Probiotic effect of fermented milk from *Pediococcus acidilactici* BK01 in fecal wistar rat microflora

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Abstract. Microorganisms that provide health benefits to the host when consumed in the right proportions are called Probiotics. Certain lactic acid bacteria (LAB) have been considered probiotics with proven health benefits. These probiotics have been used extensively for human health. In this study, in vivo evaluation was conducted to determine the count of microflora in the fecal of Wistar rats, which were given fermented milk with probiotic *Pediococcus acidilactici* BK01. *Pediococcus acidilactici* BK01 is a LAB with probiotic use, isolated from Bekasam (fermented fish). This research was conducted in vivo on 24 male rats (Wistar Rat). This study contain in 4 treatment groups. Each group contains six male rats. The results showed a significant increase in the group of rats given fermented milk compared to the control group (without fermented milk). The probiotics of fermented milk have significantly reduced the count of *E.coli* and not on the total aerobic bacteria. The conclusion of this study, probiotic of fermented milk *Pediococcus acidilactici* BK01, can increase the total microflora of lactic acid bacteria and reduce the number of pathogenic bacteria.

Keywords: Lactic acid bacteria, fermented milk, *E.coli*, microflora, *Pediococcus acidilactici* BK01

1. Introduction

Lactic acid bacteria are gram-positive bacteria that have beneficial properties for health. This bacteria live in human digestive tract in naturally. They have a vital function in control the microbial ecosystem of the large intestine [1]. The commensal intestinal flora of humans and animals includes the genera *Lactobacillus, Pediococcus* and *Lactococcus* [2] and [3]. When ingested in sufficient quantities, probiotics are live microorganisms that can provide health benefits to the host and can be used as an alternative in conventional medicine in various intestinal diseases [4]. Diarrhea is one of the leading causes of childhood morbidity and mortality in developing countries. It is also reported that diarrhea caused by *Salmonella, Shigella Escherichia coli* pathogens, *Vibrio cholera*, and *Campylobacter* is a general cause of infant and child death in developing countries [5]. There are several studies that state that many probiotic strains can inhibit the growth and activity of some disease-causing bacteria [6] and
[7]. Probiotics, some of Lactobacillus strains, is currently being studied for its probiotic properties and is recommended for the prevention therapy of acute diarrheal infections in children [8]. [9] reported L. fermentum L23, able to inhibit Listeria monocytogenes bacteria. Then there are several Lactobacillus strains isolated from various milk sources that are probiotic and able to inhibit E.coli [10], [11], and [12].

Furthermore, this type of lactic acid bacteria is a potential microorganism and has been applied to various fermented products in the world. The milk fermentation process involves LAB activity which converts milk into quality fermented milk. The activity of this bacteria in fermented dairy can be a spontaneous starter culture or inoculated. The role of LAB in milk fermentation to produce organic acids that function as preservatives and flavor enhancers. They also make exopolysaccharides that are important for texture formation. Considering the existing reports regarding the health benefits of LABs and the status of LABs that are generally recognized as safe (GRAS), they can be widely used in the development of fermented dairy products [13].

One of the LAB used in this research is Pediococcus acidilactici BK01, which results from a selection of probiotics isolated from tamarind (fermented fish). This bacterium can inhibit the growth of E.coli O157 in vitro, with an inhibitory power of 21.26 ± 0.03 mm [12]. Pediococcus acidilactici, Gram-positive, facultative anaerobic, cocci-shaped, homofermentative are widely used in commercial fermentative processes to make popular food products [14]; [15]; [16].

In a previous study, [17] applied Pediococcus acidilactici BK01 as a starter in processing fermented milk from goat milk. This fermented goat milk had a number of lactic acid bacteria of 10.38 Log CFU/ml. and pH 4.3 – 4.5. In this study, in vivo evaluation was conducted to determine the amount of microflora in the Fecal Wistar rats given the probiotic fermented milk Pediococcus acidilactici BK01.

2. Material and methods

2.1. Animal treatment
The guidelines established by The Health Research Ethics Committee Andalas University Padang (No.009/ laiketik/ KEPKFKEPUNAND) were followed during all experimental procedures. Many 24 male Wistar rats (average weight of 200g) adapted to the environment for one week in a cage, receiving standard Rodentia feed and drinking ad libitum. After that, the rats were divided into four groups: Control Group (without treatment), Group P1 (fermented milk 0.35 mL), Group P2 ((fermented milk 0.70 mL), and Group 4 (fermented milk 1.05 mL) for four weeks.

2.2. Fermented milk production
Goat milk was pasteurized at a temperature of 65-67°C for 30 minutes, and then the milk temperature reaches 37°C [18]. Starter Pediococcus acidilactici BK01 BK01 was added as much as 5%, then incubated for 12 hours at 37°C. Pediococcus acidilactici BK01 fermented milk was stored.

2.3. Fecal sampling and bacterial analysis
Fecal were collected before and after treatment for four weeks, and the total fecal microflora was calculated, namely total lactic acid bacteria [19], total E.coli, and total aerobic bacteria. Fecal samples were taken directly from the rectum by rectal stimulation and immediately transferred into sterile tubes and stored at 4°C.

