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

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ABSTRAK

Tujuan dari penelitian ini adalah untuk mengevaluasi susu fermentasi probiotik *Lactobacillus acidophilus* (L.a) dan aktivitas penghambatan *Lactobacillus casei* (L.c) terhadap *Eschericia coli* dan *Staphylococcus aureus*. Susu fermentasi disiapkan dengan menginokulasi setiap probiotik dalam susu segar yang dipasteurisasi pada suhu 43ºC dan kemudian diinkubasi pada suhu 37oC selama 24 jam. Metode difusi agar dengan menggunakan media nutrient agar digunakan untuk menilai konsentrasi penghambatan minimum (MIC). Hasil penelitian menunjukkan bahwa pH susu probiotik fermentasi mencapai 5. Diameter zona hambat susu fermentasi *L. acidophilus* menunjukkan penghambatan optimal terhadap *Eschericia coli* dan *Staphylococcus aureus* dengan nilai MIC masing-masing sebanyak 14 mm. Diameter zona hambat susu fermentasi *L.casei* menunjukkan populasi yang optimal terhadap Eschericia coli dan Staphylococcus aureus dengan masing-masing MIC masing-masing sebanyak 12 mm dan 13 mm. Susu fermentasi L. acidophilus probiotik memiliki aktivitas penghambatan yang kuat terhadap *Eschericia coli* dan *Staphylococcus aureus* daripada susu probiotik Lactobacillus casei.

Keyword : Aktivitas hambat, *Lactobacillus acidophilus, Lactobacillus casei, Eschericia coli* dan *Staphylococcus aureus.*

Kata kunci: 3-5 kata kunci, urut abjad, masing-masaing kata kunci dipisahkan dengan semi kolon

ABSTRACT

The aims of this research are to evaluation of probiotic fermentation milk of *Lactobacillus acidophilus (L.a)* and *Lactobacillus casei (L.c)* inhibitory activity against *Eschericia coli* and *Staphylococcus aureus*. The fermented milk was prepared by inoculating each probiotic in fresh pasteurized dairy milk at 43ºC and then incubated at 37ºC for 24 hours. Diffusion agar method by using nutrient agar media was used to assess the minimum inhibition concentration (MIC). The results showed that pH of fermentation probiotic milk reached 5. Inhibition zone diameter of *L. acidophilus* fermentation milk showed an optimum inhibition against *Eschericia coli* and *Staphylococcus aureus* rwith MIC value of each as much as 14 mm. The inhibition zone diameter of *L.casei* fermented milk showed an optimum inhabitation against *Eschericia coli* and *Staphylococcus aureus* with each MIC as much as 12 mm and 13 mm respectively. *The L. acidophilus* probiotics fermented milk had strong inhibitory activity against *Eschericia coli* and *Staphylococcus aureus* than *Lactobacillus casei* probiotics milk.

Key words: *Inhibitory activity, Lactobacillus acidophilus, Lactobacillus casei, Eschericia coli, Staphylococcus aureus.*

INTRODUCTION

Probiotics characterized as live microorganisms, when administered in adequate amounts, confer a health benefit to the host. Probiotics are living organisms that are 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, Bifido-bacterium, Pediococcus,* and *Streptococcus* (Tafvizi, 2012).

The LAB strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control biofilm formation and the growth of the pathogens. The bacteriocins are also present in species of genus Lactobacillus. The *L.acidophilus* produce lactacin B or 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 able to inhibit various types of bacteria pathogens like *Salmonella, Vibrio, Listeria, Shigella* and *Staphylococcus*.

The LAB can produce antimicrobial component called bacteriocin, for example, acidolin, acidophilic nor is lactosidine thought to have broad-

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spectrum good against Gram positive or negative bacteria (Ahmed, 2010). This study aims to evaluate the inhibitory activity of probiotic *L. acidophilus* and *L. casei* fermented milk against *S. aureus ATCC 8739* and *E. coli ATCC 6538.*

METHODS

Analytical balance, colony counter, digital shutter speed, spectrophotometer, shaker incubator (Mermet), incubator (Mermet), and autoclave were equipments and instruments used in this study.

Materials. The probiotics of *Lactobacillus acidophilus* FNCC 0051 and *Lactobacillus casei* were obtained from isolate reference at Faculty of Science & Technology and Pharmaceutical Chemistry Department, Faculty of Pharmacy, Airlangga University, inoculated in deMann Rogosa and Sharpe (MRS) *broth* media. *Escherichia coli* ATCC 6538 and *Staphylococcus aureus ATCC8739* which were used as test bacteria on this research gained from inventory culture at Microbiology Practicum Room, Pharmaceutical Chemistry Department, Airlangga University, inoculated on *Nutrient Agar* media.

