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Antibacterial Activity of Fermented Milk Cultured with Yeast-LAB and added Sweet corn puree(*Zea mays var. Saccharata*) Against Pathogenic Bacteria

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ABSTRACT : Fermented milk is capable of suppressing the growth of pathogenic bacteria that can be harmful to human health. The objective of this research was to evaluate antibacterial activity of fermented milk added sweet corn and cultured with lactic acid bacteria (LAB) and yeast Kluyveromyces lactis. The LAB used in this research were *Lactobacillus bulgaricus* and Streptococcus thermophilus. The treatments were arranged in a factorial 2x2 and allocated in completely randomized design with four replications. The treatments were consist of two factors, A as level of sweet corn (A1= 25% and A2= 75%) and factor B as level of yeast (B1= 0,5% and B2= 1%) and LAB 5% for all treatment with four replications. There were two bacterial test (*Escherichia coli* and *Staphilococcus aureus*) used in the assay test. Antibacterial activity assay of product towards pathogenic bacteria was confirmed using disc paper difusion method. The result showed that E. coli sensitivity was highly significant different (P<0,01) towards sweet corn fermented milk using yeast 1% and 75 % addition of sweet puree with the avarage of wide of zone of inhibition was 5 mm. Furthermore, in the antibiotic sensitivity test for two antibiotics (tetracycline and chloramphenicol 30µg) showed that E. coli was highly sensitive to both antibiotics. On the other hand, S.aureus was resistance to fermented product but sensitive to both antibiotics that indicated by wide of zone of inhibition formed. As a conclusion, fermented milk cultured with yeast-LAB able to inhibit the growth of E coli in the invitro test.

Keywords: antibacterial, LAB, yeast, pathogenic, zone of inhibition

INTRODUCTION

Lately, the need for food of animal origin that is capable of reducing the pathogenic bacteria increasingly becoming a concern. Direct source of contamination, animal enteric pathogens, was cause of food-borne deseases occured. Along with the increasing community awareness and understanding towards healthy food and beverage or so-called functional food, then the demand for such products increased rapidly both in quality and quantity. One of is a fermented milk which is known as yogurt that in the fermentation process using lactic acid bacteria (LAB) as starter culture or also as source of probiotic. The benefits of probiotics have been recognizing and exploring for over a century. Among a number of functional compounds recognized, bioactive components from fermented food and probiotics certainly take the center stage due to their long tradition of save and beneficial effects (Ahmed and Wang 2009).

Another milk cultured product known is kefir. Kefir is self-carbonated refreshing fermented milk with slight acidic taste made from kefir grains, a complex and spesific mixture of lactic acid bacteria (LAB) and yeast held together by a polysaccaride matrix. The micro-organisms contained within the kefir grain typically produce lactic acid, antibiotics and several kinds of bactericide, such products inhibiting the proliferation of both degrading and pathogenic microrganisms in kefir milk (Angulo 1993).

Unlike kefir, sample used in this study is fermented milk similar to kefir using only two types

of LAB (*Lactobacillus bulgaricus and Streptococcus thermophilus*) and *yeasts Kluyveromyces lactis*. While the addition of sweet corn aims to increase the variety of flavors and also as sources of carbohydrate or sugar for LAB and yeast culture that involved in the fermentation process. It is expected that the product able to suppressed the growth of pathogens in the invitro, and also beneficial to the health of the digestive tract. Therefore, the aim of current research was to study the antibacterial activity of fermented milk added sweet corn puree and cultured with LAB and *yeast Kluyveromyces* lactis on pathogenics bacteria growth as one of fermented milk benefits.

