

# Isolation and Characterization of Lactic Acid Bacteria from Goat Milk Kefir and Coconut Milk Kefir

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**Keywords:** Kefir, Goat Milk, Coconut Milk, Antimicrobial Activity.

**Abstract:** Research about isolation and identification of LAB from goat milk kefir and coconut milk kefir has been completed. The making of goat milk kefir was used 3% kefir granule (HK) and 5% kefir granule (KK) from total volume of goat milk. As well as coconut milk kefir was used 3% kefir granule (HS) and 5% kefir granule (KS) from total volume of coconut milk. The kefir beverage, conducted quality analysis with result pH 4.2% – 4.6%, water content 89.5% (HK), 89.1% (KK), 79.2% (HS) and 78.9% (KS). Protein content 3.3% (HK), 3.1% (KK), 1.7% (HS) and 1.6% (KS), fat content 3.58% (HK), 15.2% (HS), 3.61% (KK), dan 15.8% (KS), ash content 0.79% (HK), 0.54% (HS), 0.81% (KK) and 0.9% (KS). Antibacterial test was conducted by observing the clear zone for 48 hours. Using *Escherichia coli* and *Staphylococcus aureus* as the test bacteria, resulted that sample HK showed the highest level of resistance compare to other samples. Total plate count of LAB showed that sample KK has the highest number of LAB counts for  $1.7 \times 10^8$ . Morphology characterization of those colony are round shape, convex elevation, off-white and also gram positive bacteria with six isolate rod shape cell, and two isolate coccus shape cell. The catalase and oxidase test showed that all isolate are negative catalase and negative oxidase.

## 1 INTRODUCTION

Kefir is a symbiotic cultures from many microorganism such as lactic acid bacteria and yeast that taken shape of grains (Bensmira & Jiang, 2012). “Milk” kefir have white/ yellowish in color (Magalhães et al., 2011). In other hand “water” kefir have color more brownish (Laureys & De, 2014). Both type of kefir has anti-inflammatory, anti-fungi, immunomodulatory dan hypocholesterolemic character (Hsieh et al., 2012).

Kefir grain that we used in this research is obtained from kefir grain seller in Tangerang, Indonesia. Using this kefir grain, we can make milk kefir and water kefir beverage. “milk” kefir is a kefir beverage that’s made from mixture of kefir grains and milk. While “water” kefir is a beverage that’s made from mixture of kefir grain and other liquid except milk, such as water, coconut milk, coconut water, fruit juice, etc (Anonim, 2013).

The goat milk produced has superior nutritional content, besides that the fat and protein in goat milk is more easily digested and its vitamin B1 content is higher than cow's milk. (Mustofa, 2009). Another

advantage of goat milk compared to cow's milk is not causing allergic reaction compare to cow's milk. Therefore, goat milk can be chosen as an alternative drink for people who are allergic to cow's milk.

While coconut milk is a food that has a high content of water, protein and fat as well as cow's milk. The advantage of coconut milk from cow's milk is that it does not contain lactose as in cow's milk so that coconut milk can be consumed by sufferers of lactose intolerant. Protein in cow's milk has the potential to cause a higher allergy than protein in coconut milk. (Gea & Sebayang, 2016).

Lactic acid bacteria and their derivatives are able to prevent the emergence of various diseases such as preventing enteric pathogenic bacteria, reducing cholesterol levels in the blood, preventing colon cancer, anti-mutagen, anti-carcinogenic and increasing endurance (Suryono, 2003).

## 2 MATERIAL AND METHOD

### 2.1 Making Goat's Milk Kefir

Fresh milk is pasteurized, which is heated at 60-65 °C for 30 minutes, then cooled to room temperature ( $\pm 28$  °C), pasteurized milk is put in 3% and 5% grains of kefir and stirred evenly. Furthermore, allowed / incubated for 20-24 hours (overnight) at room temperature (25-37°C) so that the fermentation process takes place. When the milk has coagulated, strained the milk using a plastic strainer to get the kefir grains back (Usmiati, 2007).

