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Research Article

Inhibitory Activity of Probiotic Milk Against *Eschericia coli ATCC 6538 and Staphylococcus aureus ATCC 8739*

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ABSTRACT

The aim of this research is to evaluate inhibitory activity of *Lactobacillus acidophilus (L.a)* and *Lactobacillus casei (L.c)* probiotic milk against *Escherichia coli* and *Staphylococcus aureus* growth. The fermented milk was prepared by inoculating each probiotic in milk at 43ºC and then incubated at room temperature for 24 hours. Diffusion agar method by using nutrient agar media was used to assess the minimum inhibition concentration (MIC). Obtained results showed pH of fermentation probiotic milk 5 $\pm$ 0,05. Optimum inhibition zone diameter of *L. acidophilus* fermented milk against *Escherichia coli* and *Staphylococcus aureus* at MIC value of 14 mm. Inhibition zone diameter of *L. casei* fermented milk obtained an optimum against *Escherichia coli* and *Staphylococcus aureus* at MIC value of 12 mm and 13 mm respectively. *The L. acidophilus* and *Lactobacillus casei* probiotics fermented milk exhibited considerable antibacterial agent against *Escherichia coli* and *Staphylococcus aureus*.

Keyword : Inhibitory activity, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Escherichia coli* dan *Staphylococcus aureus.*

Introduction

Probiotics defined as live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host. The probiotics are living organisms that commonly used as food additives with beneficial effects on the healthy body by setting microbial balance in gastrointestinal tract. (Hassanzadazar, 2012). Lactic acid bacteria (LAB) as protective cultures are common probiotic organisms that are considered safe due to having specific characteristics. Main genera of LAB are Leuconostoc, Enterococcus, Lactobacillus,Lactococcus, Bifidobacterium, Pediococcus, and Streptococcus (Tafvizi, 2012).

The LAB strains are potentially promising, because of their ability to generate bactericidal bioactive peptides (bacteriocins) and enzymes to control biofilm formation and the growth of pathogens. The bacteriocins are also present in species of Lactobacillus. The *L. acidophilus* produce lactacin B or lactacin F, whereas *L. casei* B80 produce casein 80. Certain LAB strains have been reported to be highly antagonistic to biofilm-forming *S. aureus* (Wysocki, 2010).

The Lactobacillus is to be able to inhibit various types of bacteria pathogens like Salmonella, Vibrio, Listeria, Shigella and Staphylococcus. This LAB produces bacteriocins, such as acidolin, acidophilic nor is lactosidine thought to have a good broad-spectrum against Gram positive or negative bacteria (Ahmed, 2010). This study aims to identify the inhibitory activity of probiotic milk *L. acidophilus* and *L. casei* against *S. aureus ATCC 8739* and *E. coli ATCC 6538*.

Material and Method

Preparation of Lactic Acid Bacteria culture

Lactobacillus acidophilus FNCC 0051 and *Lactobacillus casei* was obtained from culture collection of Faculty of Science and Technology, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University respectively. The probiotics were inoculated in de Mann Rogosa and Sharpe (MRS) (Difco) fresh agar media. *Escherichia coli* ATCC 6538 and *Staphylococcus aureus ATCC 8739* were used as test bacteria gained from culture collection of Microbiology Practical Room, Department of Pharmaceutical Chemistry, Faculty of Pharmacy Airlangga University. The test bacteria were inoculated in *Nutrient Agar* (Oxoid) fresh media.

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Preparation of probiotics milk: One Ősе of fresh cultures of *L. acidophilus* and *L. casei* 24 hours was taken from the stock culture, inoculated in MRS slant agar media and incubated at 37°C for 24 hours. Each LAB culture was added by10 mL of MRS broth and shaken with vortex until the whole colony on the surface to be detached. The density of the starter inoculum was measured at a wavelength of 580 nm with dilution, if necessary, until a 25% transmittance was obtained. One litre of pasteurized dairy milk at 80-85°C for 30 minutes then cooled to 45°C. One mL of BAL inoculum in MRS broth was inoculated in 10 mL of the pasteurized milk and stirred until homogeneous then incubated at room temperature for 24 hours. Furthermore, 10 ml of LAB starter in milk was put into 200 mL of milk, incubated until the number of LAB $\geq$ 107 CFU / ml and pH of 4.0 to 4.5.

Determination of Total Plate Count (TPC): Probiotics fermentation milk as many as 1 ml diluted in 10 times with saline solution until obtained 10^{10} dilution. One ml of each dilution was taken transfer into sterile petri dish, added MRS agar media melted at 45-50°C, been homogenously, allowed until solidified, then incubated for 48 hours at 37°C. The total of colony on petri dish was counted. The petri dish contained 30-300 colonies was chosen and multiplied with the dilution factor to obtain the colony forming unit (CFU) /ml.

Inhibitory Activity Test: For inhibitory activity test of the probiotic fermented milk, *Nutrient agar* was used as test media with 10 ml of base layer and 7 ml seed layer using 25% transmittance (580 nm) of *E. coli* and *S. aureus* inoculum as test bacteria. As many as 5 µL of inoculum the test bacteria was added into seed layer media, shaken with vortex, poured on the surface of base layer on petri dish, allowed until solidified. Made hole print, filled with 50 µL sample (probiotic milk) and kanamycin standard solution, then incubated for 24 hours at 37ºC. Diameter of zone inhibition formed around the proposer was measured (mm).

