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Selection of Lactic Acid Bacteria Isolated from Traditional Dadih for the Use of Producing Commercial Fermented Milk

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Abstract: Dadih is a traditional food from West Sumatra, Indonesia, which is made by buffalo milk that naturally fermented in bamboo tubes. Commercially producing dadih requires stable and uncontaminated starter culture rather than nature starters. This study aimed to identify the capability of isolates lactic acid bacteria (LAB) as starter for producing fermented skim milk. To make dadih, goat milk was pasteurized 85^{o}C for 30 min, cooled until until 30^{o}C, poured into thorny bamboo (*Bambusa stenostachya* Hackel), and fermented at 37^{o}C in incubator for 30 h. To isolate starters, a serial dilution of dadih was carried out and fermented at 37^{o}C in incubator for 48 h anaerobically. Then, selecting and evaluating potential isolates from dadih that have similar characteristic with LAB. The identification included selecting and evaluating was purposed to ensure that the isolates were from LAB, isolates were tested with microscopic identified, motility test, Gram staining test, and catalase test. After ensuring isolates were similar characteristic with LAB, isolates were tested their ability to be a starter with tested titratable acidity, pH, viability of LAB and curd formation abilities of isolates respectively during fermentation. The characteristics of isolates were rod shape, Gram stain positive, and negative motility test and catalase test. Results showed that pH value, TA (titratable acidity), viability of LAB, and curd formation of fermented skim milk were significantly different. During fermentation, pH of fermented skim milk decreased, TA and viable counts of LAB increased. There was curd formation and syneresis towards the end of fermentation. This study shows that isolate 11, isolate 21, isolate 25, isolate 29, and isolate 41 that isolated from dadih have ability as starter culture based on pH value, TA, viability of LAB, and curd formation. Then isolates can use commercially.
Keywords: pH, titratable acidity (TA), viability of LAB, curd formation, fermentation time.

INTRODUCTION

Dadih is a traditional food from West Sumatra, Indonesia, which is made by buffalo milk that naturally fermented in bamboo tubes. Indonesian dadih is still processed traditionally with natural starter from bamboo. Natural starter of dadih needs to be changed with artificial starter (starter culture) then dadih can be produced commercially.

Fermented milk product is produced by dairy industry with controlled conditions to make a good product [1]. To make starter cultures, LAB from dadih must be isolated and identified.

Dadih contains *Lactobacillus casei* subsp. *casei, Leuconoctoc paramasentereroides, Enterococcus faecalis* subsp. *liquefaciens,* and *Lactococcus lactis* subsp. *lactis* [2]. During fermentation process, isolates can identifify the capability as starter culture to ferment milk with determined pH value and viability of LAB [3]. This study aimed to identify the capability of isolates LAB from dadih to ferment milk that determined with pH value, Titratable Acidity (TA), viability of LAB, and curd formation during fermentation process.

MATERIALS AND METHODS

Preparation of the isolates

Isolates was obtained from Dairy Labolatory, Department of Animal Science, National Pingtung University of Science and Technology. To make dadih, goat milk was pasteurized 85oC for 30 min, cooled until until 30oC, poured into thorny bamboo (Bambusa stenostachya Hackel), and fermented at 37oC in incubator for 30 h. To isolate starters, a serial dilution of dadih was carried out and fermented at 37oC in incubator for 48 h anaerobically. Then, selecting and evaluating potential isolates from dadih that have similar characteristic with LAB. The identification included selecting and evaluating was purposed to

ensure that the isolates were from LAB, isolates were tested with microscopic identified, motility test, Gram staining test, and catalase test. After ensuring isolates were similar characteristic with LAB, isolates were tested their ability to be a starter with tested titratable acidity, pH, and viability of LAB and curd formation abilities of isolates respectively during fermentation. Isolates were isolated from Dadih. Isolates have characteristic rod shape, Gram stain positive, and negative motility test and catalase test. LAB have characteristic Gram stain positive and negative motility test [4]. Isolates that used in this experiment were isolate 11, isolate 21, isolate 25, isolate 29, and isolate 41. Before using, isolates were subculture in the broth (Merck, Darmstadt, Germany) and incubated at 37^{o}C for 20 h.

Preparation of the sample

Preparation of sample was determined from Wardani *et al.* [5] with some modification. To preparation skim milk, skim milk powder (Fonterra, Amsterdam, Netherlands) 10% (v/v) was autoclaved, added with 3% of each isolates, incubated at 37^{o}C, and measured of pH, TA, viability of LAB, and curd formation of each 6 h until 30 h.

