Abstract

Lactic Acid Bacteria Identification Of Dadih Produced in Bamboo *Petung* Bali (Dendrocalamus Asper)

By:

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Dadih is a traditional Minangkabau fermented buffalo milk. Traditionally , during fermentation involves various types of interacting microorganisms. Microorganisms that play a role in the fermentation process are thought to originate from the inner surface of bamboo tube, the surface of banan leaves, and from the milk used. In Bali bamboo *petung* is one of the largest and thickest size , available at some area.. The best dadih produced in dried bamboo *petung* (24.06% moisture content) contains (0.9 -1.5) x 10^{10} LAB identified based on: morphology, gram staining, catalase test, growth at : different temperatures, pH ,and bile salt were obtained 7 isolates. By using Kit API 50 CH were isolates identified that potentially as probiotic starter of dadih, such as: . *Lactobacillus pentosus* dSGT 1 – 5 ; 7; and *Lactobacillus rhamnosus* dSGT 6.

Key words, *Bamboo petung, starter probiotic of dadih*

INTRODUCTION

Dadih is a Minangkabau fermented buffalos milk . According to Sirait (1993) dadih is a fermented milk product that resembles yogurt and kefir. The traditional fermentation process takes place spontaneously, where there is no addition of special microbes that are used as inoculums or starters, its fermentation of involves various types of interacting microorganisms. Microorganisms that play a role in fermentation process are thought to originately from the inner surface of bamboo tube, the inner surface of the cover leaves, and from the milk used (Sugitha, 1995).

Bali is an area that is rich in biodiversity. One of the biodiversity in Bali is a bamboo plant or its local name was known as "tiying". The use of bamboo plants in Bali is only limited to religious purposes, handicrafts, even though the potential of bamboo plants is still much deeper, such as shoots (bamboo shoots) can be used as vegetables and bamboo can also be a natural habitat various microorganisms that play a role in the process of making dadih.

Traditionally, the process of making dadih is still available in West Sumatra, while the potential of bamboo plants is almost all over Indonesia, including in Bali. Given the magnitude of the benefits of dadih for health it does not rule out the possibility of this food product can be made in various regions in Indonesia, especially Bali. One type of bamboo that thrives in Bali is *bambu Petung*. Fermentation of milk to dadih in a bamboo tub was caused by the presence of natural bacteria in bamboo such as lactic acid bacteria. But the freshness of bamboo culms which includes fresh bamboo (harvested with fresh green stems), semi-dried bamboo (bamboo harvested after 24 hours) and dried bamboo (harvested in dry conditions with yellow stem color characteristics) will affect the number and type of microbes contend. Based on this, it is necessary to characterize the curd produced in dried petung bamboo as a isolates source of indigenous lactic acid bacteria.

RESEARCH METHODS

Place and time of research . The study was conducted in the Laboratory of Food Microbiology, Faculty Agricultural Technology, Udayana University. The time for conducting research is May - October 2018.

Materials and research tools. The importance materials used in this study include: dried petung bamboo, fresh cow's milk, banana leaf, MRS Broth (Oxoid), MRS Agar (Oxoid), Kit API 50 CH, NaCl (Merck), NLP, Rogosa, crystal violet , lugol, tissue paper, 95% ethanol, safranin, immersion oil, H2O2 (3%), Gibson's semi-solid medium, semi-solid liquid tomato juice (four parts of 10% skim milk plus one part of Nutrient so that 10%, 0, 25% yeast exstract, 5% glucose and 10% tomato juice), Sterile SA media (trypton, yeast extract, K2HPO4, triamonium citrate, sucrose, and bacto agar alcohol 70%.

Research activities. Overall the activities are: Identification and isolation of Lactic Acid bacteria which play a role in the fermentation process of milk as a dadih starter. To obtaind the output of pure culture *Lactobacillus* Isolate from dried petung bamboo (1 week sun dry:

25.77% wc). Then the identification of the results is absolutely carried out, including: Total LAB, morphology, Gram staining (Harrigan and McCance, 1998), Catalase test, Growth at different temperatures (Nuraida, 1988), Growth at pH (Modification of Chou and Weimer, 1999; Zavaglia et al., 1998); and To identify the species of LAB used the standard Analytical Profile Index (API) 50CH (microbiology kit and medium API 50CH version 5.1 containing 49 types of sugars and their derivatives) (Biomerieux). This study consisted of 3 stages: the production of dadih in dried petung bamboo tubes followed by an identification and isolation test of LAB that plays a role in making dadih. In detail, the research plan can be seen in Figure 1.