Preparation of probiotic milk. One Öse of 24 hours fresh cultures of *L. acidophilus* and *L. casei* were taken from the stock culture, each inoculated in an MRS slant and incubated at 37°C for 24 hours, after incubation with 10 mL of MRS broth and shaken with vortex until the whole the colony on the surface to be detached. The density of the starter inoculum is measured at a wavelength of 580 nm, if necessary, until a 25% transmittance was obtained. One litre of dairy milk was pasteurized at 80-85°C for 30 minutes then cooled to 45°C, inoculated 1 mL of BAL inoculum in MRS into 10 mL of the pasteurized milk and stirred until homogeneous then incubated at room temperature for 24 hours. Furthetmore, 10 ml of LAB starter in milk was put into 200 mL of milk, incubated until the number of the LAB $\geq 10^7$ CFU/ml with pH value of 4.0 to 4.5.

Determination of Total Plate Count (TPC). Probiotics fermented milk as many as 1 ml diluted in 10 times with saline solution until obtained 10^{10} dilution. From each dilution it was taken 1 ml, transfered into sterile petri dish, added MRS agar media melted at 45-50°C, been homogenously, allowed until solidified, and incubated for 48 hours on 37°C. Counted the total of colony on the petri dish contained 30-300 colonies, and multiplied with dilution factor.

Inhibitory Activity Test. The bioassay was performed on Nutrient agar media with 10 ml of base layer and seed layer as many as 8 ml using bacteria test of *E. coli and S. aureus* which were made an inoculums with transmit 25% on wavelength as many as 580 nm. As many as 5 µL of inoculums bacteria test 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 solution and kanamycin standard, then incubated for 24 hours. Diameter of zone which was formed around the sample solution reservoir was measured (mm).

RESULTS AND DISCUSSION

The study was conducted by characterizing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* fermented milk. The *Lactobacillus acidophilus* and *Lactobacillus casei* probiotic starters were added to fresh cow's milk and incubated at 37°C for 24 hours to become probiotic milk. Characterization of probiotic milk based on organoleptic milk can be known as probiotic milk in the form of thick liquid with white solid, has a distinctive odour like milk and has a sour taste with pH value of *L. acidophilus* and *L. casei* fermented milk were the same, namely five and the viscosity of *Lactobacillus acidophilus* was 539.65 centipoise (cps) or 5.39 dPas. According to SNI (2009), the fermented probiotic milk has a distinctive odour, a viscous-solid liquid form, a distinctive taste. (Table 1)

The TPC examination results of Lactobacillus acidophilus and Lactobacillus casei probiotics were $1.3x10^7$ CFU/ml and $20.7x10^{13}$ CFU/ml, respectively. According to SNI (2009), the number of starter bacteria for probiotics was at least ≥ 107 colonies / g. This showed that probiotic fermented milk met the criteria. Characteristic of probiotic milk has similar with characteriistic results of other research reports, in which the probiotic milk *L.acidophilus, L. plantarum, L. casei* has a thick foam with a white colour, a characteristic odor like milk, sour taste. (Collado et al., 2007)

The minimum inhibitory concentration of the probiotic milk was *Lactobacillus acidophilus* were 3.125% against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 with 11.5 mm and 11 mm of growth inhibitory zone diameter respectively (Table 2 and Figure 1). The minimum inhibitory concentration of probiotic milk of *Lactobacillus casei* was 2% against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 with 10 mm and 10 mm of growth inhibitory zone diameter respectively.This means that if the *Lactobacillus acidophilus* probiotic milk concentration was less than 3.125%, it cannot inhibit the growth of *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538. If the concentration of *Lactobacillus casei* milk was less than 2%, it cannot inhibit the growth of *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538. If the concentration of *Lactobacillus casei* milk was less than 2%, it cannot inhibit the growth of *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538. Each type of probiotic bacteria has a specific of compounds including different bacteriocin. *Lactobacillus acidophillus* is known to be able to produce bacteriocin called lactacin B and acidocin A, while *Lactobacillus casei* produces casein. The presence of more than one kind of bacteriocin could protect and strenhtener the producing the effects of antimicrobial against pathogenic bacteria. According to other studies the average inhibitory activity of probiotic milk in

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Escherichia coli and Staphylococcus aureus. (Abd El-Gawad, 2014).

The bacteriocins producing LAB had characteristics as bactericidal which was very useful to develop safety of food fermentation product. Until now, there was only one bacteriocin (nisin) which was generated by LAB and had been used on food product. *L. acidophillus* can produce *Acido-philucin A.* The effect of antimicrobial *L. acidophillus* could be caused by bacteoricins' activity and others organic acids also there was microbial competition with others bacteria. Several activities which was showed by probiotics were cholesterol-lowering (Belviso, 2009).

This 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 intestinal target. The requirement of probiotics to create health effect for host by intestinal 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 pattern, especially *L. acidophillus* and *L. casei*.

CONCLUSION

Lactobacillus acidophilus probiotics fermented milk exhibited inhibitory activity against *Eschericia coli* and *Staphylococcus aureus* as well as *Lactobacillus casei* probiotics milk.

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