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Probiotics and Strong Antimicrobial of Buffalo Milk Fermentation (Dadih) From Different Places in West Sumatera Indonesia.

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ABSTRACT

Dadih is a traditional food from West Sumatera made by natural fermentation of buffalo milk in bamboo. Dadih is also known contain lactic acid bacteria which have probiotic properties. In this study, samples of dadih which investigated were origin from Solok, Sijunjung and Payakumbuh. To investigate the resistance of LAB in acidic environment, LAB was grown in MRS medium with a pH 3 and 4. The antimicrobial activity was carried out using agar diffusion method against *S.aureus*, *E.coli* and *S.typhi*. All LAB isolates are still grow in acidic environment. For antimicrobial activity, LAB from dadih origin from Payakumbuh showed the strongest activity against pathogen bacteria, which is 22 mm against *S.aureus*, 21 mm against *E.coli* and 18 mm against *S.typhi*. From biochemical characteristic and morphology, it is assumed that the LAB was classified as *Lactobacillus plantarum*

Keywords: Probiotic, Antimicrobial, *Lactobacillus plantarum*, dadih

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INTRODUCTION

Lactic Acid Bacteria (LAB) refers to a large group of bacteria, which produce lactic acid as a by product of digesting their food source. LAB accumulates to ferment the food and these bacteria could survive to live in an acidic environment. LAB are spread in nature and useful to our digestive system because it is a probiotic bacteria [1]. Various sources of LAB are from fermented milk products [2,3,4], raw goat's milk [5] fruits [6] and fermented milk [7] . LAB producing some antimicrobial compounds such as organics acids, diacetyl, hydrogen peroxide and bacteriocins. These substancs has ability to inhibit the growth of pathogenic bacteria [8]. In addition, many LAB classified as probiotics due to beneficial effects for human health, particularly digestive tract [7].

Dadih, is a traditional food of West Sumatera, which is made from raw buffalo milk, which naturally fermented in bamboo tubes. It was consumed by the West Sumatran Minangkabau ethnic group of Indonesia as one of the characteristic traditional foods of Minangkabau culture. Native people named it as "dadiah" and it is very popular dairy product in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota and Tanah Datar. In West Sumatera, dadih is still a homemade product by traditional way, no heat is applied to buffalo milk and also not using starter culture. Protein content in dadih was 39,8% and it also contain essential amino acids, calcium and vitamins B and K, that produce from fermentation process [9]. Dadih is also known contain lactic acid bacteria which have probiotic properties [7].

Even though the process did not implement the hygiene practice, there was no food poisoning was reported among people who consume dadih. Various indigenous LAB involved in the dadih fermentation may vary from time to time as well as from one place to another due to the natural fermentation [10]. Due this fact it is very interesting to investigate the indigenous LAB in dadih which taken from several areas in West Sumatera. The aim of this study was to investigate the antimicrobial activity and probiotics of LAB isolated from dadih from various regions in West Sumatera includes Solok, Sijunjung and Payakumbuh.

MATERIAL AND METHODS

Location Samples

Dadih were taken from 3 areas in West Sumatera, which is derived from Sungai Janiah village, Solok ($00^{o}47'59"S$ $100^{o}39'58"E$), Pematang Panjang, Sijunjung ($1^{o}00'S$ $100^{o}30'E$) and from Lareh Sago Halaban, Payakumbuh ($0^{o}14'S$ $100^{o}38'E$). Dadih samples obtained from local traders in local market in each region, and immediately taken to laboratory. Samples were stored in refrigerator at $4^{o}C$.

Isolation and characterization of lactic acid bacteria from dadih

Serial dilution method was used for isolation of LAB from dadih. Briefly, 1 gram sample was dissolved into 9 mL de Mann Rogosa Sharpe (MRS) broth and incubated for 24h at $37^{o}C$ in anaerobic condition. Isolation of LAB was conducted by serial dilution and spread onto MRS agar supplemented with 1,5% $CaCO_3$. 6 colonies LAB which showed clear zone on MRS agar were purified by re streaking into new MRS agar. The Gram staining and catalase test were conducted in each isolates.

pH tolerance

LAB isolates were grown in MRS broth and incubated for 24 hours at temperature $37^{o}C$. 1 mL of the culture was inoculated into 9 mL MRS broth with pH 3 and 4. The growth of LAB was observed using spectrophotometer at wave length 600 nm at 0, 2, 4, 12 and 24 hours.