Test for TA

TA was determined with 9 g samples that diluted with 9 ml sterile water, then sample was titrated with 0.1 N NaOH using 2 drops phenolphthalein solution (Nalacai Tesque, Inc, Kyoto, Japan) [6].

Test for pH value

pH meter (Suntex pH/ION meter SP-2500, New Taipei City, Taiwan) was used for determining pH. Before determination, pH meter was calibrated by using buffer solutions no. 4 and 7 (Mettler Toledo, Greifensee, Langacher) [7].

Test for viability of LAB

Five mililiters of samples were added to 45 ml of sterile 0.1% peptone (Merck, Darmstadt, Germany) and homogenized in a stomacher lab blender (400) (AES Labolatory, Combourg, France) for 1 min, solution was obtained to make 10^{-1} dilution. One mililiter from dilution (10^{-1}) was aseptically transferred to 9 ml sterile distilled water [7]. One mililiter of sample from tubes 10^{6}-10^{8} was pipetted with micropipet to petri dishes (Alpha Plus Scientific Corp, Taoyuan Hsien, Taiwan), then 15 ml of sterilized MRS (Merck, Darmstadt, Germany) supplemented with 1.5% $CaCO_3$ medium (Nihon Shiyaku reagent, Taipei, Taiwan) was poured into petri dishes. The plates were incubated at 37^{o}C for 48 h. The existence of LAB was clear zone surrounding colonies then colonies were counted by SUNTEX colony counter 570 (Suntex Instruments Co., LTD., New Taipei City, Taiwan).

STATISTICAL ANALYSIS

Statistical Analysis System (version 9.3, SAS) was used to perform data analysis and if there was a significantly different then continued by Duncan's Multiple Range Test (DMRT).

RESULTS

Capability of isolates to ferment milk was determined with measuring pH value, TA, viability of LAB, and curd formation during fermentation process.

pH value and TA

pH value of skim milk that adding each isolate was measured during fermentation process. There was a significant difference ($p<0.05$) during fermentation process on pH value and TA. Isolate 21 was the stongest to decrease pH value from 5.25 to 4.61. In the other hand, isolate 41 was the weakest ranged from 5.63 to 4.85. Additionally, isolate 11 from 5.18 to 4.68, isolate 25 was decreased from 5.63 to 4.65, and isolate 29 from 5.38 to 4.89. pH and TA had negative correlation. When pH was decreased, TA was increased during fermentation process. In the end of fermentation process, isolate 21 was the lowest pH while TA value was the highest 0.68.

Viability of LAB

There was a significant difference ($p<0.05$) during fermentation process. Viability of LAB was varied during fermentation. Increasing of fermentation time was effected to increasing viability of LAB. At the end of fermentation, isolate 21 was the highest viability of LAB 8.98 (cfu/ml) while it was the highest of TA and the lowest of pH value. Moreover, isolate 41 was the lowest 4.93 (cfu/ml) while it was the lowest of TA and the highest of pH.

Curd formation

There was a significant difference ($p<0.05$) on curd formation in skim milk during fermentation. Curd formation was changed by isolates. All of the skim milk was still liquid in 6 h. After 12 h, skim milk that added isolates (11, 21, and 29) began to make a curd that showed in Figure-1. Curd formation was correlation with decreasing of pH and increasing viability of LAB and TA. On the other hand, skim milk in isolates 25 and 41 were still liquid because pH was still high while viability of LAB and TA were still low.

At the 18 h fermentation time, curd formation was appeared when pH value was ranged from 4.78 (isolate 21) to 4.93 (isolate 41). At the 24 h fermentation time, skim milk in isolates 11, 21, and 25 had stronger to make a curd, but isolates 29 and 41 still had same curd with at the 18 h because isolate 29 and 41 had similar pH value, TA, and viability of LAB between 18 and 24 h. After 30 h fermentation time, isolates 11, 21 and 25 began to make a syneresis because they had low pH and high TA and viability of LAB. However, isolates 29 and 41 still had medium

curd formation because they had high pH and low TA and viability of LAB.

