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Isolation of Lactic Acid Bacteria as a Potential Probiotic in Dangke, a Traditional Food from Enrekang, Indonesia

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Abstract

This research is a preliminary study to explore potential probiotics of Lactic Acid Bacteria (LAB) in dangke, a traditional food from cow milk in Enrekang, Indonesia. Isolation of LAB performed using the selective medium de Man Rogosa Sharpe Agar. LAB will show clear zone on MRS medium after the addition of the indicator in the form of $CaCO_3$ 1% and incubated for 24 hours. Selection is done by observing the cell morphology and Gram staining. Further testing with the biochemical properties of sugar fermentation test. To assess their probiotic ability, tolerance of low pH were examined. The results showed that isolates of lactic acid bacteria detected were genera of Lactobacillus and could survive at pH 2. Isolates of Lactobacillus in dangke are derived from cow milk showed potential as a candidate probiotic bacteria.

Keywords: Lactic Acid Bacteri; Lactobacillus; Dangke, Probiotic.

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1. Introduction

Probiotic bacteria are defined FAO/WHO as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' [1]. The research of invitro and invivo, toward experimental animal or human, has been conducted many times to explore the role of probiotic in prevention or therapy of disease, for instance diarrhea [2], Inflammatory Bowel Disease [3], Irritable Bowel Syndrome [4, 5], asthma [6, 7], allergy [8, 9], obesity [10, 11], type 2 of diabetes [12, 13], colorectal cancer [14-16], intolerant lactose [17, 18], depression [19-21], osteoporosis [22, 23], reduction of cholesterol level [24, 25], and binding of heavy metal ion [26, 27].

The role of probiotic to prevention and therapy of some diseases has been proved, but its price is still relatively expensive. Therefore, the searching of new probiotic source needs to do. Research about isolation of probiotic potential bacteria has been conducted in many times such as from adult humans feces [28], infant feces [29], pickles of Kyoto [30], kefir [25], kimchi [31], and sake [32]. Some other researches conducted bacteria isolation from milk and milk processing, such as from ASI [33], cow milk [34], buffalo milk [35], horse milk [36], fermented milk [37], dadih [38], and dangke [39].

Dangke is milk processing product of buffalo or cow traditionally which comes from South Sulawesi especially the district of Enrekang. Dangke is processed by being heated with small fire until it boils, then it is added coagulant such as papaya sap (papain) so there will be clotting. The clot is taken into special mold made of coconut shell while being pushed so the liquid gets separated [40].

The aim of this research is to explore potential probiotics of Lactic Acid Bacteria (LAB) in dangke, a traditional food from cow milk in Enrekang, Indonesia. In this research, LAB isolation is conducted by using selective medium de Man Rogosa Sharpe Agar, observing the cell morphology, Gram staining, biochemical properties test, and testing of bacteria viability on low pH.

2. Material and Methods

Sample Preparation of Dangke

There was 10 gram dangke suspended into sterile physiological liquid (NaCl 0.9%) as many as 90 ml and it was taken into homogene. From the suspension, it was taken 1 ml and put into thinner tube which contained 9 ml sterile aquades, it was taken into homogene producing dilution of 10^{-1}. The dilution was continued until dilution of 10^{-3}.

Isolation and Selection of Lactic Acid Bacteria from Dangke

Dangke suspension inoculated to liquid medium of MRS Broth then it was incubated for 24 hours by temperature 37^{o}C. The culture of MRS Broth was inoculated to MRSA medium then it was added $CaCO_3$ 1%, next it was incubated for 48 hours. The colony around it was formed transparent zone purified again on MRSA medium by continuous scratch method then it was incubated for 24 – 48 hours. Kultivation was conducted many times over on the same medium and condition until we got single colony. Then the pure isolate was moved to slant agar as stock, it was kept in refrigerator by temperature 4^oC.

Bacteria Identification by Gram Staining and Biochemical Test

The pure isolate was taken asepticly and put on object glass cleaned by alcohol 96% and it was given fixation on spirtus lamp. After cold, it was dropped Gram A (Violet Crystal) 2-3 drops for 1 minute, then it was cleaned by flowing water and dried in the air. By the same procedure, the was continued by Gram B (Iodium) for 1 minute, Gram C (Alcohol 96 %) for 30 seconds, and Gram D (Safranin) for 45 seconds. This observation was conducted by looking at the form and color of cell under microscope with certain magnification.

Biochemical test that was conducted comprised KIA test, Motility, Catalase by putting the pure isolate on the object glass then it was dropped H_2O_2, it was observed if there was gas bubble produced or not, and Carbohydrate test (glucose, lactose, sucrose, maltose, manitol and malonat) by taking in the pure isolate one ose in every medium and it was taken into homogene then incubated by temperature 37^oC for 1 x 24 hour.

Tolerance of Low pH

One ose of pure isolate was inoculated into 5 ml medium of MRS Broth, incubation on temperature 37^oC for 24 hours. Then 1% inoculum was taken into 5 ml MRS Broth which was managed its pH by using HCl with variation pH 2,0; 2,5; 3,0; 3,5 and 4,0. Incubation was conducted with temperature 37^oC for 24 hours. Counting the number of bacteria in inoculum at the beginning and the last of incubation was conducted by plating method using MRSA.

3. Results and Discussion

Isolation and Selection of Lactic Acid Bacteria from Dangke

There were three isolates derived from dangke cow milk which grew on medium of MRSA + $CaCO_3$ 1% for 1 x 24 hours by temperature 37^o C which had different colony morphology comprised form, side, elevation, and color. The different colony morphology became basic for assumption that the three isolates were different kinds of bacteria. After being conducted the purification for the three isolates and being conducted the screening by using medium of MRSA added with 1% $CaCO_3$ as a medium used to select LAB, two isolates showed transparent zone around its colony as a consequence of being produced lactic acid reacted with $CaCO_3$ forming Ca-lactic which fused in medium [33].