Antimicrobial activity of lactic acid bacteria against pathogenic bacteria

Antimicrobial activity of LAB against pathogenic bacteria was carried out using disc diffusion assay. 6 selected LAB isolates were grown in MRS broth and incubated anaerobically for 24 hours at temperature $37^{o}C$. The pathogenic bacteria (*E.coli, S. aureus* and *S.typhi*) were grown in Nutrient broth at $37^{o}C$ for 24 hours. The pathogenic bacteria then swabbed onto surface of MRS agar using sterile cotton swab. Filter paper disc (diameter 6 mm, Whatman no 1) was immersed in LAB cultur and air dried. The paper discs were placed on the

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surface of MRS agar which has been swabbed with pathogen bacteria, then incubated at 37^{o}C for 24hours. Inhibition zone which formed around the discs then measured using caliper.

RESULT AND DISCUSSION

LAB isolation, enumeration and characteristic

Total colony of LAB obtained from 1 gram each dadih from Solok, Sijunjung and Payakumbuh were 84 x 10^8 CFU/mL, 308 x 10^8 CFU/mL and 80 x 10^8 CFU/mL respectively. 6 colonies from each samples were identified as *Lactobacillus* based on morphology, catalase test and Gram staining. All characteristic of the colonies from different dadih samples give the same result. The result was showed in table 1.

Table 1: Characteristic LAB isolated from dadih

LAB	origin	Characteristic		
		Gram staining	Catalase test	Morphology
E1	Solok, West Sumatera	Positive	Negative	Creamy, smooth and round colonies
E2		Positive	Negative	Creamy, smooth and round colonies
E3		Positive	Negative	Creamy, smooth and round colonies
E4		Positive	Negative	Creamy, smooth and round colonies
E5		Positive	Negative	Creamy, smooth and round colonies
E6		Positive	Negative	Creamy, smooth and round colonies
R1	Sijunjung, West Sumatera	Positive	Negative	Creamy, smooth and round colonies
R2		Positive	Negative	Creamy, smooth and round colonies
R3		Positive	Negative	Creamy, smooth and round colonies
R4		Positive	Negative	Creamy, smooth and round colonies
R5		Positive	Negative	Creamy, smooth and round colonies
R6		Positive	Negative	Creamy, smooth and round colonies
S1	Payakumbuh, West Sumatera	Positive	Negative	Creamy, smooth and round colonies
S2		Positive	Negative	Creamy, smooth and round colonies
S3		Positive	Negative	Creamy, smooth and round colonies
S4		Positive	Negative	Creamy, smooth and round colonies
S5		Positive	Negative	Creamy, smooth and round colonies
S6		Positive	Negative	Creamy, smooth and round colonies

