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Isolation and Identification of Lactic Acid Bacteria from Dangke a White Soft Traditional Cheese from Enrekang Regency

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Abstract: ***Dangke is a White soft traditional cheese from Enrekang Regency. It have a potential to have Lactic Acid Bacteria isolate. Lactic acid bacteria could be produce a bacteriocin to inhibit pathogenic bacteria. Sample of dangke were obtained from 3 district (Enrekang, Cendana, and Bamba) in Enrekang Regency. All isolate were tested for characteristics based on Gram straining, and biochemical tested. Isolation and identification of Lactic Acid Bacteria from dangke a white soft traditional cheese were suspected to be Lactobacillus lactis, Lactobacillus bulgaricus, Leuconostoc dextranicum, and Streptococcus thermophillus.***

Index Terms: Dangke, White soft cheese, Lactic acid bacteria

I. INTRODUCTION

Dangke is a White soft traditional cheese originating from the Regency of Enrekang. Used of sap papaya for the coagulation process of milk protein, and salt in the preservation process. The storage period at room temperature is around 3 days. Zakariah *et al.* [1], showed dangke was storage 1 week by plastic material have contamination by *E. coli and coliform.*

Products derived from milk have the potential to have lactic acid bacteria isolates. *Lactobacillus fermentum* [2,3] *Lactobacillus plantarum* [4] *Lactococcus, Leuconostoc, Streptococcus, Pediococcus, Enterococcus* [5], and genera *Lactobacillus* [6] in dangke a white soft traditional cheese. Lactic acid bacteria that have potential as a biopreservation agent because they can produce bacteriocin products that can inhibit pathogenic bacteria that cause decay processes in these dangke products. Some strains of lactic acid bacteria are known to produce bacteriocins which can reduce the risk of contamination, and increase the shelf life of dangke products.

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Identification of lactic acid bacteria can use macroscopic, microscopic and biochemical tests. This macroscopic test includes the form of colonies that grow on MRSA, microscopic tests include gram staining, whereas biochemical tests can use triple sugar iron agar test, urea test, sulfide indole motility test, metal red voges proskeuer test, citrate test, urea test, glucose test , lactose test, sucrose test, maltose test.

Research on identification of Lactic Acid Bacteria originating from traditional Enrekang cheese Dangke is still limited, so research is important information to obtain candidates for lactic acid bacteria isolates as biopreservation agents in dangke products.

II. MATERIAL AND METHODS

A. Sample preparation

Samples were obtained from 3 district (Enrekang, Cendana, and Bamba) in Enrekang Regency, which were made from cow's milk.

B. Isolation of Lactic Acid Bacteria

Twenty-two grams of dangke samples were homogenized with 225 ml of physiologically sterile NaCl solution and then diluted with 10^{-4}, 10^{-5}, 10^{-6} were spread on MRS agar, then incubated at 37°C for 48 hours. After incubation, the total colonies of Lactic Acid Bacteria were calculated, and macroscopic differentiation of colonies was carried out based on size, shape, color, surface, and sensitivity. Next, 1 ose of sample was cultured on sloping agar.

C. Identification of lactic acid bacteria.

All isolates were tested for characteristics based on gram straining, then biochemical tested with triple sugar iron agar test, sulfide indole motility test, metal red voges proskeuer test, citrate test, urea test, glucose test, lactose test, sucrose test, maltose test.

III. RESULT AND DISCUSSION

The macroscopic observations of the dangke samples were presented in Table 1.

Table 1. Macroscopic Observation of Lactic Acid Bacteria isolated from Dangke a Whites Soft Traditional Cheeses.

