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## Research Article

# Characterization of the Antimicrobial Activity of Lactic Acid Bacteria Isolated from Buffalo Milk in West Sumatera (Indonesia) Against *Listeria monocytogenes*

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## Abstract

**Background and Objective:** *Listeria monocytogenes* is an important pathogenic bacteria in various cases of poisoning in the food industry due to its ability to grow in cold temperatures and to survive in freezing temperatures. Lactic acid bacteria have important probiotic attributes including their antimicrobial effect against this pathogen. Therefore, this study aimed to isolate lactic acid bacteria from buffalo milk and characterize its antimicrobial activity against *Listeria monocytogenes*. **Materials and Methods:** Buffalo milk was collected from four districts in West Sumatera, Indonesia and its composition analysed. A total of 88 lactic acid bacteria strains were isolated and grown at De Man Rogosa Sharpe Agar (MRSa). The strains were identified based on morphology (shape, size and colour) and their biochemical characteristics (catalase test and the fermentation type) and then screened for antimicrobial activity against *L. monocytogenes*. The species were further identified based on 16S rRNA gene sequence analysis. **Results:** As a result of isolation and identification, 19 strains of lactic acid bacteria were screened against *L. monocytogenes*, but only three isolates (A 3.2, A 3.3 and TD 7.2) showed high inhibition against *L. monocytogenes*. They were identified using 16S rRNA gene sequence analysis. **Conclusion:** The BLAST results of the identification procedure showed that the isolated bacteria from buffalo milk belonged to *Lactobacillus fermentum* strain L 23 (A 3.3), *Lactobacillus fermentum* strain 6704 (TD 7.2) and *Lactobacillus oris* strain J-1 (A 3.2).

**Key words:** Lactic acid bacteria, buffalo milk, antimicrobial activity, 16S rRNA, inhibition zone and *Listeria monocytogenes*

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.



1.5 kb) were purified with a Fast Gen Gel/PCR Extraction Kit (Nippon Genetics, Germany) according to manufacturer's instructions and the sequenced data were analysed and processed using BioEdit software. The sequences were compared with the sequences available in GenBank using BLAST (the Basic Local Alignment Search Tool) and all sequences were aligned using ClustalW (<http://clustalW.ddbj.nig.ac.jp>).

## RESULTS AND DISCUSSION

The chemical composition of buffalo milk from some districts in West Sumatera are listed in Table 1, the buffalo milk was found to contain 7.22-7.83% protein, 7.18-7.88% fat and 80.62-81.03% moisture with a pH range from 6.06-6.39. The moisture and fat contents found in this study were similar to those of swamp buffalo milk (81% moisture and 7.0%) fat<sup>18</sup>. The pH range obtained in this study was lower than that reported for buffalo milk from the region of Cantal, France (pH 6.81)<sup>19</sup>.

**Total lactic acid bacteria in buffalo milk:** As can be seen in Table 2, the total number of lactic acid bacteria from Agam and Tanah Datar was higher than that of 50 Kota and Solok. Moreover, the total LAB from this study was higher than that reported from Bulgarian Murrah buffalo ( $3.22 \times 10^5 \text{ cm}^{-3}$ )<sup>20</sup>.

The support of LAB in dairy products microbiota can be viewed applicable since these microorganisms are naturally show in milking and processing environment, facilitating the contamination of raw milk and processed products<sup>21</sup>. The LAB counts from raw milk were  $8 \times 10^5 \text{ C}^{22}$ .

**Lactic acid bacteria strains from buffalo milk:** As can be seen in Table 3. Eighty-eight LAB strains from buffalo milk were evaluated in this study and 19 were screened for antimicrobial activity against *Listeria monocytogenes*. In general, such strains were rod-shaped, gram-positive, 1-4 mm in size, beige and catalase-negative. In addition, they did not show the ability to form CO<sub>2</sub> and were thus classified as homofermentative; if bacteria can produce CO<sub>2</sub>, they are classified as heterofermentative. Homofermentative LAB have mostly been found in cow milk, cheese and

fermented milk<sup>23</sup>. Other reports have found homofermentative LAB strains in milk including *Streptococcus cremoris* and *S. lactis*<sup>24</sup>.

Previous researchers successfully isolated *Lactobacillus acidophilus*, *L. delbrueckii* ssp. *bulgaricus*, *Lactococcus lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis* and *Streptococcus thermophilus*<sup>25</sup>, *Bifidobacterium* spp. and *Lactobacilli* spp.<sup>9</sup>, *Lactococcus lactis*<sup>26</sup>. Lactic acid bacteria were isolated from various types of buffalo milk along with *Lactobacillus plantarum*, *L. brevis*, *L. pentosus* and *Lactococcus lactis*<sup>27,28</sup>. In addition, *Lactococcus lactis*, which can produce lactic acid, which is 57.61% of the lactic acid bacteria found in buffalo milk from North Sumatera along<sup>28</sup>.

**Antimicrobial activity against *Listeria monocytogenes*:** As shown in Table 4 of the 19 screened LAB strains from buffalo milk, 3 were found to have higher inhibitory activity against *L. monocytogenes*, i.e., strain A 3.3 (19 mm) and A 3.2 (18 mm) from Agam and strain TD 7.2 (19 mm) from Tanah Datar. Martinez and de Martinis<sup>29,30</sup> reported that *Lactobacillus sakei*, which produces bacteriocin, could decrease *L. monocytogenes* at 8°C. The bacteriocin of *L. mesenteroides* 11 partially inhibited *L. monocytogenes* at 8°C but at 15°C, it was unable to prevent the growth of the pathogen. Amezcuita and Brashears<sup>31</sup> found that the strain identified as *Pediococcus acidilactici* was a possible bacteriocin producer with antilisterial activity. Some of the studied LAB have antimicrobial activity against *L. monocytogenes* and probiotic potential<sup>32,33</sup>; the LAB from Dadih Solok could inhibit *L. monocytogenes* given its 8-14-mm clear zone<sup>34</sup>.