DISCUSSION

During fermentation process, isolates had ability to change pH value, TA, viability of LAB, and curd formation. Increasing of TA and decreasing of pH were indicated that isolates had ability as starter culture. pH value was decreased that caused by lactic acid prodution. pH value during incubation was reduced by proteolytic activity of isolates [3]. RNA and protein synthesis were affected by pH value [8]. Viability of LAB was increasing during incubation while increasing of TA because lactic acid was produced by LAB from lactose in dairy products [9]. Curd formation in the end of fermentation because lactic acid was converted by LAB from lactose, then precipitated a milk protein, formed curd, and other metabolites [10].

Table-1: Quality of skim milk with adding isolates during fermentation

Isolates	Fermentation time hour (h)[1]	pH value[1]	TA (%)[1]	Viability of LAB (cfu/ml) [1]	Curd formation[3]
11	6	5.18 ± 0.01^{a2}	0.32 ± 0.00^{e}	8.81 ± 0.01^{d}	-
	12	4.97 ± 0.01^{b}	0.41 ± 0.00^{d}	8.87 ± 0.01^{c}	+
	18	4.83 ± 0.00^{c}	0.46 ± 0.00^{c}	8.89 ± 0.00^{b}	++
	24	4.78 ± 0.00^{d}	0.50 ± 0.00^{b}	8.92 ± 0.00^{a}	++++
	30	4.68 ± 0.00^{e}	0.63 ± 0.00^{a}	8.93 ± 0.00^{a}	*
21	6	5.25 ± 0.01^{a}	0.27 ± 0.01^{e}	8.72 ± 0.02^{d}	-
	12	4.96 ± 0.00^{b}	0.42 ± 0.00^{d}	8.89 ± 0.00^{c}	+
	18	4.78 ± 0.01^{c}	0.51 ± 0.00^{c}	8.92 ± 0.00^{b}	+++
	24	4.71 ± 0.01^{d}	0.61 ± 0.00^{b}	8.94 ± 0.00^{b}	++++
	30	4.61 ± 0.01^{e}	0.68 ± 0.00^{a}	8.98 ± 0.00^{a}	*
25	6	5.63 ± 0.01^{a}	0.14 ± 0.00^{e}	8.49 ± 0.02^{d}	-
	12	5.45 ± 0.01^{b}	0.19 ± 0.00^{d}	8.56 ± 0.00^{c}	-
	18	4.83 ± 0.03^{c}	0.47 ± 0.02^{c}	8.88 ± 0.00^{b}	++
	24	4.71 ± 0.01^{d}	0.61 ± 0.00^{b}	8.94 ± 0.00^{a}	++++
	30	4.65 ± 0.00^{e}	0.65 ± 0.00^{a}	8.96 ± 0.00^{a}	*
29	6	5.38 ± 0.02^{a}	0.22 ± 0.01^{d}	8.58 ± 0.01^{b}	-
	12	5.04 ± 0.01^{b}	0.40 ± 0.00^{c}	8.71 ± 0.01^{a}	+
	18	4.89 ± 0.01^{c}	0.43 ± 0.00^{b}	8.72 ± 0.01^{a}	++
	24	4.90 ± 0.01^{c}	0.43 ± 0.00^{b}	8.72 ± 0.01^{a}	++
	30	4.89 ± 0.00^{c}	0.44 ± 0.00^{a}	8.73 ± 0.01^{a}	++
41	6	5.63 ± 0.01^{a}	0.15 ± 0.01^{d}	8.49 ± 0.02^{d}	-
	12	5.33 ± 0.01^{b}	0.24 ± 0.00^{c}	8.59 ± 0.00^{c}	-
	18	4.93 ± 0.00^{c}	0.43 ± 0.00^{b}	8.67 ± 0.02^{b}	++
	24	4.92 ± 0.00^{c}	0.43 ± 0.00^{b}	8.72 ± 0.02^{ab}	++
	30	4.85 ± 0.01^{d}	0.45 ± 0.00^{a}	8.74 ± 0.00^{a}	++

[1]Data are expressed as means values ± standard deviation (n = 3).

[2]Mean values in same column with difference letter are statistically significant ($p<0.05$).

[3]Curd formation: (-): liquid, (+): little curd, (++): medium curd, (+++): strong curd, (++++): very strong curd, (*): curd and made a syneresis

Fig-1: Curd formation of skim milk

CONCLUSION

This study shows that isolates 11, 21, 25, 29, and 41 that isolated from dadih have ability as starter culture based on pH value, TA, viability of LAB, and curd formation. Then isolates can use commercially.

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