



Isolation and characterization of lactic acid bacteria from Etawa crossbreed goat's milk

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ABSTRACT

Goat's milk is white liquid derived from ruminant types of dairy goats. Milk is one of habitats of lactic acid bacteria (LAB). LAB have a potential as antimicrobial because capable to kill the pathogenic bacteria. LAB isolated from Etawa crossbreed goat's milk were characterized to stipulate the genus of the isolates. Characterization of LAB consists of colony, morphology and biochemical assay. The morphological examination of the colony, cell morphology and biochemical assay showed that three isolates were identified as *Leuconostoc*, *Enterococcus* and *Lactobacillus*. The antimicrobial activity assay showed that those isolates exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, but those isolates did not exhibit antifungal activity against *Candida albicans*.

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1. Introduction

Recently, the products of goat milk and its derivatives in worldwide tends to increase due to their nutritional contents and organoleptic properties (Schirru et al., 2012). Goat milk nutritional value is better than the dairy cattle. In Indonesia, especially in Aceh Province, utilization of goat milk as additional nutrition for the family is still limited due to Aceh does not have a milk processing industry at farm level.

In Aceh, Etawa crossbred (Etawa crossed with local Kacang goat) which is known as a dairy goat, while Kacang goat is known as indigenous goat and sold for meat. Kacang, Etawa and Etawa crossbreed goats have been known as indigenous to Southeast Asia (Devendra, 1993).

Besides liquid milk, goat's milk can be processed into various commercial products as ice cream, capsule, cheese, yogurt, and butter. The presence of lactic acid bacteria (LAB) in milk fermentation can be used spontaneously as starter for cultures inoculation.

LAB is Gram-positive bacteria of bacilli (rod-shaped) or cocci (spherical), acid-tolerant properties, and has low guanine-cytosine (GC) contents. LAB usually found in milk product, as the major metabolic from the end product of carbohydrate fermentation (Aly et al., 2004). LAB plays important role in the production of fermented food. Some of these bacteria can be used as antimicrobial and able to inhibiting the growth of several microorganisms (Lindgren and Dobrogosz, 2006). Therefore, LAB is very important as

source of inhibitory compounds to improve the quality of food. LAB can be classified based on morphology, sugar utilization, mode of glucose fermentation, and certain temperatures. LAB is a group of bacteria that have physiological, morphological, and metabolic similarities. Phylogenetically, LAB also a group of bacteria relatively closely and these bacteria under the family of *Lactobacillaceae*. The aims of this research was to isolate, characterize and identify of LAB from Etawa crossbreed goat's milk. Another purpose of this research is to determine the activity of the isolate of LAB against *Staphylococcus aureus* ATCC 29923, *Escherichia coli* ATCC 38218, and *Candida albicans*.

2. Materials and Methods

2.1. Samples collection

Etawa goat milk as much as 250 mL were collected in sterile plastic aseptically from dairy farm containers, Darussalam, Aceh Besar District, Aceh Province. The sample were directly kept in ice-cooled box under low temperature and then immediately brought to the laboratory for next analysis.

2.2. Isolation of lactic acid bacteria (LAB)

Fresh Etawa goat milk sample plated into Mann Rogosa Sharpe (MRS) agar plate media to isolate of LAB. The MRS agar media were used to distinguish from other bacteria. One percentage of CaCO₃ were added to MRS-agar media plates, then incubated under anaerobic condition for 5 days at 30°C. The obtained colonies were identified by inhibition zone around each colony, then the colony were randomly selected from MRS-agar plate media. The colony then purified by replacing it on other MRS-agar plates media. The strains of LAB were identified through morphological examination by macroscopic and microscopic assays. A single colony was purified by streaking method, followed by identification of the morphology, spore formation, biochemical, motility, catalase assay, Gram-staining, and their growth ability on the carbon source. If strains of the colony possess Gram positive and catalase negative, the selective-strains then kept in 10% skim milk broth and store at cold storage at -80°C.

2.3. Antimicrobial activity test

The antimicrobial activity of LAB were determined against *S. aureus*, *E. coli* and *C. albicans* using disc diffusion method. The antibacterial activity of *S. aureus* and *E. coli* were performed on Tryptone Soya Agar (TSA) media, while the antifungal activity of *C. albicans* were examined on Sabouraud Dextrose Agar (SDA) media. Each microbes was suspended in aquadest sterile and standardized to approximately 10⁸ CFU/mL or suitable to 0.5 of McFarland. A sterile swab was soaked in the suspension containing the microbes and applied on the surface of the plates containing of TSA for the bacteria, and SDA media for the fungi and allowed to absorb. A sterile paper discs (Whatmann No. 1) humidified with 20 ml of supernatant solution from the exponential phase of each isolate of LAB. In this assay, ciprofloxacin and nystatin were used as positive control. The antimicrobial activities were determined by measuring the diameters of inhibition growth around the disc after time incubation. The experiments were performed in triplicates.

3. Results and discussion

3.1. Isolation and identification of LAB from Etawa goat milk

There are three isolates with differences in morphology and colony. Identifications of the isolates were done through morphological examination by macroscopic and microscopic assays. The macroscopic assay was performed by shape and colour colony, while the microscopic assay was determined through Gram staining. In this study, the biochemical and physiological characteristics were also tested. The biochemical properties in this study including catalase and motility. Meanwhile, methyl red (MR) and voges proskauer (VP) assays were performed on De Man, Rogosa and Sharpe (MRS) agar media at 14 and 37 °C. The carbon dioxide (CO₂) production from the glucose in the presence of 5; 6.5; and 10% of NaCl also determined. The carbohydrate utilization such as glucose, lactose, sucrose were also performed.