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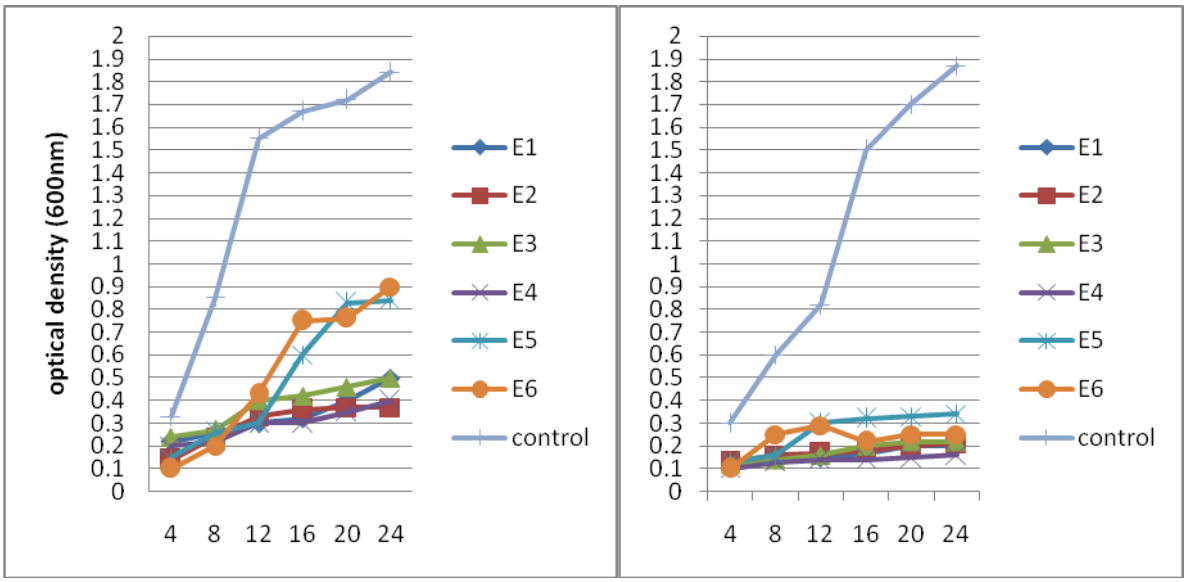
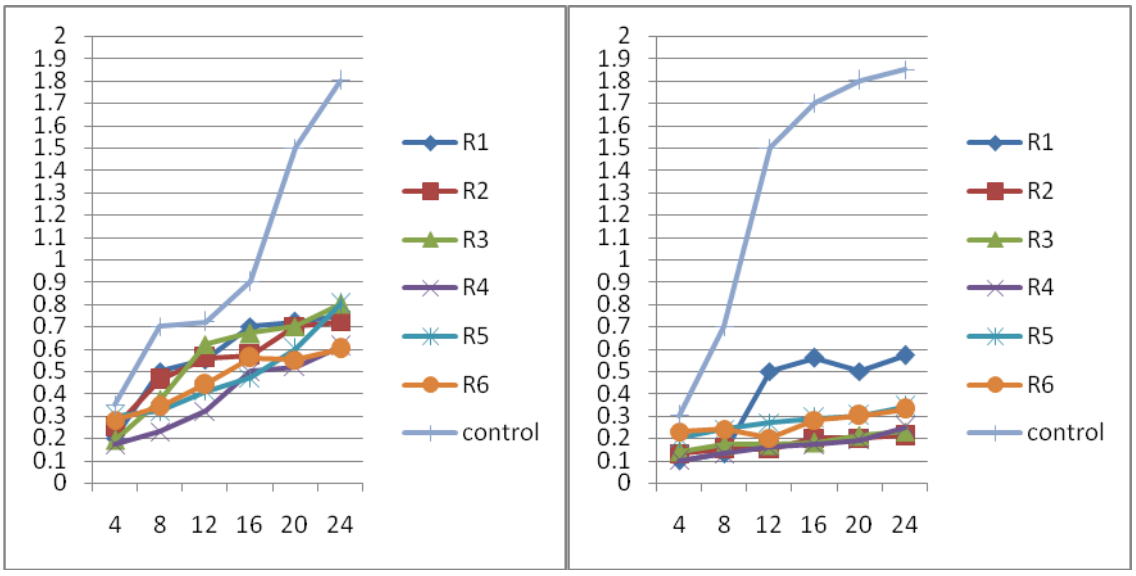
pH tolerance

In this study, it was observed that LAB from dadih has ability to growth in an acidic environment. At pH 4, the cell density which indicates absorbance, has greater value than pH 3, so it could be concluded that LAB growth was strongly influenced by pH. One of the requirement to be categorized as probiotic is the LAB should be able to survive in acidic environment. LAB should be resists in acidic condition of the stomach and upper intestine which contain bile salts [11]. Acidic conditions is one of the most detrimental factor affecting the growth of LAB, because in general, bacterial growth is significantly decreased at pH below 4,5 [12]. The result was showed in Figure 1



