Microbiological Characteristic and Antimicrobial Activity of Koumiss Against *Salmonella typhimurium* and *Mycobacterium tuberculosis*

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Abstract

Koumiss is a traditional fermented milk product originated from the Central Asian steppes and mostly produced from mare milk by spontaneous fermentation of lactose to lactic acid and alcohol. Koumiss's starter cultures consist of lactic acid bacterias (Lc. lactis and Lb. acidophilus) and yeast (Sc. cereviceae). Koumiss is believed to pose health promoting properties, which is mainly related to the ability of the starter to produce vitamins of the B-group and antimicrobials. Koumiss could also served as functional food that was showed by its capability to produce the antimicrobial substrate that inhibit pathogenic bacterias. The objectives of this research were to identify microbiological characteristics of koumiss and to study its antimicrobial activity towards pathogenic bacterias such as S. Typhimurium ATCC 14028 and M. tuberculosis H37RV. The experimental design used on this research were non parametric of cohran test for M. tuberculosis H37RV and descriptive analysis for S. Typhimurium ATCC 14028. Variables observed were diameter of inhibition zone of the antagonistic assay used well diffusion method agar for S. Typhimurium ATCC 14028. In addition, the Lowenstein Jensen (LJ) modification agar was used for study the inhibition of M. tuberculosis H37RV. The result showed, koumiss could decrease the total of coliform. The average of koumiss's inhibition zone in different storage time toward S. Typhimurium ATCC 14028 was ± 7.801 mm. It was bigger than filtrate which was ± 6.002 mm. The average of diameter showed the antimicrobial activity of koumiss against S. Typhimurium ATCC 14028. The result of Cohran test showed the growth of M. tuberculosis H37RV could obstructed with modification of LJ extra koumiss stored for 4, 6, and 8 days. The conclusion of this research that koumiss was effective to against pathogenic bacterias such as S. Typhimurium ATCC 14028 and M. tuberculosis H37RV.

Keywords: antimicrobial activity, koumiss, mare's milk, pathogenic bacteria

Introduction

Milk is animal products contain a variety of potential nutrients that the body needs. Physical and chemical composition of mare's milk is different from cow's milk, goat, buffalo and camels. Mare's milk has a low fat content is 1.6% and high lactose of 6.1% Chandan et al. (2008). in Indonesia mare milk called wild horse milk is widely produce in West Nusa Tenggara (NTB). This milk is a naturally fermented milk product that has a liquid consistency without pasteurization treatment.

Koumiss is made by fermentation with a mixed microflora, which contains different lactic acid bacteria and yeasts that use for the treatment of tuberculosis in Russia. Bacteria are commonly used as starter cultures are producing antimicrobial substrates that have antagonistic properties against pathogenic bacteria. The number of high antimicrobial substrates will play a more powerful in inhibiting pathogenic bacteria, especially *Salmonella typhimurium* and *Mycobacterium tuberculosis* bacteria.

Materials and Methods

Preparation of Koumiss starter culture

The first step for making a starter koumiss is pasteurized mare milk at 65 °C for 30 minutes, then cooled to a temperature of 28 °C. Koumiss starter culture made by dividing the three equal parts, one part milk with *Lc. lactis* D-01, one part milk inoculated with *Lb. acidophilus* Y-01 and then incubated at 37 °C for seven hours and one part milk inoculated with *Sc. cereviceae* at 25 °C for five hours. *Lc. lactis* D-01, *Lb. acidophilus* Y-01 and *Sc. cereviceae* as much as 3%-5% (v/v) mived into the mare pasteurize milk. The results of a mixture of milk and starter cultures were incubated at 28 °C for 24 hours to form the desired starter (modified Rahman et al., 1992).

Koumiss manufacture

Koumiss made by pasteurized at 65 °C for 30 minutes and after the temperature reached 28 °C were inoculated with a starter (30%). Incubation was performed again at 28 °C for 42 hours (Rahman *et al*, 1992).

Characteristics of microbiological koumiss

Pipetted one ml koumiss, put in a petri dish, then poured with 15-20 ml of sterile medium and homogenized. Petri dishes were incubated with the situation reversed in an incubator temperature of 37 °C for 24 hours (Fardiaz, 1992). Microbial colonies formed was calculated based on the Standard Plate Count (SPC).

The antimicrobial activity of Koumiss against Salmonella Typhi-murium

The inhibition of antimicrobial activity against the *Salmonella* typhi-murium made by the well agar diffusion method (Wiryawan *et al.*, 2009). This method performed by spreading the bacteria standard 0.5 Mc. Farland without equally diluted, cut well with a hole punch or cork borer (5 mm), coated with Bacteriological media koumiss used to avoid seep at the bottom of the well. A total of 50 µl koumiss pipetted into the well, then the cup is placed in the refrigerator and then incubated at 37 °C for 24 hours.

The antimicrobial activity of Koumiss against Mycobacterium tuberculosis

Inoculation of bacterial suspension begins with the preparation of *Mycobacterium tuberculosis* H37RV with a standard concentration of 0.5 Mc. Farland and diluted to 10^3 cfu/ml. Dilution made by adding 1% of bacteria (v/v) into five ml of NaCl sterile. A total of 100 ml bacterial suspension was inoculated into the media of resistance that has been prepared. Incubation media tubes with horizontal position with the angle of 30^o to the incubator at 37 °C for one night with the lid loose. After incubation, the tube caps are tightened and enforced tube into a vertical position. Colony growth readings performed at day 28 and 42 (Sjahrurachman, 2008).