RESULTS AND DISCUSSION

In this study, which is a continuation of research (Sugitha and Puspawati, 2017), namely the manufacture of dadih in a dry bamboo tube. From the results of the research carried out in dried petung bamboo tubes (1 week sun dry) produce dadih with quality seen in Table 2 and Table 3. Mean bamboo moisture content(24,06),dadih water content(75.17%) and the resulting curd pH(6.30); it mean bamboo water content was largely determined by the weather during drying, so that dryness affects the whey content as well as the pH. Increased water levels of bamboo tube cause an increase in whey levels and pH.

Table 2. Bamboo tube moisture content and moisture content and pH of dadih produced in dried of petung bamboo

Replication	Water content of Bamboo tube BAMBU	Water content DADIH (%)	pH. DADIH
Dadih 1	23,14	72,24	6,0
Dadih 2	24,71	75,19	6,2
Dadih 3	24,32	75,07	6,7
Average	24,06	75,17	6,30

Other components of dadih nutrition such as: lactic acid, protein, fat and total LAB which are important in the process of lactose fermentation to lactic acid (Table 3). In this study were more concerned to the content of LAB than other components; thus each sample of curd (D1, D2, D3) was possibility as a candidate for LAB isolation of dry bamboo curd (KIDBPK 1; 2; 3) in the next stage.

The nutritional component of dried bamboo curd (Table 3 states that the mean of lactic acid (0.31%), protein (8.70%), fat (2.91%) and the average LAB starting from $1.1x10^{10}$

to $9.4x10^{10}$ cfu / g. Based on the LAB population the dadih can be used as functional food.The characteristics of dadih produced can be seen in Table 4 and Figure 3;

Table 3. Nutritional component of Dadih produced in Dry bamboo petung bali

Ulangan	Lactic acid (%)	Protein (%)	Fat(%)	Total LAB (cfu/g)
Dadih 1	0,37	8,68	3,39	9.4×10^9
Dadih II	0,33	8,31	2,72	1.5×10^{10}
Dadih III	0,22	9,12	2,63	1.1×10^{10}
Rata-rata	0,31	8.70	2,91	

Figure 3 Performance of Dadih produced in Dry bamboo petung bali

		Flavor and taste Specifict Milk taste, rather bitter and astringent in taste; soft in texture, hollow on surfaces and smooth the edge of surface and yellowish white in color.

Table 4. Sensory Evaluation Performance of Dadih produced in Dry bamboo petung bali

Ulangan	Aroma	Warna	Textur	Rasa
Dadih 1 (KID 1)	Agak asam	Putih	Agak Padat	Hambar,asam, agak pahit, rasa susu sedikit
Dadih 2 (KID 2)	Khas Susu	Putin	Agak Lembek	Hambar,agak asam, sedikit pahit, rasa susu
Dadih 3 (KID 3)	Khas susu	Putih	Agak Lembek	Hambar, agak asam, sangat pahit, rasa susu

Physical Characteristics and Biochemistry of Dadih produced in Dry bamboo petung bali

At the beginning of identification, there were 21 KIDBPK selected through morphological characteristics: colony shape, colony position, cell shape, gram stain reaction, Catalase test and resistance growth at various temperatures starting by 10^{o} C; 25^{o}C; 37^{o}C and 45^{o}C; pH (2; 4; 6); and on the media bile solutiom content, can be seen in Table 5. Gradual testing based on the characteristics of KIDBPK, the yellow in color mean catalase positive, it did not followed by a growth test at pH. Growth resistance test for pH against 13 KIDBPK, resulting in 3 KIDBPK whose population is reduced by more than 2 log cycles, namely: IBK (P); IILK (P); and IIBK (M) (in red color) were not followed by endurance tests on bile media (Table 6). .The rest Of the 10 KIDBPK tested for growth resistance in bile salts (Table 7) produced 3 negative isolates growth (green in color). In the last stage of isolate selection performance, only 7 KIDBPKs were identified with the API 50 CH Kit, or which were fully genus and species identified (Table 8)