(a) (b)



(a) (b)

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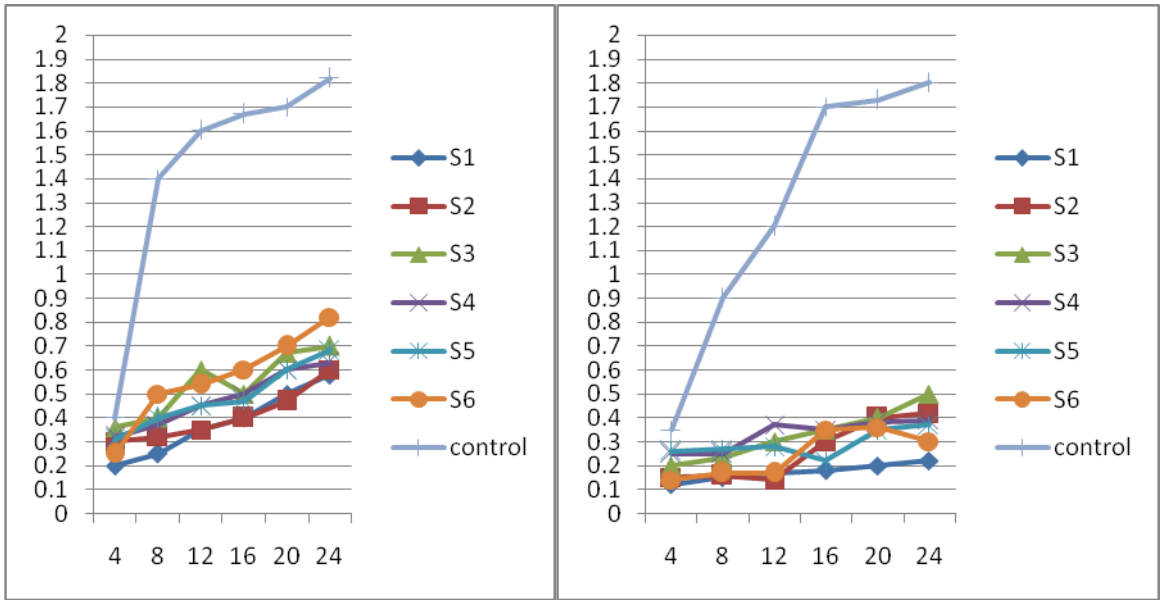

(a) (b)

Figure 1: Effect pH on growth of LAB : (a) pH 4, (b) pH 3

Activity of isolated Lactic acid bacteria against pathogen bacteria

The antimicrobial activity of LAB isolated from dadih against pathogen bacteria was showed in Table 2. For LAB from dadih from Solok, generally all the isolates showed strong antimicrobial activity; 14mm against *S.typhi*, 14mm against S.aureus and 12mm against *E.coli*. Different result showed by LAB isolated from dadih from Sijunjung, only 3 isolates (R2, R3 and R5) showed strong antimicrobial activity; 12 mm against *E.coli*, 13,5mm against *S.aureus* and 12 mm against *S.typhi*. For LAB from dadih origin from Payakumbuh, 5 of 6 the isolates showed strong anti microbial activity against pathogen bacteria compared from dadih origin from Solok and Sijunjung . The strongest antimicrobial activity against *S.aureus* was 22mm; the strongest antimicrobial activity against *E.coli* was 21mm, and the strongest antimicrobial activity against *S.typhi* was 18 mm. The result of antimicrobial activity showed in Figure 2 and Table.2.

