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The Utilization of Microorganisms Isolated From Fermented Coconut Milk For The Production of Virgin Coconut Oil

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ABSTRACT

The fermented coconut milk was analyzed for numbers and types of Lactic Acid Bacteria (LAB) at duration of 0, 6, 12, 18 and 24 hours and incubated at 25°C, 30°C and 40°C. The isolates were then characterized based on morphology and biochemical characteristics, followed by proteases and amylase enzymes activities assayed. The results indicated that all samples contained high numbers of LAB (Lactic Acid Bacteria). Fresh coconut milk harbored $6{,}4x10^6$ cfu/g LAB. The population of LAB increased during the fermentation period of 18 h reached up to $1{,}9x10^9$ cfu/g and at 24 h decreased to $1{,}5x10^9$ cfu/g. The bacteria could grow well at 25°C, 30°C and 40°C. The best temperature of growing was 30°C. The selected LAB have the proteolysis and amyl lytic enzyme activity indicated that these selected LAB has the potential to be used as pure culture for the production of VCO (Virgin Coconut Oil) of high quality oil.

KEYWORDS: Coconut milk, Lactic Acid Bacteria, Virgin Coconut Oil.

1. INTRODUCTION

Production of Virgin Coconut Oil (VCO) has been practiced using traditional method. Typically, the process start by mixing the freshly extracted coconut with boiled water at 1:1 (w/w) ratio and allowing to stand at room temperature for 24 h [1]. However although this method is chip and easy to perform but the length period of time left the unprotected freshly coconut milk under the unhygienic environmental condition is considered resulted in Virgin Coconut Oil of low quality. The wild organism may grow producing several compounds, which influenced the quality of the oil.

Coconut milk raw material used for virgin oil production mainly consists of (w/w) 21.3% fat, 2.0% protein, 2.8% carbohydrate and 2.1% of sugar [2] together with some vitamins and trace elements. Virgin Coconut Oil (VCO) consists of C8, C10, C12 and C14 fatty acid with lauryl acid has the most composition of 47-53% among all saturated fatty acid [3]. This oil found in the matrix tissue of coconut fresh, which bounded with protein, fat, carbohydrate. Presently the commercial method using of high temperature for the processing of oil from copra and it was extracted the oil by physically and chemically method. The implementation of physical and chemical treatment to extract the oil may decompose lauryl acid thus the method above could not be applied in the production of Virgin Coconut Oil.

Microbes isolated from fermented coconut milk is an alternative method for producing VCO since the organism presence in the coconut milk have adapted with the environments condition and could actively breakdown the compounds in coconut milk. The use of commercial yeast for making VCO could break down the carbohydrate and sugar but not the protein and fat [4].

Breakdown the linkage of the protein, fat and carbohydrate could be carried out by the inoculation of effective microbes having proteolysis and amyloid enzymes on to the freshly extracted coconut milk to coagulate the protein and further centrifugation the protein can be separated from fat, carbohydrate water. With this method, lauryl acid will not be decomposed.

Isolation, selection and characterization of microbes at interval period of time at different incubation temperature is expected to find out the best effective microbes which could be applied to produce Virgin Coconut Oil at small scale industry.

2. MATERIALS AND METHODS

Fermented coconut milk preparation

Coconut fruits were purchased from the local market, Malang region East Java Province, Indonesia. The flesh was shredded and then pressed using hydraulic press machine to obtain coconut milk. The milk was incubated at room temperature for 24 hours to allow natural fermentation occurred. Sampling of fermented coconut milk (l0 ml) was dispensed in 90 ml of Buffered Peoton Water and l.0 ml was removed diluted was every 2 hours to count and isolate lactic acid bacteria.

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Isolation of Lactic Acid Bacteria (LAB)

Total number of LAB was examined using MRS agar supplemented with 2% (w/v) fructose. Colonies of the bacteria were selected based on catalase test [5]. The catalase negative colonies of LAB were then isolated and characterized [6]. The selected LAB were purified, grown in MRS+ 2% (w/v) fructosa for 72 hours at 30°C and used as stock culture for further analysis.

1. Selection, Isolation, Characterization of LAB Producing Proteolysis and Amyl lytic Enzymes

a. Protease Test

The proteolysis test of the organisms was detected [7]. The MRS Agar was added with skim milk (Oxoid, CM), poured in steriled Petridis (20 ml) leaf and cooled at room temperature (± 30°C). The colony having a clear zone were selected for further study.

b. Amylase Test

This test was conducted to analyze the amyl lytic activity (8). The MRS Agar was added with 0.5% (w/v) starch (oxoid) then inoculated with the selected organisms. The colony having a clear zone (3 isolates) were used for further study. All the isolates chosen were then examined their total protein LAB and yeast using the method.

