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Characteristics of Indigenous Probiotic from River Buffalo Milk in North Sumatera Indonesia

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Abstract

The research aimed to know the probiotic characteristics of Lactic Acid Bacteria (LAB) was isolated from river buffalo milk in North Sumatera Indonesia. A total of 41 LAB isolated from river buffalo milk, were tested for bile salt condition, antimicrobial activity and ability to attach on intestinal mucosa. Result showed all of the isolates were able to survive in the presence of 0.5% bile salt and there were decreasing viability cell number from 1.1 to 3.3 log cfu/ml. The ten isolates have antibacterial effect on the indicator microorganisms and were able to attach on intestinal mucosa. An increasing of LAB cell numbers attach on intestinal mucosa ranged from 0.7 to 1.7 log cfu/cm^2.

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In conclusion, ten isolates from river buffalo milk have some probiotic properties and potential as a candidate probiotic bacterium.

Keywords: buffalo milk; lactic acid bacteria; probiotic

1. Introduction

Lactic Acid Bacteria (LAB) are widely distributed in the nature. Milk from different mammalian animals can be used as source of LAB. Buffalo milk is a source of various lactic acid bacteria (LAB) which is potentially as culture starter as well as probiotic [1,2]. Some strains of LAB can be considered as probiotic bacteria. Probiotics have been defined in many ways over the years. The most widely accepted definition is live microorganisms which when administered in adequate amounts confer a health benefit on the host [3]. Probiotics are beneficial bacteria in that they favorable alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection [4].

Research about characterization of probiotic from milk have been conducted in many times such as milk goat [5, 6], Bima horse milk [7], wild horse milk [8], Sumbawa horse milk [9], sheep milk [10] breast milk [11] and buffalo milk [1, 2, 12, 13]. Buffalo milk represents an important animal product, due to its nutrient. Buffalo milk also being natural niche and habitat of lactic bacteria which can produse antimicrobial metabolite compounds such as lactic acid, bacteriocins (e.g. acidocin, acidophilin, lacticin, nisin), and hydrogen peroxide [14].

There are also surprisingly studies on the characterization of potential probiotic bacteria from buffalo milk. Previous research showed the species identified probiotic from buffalo milk were, *Lb. acidophilus, Lb. delbrueckii ssp. bulgaricus, Lc. lactis ssp. Cremoris, Lc. lactis ssp. Lactis, Str. thermophilus* and *Lb. acidophilus* [13].

Viability and survival of probiotic bacteria are important characteristics in order to provide health benefits. Probiotic should survive the gastro-intestinal transit to finally colonize the gut. Natural resistance to gastro-intestinal transit varies between LAB species. Indeed, certain strains have the capacity to resist more easily to the extreme acidity of stomach. Another desirable characteristic of probiotics are their capacity to modulate the intestinal microbiota and the ability to produce antimicroba. In order to be able to exert its beneficial effects, a good potential probiotic strain is expected to have a number of desirable properties [15, 16].

This study will be focused on the probiotic characteristics of isolated from river buffalo milk in North Sumatera Indonesia.

2. Materials and Methods

The river buffalo milk samples were collected from different farm in North Sumatera, Indonesia (Lubuk pakam, Medan, Patumbak, and Siborong-borong). It was obtained from healthy buffalo under good condition. The sample was collected in sterile bottle. The samples were transferred to the laboratory in chilled condition (10 – 12°C). Isolation of LAB from buffalo milk was carried out by De Man Rogosa Sharpe (MRS). Morphological,

physiological and biochemical characteristics were conducted to identify LAB and to obtain the probiotic candidate will be done the following steps.

2.1. Tolerance bile salt

The number of 41 isolates were inoculated into MRS Broth with concentrations of bile salt 0.5% (*oxgall*), MRS broth without bile salt was a control, and incubated at 37 ºC for 6h. Then 0.1 mL inoculums was transferred to MRS agar by pour plate method and incubated at 37 ºC for 24h. The growth of LAB on MRS agar plate was used to designate isolates as bile salt tolerant [17].

2.2. Antimicrobial activity

Cell-free culture supernatants for antibacterial assay was prepared by growing the isolates in MRS broth at 37 °C and centrifuged at 12,000 x *g* for 10 min at 4 °C. The antimicrobial activity of the cell-free culture supernatant was determined by the agar well diffusion assay. Aliquots of supernatants (100 µL) were placed in wells (5 mm diameter) cut in cooled soft nutrient agar plates (25 mL) previously seeded (1% v/v) with the appropriate indicator strains, which were *Eschericia coli, Salmonella typhimurium, Sthapilococcus aureus, Bacillus cereus* and *Pseudomonas aeroginosa*. The plates were incubated under optimal conditions for growth of the target microorganisms. After 24 h, diameters of the growth inhibition zones were measured. The inhibitory spectrum of the antibacterial agent produced by isolates of lactobacilli against different gram-positive and gram-negative bacteria was determined by agar-well diffusion assay [18].