### 2.2 Making Coconut's Milk Kefir

Grated old coconut to separate the coconut meat from the coconut shell. Then add hot water (90-95°C) to the shredded coconut meat with ratio of water to coconut meat is 1.5: 1. The mixture of coconut meat and hot water is allowed to stand for one minute, then the mixture is blended for 5 minutes. After that, pour the mixture into a cheesecloth and filter coconut meat from coconut milk. Coconut milk that has been obtained is pasteurized by heating coconut milk at 85-90 °C for 15 seconds. After pasteurization, cool the coconut milk to room temperature of 28 °C, then add kefir grains as much as 3% and 5% and stir evenly. Furthermore, allowed / incubated for 20-24 hours (overnight) at room temperature (25-37 °C) so that the fermentation process takes place. When the mixture has coagulated, strained the coconut milk using a plastic strainer to get the kefir grains back (Anonim, 2013).

### 2.3 Quality Analysis of Probiotic Beverages (Kefir)

Analyses of pH, water content, protein content, fat content and ash content of each sample were performed according to the standard method by SNI 01-2891-1992.

### 2.4 Antibacterial Activity Test of Goat Milk Kefir and Voconut Milk Kefir

Mueller Hinton Agar was poured as much as 15 ml into a petri dish, after it was left to solidify, streak *Escherichia Coli* and *Staphylococcus aureus* inoculums into different petri dishes. Inoculum scraping is done continuously. Dip sterile paper discs into each sample and inserted into a petri dish.

Incubated in an incubator at  $\pm 35$ °C for 18-24 hours. Furthermore, measured the diameter of the clear zone around the paper disk with calipers.

### 2.5 Isolation of Lactic Acid Bacteria from Goat Milk Kefir and Coconut Milk Kefir

#### Total plate count of lactic acid bacteria

A total of 1 gram of kefir sample was diluted in 9 mL MRS Broth, vortexed until homogeneous. A total of 0.1 mL of the dilution results was put into an eppendorf tube containing 0.9 mL of MRS Broth media. Make the dilution up to 10<sup>-6</sup> dilution. At the last dilution, 0.1 mL was taken and then sprayed onto the MRS agar medium and flattened with a hockey stick. Then put in anaerobic jar and incubated for 48 hours at 37°C. Calculate the total growing colonies using the CFU / g formula (Purwati et al., 2005).

#### Isolation of lactic acid bacteria

Using a sterile spoon and curd aluminum foil weighed as much as 1 g sample, then dissolved with 9 ml of MRS Broth solution in a test tube, then vortex until homogeneous. This result is called dilution 1, put into an anaerobic jar, then incubated for 24 hours in an incubator at 37°C. After 24 hours, take 1 ml and put into a test tube containing 9 ml of MRS Broth solution, then vortex until homogeneous. Do this serial dilution until 7 times. From the last dilution 100  $\mu$ l sample is taken and planted with the spread method on petridish which contains MRS Agar media, then flattened with a hockey stick that was previously sterilized with alcohol and burned with Bunsen and then aerated. The inoculum was stored in an anaerobic jar then incubated in an incubator for 48 hours at 37°C.

In order to purify the colony, using the streak method, by using an ose needle take one colony and then streak on to the new MRS Agar media simultaneously, and then incubated for 24 hours at 37°C. (Purwati et al., 2005).

### 2.6 Identification of Lactic Acid Bacteria from Goat Milk Kefir and Coconut Milk Kefir

#### Macroscopic Identification

This identification activity was carried out on BAL isolates which observe the shape, color, and edges, lactic acid bacterial cells (Purwati et al., 2005).

### Gram staining

Colonies that characterize LAB are stained with gram as follows: 1) Bacteria and bacteria are taken flattened on a glass object (preparation) that has been cleaned with aquadest, 2) then dried on a bunsen or dryer, 3) dripped with dye violet crystals, 4) then wait for 1 minute for the dye to seep in by bacteria, 5) then rinse with running water and drop with a complex iodine solution, then wait for 1 minute, then rinse with running water, 6) wash with alcohol by means of dipping into dilute alcohol, 7) drops with safranin dye, then waited 30 seconds, 8) after it is dried and examined under a microscope (Dwidjoseputro, 1989).

### Catalase test

The procedure performed in this catalase test is first to apply a small amount of bacterial isolate on a glass object. Then add a few drops of H<sub>2</sub>O<sub>2</sub>, if necessary stir using a toothpick. After that positive catalase was observed by the presence of many air bubbles and negative catalase was shown by the absence of air bubbles.

### Oxidase test

The procedure used in this oxidase test is to use a sterile swab, a little isolate is taken from the agar medium. Then one drop of reagent was added to the isolates in the swab, after which it was observed that a positive reaction was marked by changing the bacterial isolate to a purple color.

