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Comparison of the Influence of Trypsin on the Number of Colonies Lactobacillus spp. on the Soy Milk and Cow's Milk Medium

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***Abstract*—Soy milk has high carbohydrate levels, so soy milk can be used as an alternative medium for the growth of Lactobacillus spp. In addition, in vivo, to be able to play an optimal role, Lactobacillus spp. must be able to survive the influence of enzymes in the digestive tract, including the trypsin enzyme. This research is an experimental laboratory that aims to compare the effect of trypsin addition on the number of Lactobacillus spp colonies using the TPC (Total Plate Count) method on soy milk and cow milk media. There are four treatment groups namely soy milk and cow milk with and without the addition of trypsin enzymes, each with 3 repetitions. Calculation of the number of colonies was carried out at the 36th hour. Data were analysed statistically using the t-independent test. The results showed that the average number of colonies of Lactobacillus spp. in soy milk is higher than cow milk, with or without the addition of trypsin. Amount of bacterial colony in the medium of soy milk is 2.5 x1018 CFU/ml, while in cow milk 4.5 x 1012CFU/ml. The percentage average of Lactobacillus spp mortality in soy milk is lower (54.64%) than cow milk (73.04%). There was a significant difference between the percentage of Lactobacillus spp deaths. in the media of soy milk and cow milk with p-value = 0.00 (p <0.05). The conclusion of this study shows that soymilk media is better at growing Lactobacillus spp. and more resistant to trypsin enzymes when compared to cow milk.**

Keywords*—*lactobacillus spp., soy milk, cow milk, trypsin

I. INTRODUCTION

The growth media of probiotic bacteria circulating in society at this time is dominated by cow's milk [1]. The use of cow's milk as a medium of growth of probiotic bacteria has several drawbacks, the carbohydrate content is only 47%, and the fat content is quite high, which is 33% [2]. Based on this, alternative media is needed for the growth of probiotic bacteria. One alternative growth medium that can be used is soy milk. Soy milk has several advantages compared to cow's milk, which has a very high carbohydrate content: 62.9%, and the fat content is low: 1.2%. High carbohydrate levels in soy milk can be used as a good growth medium for Lactobacillus spp. [3,4]. In addition, there are other benefits owned by soy milk that is not owned by cow's milk that contains soybean trypsin inhibitors (SBTI) that can inactivate trypsin [5].

One of the requirements of probiotics is the resistance to living in the gastrointestinal tract. Thus, Lactobacillus must be able to survive in the gastrointestinal tract and maintain its standard amount in the intestines by 10^8 colony forming units/mL (CFU/ml) in order to play an optimal role [6,7]. One of the substances that can inhibit the growth of probiotic bacteria is digestive enzymes. Among digestive enzymes there is a proteolytic enzyme, which is an enzyme that is able to break down proteins into smaller molecules [8]. Trypsin can represent a group of proteolytic enzymes. This enzyme is a protease enzyme produced by the pancreas and secreted into the duodenum. This enzyme hydrolyses proteins into peptides. The concentration of trypsin in the body is 0.5-1% [9]. Need a growth medium that can support carbon needs and that can increase the resistance of probiotic bacteria to the influence of trypsin in order to maintain optimal amounts until it reaches the intestines. This study aims to calculate the number of colonies and the percentage of lactobacillus spp. deaths in soymilk media, compared to cow's milk media.

II. METHODS

The material in this study consisted of trypsin (15000 U/ml) in 0.05 M buffer Tris HCl pH 8, deMan Rogosa Sharp Agar (MRSA) and test bacteria, namely Lactobacillus spp. from the

Microbiology Laboratory of the Faculty of Medicine, Jenderal Achmad Yani University.

