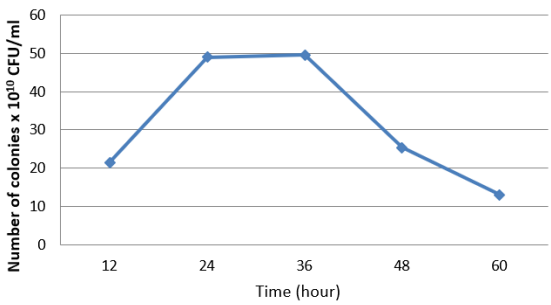

Fig. 1. Growth curve of Lactobacillus spp in the medium of pink guava juice (Psidium guajava Linnaeus) in incubation temperature 37ºC.

According to Figure 1, there is no lag phase or adaptation phase. This suggests that Lactobacillus spp bacteria have adapted during the rejuvenation process in the MRSA medium and have actively synthesized enzymes necessary for metabolism [12].

The log phase starts at the 0th hour until less than the 24th hour. In this phase Lactobacillus spp mostly uses simple carbohydrates in the medium of guava juice, namely glucose, fructose, and sucrose as a source of energy for cell division compared to other phases. Lactobacillus spp colonies that grow

at the 12th hour amounting to 21.5 x 10^{10} CFU/ml have exceeded the minimum TPC limit of probiotic drinks which is 10^7 CFU/ml [13,14].

In the 24th to 36th hours, bacteria begin to enter the stationary phase of the phase at which bacteria reach the maximum population with a colony count of 49.5 x 10^{10} CFU/ml. In this phase, the amount of nutrients has decreased, competition between bacteria is increasing and there has been an accumulation of metabolite products namely lactic acid that causes low media pH and is no longer suitable for its growth, so that its growth is inhibited. In this phase cell division is still ongoing but the number of cells that are still able to divide (living cells) is proportional to the number of dead cells [15].

By the time, the amount of nutrients especially simple carbohydrates has been reduced even depleted, then the new bacteria will use a more complex source of carbohydrates. This shift in the use of carbon sources is usually characterized by slow growth or even death, as bacteria take time to activate the genes needed to synthesize enzymes that can turn complex carbon sources into simpler forms [15].

In the medium that has been commonly used for probiotic drinks, namely cow's milk, TPC Lactobacillus spp obtained in the stationary phase (24-48 hours) amounted to 10^9-10^{11} CFU/ml. Research previously conducted by Melia Nurmalita in 2006 in the medium of white guava juice fermented by bal group, obtained by TPC during the stationary phase amounted to 72 x 10^6 CFU / ml [16]. Similarly, in research conducted by Sri Mutiar in 2011 on dragon fruit juice fermented by Lactobacillus spp, TPC obtained during the stationary phase (24th-36th hour) amounted to 56.80 x 10^6 CFU/ml. Judging from the results of these three studies, it can be concluded that pink guava juice can be an alternative probiotic drink [17].

Based on Figure 6 the phase of death begins to occur after the 36th hour which is characterized by a fairly drastic decrease in the number of cells. In this phase there is cell death in addition to the depletion of nutrients also due to environmental conditions that do not support the growth of bacteria, such as low media pH caused by the accumulation of metabolite products namely lactic acid [13,15].

Too acidic pH media will decrease the activity of hexokinase enzymes and inhibit the growth of Lactobacillus spp because such enzymes are necessary to decompose glucose as an energy source to grow. If the pH of the media is no longer tolerated and the hexokinase enzyme is no longer functioning, then Lactobacillus spp begins to enter the death phase [13,15].

C. Comparison with SNI TPC Probiotic Drink

Based on observations until the 60th hour, TPC Lactobacillus spp in the medium of pink guava juice during the stationary phase (24th to 36th hour) amounted to 49.5 x 10^{10} CFU/ml which does not differ much from TPC cow's milk media during the stationary phase (24th to 48th hour) which is an average of 10^9-10^{10} CFU/ml. The amount has exceeded the SNI limit for TPC probiotic drinks which is a minimum of 10^7 CFU/ml [18]. and can be considered as an alternative growth medium for probiotic drinks from the medium of guava juice.

IV. CONCLUSION

- Lactobacillus spp growth curve in pink guava media is drawn from the log phase until the death phase starting at 12 o'clock, 24, 36, 48 to 60 with all TPC counts of 21.5 x 1010 CFU/ml, 49 x 1010 CFU/ml, 49.5 x 1010 CFU/ml, 25.5 x 1010 CFU/ml, and 13 x 1010 CFU/ml respectively. The stationary phase of the curve is drawn at the 24th to the 36th hour.
- All the amount of TPC exceeds the SNI standard of probiotic drinks at all incubation times.

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