(a) (b) (c)

(d) (e) (f)

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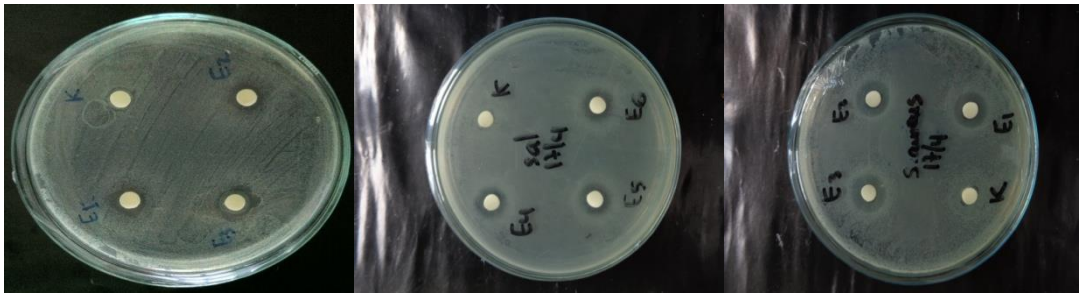

(g) (h) (i)

Figure 2: (a) antimicrobial activity of LAB from dadih of Sijunjung against *E.coli*, (b) against *S.typhi*, (c) against *S.aureus*, (d) antimicrobial activity of LAB from dadih of Solok against *E.coli*, (e) against *S.typhi*, (f) against *S.aureus*, (g) antimicrobial activity of LAB from dadih of Payakumbuh against *E.coli*, (b) against *S.typhi*, (c) against *S.aureus*

Table 2: Inhibition zone of LAB from dadih against pathogen bacteria

isolates	origin	Inhibition zone against (mm)		
		S.typhi	S.aureus	E.coli
E1	Solok, West Sumatera	11	12	10
E2		12	12	10
E3		12.5	13	11
E4		12	13.5	11
E5		14	13	10
E6		12	14	12
R1	Sijunjung, West Sumatera	-	-	-
R2		10	12	12
R3		10	13.5	11
R4		-	-	-
R5		12	10	11
R6		-	-	-
S1	Payakumbuh, West Sumatra	18	14	12.5
S2		12	15	14
S3		13	18	21
S4		12	18	18
S5		15	22	11
S6		11	12	8

From Figure 2 could be seen that LAB could inhibit the growth of pathogenic bacteria which is characterized by a clear zone around the paper discs. Generally the inhibition zone against Gram positive bacteria was greater than Gram negative bacteria. It is due to several mechanisms of resistance including permeability barrier properties on exterior layer that could inhibit the entry of antimicrobial compounds, as well as the mechanisms inactivate specific resistance of the compound to prevent penetrate the cytoplasmic membrane or prevent binding to intracellular [13]. The similar result was reported by Askari et al [14]. Askari et al reported that LAB isolated from dried fruits have strong antimicrobial activity against Gram positive bacteria, because the outer membrane of this bacteria could acts as permeability barrier for the cell to prevent the molecules such as antibiotics, detergent and dyes, to reach the cytoplasmic membrane. The antimicrobial activity of LAB might be due by several factors such as reduced pH, competition of the substrate and production of bacteriocin, a bactericidal protein which is an extracellular protein of LAB [15].

Syukur [7,8,9] has reported that the molecular characterization of LAB from dadih origin Sijunjung, using amplification gene 16SrRNA refers to *Lactobacillus plantarum* FL11-3. Due to the geographic proximity, allows that species of LAB in Solok and Payakumbuh were also *Lactobacillus plantarum*. Siezen and Hylckama Vlieg [16] reported that *Lactobacillus plantarum* occupies a diverse range of environmental niches and has an

enormous diversity in phenotypic properties. *L.plantarum* is highly versatile and found in many different ecological niches such as vegetables, meat, fish and dairy products. Further antimicrobial compound will determined structure and relationship among different places.

CONCLUSION

From 3 samples of dadih taken from 3 different areas in West Sumatera namely Solok, Sijunjung and Payakumbuh, all LAB isolates have resistant properties in acidic environment. The strongest antimicrobial activity was shown by LAB isolated from dadih origin from Payakumbuh, followed by LAB from dadih origin from Solok and Sijunjung. Possible *L.plantarum* is the potential probiotic from West Sumatera

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