Table 5 Morphology and Physiology isolate Candidate of Dadih Produced in Dry Bamboo Petung Bali

No	Candidste isolate code	Morphology characterystic				Physiology characterystic										
						Growth at (^{0}C)				Growth at pH-			Growth at salt			
		Colony shape	Colony position	Gram	Katalase	10^{o}C	25^{o}C	37^{o}C	45^{o}C	2	4	6	5%	8%	10%	18%
1	IBB (P)	Round big	Surface	+	-	tio+	++	++	++	+	+	++	++	-	-	-
2	IBK (M)	Round small	foating	+	+			+								
3	ILK (D)	Oval small	base	+	-	+	++	++	+++	+	++	++	++	+	-	-
4	IBK (P)*	Round small	Surface	+	-	+	++	+	++	+	+	+	++	+	++	-
5	ILK (M)*D	Oval small	Floating	+	-	+	++	+	++	+	+	+	++	++	+	-
6	IBK (D)*	Round small	base	+	-	+	++	+	++	+	+	+	++	+	+	-
7	IIBB (P)	Round big	Surface	+												
8	IILK (M)	Oval smaall	floating	+	-	+	++	++	+++	+	+	++	++	++	++	-
9	IILK (D)	Oval small	Base	+												
10	IILK (P)*	Oval small	surface	+	-	+	++	+	++	+	+	+	++	++	+	-
11	IIBK (P)*	Oval small	surface	+												
12	IIBK (M)*	Roung small	floating	+	-	+	++	+	++	+	+	+	++	++	+	-
13	IIBK (D)*	Round small	Dasar	+	-	+	++	++	++	+	+	++	++	++	++	-
14	IIBK(D)S			+		++	++	+	++	+	+	+	++	++	++	-
15	IIIBS (P)K	Round med.	surface	+	-	+	++	+	++	+	+	+	++	-	-	-
16	IIIBS(P)S			+		++	++	+	++	+	+	+	++	++	++	-
17	IIIBK (M)	Round small	floating	+	+			+								
18	IIIBB (D)	Round big	Base	+	+			++								
19	IIILK (P)*	Round small	Surface	+												
20	IIIBK (M)*	Round small	Floating	+				+								
21	IIIBK (D)*	Round small	base	+	-	+	++	+	++	+	+	+	++	++	+	-

Tabel 6 Isolate Population Growth of Dadih produced in Dry bamboo petung bali on pH. 2.0

Candidate Isolate code	Population Growth at pH 2.0						
	Incubation (0 hr)			Incubation(2hrs)			Different (log cycle cfu/g)
	cfu/g		log cfu/g	cfu/g		log cfu/g	
IBB (P)	1.0 x 10^7	10000000	7.0000	1.2 x 10^6	1200000	6.0792	-0.9208
IBK (M)							
ILK (D)	3.4 x 10^8	340000000	8.5315	3.5 x 10^6	3500000	6.5441	-1.9874
IBK (P)*	7.2 x 10^8	720000000	8.8573	2.6 x 10^6	2600000	6.4150	-2.4424
ILK (M)*D	5.6 x 10^8	560000000	8.7482	2.6 x 10^7	26000000	7.4150	-1.3332
IBK (D)*	1.7 x 10^7	17000000	7.2304	2.0 x 10^6	2000000	6.3010	-0.9294
IIBB (P)							
IILK (M)	2.8 x 10^8	280000000	8.4472	6.0 x 10^6	6000000	6.7782	-1.6690
IILK (D)							
IILK (P)*	2.5 x 10^8	250000000	8.3979	1.5 x 10^6	1500000	6.1761	-2.2218
IIBK (P)*							
IIBK (M)*	6.5 x 10^8	650000000	8.8129	3.5 x 10^6	3500000	6.5441	-2.2688
IIBK (D)*	1.4 x 10^9	1400000000	9.1461	2.3 x 10^7	23000000	7.3617	-1.7844
IIBK(D)S	1.0 x 10^8	100000000	8.0000	5.5 x 10^6	5500000	6.7404	-1.2596
IIIBS (P)K	1.4 x 10^7	14000000	7.1461	5.0 x 10^6	5000000	6.6990	-0.4472
IIIBS(P)S	7.4 x 10^8	740000000	8.8692	3.1 x 10^7	31000000	7.4914	-1.3779
IIIBK (M)							
IIIBB (D)							
IIILK (P)*							
IIIBK (M)*							
IIIBK (D)*	3.6 x 10^8	360000000	8.5563	2.4 x 10^7	24000000	7.3802	-1.1761

Table 7 Isolate Population Growth of Dadih produced in Dry bamboo petung bali on media of 3% bile salt.