**Molecular identification using 16S rRNA\*\*\*:** The isolates were molecularly identified by amplifying and sequencing the 16S rRNA genes and comparing the results to the database of known 16S rRNA sequences. The BLAST results of the identification procedure showed that the isolated bacteria belonged to *Lactobacillus fermentum* strain L 23 (A 3.3), *Lactobacillus fermentum* strain 6704 (TD 7.2) and *Lactobacillus oris* strain J-1 (A 3.2). According to Pascual *et al.*<sup>35</sup>, *Lactobacillus fermentum* strain L 23 produces bacteriocins and it is heat-stable with a low-molecular-mass

Table 1: Composition of buffalo milk

District	Water (%)	Fat (%)	Protein (%)	pH
50 Kota	81.44±1.17	7.18±0.09	7.22±0.39	6.06±0.04
Agam	80.62±0.93	7.34±0.50	7.57±0.21	6.39±0.25
Tanah datar	80.48±1.12	7.73±0.22	7.23±0.76	6.10±0.02
Solok	81.03±1.91	7.88±0.98	7.83±0.19	6.08±0.03

Value represent Mean±SD, n = 3

Table 2: Total lactic acid bacteria of buffalo milk

District	Total LAB ( $1 \times 10^6$ CFU g <sup>-1</sup> )
50 Kota	3.0 ± 0.820
Agam	190.0 ± 30.51
Tanah datar	257.0 ± 8.540
Solok	24.0 ± 34.04

Table 3: Morphological and biochemical characteristic of the LAB

		Characteristic					
		Morphology				Biochemical	
District	Strain of LAB	Form	Gram stain	Size	Color	Catalase test	Fermentatif type
50 Kota	P 1.1	Rod	+	1 mm	Cream	-	Homo fermentatif
	P 1.2	Rod	+	1 mm	Cream	-	Homo fermentatif
	P 3.1	Rod	+	2 mm	Cream	-	Homo fermentatif
	P 3.5	Rod	+	4 mm	Cream	-	Homo fermentatif
Agam	A 1.2	Rod	+	2 mm	Cream	-	Homo fermentatif
	A 3.1	Rod	+	2 mm	Cream	-	Hetero fermentatif
	A 3.2	Rod	+	3 mm	Cream	-	Hetero fermentatif
	A 3.3	Rod	+	3 mm	Cream	-	Homo fermentatif
	A 3.4	Rod	+	3 mm	Cream	-	Homo fermentatif
Tanah Datar	TD 2.2	Rod	+	3 mm	Cream	-	Homo fermentatif
	TD 2.3	Rod	+	1 mm	Cream	-	Homo fermentatif
	TD 3.1	Rod	+	1 mm	Cream	-	Homo fermentatif
	TD 3.2	Rod	+	1 mm	Cream	-	Homo fermentatif
	TD 6.1	Rod	+	3 mm	Cream	-	Homo fermentatif
	TD 6.3	Rod	+	3 mm	Cream	-	Homo fermentatif
	TD 7.1	Rod	+	5 mm	Cream	-	Homo fermentatif
	TD 7.2	Rod	+	1 mm	Cream	-	Hetero fermentatif
Solok	S1.1	Rod	+	5 mm	Cream	-	Homo fermentatif
	S1.3	Rod	+	3 mm	Cream	-	Homo fermentatif

Table 4: Antimicrobe activity of LAB strains against *Listeria monocytogenes*

No.	District	Strain of LAB	Inhibition zone (mm)
1	50 Kota	P 1.1	17
2		P 1.2	17
3		P 3.1	12
4		P 3.5	14
5	Agam	A 1.2	17
6		A 3.1	14
7		A 3.2	18
8		A 3.3	19
9		A 3.4	15
10	Tanah Datar	TD 2.2	17
11		TD 2.3	15
12		TD 3.1	12
13		TD 3.2	15
14		TD 6.1	14
15		TD 6.3	15
16		TD 7.1	15
17		TD 7.2	19
18	Solok	S1.1	11
19		S1.3	11

Value represent Mean ± SD, n = 3

(<7000-Da) peptide. Yavuzdurmaz and Sebnem<sup>36</sup> reported that *Lactobacillus fermentum* showed positive fermentation

results for xylose, ribose, arabinose, melibiose, raffinose, galactose, maltose, sucrose, fructose and lactose; the fermentation result for *Lactobacillus oris* strain J-1 included ribose, arabinose, trehalose, melibiose, raffinose, galatose, maltose, sucrose, fructose and lactose. According to the classification, these biochemical characteristics indicate that the bacteriocin produced by *Lactobacillus fermentum* strain L 23 belongs to the class II lactic-acid bacterium bacteriocins<sup>37</sup>. Finally, the PCR products were sequenced and analysed using the basic local alignment search tool (BLAST, <http://blast.ncbi.nlm.nih.gov/>).

## CONCLUSION

Isolation and identification resulted in 88 strains of LAB from buffalo milk, which were mostly characterized as rod-shaped, gram-positive, catalase-negative, homo-fermentative and heterofermentative. Of the isolates, only three (A 3.3, A 3.2 and TD 7.2) showed high inhibition against *Listeria monocytogenes* and were

identified as *Lactobacillus fermentum* L23 (A 3.3), *Lactobacillus fermentum* 6704 (TD 7.2) and *Lactobacillus oris* strain J-1 (A 3.2).

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