2. Growth Characterization

The Effect of Temperature on the Growth of The Organisms

The selected isolated were grown in MRS Fructose broth for LAB isolation. All the organisms were incubated at different level of temperature (20°C, 30°C, 40°C and 50°C) for 48 hours. The growth profile of the selected organisms was measured with spectrophotometer (9) at 610 nm wavelength. The evaluation was taken every two hours. Colonies representing different morphologies were selected off and classified as Type I, Type II etc.

3. RESULTS AND DISCUSSION

1. Lactic Acid Bacteria (LAB) explored from coconut milk kept for 24 Hours

a. LAB Total

Sampling was carried out during 0, 6, 12, 18 and 24 hour. 24 hour fermented LAB exploration result is shown in Table 1. Since 0 hour, the initial count of LAB was of $6.4x10^6$ cfu/ml LAB then growing very rapidly until the 12^{th} h and reached stationer phase at the 24^{th} hour. This showed that LAB growth is maximum in coconut oil after being fermented for 12 hours.

b. Amount and Types of LAB

Observation of type and amount of LAB presented in Table 1 shows that since the beginning there are already 10^5 cfu/ml and 4 types of LAB existed, except for type 4, where the amount is 10^6 cfu/ml. During the 6 hours fermenting period, the amount of LAB type IV colony increased. This showed that LAB type IV is the dominant organisms during growing process until it reached 10^9 cfu/ml at the end of 24-hour fermentation.

Table 1 The total of Lactic Acid Bacteria (LAB) in fermented coconut milk.

Fermented period (hour)	**LAB total (cfu/ml) ***			
	Repetition			**Average**
	I	**II**	**III**	
0	$1,2x10^7$	$4,7x10^6$	$2,3x10^6$	$6,4x10^6$
6	$1,1x10^8$	$1,2x10^9$	$1,0x10^9$	$7,8x10^8$
12	$1,1x10^8$	$2,9x10^9$	$2,0x10^9$	$1,7x10^9$
18	$4,2x10^8$	$2,6x10^9$	$2,6x10^9$	$1,9x10^9$
24	**$1,1x10^9$**	**$1,8x10^9$**	**$1,5x10^9$**	**$1,5x10^9$**

*the total of LAB for each sample is the average of colonies from two petri dishes.

Table 2. Amount and type of Lactic Acid Bacteria (LAB) in fermented coconut milk

Fermented Period (hour)	**Average amount for each LAB (cfu/ml) ***			
	Type of LAB			
	I	II	III	**IV**
0	$2,2x10^5$	$8,4x10^5$	$8,5x10^5$	$4,5x10^6$
6	$1,2x10^6$	$7,5x10^7$	$4,0x10^7$	$6,7x10^8$
12	$9,0x10^5$	$1,0x10^8$	$9,1x10^7$	$1,5x10^9$
18	$6,2x10^6$	$8,2x10^7$	$1,1x10^8$	$1,7x10^9$
24	**$8,5x10^6$**	**$7,3x10^7$**	**$1,1x10^8$**	**$1,3x10^9$**

*Average of LAB for each type is the average of colonies amount in two petri dishes with 3 repetition

Selection, Isolation and Characterization of Protease and Amylase Producing LAB

a. Characterization of LAB obtained from the experiment resulted in 45 isolates, then 15 isolates are chosen out based on its gram stain, morphology and catalase test, its ability to produce protease enzyme, amylase, oxidase, test glucose fermentation and its way of fermentation whether its oxidative or motility fermentative. Table 3 shows that all LAB isolates are coccus, gram +, not motile, anaerobe and fermentative have the ability to produce enzyme protease, amylase and able to ferment glucose but cannot produce catalase enzyme.

Table 3 Characteristic of Lactic Acid Bacteria Isolates from Fermented Coconut Milk.

Isolate	Characteristics								
Code no.	Catalase	Oxidative	O/F	Ferm glu	Motil	Proteolysis	Amylolytik	Gram	Morphology
02	-	-	F	+	-	+	+	+	Coccus
03	-	-	F	+	-	+	+	+	Coccus
04	-	-	F	+	-	+	+	+	Coccus
62	-	-	F	+	-	+	+	+	Coccus
63	-	-	F	+	-	+	+	+	Coccus
64	-	-	F	+	-	+	+	+	Coccus
122	-	-	F	+	-	+	+	+	Coccus
123	-	-	F	+	-	+	+	+	Coccus
124	-	-	F	+	-	+	+	+	Coccus
182	-	-	F	+	-	+	+	+	Coccus
183	-	-	F	+	-	+	+	+	Coccus
184	-	-	F	+	-	+	+	+	Coccus
242	-	-	F	+	-	+	+	+	Coccus
243	-	-	F	+	-	+	+	+	Coccus
244	**-**	**-**	**F**	**+**	**-**	**+**	**+**	**+**	**Coccus**