2.3. The ability of LAB to attach on intestinal mucosa

Selected LAB isolates incubated in MRS Broth for 24h, then harvested with deposited using a centrifuge at 5000 rpm for 15 minutes. The precipitate was washed with PBS then soluted into PBS to reach at least 9 log cfu ml^{-1}. Mucus was prepared from rat *Sprague Dawley* intestine. By scraping the inside of the intestine with a spatula, the material was removed and collected in 200 ml ice-cold phosphate buffer solution (PBS). LAB number counted with plate count. 10 ml of suspension BAL (10^8 log cfu ml^{-1}) is inserted into a petri containing rat intestinal pieces with 1 cm^2 area and incubated at ambient temperature (30 ^oC) for 60 min [19].

3. Result and discussion

In the present study obtained 41 isolates LAB from North Sumatera river buffalo milk, higher number than reported by [13] were identified 27 isolates LAB obtained from Islamabad buffalo milk. Indigenous probiotic characteristics from river buffalo milk will be explained clearly in sub-bab below.

3.1. Tolerance bile salt

Result of tolerance against bile salt showed a decreasing of cell number from 1.1 to 3.3 log cfu ml^{-1} with average 2.29±0.64 log cfu ml^{-1} (Figure 1). According to the results all of the isolates were resistant to 0.5% bile salt, L20 isolate is more tolerant than other isolates. In agreement with [2] have been found that all isolates were

resistant to 0.5% bile salt.

Ten isolates from river buffalo milk that had the highest tolerance in bile salt condition showed by L12, L16, L17, L19, L20, M10, P8, S3, S19 and S20. The survival at bile salt condition is one of the critical point for the probiotic bacteria. Several strains of *Lactobacillus* are able to hydrolyze bile salt by using specific enzimes, bile salt hydrolysis that is able to decrease the solubility of bile salt, which in turn, decreases or eliminates the toxic effect of the bile salt to the LAB [6]. Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host. This will help *Lactobacilli* to reach the small intestine and colon and contribute in balancing the intestinal microflora [20].

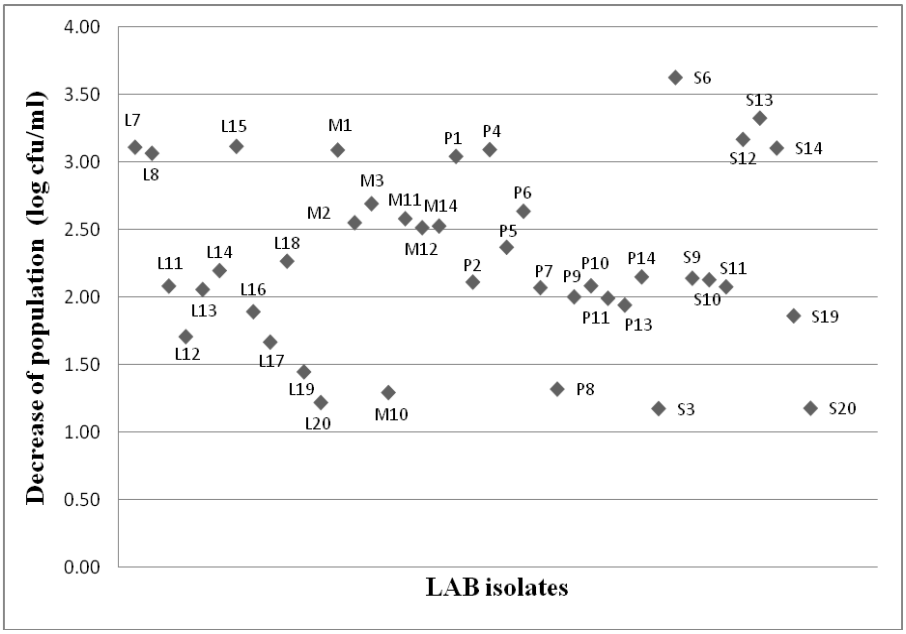

Figure 1 : Decrease of LAB isolates population after exposure to bile salt

3.2. Antimicrobial activity

The selected strains were assayed according to their antimicrobial activity. In present study, a total of 41 isolates were selected, and obtained 10 the best isolates which were showed in Table 1. The diameter of inhibition zones showed that all of the isolates have antibacterial effect on the indicator microorganisms, *Escherichia coli*, *Salmonella thypimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The antibacterial effect on the indicator microorganisms was determined by diameter of inhibition zones. The diameters of the inhibition zones were in the range of 6.96 - 13.86 mm, with the largest diameter of the inhibition zone by strain L20.