Each of two selected isolates was isolate A and B. Isolate A had small colony, medium in round form, convex elevation, flat side, bright surface, and milk white color. While isolate B showed different colony morphology with isolate A, namely medium colony to big and round colony, flat side, bright surface, and milk white color. The different from isolate A was on the bigger colony size.

Bacteria Identification by Gram Staining and Biochemical Test

Next two selected isolates were observed its Gram characteristics and biochemical activities to concern about characterization and identification.

Table 1: Biochemical test of Lactic Acid bacteria

Biochemical Test	**Bacteria Isolates**	
	A	**B**
Gram Staining	Bacil of Positive Gram	Bacil of Positive Gram
Catalase	-	-
KIA	A/A, -/-	A/A, -/-
Urea	-	-
S. Citrat	-	-
LIA	-	-
MIO	-/ /-	-/ /-
Glucose	+	+
Lactose	+	-
Sucrose	+	+
Maltose	+	+
Manitol	+	-
Malonat	-	-
	Lactobacillus A	Lactobacillus B

Gram characteristics and biochemical activities on table 1 which is suited for Bergey's Manual of Determinative Bacteriology shows that isolate are group of LAB identified as Lactobacillus [41]. Lactobacillus is rod-shaped, often long and slender. Non-motile. Gram-positive. Pigment production rare; when present, yellow or orange to rust or brick-red. Gelatin is not liquefied. Growth on potato is poor or absent. Glucose and similar aldehydic hexoses, carbohydrates which yield these simple sugars, and polyhydroxy alcohols are changed either by homofermentation to lactic acid or by heterofermentation to lactic and acetic acids, alcohol and carbon dioxide. Nitrates are not reduced except under certain conditions with Lactobacillus plantarum. Several species grow at relatively high temperatures. Poor surface growth because these bacteria are generally microaerophilic or anaerobic. Do not produce catalase. Found in fermenting animal (especially dairy) and plant products [41].

Tolerance of Low pH

In this research, Lactobacillus A and Lactobacillus B were tested their growing ability on low pH, namely pH 2.0, 2.5, 3.0, 3.5, and 4.0. Test of bacteria resistance to low pH is one of the most important characteristics in determining bacteria potential to be probiotic [1]. The result of resistance of LAB isolate toward low pH can be seen on Table 2 and 3.

Results showed that based on House Index (HI), Container index (CI) and Breteau index (BI), the most successful program to decrease larva index of Aedes aegypty mosquitoes was abate program following by fogging and health education.

Table 2: The Number of Bacteria Cell of Lactobacillus A on Low pH

Variation of pH	The First Number of Bacteria Cell (CFU/ml)	The Last Number of Bacteria Cell (CFU/ml)
2.0	1.3×10^8	2.5×10^6
2.5	1.3×10^8	5.6×10^6
3.0	1.3×10^8	8.6×10^7
3.5	1.3×10^8	7.4×10^8
4.0	1.3×10^8	8.6×10^9

Table 3: The Number of Bacteria Cell of Lactobacillus B on Low pH

Variation of pH	The First Number of Bacteria Cell (CFU/ml)	The Last Number of Bacteria Cell(CFU/ml)
2.0	1.1×10^8	8.5×10^6
2.5	1.1×10^8	1.4×10^7
3.0	1.1×10^8	6.6×10^8
3.5	1.1×10^8	9.3×10^8
4.0	1.1×10^8	7.6×10^8

Lactobacillus isolated from dangke of cow milk shows that the ability to survive on low pH. Both of isolates can grow with mortality level less than 3 log so it can be stated that the isolate is resistant on acid state. Each bacteria cell of Lactobacillus A and Lactobacillus B grew on pH 2 with amount 2.5×10^6 CFU/ml (there was a decreasing of cell number of 2 log) and 8.5×10^6 CFU/ml (there was a decreasing of cell number of 2 log). The number of first inoculum each Lactobacillus A and Lactobacillus B added was 1.3×10^8 CFU/ml and 1.1×10^8 CFU/ml. The beginning LAB used for probiotic test was $10^8$to 10^9 CFU/ml. In acid state, Lactobacilllus can maintain its sitoplasm acidity so that the protein and enzyme in cell can keep working optimally. Isolate of LAB can adapt on low pH because it has regulation system of cell internal pH (pHi). This case can be achieved with new enzymes synthesis and it produces proton (H+) from inside of cell of which release happens through hydrolysis process of ATP (H+-ATPase). LAB hold out from damage of acid because there are histidin dekarboksilase and arginin deiminasi enzyme. The tolerance of LAB toward acid is high enough because its ability to maintain sitoplasm pH has more bases than extraseluler pH [42]. Lactobacillus A and Lactobacillus B are positive Gram bacteria which have resistance toward the very extreme pH [40]. Both of bacteria can pass an interior cavity of stomach producing acid so that it can achieve down intestines to be able to push pathogen bacteria which likely stay in digestive line. Bacteria will have effect on intestine environment if the number of population of the bacteria achieves minimal 10^6 – 10^8 CFU/ml. Based on the result of tolerant test of bacteria isolate from dangke cow milk toward low pH, it showed that both of isolates have potential as candidate probiotic bacteria

4. Conclusion

This research showed that Lactic Acid Bactetia isolated from dangke, a traditional food from cow milk in Enrekang, Indonesia is Gram positive, rod shaped, non motile. The results showed that isolates of lactic acid bacteria detected were genera of Lactobacillus and could survive at pH 2. Isolates of Lactobacillus in dangke are derived from cow milk showed potential as a candidate probiotic bacteria.

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