Characteristic of growth

a. Growth in different temperature

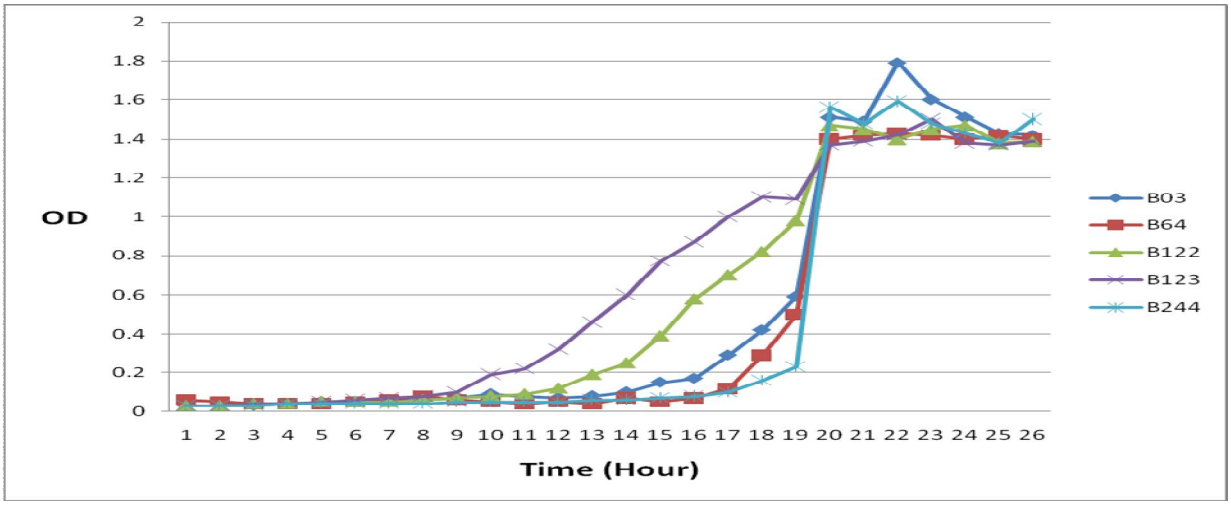

Figure 1 Growth Curve of LAB at 25°C

As shown in Figure 1 the LAB started to grow after 13 hours if the incubation time was 25°C and reached stationare phase after 20 hours of incubation. LAB with code No of B03 at 21 hours started to multiply and then the growth was decreased after 23 hours at incubation temperature of 25°C.

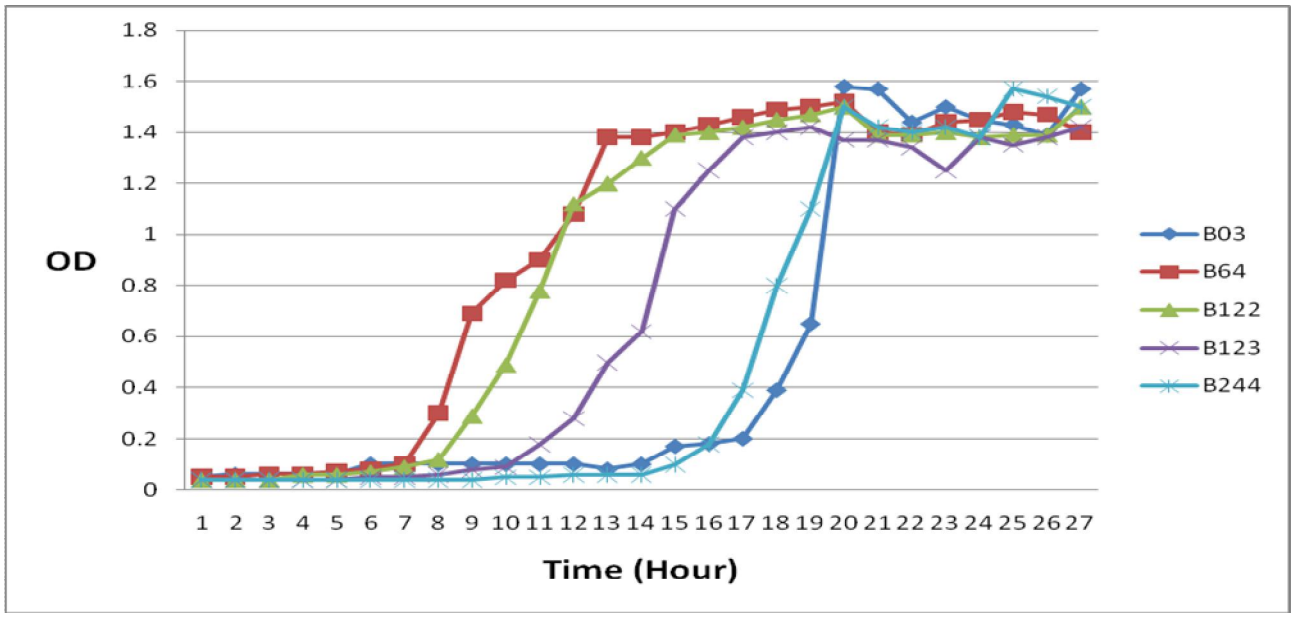

Figure 2. Growth Curve of LAB at 30°C

The growth of isolated LAB at 30°C is presented in Figure 2. The organism with code number of B64 has shorter lag phase (7th hours) than the orther organisms and reached stationair phase after the 19th hours of incubation. It might be this organisms has the incubation temperature of 30°C.