Region Sources	Colony strain	Size	Form	Elevation	Margin	Colour	Sensitivity
Bamba	Isolate 2 Bamba	Small	Circular	Convex	Entire	White creamy	Butter
	Isolate 3 Bamba	Small	Circular	Convex	Entire	White creamy	Butter
	Isolate 3.2.3 Bamba	Medium 0.3 cm	Circular with a core in the middle	Convex	Entire	White creamy	Butter
	Isolate 3.2.2 Bamba	Large 0.5 cm	Circular	Convex	Entire	White creamy	Slimy
Cendana	Isolate 6 cendana	Medium	Circular	Convex	Entire	White creamy	Butter
	Isolate 2.2.2 Cendana	Large 0.4 cm	Circular	Convex	Entire	White	Butter
	Isolate 5 cendana	Small	Circular	Convex	Entire	White	Butter
	Isolate 2.2.4 Cendana	Medium 0.2 cm	Circular	Convex	Entire	White	Butter
Enrekang	Isolate 1 Enrekang	Small	Circular	Convex	Entire	White Creamy	Butter
	Isolate 6 Enrekang	Medium 0.2 cm	Circular	Convex	Entire	White Creamy	Butter
	Isolate 1.2.4 Enrekang	Large 0.4 cm	Circular with a core in the middle	Convex	Entire	White Creamy	Slimy
	Isolate 1.2.1 Enrekang	Extra Large 1.1 cm	Circular with a core in the middle	Convex	Entire	White	Butter

Table 2. Microscopic and Biochemistry of Lactic Acid Bacteria was isolated from Dangke a White Soft Traditional Cheese

Isolat	Gram	Form	TSIA	SIM	MR	VP	C	U	Glu	Lac	Suc	Mal	Gases
1.2.4 Enrekang	+	Yeast cell	+	-	+	-	-	-	+	+	+	-	+
3.2.2 Bamba	+	Yeast cell	+	-	+	-	-	-	+	+	+	-	+
2 Bamba	+	Rods	+	-	+	-	-	-	+	+	+	+	-
1 Enrekang	+	Rods	+	-	+	-	-	-	+	+	+	-	-
6 Cendana	+	Rods	+	-	+	-	-	-	+	+	+	-	-
6 Enrekang	+	Cocci	+	-	+	-	-	-	+	+	+	-	-
1.2.1 Enrekang	+	Cocci	+	-	+	-	-	-	+	+	+	-	-
3 Bamba	+	Cocci	+	-	+	+	-	-	+	+	+	-	-
2.2.2 Cendana	+	Cocci	+	-	+	+	-	-	+	+	+	-	-
3.2.3 Bamba	+	Cocci	+	-	+	-	-	-	+	+	+	-	-
5 Cendana	+	Cocci	+	-	+	-	-	-	+	+	+	-	-
2.2.4 Cendana	+	Cocci	-	-	+	-	-	-	-	+	-	-	-

Note: TSIA=Triple sugar iron agar test; SIM= Sulfide indole motility test; MR= Metal red test, VP= Vvoges proskeuer test, C= Citrate acid test; U= Urea test, Glu=Glucose test; Lac= Lactose test; Suc= Sucrose test, Mal= Maltose test.

The results showed that the size of several colonies has a variety of diameters ranging from 0.1, 0.3, 0.4, 0.5 and 1.1 cm. This is different from the results obtained by Sulmiyati et al. [7] , that the diameter size of the lactic acid bacteria colonies obtained is 0.5-1 mm. The results of the observation of the form in the study are circular in accordance with the results of research by Yolanda and Meitiniarti [8], that lactic acid bacteria was isolated from kimchi obtained macroscopic circular morphology.

All isolates have elevation in the form of convex, while the margin is in the form of entire. The color of the colony is macroscopically yellowish and white. Sensitivity like butter, but for isolates 1.2.4 Enrekang and 3.2.2 Bamba was slimy.