MATERIALS AND METHODS

Sample sources

Milk used in this study was obtained from Lives Stock farm located at the ring road around the University of Syiah Kuala. Lactic acid bacteria as culture derived from Food Science Microbiology Laboratories, Agriculture Institute Bogor, while *yeast Kluyveromyces* lactis used was previously isolated from naturally fermented buffalo milk (Yurliasni, 2010)

Sample preparation

Fresh milk that obtained from small dairy farm in Aceh province was pasteurized at 90 to 95 C for 10 min, then cooled at 30^{o}C. Sweet corn were obtained from public market and made into puree using a blender, then filtered to produce a smooth texture and pasteurized at 90 to 95 C for 5 min. 5% of LAB was added to all pasturized mik, mix in sweet corn puree, and inoculated with yeast culture that previously growth in milk as well, in accordance with treatment designed, incubated at 30^{o}C for 24 h. This experiment using a completely randomized factorial design consisted of treatment were sweet corn puree percentage (25 % and 75 %) and yeast percentage (0.5 % and 1 %) and each treatment replied four times.

pH and lactic acid level measurement

Measurement of pH and lactic acid levels is done after an incubation period. pH was measured using a pH meter (AZ 86 502), while lactic acid levels were analyzed using Mann 's Acid Test method (Hadiwiyoto, 1994)

Total number of microorganisms

Nutrient agar media (Oxoid) was autoclave for 15 min at 15psi pressure 121^{o}C and cool to 45^{o}C. Pour-plate technique or serial dilution used for plating sample (Cappucino and Natalie 2005). Serial dilutions of sterile pepton water made up to 10^{6}. 1 mL sample was tansfered into any dilution in sequence until dilution 10^{5} and 10^{6}. 1 mL of each dilution placed into sterile petri dish in duplicate. Molten agar 45^{o}C was poured into petridishes containing diluted sample. Incubated at 30^{o}C for 24 h. Total number of cfu/mL counted on
Quebec colony counter.

Antimicrobial activity assay

The selected bacterial test (*Escheriachia coli dan Staphylicoccus aureus*) were grown in physiological NaCl at 37^{o}C for 24 h until the stationary phase, then by pour-plate tecnique and serial dilution bacterial test were plated on A (Violet Red Bile(VRB) Agar media (Oxcoid) and Vogel Johnson (VJ) Agar media (oxcoid) respectively about 10^{3} cfu/mL. Antibacterial activities against *E.coli* and S. aureus were observed by using diffusion methods as described by Bonev *et* al.(2008). Using paper disk 100 µL of sample was spotted in duplicates onto the surface of VRBA and VJA agar plate containing lawn of E.coli and S. aureus. The agar plates then incubated at 37^{o}C for 24 h and examined for clear zones of inhibition around the spots where sample were applied by using calipers.

Data analysis

The quantitative data were analyzed by using Two-way analysis of variance (ANOVA) and the differences among treatments menas were examined by Duncac Multiple range Test P<0,05 (Steel and Torrie, 1995) The total of microrganism cell (cfu/mL) from sample test was converted to logaritmic value before statistical analysis.

RESULTS AND DISCUSSION

Based on Table 1, shows that the addition of yeast *Kluyveromyces lactis* and sweet corn puree with different percentages in fermented milk does not affect the pH value .This is indicated by the absence of differences in pH values between treatments, but this value is lower than normal yogurt 4,5 to 4,6. Fleet (1990) reported that bacteria, predominantly LAB, commonly excrete organic acids which lead to a lowering in the pH, therefore, interrelationship between LAB and yeast as applied in many fermented foods and beverages, plays an essensial role in fermented product.

The treatment given significantly increase($P < 0.05$) lactic acid levels of fermented milk shown by the lower percentage adition of sweet corn puree (25%). Viljoen (2006) state that when starch-rich material such sweet corn puree mix in the milk fermentation cultured with yeast-LAB, the interaction between yeast and lactic acid bacteria create environtmental condition that protect the product from spoilage by fungi and pathogens owing to the low pH and high compostion of acetic and lactic acids.