Statistical Analysis

Testing treatment for *M. tuberculosis* H37RV is day 0, 2, 4, 6 and 8. Analysis of data for *M. tuberculosis* H37RV using non-parametric design, Cohran test. Statistical models are used as follows:

Cohran Test (Daniel, 1990)

$$Q = \frac{c\,(c-1)\left(\sum_{j=t}^{c} C_j^2\right) - (c-1)N^2}{(cN) - \sum_{i=1} R_i^{\,2}}$$

Information:

Q = Cohran statistics

C = Number of replication

N = Total number of treatment

R = Total number of replication

Results and Discussion

Microbiological characteristics of Koumiss

Koumiss microbiological charac-teristics observed a total coliform, total microorganisms (TPC), total lactic acid bacteria and yeast total. Microbiological characteristics of koumiss in this study had 9.67 $\log_{10}$ cfu TPC/ml, coliform> 1 $\log_{10}$ cfu/ml, LAB 10.13 $\log_{10}$ cfu/ml and 9.72 $\log_{10}$ cfu yeast/ml.

The number of yeast colonies during storage ranged 9-11 $\log_{10}$ cfu/ml. This amount is less than the number of LAB colony during storage ranged 8-12 $\log_{10}$

cfu/ml. LAB and yeasts grew together form a symbiosis in the koumiss like kefir grains. Yeasts in the kefir grains serves to maintain the integrity and viability of microflora populations. Essential amino acids and growth factors for lactic acid bacteria produced by yeast, whereas the metabolites of LAB is used as an energy source. Symbiosis between the LAB and the yeast is making kefir into a stable product (Farnworth and Mainville, 2003).

The inhibitory activity of antimicrobial Koumiss againts S. typhimurium ATCC 14028

The diameter inhibition of koumiss against *S. typhimurium* ATCC 14028 increased on the day of the storage (H8). Koumiss has a pH of 3.87 ± 1.77 0.004 ± 0.032 and TAT. The optimum pH value for growth of *S. typhimurium* is 6.5 to 7.5 (Cox, 2000), the growth of *S. typhimurium* ATCC 14028 koumiss been restrained by a low pH. Diameter inhibition of koumiss again *S. typhimurium* ATCC 14028 with a spread plate method is smaller than the pour plate method. The population of *S. typhimurium* ATCC 14028 on a spread plate method is 10^8 cfu/ml, whereas the pour plate method 10^6 cfu/ml.

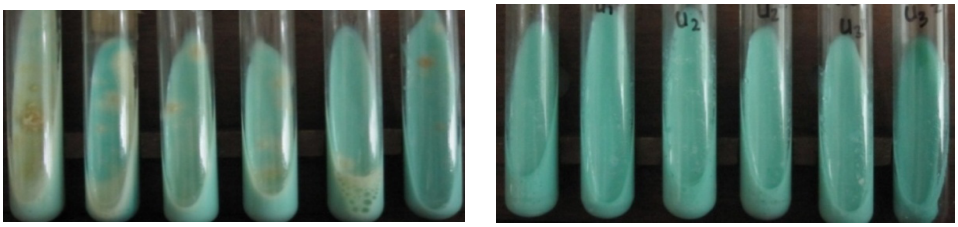
Antimicrobial activity can be observed in the diffusion test wells influenced by several factors, such as: (1) the type and size of the tube, (2) the type of agar, pH and salt content, (3) the ability of substances to diffuse into the agar, (4) characteristics of the media and (5) type of test bacteria used (Branen, 1993).

Inhibition of the filtrate and koumiss by category Morales et al. (2002) included the intermediate category. Intermediate category is the category with inhibitory response against bacterial pathogens that need to be treated with high doses of antimicro-bials. One way is by doing regular therapy with these antimicrobials.

The inhibitory activity of antimicrobial Koumiss againts M. tuberculosis H37RV on variety storage times

Control indicates the growth of *M. tuberculosis* with means proportion of 1.00 *M. tuberculosis* H37RV grown on Lowenstein Jensen media controls that are genuine growth medium *M. tuberculosis*. Growth of *M. tuberculosis* H37RV in the 8th week of observation is presented in Figure 2.

Growth of *M. tuberculosis* H37RV in the 8th week of observation for the control treatment highly significant ($P<0.01$) with the addition of koumiss treatment on day zero storage time (H0), four days (H4), six days (H6) and eight days (H8). Aditama (1999) suggest that the inhibitory properties of fermented compounds are bacteriostatic, because *M. tuberculosis* still can grow when the acidity is removed until it reaches a neutral pH.



control (n=6) *koumiss* H8 (n=6)

Figure 2. Growth of *M. tuberculosis* H37RV in the Storage Koumiss Days (H2), (H4), (H6) and (H8)

Conclusion

Koumiss with storage treatment can inhibit bacterial growth of *S. typhimurium* ATCC 14028 in the range of inhibition zones varying, while the antimicrobial activity of koumiss effective in inhibiting the growth of bacteria *M. tuberculosis* H37RV after the product has a minimum of four days of storage.

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