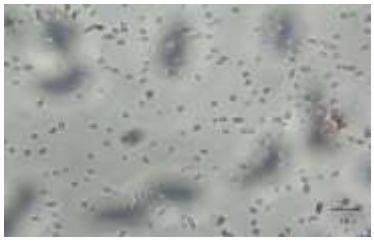
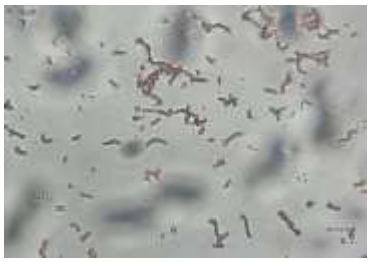
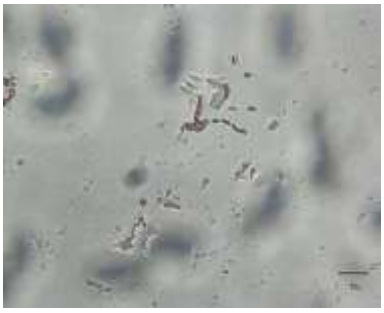
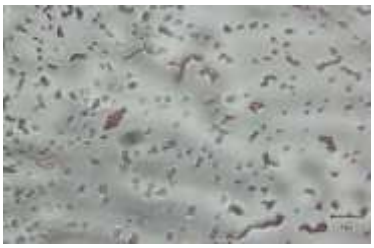
Candidate Isolate code	Population Growth at 3% Bile salt						
	Incubation (0 hr)			Incubation(4 hrs)			Different (log cycle cfu/g)
	cfu/g		log cfu/g	cfu/g		log cfu/g	
	1.0 x 10^9	1E+09	9.0000	3.5 x 10^8	3.5E+08	8.5441	-0.4559
IBB (P)							
IBK (M)	1.3 x 10^8	1.3E+08	8.1139	3.9 x 10^8	3.9E+08	8.5911	0.4771
ILK (D)							
IBK (P)*	2.6 x 10^8	2.6E+08	8.4150	1.5 x 10^9	1.5E+09	9.1761	0.7611
ILK (M)*D	1.8 x 10^8	1.8E+08	8.2553	2.7 x 10^9	2.7E+09	9.4314	1.1761
IBK (D)*							
IIBB (P)	7.8 x 10^8	7.8E+08	8.8921	1.0 x 10^9	1E+09	9.0000	0.1079
IILK (M)							
IILK (D)							
IILK (P)*							
IIBK (P)*							
IIBK (M)*	8.0 x 10^7	80000000	7.9031	3.1 x 10^9	3.1E+09	9.4914	1.5883
IIBK (D)*	5.7 x 10^8	5.7E+08	8.7559	7.0 x 10^8	7E+08	8.8451	0.0892
IIBK(D)S	1.0 x 10^7	10000000	7.0000	2.4 x 10^8	2.4E+08	8.3802	1.3802
IIIBS (P)K	5.0 x 10^8	5E+08	8.6990	2.6 x 10^8	2.6E+08	8.4150	-0.2840
IIIBS(P)S							
IIIBK (M)							
IIIBB (D)							
IIILK (P)*							
Ketaa	3.7 x 10^8	3.7E+08	8.5682	2.8 x 10^8	2.8E+08	8.4472	-0.1210
IIIBK (D)*							