## 3 RESULT AND DISCUSSION

### 3.1 Quality Analyses for Goat Milk Kefir and Coconut Milk Kefir

From the producing goat milk kefir and coconut milk kefir, the quality analyses can be seen on the Table 1 and Table 2.

Table 1: Quality analyses for kefir goat milk.

Test Bacteria	Sample	Antimicrobial Index	
		24 hrs	48 hrs
<i>E. coli</i>	HS	0.1	0.11
	HK	0.37	0.37
	KK	0.18	0.18
	KS	0.06	0.06
<i>S. aureus</i>	HS	0.35	0.35
	HK	0.51	0.6
	KK	0.6	0.63
	KS	0.25	0.28

Table 2: Quality analyses of coconut milk kefir.

Sample	pH	Water Content	Protein Content	Fat content	Ash content
HS (3% kefir grain)	4.6	79.2	1.7	15.2	0.54
KS (5% kefir grain)	4.3	78.9	1.6	15.8	0.59

From this table we able to see the pH of 3% kefir grain is different with 5% kefir grains. The 3% kefir grain is a little bit higher pH than the 5%, we believe the differences between this pH is because the fermentation process that takes place. The more lactic acid that produce by lactic acid bacteria, the acid it become, thus the 5% kefir grain have lower pH.

The water and ash content of all samples shows that the water and ash level does not cross the existing standard of SNI 7552:2009. The protein content shows that the more kefir grains we add it tends to have lower protein content. (Handoyo & Morita, 2006) stated that protein will be degrade or hydrolyzed during the fermentation process.

The fat content of coconut milk kefir is significantly higher than the goat milk kefir. The fat content of coconut milk vary between 0.9 – 34.7 % depends on the variety of the coconut and level of ripeness. The more ripe the coconut, the more higher the fat content (Gea & Sebayang, 2016). Therefore after the fermentation process with kefir grains, the fat content of coconut milk kefir will still be higher than the goat milk kefir.

### 3.2 Antibacterial Activity Test of Goat Milk Kefir and Coconut Milk Kefir

The results of bacterial growth inhibition test indicator with disc diffusion method showed the four samples demonstrate the ability to inhibit bacterial growth indicator, characterized by the formation of a clear zone around the paper discs with different sizes.

Table 3: Antimicrobial index.

Sample	pH	Water Content	Protein Content	Fat content	Ash content
HK (3% kefir grain)	4.5	79.2	3.3	3.58	0.79
KK (5% kefir grain)	4.2	78.9	3.1	3.61	0.81

In this study two species of pathogenic bacteria is *Staphylococcus aureus* and *Escherichia coli* (collections of Microbiology Laboratory of the University of North Sumatra) was used. Based on this data we able to see that the clear zone on Gram positive bacteria is more higher than Gram negative bacteria.

This is because the difference between the peptidoglycan walls between this bacteria. Gram positive bacteria only have one peptidoglycan walls, whereas Gram negative bacteria have outside cover membrane that protect cytoplasm and peptidoglycan.

### 3.3 Total Plate Count of Lactic Acid Bacteria

In this study, TPC of LAB colonies were calculated in coconut milk kefir and goat milk kefir. It aims to calculate the number of lactic acid bacteria colonies that exist at each concentration. After lactic acid bacteria were grown on MRS agar, the total colony count was calculated using the CFU / mL formula.

Table 4: Total plate count of lactic acid bacteria.

Sample	TPC
HS	$5.2 \times 10^7$
HK	$9.3 \times 10^7$
KK	$1.7 \times 10^8$
KS	$1.2 \times 10^8$

### 3.4 Isolation of Lactic Acid Bacteria from Goat Milk Kefir and Coconut Milk Kefir

Isolation of lactic acid bacteria from the sample begins by growing LAB on the selective medium, namely de Mann Rogosa Sharpe (MRS) broth. MRS broth is called a selective medium because it contains optimum nutrients and pH for LAB growth. LAB enrichment with MRS broth was continued with multilevel dilution aimed at reducing the number of LAB in the medium making it easier to isolate bacteria. Serial dilution carried out until  $10^{-7}$  also using MRS broth medium. The results of the dilution were planted into the MRS agar medium and incubated for 48 hours. After incubation, LAB colonies will appear on the MRS medium, the white or yellowish white.

The LAB isolation results are then purified into new media so that pure isolates are obtained.