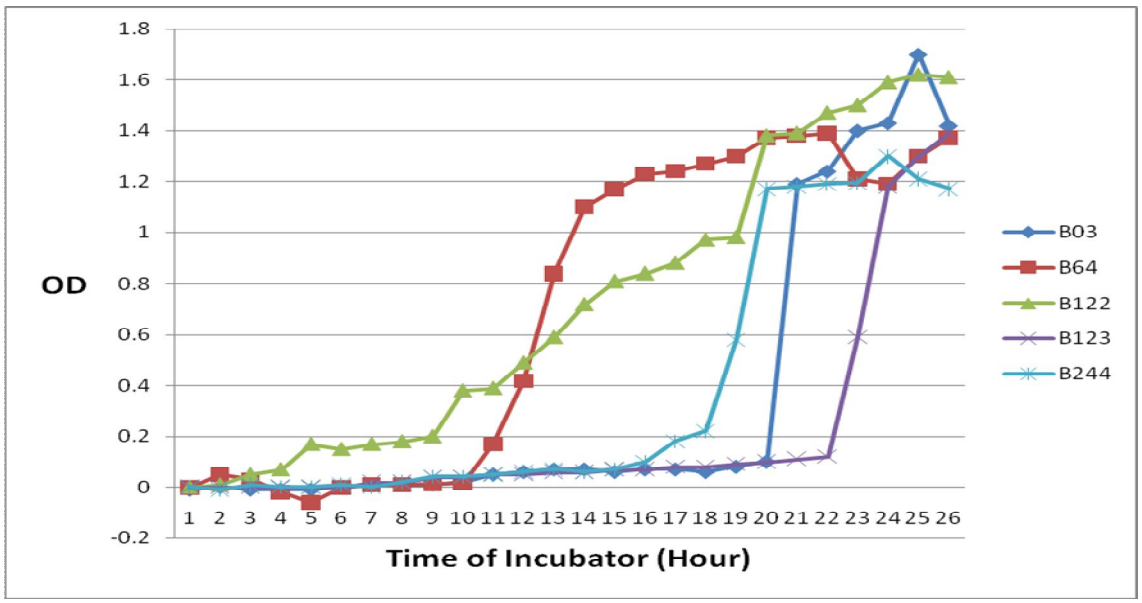

Figure 3. Growth Curve of LAB at 40°C

The increase of the incubation temperature (40°C) the LAB (B64) has longer lag phase (11st hours) than that of 30°C incubation temperature.

As expected the freshly coconut milk has a large of microbes and based on the morphology and physiology test it could be concluded that the organisms are of Lactic Acid Bacteria (LAB) due to gram positives non sporing and catalase negative [10]. At the initial stage of fermentation the fresh extracted coconut milk has already contains organisms in the range of 2,2 x 10^5 cfu/ml up to 4.5 x 10^5 cfu/ml. This probably coconut milk contains protein, fat, carbohydrate, vitamin and mineral which favored by the organism [1]. During the course of fermentation the organisms has started to developed and reached stationeries phase after 17 hour, 21 hour and 24 hour. This condition indicated that the environment supported the multiplication of the organisms. On the other hand, LAB could produce antimicrobial compound and has antagonistic activity to prevent the wild organism to grow [10].

The growth curve of the selected organisms is affected by the incubation temperature. The best incubation temperature is 30°C since this condition is similar with the environment condition at tropical country such as Indonesia. At this temperature, the LAB with co No B 64 reached the exponential phase after 10 hour of incubation and the stationaries phase at 13 hour of incubation. All the isolates tested have proteolysis and amyl lytic enzyme activity. The inoculation of this LAB (B64) pure culture on to the freshly extracted coconut milk under hygienic condition could accelerate the process for the production of virgin coconut oil having high lauric content.

CONCLUSIONS

Lactic Acid Bacteria with CO. No 64 is considered the best organisms. This organism could be used as effective microbes. Inoculation of this organisms on to fresh coconut milk and incubated at 30^{o}C for 13 hours could produce Virgin Coconut Oil. Further study on the application of LAB for the production of VCO at small scale industry should be carried out.

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