Table 3. *Type of fermentation of Lactobacillus sp.*[10]

Isolate	Glukose	Lactose	Sucrose	Maltose
Lactobacillus lactis	+	+	+	+
Lactobacillus bulgaricus	+	+	+	-
Lactobacillus brevis	+	-	-	+
Lactobacillus casei	+	+	-	+
Lactobacillus bifidus	+	-	+	+
Lactobacillus delbrueckii	+	-	+	+
Lactobacillus casei	+	+	-	-

Gram strains testing and the characteristics of the biochemical process of isolates were presented in Table 2. Furthermore, Table 3 showed type of fermentation of genera *Lactobacillus* to be distinguish between species. Biochemical test process Isolate 2 Bamba has biochemistry characteristics that can ferment glucose, lactose, sucrose, and maltose that suspected species to *Lactobacillus lactis*. Isolates 1 Enrekang, and 6 Cendana have biochemical characteristics that could be fermented glucose, lactose, and sucrose, but cannot ferment maltose. This type of suspected species to *Lactobacillus bulgaricus.*

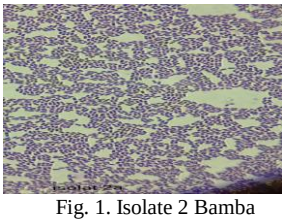

Fig. 1. Isolate 2 Bamba

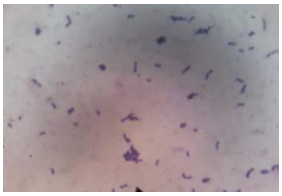

Fig. 2. Isolate 6 Enrekang

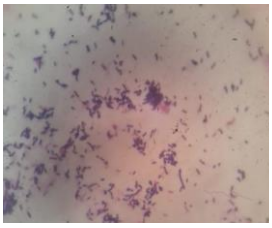

Fig. 3. Isolate 1 Enrekang

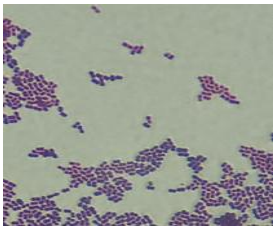

Fig. 4. Isolate 6 Cendana

Isolate 1.2.4 Enrekang and 3.2.2 Bamba has characteristics of yeast cells, based on the form of this isolate suspected *to Candida guilliermondii*. This is supported by Utama et al. [9], that yeast isolated from dangke obtained yeast / yeast isolate which has the characteristic of being able to ferment glucose, sucrose, but unable to ferment maltose and form the gram stain of its strain which is shaped like *Candida guilliermondii.*

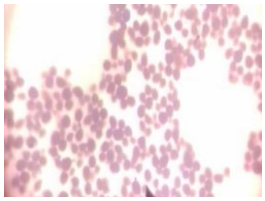

Fig. 5. Isolate 124 Enrekang

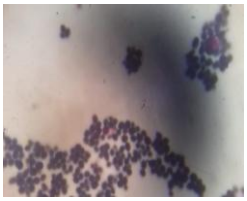

Fig. 6. Isolate 322 Bamba

Isolate 1.2.1 Enrekang and 3.2.3 Bamba which is oval shaped and can ferment Glucose, Lactose, and Sucrose. This type of isolate suspected to *Leuconostoc dextranicum*. While isolates 3 Bamba and 2.2.2 Cendana have fermented biochemical characteristics can ferment glucose, lactose, sucrose, cannot ferment maltose, have a positive MR and VP test. Positive MR testing (there is a red color change) indicates that there is lactic acid production, while positive VP testing (formed red ring) shows that the end result of acetyl methyl carbinol fermentation. This type of isolate leads to *Streptococcus thermophillus.*

Fig. 7. Isolate 121 Enrekang

Fig. 7. Isolate 323 Bamba

Fig. 8. Isolate 3 Bamba

Fig. 9. 222 Cendana

IV. CONCLUSION

Isolation and identification of Lactic Acid Bacteria from Dangke a White Soft Traditional Cheese were suspected to be Lactobacillus lactis, Lactobacillus bulgaricus, Leuconostoc dextranicum and Streptococcus thermophillus. All isolate should be identify by rRNA gene sequencing.

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