Fig. 1. One of colony LAB from Etawa goat milk (Etawa crossbred with local Kacang goat)

The results revealed that all isolates that tested in this study were non-motile, Gram positive and non-spore formers. The results also revealed that the colours of the colony were white and creamy. The pure isolates obtained were classified based on shape, cell arrangements, Gram staining, catalase, and motility. The macroscopic and microscopic assay at the genus level indicated that all isolates consisted of three genus, namely *Leuconostoc*, *Enterococcus* and *Lactobacillus*. The biochemical properties results of LAB isolated from Etawa goat milk is presented in Table 1.

Table 1. The biochemical characteristics of LAB isolated from Etawa goat milk.

Biochemical characteristics	Genus		
	<i>Leuconostoc</i>	<i>Enterococcus</i>	<i>Lactobacillus</i>
Morphology	Cocci	Cocci	Rods
Gram Staining	+	+	+
Motility	-	-	-
CO ₂ from glucose	+	-	-
Growth at 14°C	+	+	+
Growth at 37°C	-	+	+
at pH 4.5	+	+	+
at pH 9.6	-	+	+
Growth in 5% NaCl	-	+	+
Growth in 6.5% NaCl	+	+	+
Growth in 10% NaCl	-	+	-
Glucose	+	+	+
Lactose	+	+	+
Sucrose	+	+	+
Methyl Red (MR) test	-	+	+
Voges Proskauer (VP) test	-	-	-
Fermentation Test	He	Ho	Ho
Catalase test	-	-	-

Silva et al., (2013) mentioned that LAB play important roles in increasing the functional value of goat milk. Delavenne et al., (2012) and Wouters et al., (2002) stated that milk is the natural habit of LAB. LAB is widely used in milk manufacture to produce derivatives of milk product due to Lab have specific properties. LAB also used as starter and non-starter in fermented milk manufacture. In this study, we

found that *Leuconostoc* is one of LAB from Etawa goat milk. These genus are Gram-positive, non-sporulation bacteria that able to produce lactic acid. *Leuconostoc spp* bacteria such as *L. cremoris*, *L. dextranicum*, *L. mesenteroides subsp cremoris*, and *L. citrovorum* are responsible for the characteristic flavour of milk. *L. mesenteroides subsp cremoris* is bacteria that commonly used in cultured butter milk and sour cream.

Setyawardani et al., (2011) stated that strains of *Lactobacillus* such as *L. rhamnosus* and *L. plantarum* have been identified in LAB that isolated from Etawa and Saanen crossbred goat milk in Java.

Khelid (2006) mentioned that LAB also were identified as *Leuconostoc* strain isolated from camel milk in Morocco. He also identified that *Leuconostoc* strain also present in khafir. Khafir is a fermented milk drink was made from khafir grains. The presence of *Enterococci* strains bacteria in this study is typical and strictly due to this strain were grown at 37°C in 10% of NaCl at pH 9.6. *Enterococci* have been known able to convert carbohydrates into lactic acid. The strains of *Enterococci* also known as typical homofermentative of LAB (Kun, 2003). Bulut et al., (2005) mentioned that *Enterococcus* as one of indigenous LAB in Comlek Peyniri (typical artisanal cheese) in Central Anatolia.

LAB also were identified by Ashmalg et al., (2009) from camel milk in Sudan as *Lactobacillus* strain such as *L. raffinolactis*, *L. plantarum*, *L. alimentarium*, *L. fermentum*, *L. paracasei*, *L. gasserii*, *L. rhamnosus*, *L. divergens*, *L. brevis*, and *L. animalis*. Several researchers reported that LAB are contribute to human health as probiotic.

3.2. Antimicrobial activity assay of LAB from Etawa goat milk

The antimicrobial assay was determined in order to find out the ability of LAB against the pathogenic microbe such as *S. aureus* ATCC 29923, *E. coli* ATCC 38218, and *C. albicans*.

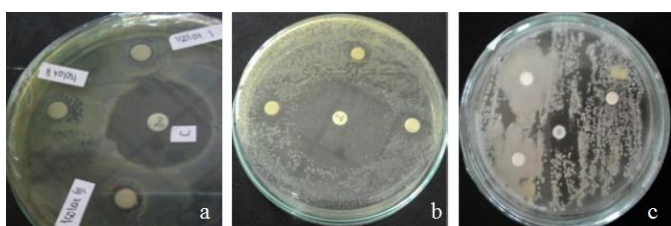


Fig. 2. The antimicrobial activity of LAB isolates against *Escherichia coli* (a), *Staphylococcus aureus* (b), and *Candida albicans* (c).

The results showed that three isolates of LAB *Leuconostoc*, *Enterococcus*, and *Lactobacillus* have antibacterial activity towards pathogenic bacteria of *S. aureus* ATCC 29923 and *E. coli* ATCC 38218, which indicated by the formation of growth inhibition zones around the disc paper. Unfortunately, all LAB isolates were used in this study did not exhibit antifungal activity against *Candida albicans* (Figure 2).

Conclusion

The macroscopic and microscopic assays at the genus level confirm that LAB that isolated from Etawa goat milk

were identified as members of the genus *Leuconostoc*, *Enterococcus*, and *Lactobacillus*. The antimicrobial activity showed that all isolates exhibited great inhibitory activity towards *Staphylococcus aureus* and *Escherichia coli*, but did not exhibit inhibitory activity against *Candida albicans*.

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