Results And Discussion

Characterization of Probiotic Milk

Table 1. Characteristic of Probiotic Milk

Organoleptic		SNI
Texture	Viscous paste	Viscous-solid liquid
Colour	white	
Distinctive smell	Smell like milk	Normal/specific
Taste	sour	-
pH *L. acidophilus*	5+0.05	-
pH *L. Casei*	5 +0.05	-

This study was conducted by fermentation technology for achieving probiotic fermentation milk. Fresh cow's milk used in this study was standardized accordingly SNI 2009. Characterizing probiotics milk was also standardized according to requirement of SNI 2009. Characterization of probiotic milk based on organoleptic milk can be known as probiotic milk in the form of thick liquid (paste) with white solid, has a distinctive odour like milk and has a sour taste (Table 1). For pH examination, the data obtained if the pH of *L. acidophilus* and *L. casei* are the same and the viscosity of *Lactobacilli fermented milk wa*s 539.65 centipoise (cps) or 5.39 dPas. According to SNI (2009), probiotic milk has a distinctive odour, a viscous-solid liquid form, a distinctive taste.

Determination of MIC against *Escherichia coli* ATCC 8739 dan *Staphylococcus aureus* ATCC 6538

Table 2. Growth inhibition zone of probiotic milk at various Concentrations (%) against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538

Consentration (%)	*Escherichia coli* ATCC 8739		*Staphylococcus aureus* ATCC 6538	
	L. acidophilus	*L. casei*	*L. acidophilus*	*L. casei*
25	14 mm	12 mm	14 mm	13 mm
12,5	12 mm	13 mm	13 mm	14 mm
6,25	11 mm	14 mm	12 mm	15 mm
3,125	11,5 mm	13 mm	11 mm	14 mm
2	9 mm	10 mm	9 mm	10 mm
1,5	9 mm	9 mm	9 mm	9 mm
1	9 mm	9 mm	9 mm	9 mm
0,5	9 mm	9 mm	9 mm	9 mm

probiotic milk had similar characterization to the research of Ranti (2014), by which the probiotic milk of *L. acidophilus*, *L. plantarum*, *L. casei* have a thick foam with a white colour, a characteristic odour like milk, sour taste, pH of the probiotics milk of probiotic were 4.60 ± 0.0057, 4.32 ± 0.0057, and 4.43 ± 0.0115 for *L. acidophilus*, *L. plantarum*, and *L. casei* respectively. The viscosity of *L.acidophilus*, *L. plantarum*, and *L. casei* probiotic milk were 0.9 ± 0.06, 0.9 ± 0.00, and 0.9 ± 0.06 (dPas) respectively.

The MIC of *Lactobacillus acidophilus* probiotic milk at 3.125% against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 was classified as weak criteria (Table 2). As well as the MIC of *Lactobacillus casei* probiotic milk at 2% against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 with inhibition diameter of 10 mm. This means that formulation by both lactobacilli probiotic milk should be more than the MIC. In the future research, inhibitory activities assay using various pathogenic microorganisms was needed to ensure the capability and prospective of the probiotics milk development as antimicrobial agents. Karimi et al (2014) had proved effect of probiotic bacterial strains isolated from different natural sources against 2 pathotypes of pathogenic *E. coli*. Various inhibitory potency of 13 probiotic colonies isolated from 20 samples of traditional dairy products was reported. Each species of probiotic bacteria has a variety of compounds including different bacteriocin. *Lactobacillus acidophilus* is known to produce bacteriocin lactacin B and acidocin A while *Lactobacillus casei* produces casein. The presence of more than one kind of bacteriocin can stronger resistance to pathogenic bacteria. According to other studies the average inhibition of probiotic milk in *Escherichia coli*, *Salmonella typhimurium*, and

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Vibrio cholera was 11.90 mm; 11.50 mm, 12.10 mm (Ranti, 2014).

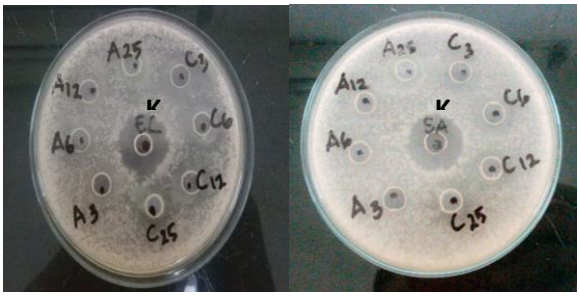

Figure 1. Inhibitory activity of *L.acidophilus* and *L.casei* probiotics milk against *Staphylococcus aureus* (a) and *Eschericia coli* (b) and 62,5 ppm kanamycin standard solution (K).

Lactic acid bacteria which produced bacteriocins had characteristics as bactericidal, which was very useful to develop safety of food fermentation product(Belviso, 2009). Until now, there was only one bacteriocin generated by lactic acid bacteria and had been used on food product, which was nisin. The *L. acidhopillus* can produce *Acido-philucin A.* The effect of antimicrobial *L. acidophillus* may be caused by bacteriocins activity and others organic acids for microbial competition with other bacteria. Several activities which was showed by probiotics were cholesterol-loweringThis phenomenon was supported by TPC data of *L. acidophillus* and *L. casei* probiotics milk for each 10^7 cfu/ml and 10^{13} cfu/ml which met preparation requirements with recidence target intestine. The requirement of probiotics to create health effect for host by intestine target and inhibit pathogen bacteria was minimal 10^6 cfu/ml (Cook, 2012). This fact explained that there was acid production and or others compound such as bacteriocins which reached the most at 24-periode, even though it was not linear with total of probiotics cell which; entering into decline phase, so total of cell was decrease at 24-periode. Correlation between metabolite production and biomass which followed non-growth associated pattern can be formed as reference when making probiotics milk preparation, especially *L. acidophillus* and *L. casei* both as single and multi-strain formula.

Conclusion

Lactobacillus acidophilus and *Lactobacillus casei* probiotics milk exhibited considerable antibacterial effect against *Eschericia coli* and *Staphylococcus aureus*.

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