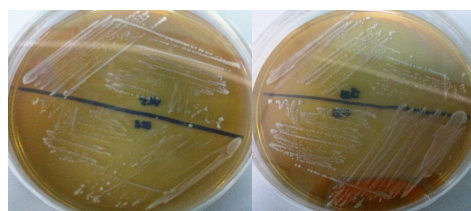


Figure 1: The results of isolation of lactic acid bacteria.

### 3.5 Morphological Identification of Lactic Acid Bacteria

Based on the identification of the shape of the LAB colony, the appearance of the LAB colony on MRS agar is circular, milky white that does not change the color of the MRS agar (brownish yellow), with smooth edges and convex elevation (Figure 1).

LAB colonies on MRS agar found in this study are in accordance with research conducted by (Komang, 2005) stating that morphological characterization of LAB isolates based on color indicates that the colonies are milky white with a round shape.

#### Gram stain test

Gram stain testing on a single colony (LAB). Figure 2 shows the results of Gram staining of LAB bacteria isolated from goat milk kefir and coconut milk kefir with concentrations of 3 and 5%. From these four samples, 8 isolates were taken. Of the eight isolates available, there were six isolates in the form of bacilli, and two isolates in the form of cocci, and the eight isolates were gram-positive bacteria that were purple.

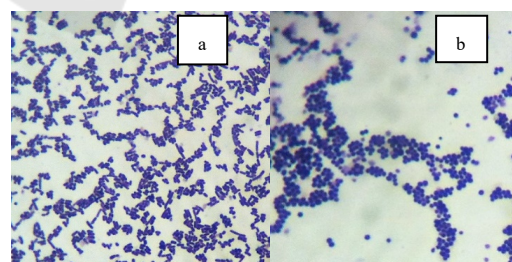


Figure 2: Gram staining (gram positive) from LAB (a) rod-shaped, and (b) coccus-shaped.

It can be seen the results of Gram stain tests and morphological observations under microscope with a magnification of 100x of 8 LAB isolates.

Table 5: Gram stain and morphological test results of LAB isolates.

Isolates	Gram	Morphology
HK1	+	Basil
HK2	+	Kokus
KK1	+	Basil
KK2	+	Basil
HS1	+	Basil
HS2	+	Basil
KS1	+	Kokus
KS2	+	Basil

Based on the identification of the shape of the LAB colony, the appearance of the LAB colony on MRS agar is circular, milky white that does not change the color of the MRS agar (brownish yellow), with smooth edges and convex elevation (Figure 1).

#### Catalase test

Catalase test was conducted to determine the presence of the enzyme catalase in bacterial isolates which were tested using hydrogen peroxide reagents ( $H_2O_2$ ). Bacteria that have the catalase enzyme are able to break down  $H_2O_2$  into  $H_2O$  and  $O_2$ . Based on the test results (Figure 3) below shows that the eight isolates showed negative results on the catalase test.

This is evidenced by the absence of air bubbles formed when the isolate was added to hydrogen peroxide. This is in accordance with Djide & Sartini (2008) who suggested that the results of biochemical tests in the form of catalase tests on lactic acid bacteria showed negative results.



Figure 3: Catalase test on LAB isolates.

#### Oxidase test

In the oxidase test, all 8 isolates show no sign of color change. Therefore all 8 isolates are negative oxidase. The result can be seen on figure 4.

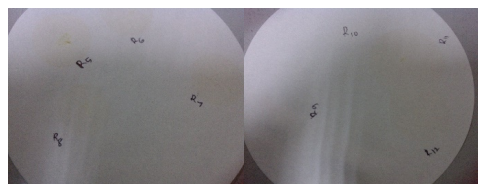


Figure 4: Oxidase test on LAB isolates.

Based on this result, LAB cultures were not able to carry out the oxidation Gaby Hadley reagent, to

yield the purple compound. This might be due to the fact that the isolates didn't possess the cytochrome c (which means isolates might be anaerobic organism) that enables them to use free oxygen in their energy metabolism (Thakur et al., 2017).

## 4 CONCLUSIONS

The quality analyses of kefir goat milk and kefir coconut milk shows that the quality meets the standard requirements of SNI 7552: 2009. Variations in the concentration of grains of kefir against goat milk kefir and coconut milk kefir affect the total lactic acid bacteria present antimicrobial values. Where the highest type of kefir has a total lactic acid bacteria is goat milk kefir with 5% kefir grain concentration that is equal to  $1.7 \times 10^8$ . Goat milk kefir and coconut milk kefir can be used as antibacterial because it can be seen in kefir with variations in the concentration of kefir grains affecting antimicrobial values.

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