Changes in measures of diameter of the inhibition zone and consequently the sensitivity of the revealing strains tested, observed when the action of lactic acid bacteria strains of the same species or different species may also be related to the existence of different mechanisms of inhibition or nature inhibitory chemical substance that

influences its diffusion in the culture medium [22].

Antimicrobial activity is important criteria for *Lactobacillus spp.* to use against various diseases caused by pathogens. Our study reveals that the identified *Lactobacillus brevis* can be used against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and *Lactobacillus fermentum* against *Bacillus subtilis* and *Shigella dysenteriae* [1].

Table 1: Inhibitory diameter average of selected strains of antibacterial-producing LAB

	Inhibitory diameter average (mm)				
Isolates	*E.Coli*	*Salmonella*	*S. Aureus*	*Bacillus*	*Pseudomonas*
L12	8.26 [a]	7.28 [a]	8.31 [a]	6.96 [a]	8.34 [c]
L16	7.93 [a]	7.32 [a]	10.09 [b]	7.47 [a]	8.84 [d]
L17	8.06[a]	8.11 [a]	8.02 [a]	8.30 [b]	7.69 [b]
L19	7.37 [a]	8.25 [a]	8.34 [a]	8.34 [b]	9.13 [d]
L20	13.50 [c]	12.98 [c]	12.45 [c]	13.69 [d]	13.86 [g]
M10	8.09 [a]	7.59 [a]	8.00 [a]	6.94 [a]	7.50 [ab]
P8	7.65 [a]	7.68 [a]	9.84 [b]	7.02 [a]	7.29 [a]
S3	13.22 [c]	11.84 [b]	12.31 [c]	13.53 [d]	12.54 [f]
S19	8.53 [a]	7.55 [a]	9.50 [b]	8.34 [b]	8.27 [c]
S20	10.20 [b]	8.09 [a]	9.82 [b]	11.22 [c]	11.65 [e]

Note: Means in the same column with different letter differ significantly ($P<0.05$) L: buffalo milk from Lubukpakam; M: buffalo milk from Medan; P : buffalo milk from Patumbak, S: buffalo milk from Siborong-borong.

Table 2: Probiotic population on intestinal mucosa

No	Isolates	Increasing cell number (log cfu cm^{-2})
1	L12	1.42 [d]
2	L16	1.19 [c]
3	L17	0.78 [a]
4	L19	0.98 [b]
5	L20	1.72 [e]
6	M10	1.63 [e]
7	P8	1.67 [e]
8	S3	1.64 [e]
9	S19	1.37 [d]
10	S20	1.46 [d]

Note: L: buffalo milk from Lubukpakam; M: buffalo milk from Medan; P: buffalo milk from Patumbak, S: buffalo milk from Siborong-borong

3.3. The ability of LAB to attach on intestinal mucosa

Test of the ability of lab to attach on intestinal mucosa was done in 10 the best isolates, the results were showed in Table 2. An increasing of LAB cell number attach on intestinal mucosa ranged from 0.7 to 1.7 log cfu / cm^2. Probiotics effects on the microbiota and pathogens of the gastrointestinal tract, selected probiotic strains have been suggested to affect also pathogens of the oral cavity and reduce the colonisation level and activity of cariogenic *Streptococci* and reduce levels of *Candida* [23].

Probiotics in the gastrointestinal tract decrease adhesion of both pathogens and their toxins to the intestinal epithelium. Several strains of *Lactobacilli* and *Bifidobacteria* are able to compete with pathogenic bacteria for intestinal epithelial cell binding, and they can displace pathogenic bacteria even if the pathogens have attached to intestinal epithelial cells prior to probiotic treatment. However, specific probiotics or probiotic combinations should be selected based on their ability to inhibit or displace a specific pathogen [24].

4. Conclusion

The present study revealed that LAB from river buffalo milk in North Sumatera, were able to survive in the presence of 0.5% bile salt concentration, isolates have antibacterial effect on the indicator microorganisms and were able to attach on intestinal mucosa. A total of 10 the best isolates are derived from river buffalo milk in North Sumatera showed potential as a candidate probiotic bacteria.

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