Table 1. The avarage of pH value and lactic acid level

Fermented milk samples	Parameter	
	pH	Lactic acid level (%)
$A_1 B_1$	4.010±0.00	1.53±0.06[a]
$A_1 B_2$	4.010±0.00	1.54±0.04[a]
$A_2 B_1$	4.010±0.00	1.29±0.08[b]
$A_2 B_2$	4.010±0.00	1.30±0.03[b]

Note: means with different superscript at the same column differ significantly (P<0,05)

Data in Table 2 indicates that the treatment significantly inhibit ($P < 0.05$) the growth of *E. coli* which was can be seen by zone of inhibition formed but not inhibit S. *aureus*. In contrast to other studies have shown that kefir inhibited the growth of S. *aureus* (Yusekdag et al 2004a). The reason is kefir in this study contains some lactic acid bacteria such as *L. cremoris , Lc. Lactis, Str. thermophilus* dan *Str. Durans*. Antibacterial activity in fermented milk sample was produced by extracellular metabolites during milk fermentation. Previous research states that antimicrobial effects present in fermented food and beverages are attributed to organic acids, antibiotic factor, volatil acids, hydrogen peroxide and and a number of substrates excrete in the product (Bull and Slater 1982). Silva *et al*. (2008) studied the effect of antimicrobial activity of broth (added with different sugars) with kefir grain and he found that kefir grains promoted the hydrolisis of non reducing sugar, which were converted into organic acid and substances capable of producing inhibition halos in experiments with pathogenic microorganisms included *E.coli* and *S. aureus* The results showed that inhibition of sampel on E. coli was higher than on S. aureus and it means that S. aureus resistant to the fermented milk samples. Fermented milk treated with the addition of 25 % sweet corn puree and of 0.5 and 1 % yeast culture produces the largest zone of inhibition, but there is no difference between the two treaments. Table 2 also shows that the susceptibities of two bacteria test to two different antibiotics. The result of antibiotic sensitivity assay showed that

both of bacteria test had different resistance level on each tested antibiotic. Furthermore, E. coli and S.aureus was highly sensitive to chloramphenicol but less sensitive to tetracycline based on the size of zone of inhibition.

Table 2. Antibacterial activity assay of sample

Clear zone of growth inhibition (mm)		
Fermented milk samples	*E.coli*	*S. aureus*
A1 B1	0.50±0.00[a]	0.00
A1 B2	0.47±0,03[a]	0.00
A2 B1	0.42±0.03[b]	0.00
A2 B2	0.41±0.04[b]	0.00
Antibiotics		
Chloramphenicol	1.50±0.03	1.50±0.00
Tetracycline	0.40±0.04	0.45±0.01

Note: means with different superscript at the same column differ significantly (P<0.05)

Basically the total amount of bacteria in fermented milk such as yogurt immediately after 24 h incubation is 10^8 cfu / mL, but very different from fermented milk that used mixed cultures.

Tabel 3 shows that the addition of yeast culture and sweet corn puree does not effect the total amount of microorganism. Previous study by Witthuhm *et al*. (2004) state that the content of bacteria in kefir varied from 6.4 x10^4 to 8.5 x 10^8 cfu/g, and yeast from 1.5 x 10^5 to 3,7 x 107 cfu/g. Iregoyen *et al*. (2005) also reported that besides a viable population of 10^8 cfu/mL of lactobacilli, lactococci and 10^5 cfu/mL of yeast, kefir also had 10^6 cfu/mL of acetic acid bacteria after 24 h fermentation.

Table 3 . Total number of microorganism

Fermented milk samples	CFU/mL
A_1 B_1	5.593 ± 0.35
A_1 B_2	5.693 ± 0.45
A_2 B_1	5.060 ± 0.31
A_2 B_2	5.973 ± 0.16

When related to pH value and lactic acid levels obtained after the fermentation, as states by Nout (1991) that excessive acid production by lactic acid bacteria will result in decline in the number of surviving yeast, which consequently leads to deficiency of growth factors. As a result of such deficiency the lactic acid bacteria would produce less acid and in turn allow an increase in yeast numbers.

CONCLUSION

Fermented milk with high lactic acid levels of all treatment given as well as the addition of sweet corn and yeast LAB able to suppress the growth of pathogenic bacteria better with a wider zone of inhibition formed.

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