2.4. Statistical analysis
The data obtained were tested for differences in the number of microflora in the rat's fecal before and after administering the probiotic fermented milk Pediococcus acidilactici BK01 using the paired t-test. Differences in the effect of fermented milk on microflora in the rat's fecal of all groups were analyzed by the Kruskall-Wallis test and followed by the Mann-Whitney test to see the differences between groups.
3. Result and discussion

3.1 Count of Lactic Acid Bacteria (LAB)
The addition of probiotic fermented milk *Pediococcus acidilactici* BK01 for four weeks in rats to the total of LAB can be seen in the fecal Table 1. There was a significant increase (P<0.01) in number of lactic acid bacteria compared to the control group after giving *Pediococcus acidilactici* BK01 probiotic fermented milk.

| Group     | Before  | After   | Δ       | Δ (%)  | P    |
|-----------|---------|---------|---------|--------|------|
| Control   | 7.74 ± 0.26 | 7.92 ± 0.05 | 0.18*   | 2.32   | 0.004|
| Group M1  | 10.17 ± 0.10 | 7.57 ± 0.09 | 2.60*  | 34.34  |
| Group M2  | 11.03 ± 0.08 | 7.59 ± 0.04 | 3.44*  | 45.53  |
| Group M3  | 11.24 ± 0.14 | 7.84 ± 0.14 | 3.40   | 43.37  |

Based on statistical tests, the increase of total lactic acid bacteria showed significant differences in the M1, M2, and M3 groups. The highest increase in total lactic acid bacteria was M2 of 3.44 Log CFU/mL, followed by M1 2.60 Log CFU/mL and control group 0.18 Log CFU/mL. These results are in line with previous studies [23]. The increase in anaerobic microorganisms also occurred in the fecal minipig fed a high cholesterol diet followed by a diet containing a mixture of three Lactobacillus strains.

3.2. Count of E.coli
The administration of the probiotic fermented milk *Pediococcus acidilactici* BK01 significantly (P<0.01) decreased the number of E.coli in the fecal, compared to conditions before the administration of fermented milk (Table 2). There was a decrease in E. coli in the fecal, increasing the dose of fermented milk consumption after four weeks. The results of this research are in line with the previous studies of [23]. The increase in anaerobic microorganism also occurred in the fecal minipig fed a high cholesterol diet followed by a diet containing a mixture of three Lactobacillus strains.

| Group     | Before  | After   | Δ       | Δ (%)  | P    |
|-----------|---------|---------|---------|--------|------|
| Control   | 7.48 ± 0.48 | 7.40 ± 0.20 | 0.08*   | 1.07   | 0.001|
| Group M1  | 5.59 ± 0.11 | 7.46 ± 0.46 | 1.87*  | 26.07  |
| Group M2  | 5.24 ± 0.23 | 7.41 ± 0.07 | 2.17*  | 29.28  |
| Group M3  | 4.30 ± 0.30 | 7.52 ± 0.22 | 3.22*  | 42.82  |

The decrease of *E. coli* with the provision of probiotic fermented milk *Pediococcus acidilactici* BK01 was due to lactic acid bacteria producing antimicrobial compounds such as lactic acid and other organic acids, bacteriocin, hydrogen peroxide, ethanol, and carbon dioxide that can inhibit pathogenic bacteria such as *E. coli*. [24] explained that LAB could release antimicrobial metabolites called bacteriocins. Pediocin produced by *Pediococcus acidilactici* is also an attractive antimicrobial agent against many bacterial pathogens and has pharmaceutical applications [25]. Organic acids and hydrogen peroxide produced by lactobacilli are reported to inhibit the growth of gram-positive and gram-negative bacteria, while bacteriocins are very influential on gram-positive [26].

In addition, lactic acid bacteria can also inhibit the proliferation of pathogenic bacteria [27] and [28]. [29] add this may involve stimulation of the immunological defense or secretion of antimicrobial compounds. *Lactobacillus* and *Bifidobacterium* counts were significantly higher in LAB-treated rats, indicating that the two strains successfully tolerated gastric acid and bile salts [30] and [31].
3.3. Count of aerobic bacteria

The count of aerobic bacteria in the control group, M1, M2, and M3, after giving fermented milk Pediococcus acidilactici BK01 did not increase significantly (P>0.05) compared to the control (Table 3). Generally, natural microflora presented in the gastrointestinal tract of the rat includes facultative aerobic bacteria such as *Coliform, Pseudomonas, Lactobacilli*, and yeasts such as Candida and other microbes.

| Group     | Before    | After     | ∆        | ∆ (%)    | P        |
|-----------|-----------|-----------|----------|----------|----------|
| Control   | 7.53 ± 0.07 | 7.16 ± 0.17 | 0.37     | 4.91     | 0.067    |
| Group M1  | 7.61 ± 0.03 | 7.30 ± 0.01 | 1.31     | 4.07     |          |
| Group M2  | 7.48 ± 0.12 | 7.10 ± 0.39 | 0.38     | 5.08     |          |
| Group M3  | 7.46 ± 0.23 | 7.09 ± 0.08 | 0.37     | 4.91     |          |

4. Conclusion

The results showed a significant increase in the group of rats given fermented milk compared to the control group (without fermented milk). The probiotics of fermented milk have significantly reduced the count of *E.coli* and not on the total aerobic bacteria in fecal Wistar rat. The conclusion of this study, probiotic of fermented milk *Pediococcus acidilactici* BK01, can increase the total microflora of lactic acid bacteria and reduce the number of pathogenic bacteria.

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