This research is an experimental laboratory research with posttest-only control research design. The study used a complete randomized design (RAL) with 3 repetitions in each treatment group. Number of lactobacillus spp. colonies. calculated using the total plate count method. The data obtained was analysed with a t-independent test. In this study there were 4 treatment groups, namely:

- Group 1: Lactobacillus spp. culture without the addition of trypsin enzyme in soymilk medium.
- Group 2: Lactobacillus spp. culture with the addition of trypsin enzyme 1% in soymilk medium.
- Group 3: lactobacillus spp. culture of the addition of the enzyme trypsin in the milk media of cows.
- Group 4: Lactobacillus spp. culture the addition of trypsin enzyme 1% in cow's milk media.

A. *Lactobacillus spp. Culture Manufacturing*

Lactobacillus spp. are mixed into a solution NaCl 0.9% sterile until obtained turbidity in accordance with McFarland standard 0.5 (10^8CFU/ml bacteria) [10].

B. *Lactobacillus spp. Culture on Medium Soy Milk and Cow's Milk Without and With the Addition of Tripsin Concentration 1%*

Inoculation 1 ml Lactobacillus spp. media into two test reaction tubes containing 9 ml of soy milk and two reaction tubes containing 9 ml of cow's milk. After that, prepare trypsin (15000 U/ml) inside 0.05 M Tris buffer HCl pH 8 with a concentration of 1%. Trypsin is added to group 2 and group 4. Each reaction tube is then heated in a 40°C water heater for 2 seconds while being shaken gently until the trypsin dissolves and compacted. The solution is incubated at a temperature of 37°C for 10 minutes. After that, the solution is diluted with NaCl 0.9% sterile until it reaches a dilution of 10^{-10}. The dilution of solution is then transferred into a petri dish. At each dilution, 12 to 15 ml of sterile MRS-A is added at 45°C ± 1°C, then homogenized until compacted. This is the first layer. After that made a second layer by adding 5 ml MRS-A until compacted. This was done three repetitions in each experimental group. Incubation of petri dish at 37°C for 24 hours. Then after 24 hours, the number of colonies is calculated using the Total Plate Count (TPC) method [11].

C. *Calculation of the Number of Colonies by TPC Method*

Calculation of the number of bacterial colonies, using the TPC method. Selected petri dish from each petri dish showing the number of colonies between 25-250 colonies using the colony counter tool [11].

III. Results and Discussion

A. *The Total Number of Lactobacillus spp. Colonies in Soy Milk and Cow's Milk Media with/without Tripsin Addition Concentration 1% Incubated for 36 Hours*

The calculation of the number of bacterial colonies in this study was conducted using dilution techniques with dilution from 10^7 to 10^{16} CFU/ml, then calculated by TPC method. The results of the observations calculated the number of colonies of lactobacillus spp. bacteria. On the medium of soy milk and cow's milk without and with the addition of trypsin seen in Table 1.

TABLE I. Number of Bacterial Colonies of Lactobacillus spp. on the Medium of Soy Milk and Cow's Milk

Dilution	Colonies number	
	Soy milk	***Cow's milk***
10^7	TMTC	250
10^8	TMTC	250
10^9	TMTC	173,3
10^{10}	TMTC	108
10^{11}	TMTC	45,3
10^{12}	TMTC	22,3
10^{13}	TMTC	22
10^{14}	TMTC	20
10^{15}	TMTC	18,5
10^{16}	250	8,3

Description: TMTC: too much to count (>250 colonies)
Number of colonies that can be counted : 25-250 colonies

Based on table 1, the number of bacterial colonies of Lactobacillus spp. on the medium of soy milk, more than cow's milk. The number of colonies in soymilk media is 2.5 x10^{18} CFU/ml, while in cow's milk 4.5 x 10^{12} CFU/ml. The results of this study show that soy milk is a good substrate for the growth of Lactobacillus spp. This bacterium can assimilate oligosaccharides (prebiotics) that are abundant in soy milk as an energy source. With the help of the enzyme b-galactosidase growth and activity of stimulated bacteria [12]. In addition to be fermented by lactobacillus spp. bacteria. Prebiotic ingredients will also produce short-chain fatty acids that can be used as energy sources [13]. In addition, soy milk contains the main carbon sources of fructose and sucrose, in contrast to cow's milk, which has only the main carbon source of lactose alone [14]. The other side, probiotic bacteria Lactobacillus spp. less effective at fermenting lactose in cow's milk [15].