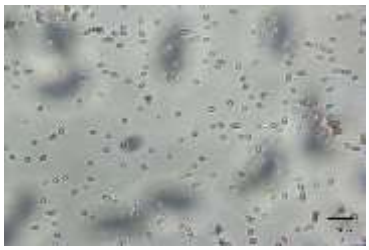
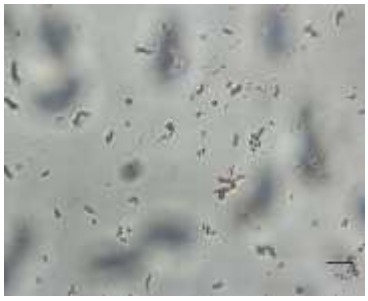
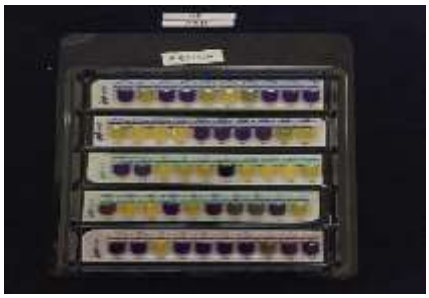
Table 8 Isolat Name of Dadih produced in Dry bamboo petung bali through Kit API 50 CH

No	Isolate Candidate Code	New Isolat Code	Identification	% ID
1	ILK(M)*	dSGT1	*Lactobacillus pentosus* dSGT1	98,6 %
2	ILK(D)	dSGT2	*Lactobacillus pentosus* dSGT2	98,7 %
3	IBK(D)*	dSGT3	*Lactobacillus pentosus* dSGT3	67,3 %
4	IIBK(D)*S	dSGT4	*Lactobacillus rhamnosus* dSGT4 **atau** *Lactobacillus pentosus* dSGT4	50,4 % 49,2 %
5	IIBK(D)*B	dSGT5	*Lactobacillus pentosus* dSGT5	94,6 %
6	IILK(M)	dSGT6	*Lactobacillus rhamnosus* dSGT6	99,8 %
7	IIIBS(D)K	dSGT7	*Lactobacillus pentosus* dSGT7	98,6 %

The fermentation results of 49 types of sugar in the API 50CH Kit showed that 7 isolates dadih of dry petung bali bamboo , were identified as genus *Lactobacillus* consisting of 2 species: *pentosus* and *rhamnosus* (Table 8). After going through morphological and biochemical tests (Table 5; 6 and Table 7); It turned out that isolate number 1 (ILK (M) * was identified as *Lactobacillus pentosus* dSGT 1 with a level of 98.60%. Likewise with isolates 2 = *Lactobacillus pentosus* dSGT 2; isolates 3 = *Lactobacillus pentosus* dSGT 3; isolates 5 = *Lactobacillus pentosus* dSGT 5; and isolates 7 = *Lactobacillus pentosus* dSGT 7, but isolates 6 = *Lactobacillus rhamnosus* dSGT 6 (99.80%), while isolates 4 were identified double: *Lactobacillus pentosus* dSGT 4 (49.20% or *Lactobacillus rhamnosus* dSGT 4 (50.40%) The ability Isolate of Dadih produced in Dry bamboo petung bali to ferment 50 types of sugar (API Kit 50CH) can be seen in Figure 4 (photo strips and evaluation results).

Figure 4 Characteristic Performance of Dadih isolates produced in Dry bamboo petung bali through Kit API 50 CH

No	Isolate code	Isolate name	Cell shape	Sugar fermentation
1	dSGT1	*Lactobacillus pentosus* dSGT1		
2	dSGT2	*Lactobacillus pentosus* dSGT2		
3	dSGT3	*Lactobacillus pentosus* dSGT3		
4	dSGT4	*Lactobacillus rhamnosus* dSGT4 **atau** *Lactobacillus pentosus* dSGT4		
5	dSGT5	*Lactobacillus pentosus* dSGT5		

6	dSGT6	*Lactobacillus rhamnosus* dSGT6		
7	dSGT7	*Lactobacillus pentosus* dSGT7		

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the study it can be concluded that:

1. Dry petung bamboo plants (24% moisture content) can be used in making dadih / fermented milk (different in characteristics).
2. The resulting dadih contained lactic acid bacteria of 10^9-10^{10} CFU / g produced 23 candidate isolates tested for morphology, biochemistry test, growth resistance at temperature, pH and bile salt media, so that 7 candidate isolates were identified with API 50CH KIT.
3. Identification using API 50 CH Kit to produce isolates dSGT 1-5; 7 and *Lactobacillus rhamnosus* dSGT 6

Suggestion

Associated with *Lactobacillus rhamnosus* dSGT 6 isolates identified (50.40%) and *Lactobacillus pentosus* dSGT 6 (49.20%) need to be purified to obtain pure isolate. Necessary to apply the best isolate in the process of dadih making.,

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