Based on the theory also states that soy milk contains Soybean Trypsin Inhibitor (SBTI) which is a trypsin inhibitor contained in soybeans. Soybean trypsin inhibitors can inhibit the action of trypsin by bonding to the reactive protease site on trypsin which will form a single chain of polypeptides on disulfide bonds [16].

B. *Percentage of Lactobacillus spp. on The Medium of Cow's Milk and Soy Milk*

Here is the percentage of lactobacillus spp. deaths. On the medium of cow's milk and soy milk.

TABLE II. PERCENTAGE OF LACTOBACILLUS SPP. DEATHS ON THE MEDIUM OF COW'S MILK AND SOY MILK

Dilution	Percentage of lactobacillus spp. deaths	
	Cow's milk	*Soy milk*
10^7	0%	0%
10^8	0%	0%
10^9	76,5%	0%
10^{10}	87,3%	0%
10^{11}	82,3%	79,3
10^{12}	94,1%	92,2%
10^{13}	95,4%	92,6%
10^{14}	96,5%	93,2%
10^{15}	98,3%	94,1%
10^{16}	100%	95%

Based on Table 2, the average percentage of Lactobacillus spp. deaths in soy milk is lower (54.64%) than cow's milk (73.04%). Trypsin can inhibit bacterial growth because cell surface proteins that have molecular weight 250-k are highly sensitive to the action of trypsin. Trypsin can eliminate 250-k of cell surface proteins that can play a direct role in bacterial cell growth. Soy milk contains Soybean Trypsin Inhibitors (SBTI) that can inhibit trypsin by bonding to reactive site protease on trypsin that forms a single chain of polypeptides in disulfide bonds [17,18].

In addition, the content of soy milk is high in carbohydrates (especially sucrose and fructose) when compared to cow's milk which has lower carbohydrate levels. Soy milk has oligosaccharides (prebiotics) as an energy source that stimulates the growth and activity of Lactobacillus spp. [19].

C. Analysis of Lactobacillus spp. Mortality Data on Cow's Milk and Soy Milk Media

To determine a significant difference in the percentage of deaths in the media group of soy milk and cow's milk, a t-independent test was conducted. The results of the analysis can be seen in Table 3.

TABLE III. RESULTS OF ANALYSIS OF LACTOBACILLUS SPP. DEATH PERCENTAGE DATA ON THE MEDIUM OF COW'S MILK AND SOY MILK

Percentage of death	N	Mean	Sig. Uji Normality	Sig. *t-Test*
Soy milk	10	54,64%	1,000	0.00
Cow's milk	10	73,04%	1,000	

In table 3, the T-independent test result was $p<0.05$, indicating that there was a significant difference between the percentage of Lactobacillus spp. deaths. On the medium of cow's milk and soy milk. This suggests that Lactobacillus spp. in soymilk media is more resistant to exposure to the enzyme trypsin when compared to cow's milk media. Therefore, soy milk can be used as an alternative probiotic medium.

IV. CONCLUSIONS

- Average number of lactobacillus spp. colonies. with/without trypsin in soymilk media more ($2.5x10^{15}$CFU/ml) when compared to cow's milk media ($4.7x10^{10}$CFU/ml).
- Average percentage of Lactobacillus spp. deaths in soymilk media is less (54.64%) when compared to cow's milk media (73.04%).
- There is a significant difference between the percentage of Lactobacillus spp. deaths in cow's milk and soy milk ($p<0.05$). Soymilk media is better at growing Lactobacillus spp. and more resistant to trypsin when compared to cow's milk.

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