Identification of lactic acid bacteria from etawa goat milk kopelma Darussalam Village, Banda Aceh

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Abstract. Identification of Lactic Acid Bacteria (LAB) has been carried out based on the catalase test and Gram staining of Etawa pure Goat Milk samples that obtained from Kopelma Village, Darussalam District, Banda Aceh City, Aceh Province. De Man, Rogosa, Sharpe Agar (MRSA) media was used for LAB isolation. There were 10 isolates of goat milk bacteria that used for microscopic characteristics, morphological observations, catalase test and Gram staining. Based on the microscopic test, it was explained that all colonies had similar characteristics, that having a round shape, convex surface shape, 1-2 mm diameter and cream color. In the catalase test, the results were negative, because all isolates did not produce gas bubbles after dripped with H₂O₂ solution. The morphological results showed that all isolates were Gram positive, marked by purple cells and bacilli cells. Based on the Gram staining and the shape of the bacillus cells, it were suspected that milk isolates are candidates for the genus Lactobacillus. Microscopic diagnosis is only a provisional presumption. Therefore, it needs a conclusive diagnosis, such as biochemical properties which are important factors that must be carried out for the next stage.

1. Introduction
Goat milk is a type of milk that contains complete nutrition so it is an ideal growth medium for microorganisms [1]. One of the main groups of bacteria that grow in milk is Lactic Acid Bacteria (LAB). The LAB is a microorganism that is classified as Generally Recognized as Safe (GRAS) which produces antimicrobial compounds including organic acids, diacetyl and bacteriocin. Antimicrobial compounds produced by LAB can inhibit the growth of food spoilage bacteria and pathogenic bacteria [2]. Therefore, LAB have been widely used to extend the shelf life of a product, so it is often used as an alternative for food preservation [3].
Lactic acid bacteria are classified as a type of probiotic microflora that can produce lactic acid mainly from the class of Lactobacilli and Bifidobacteria through fermentation [4]. One of the processed milk products through the fermentation process is yoghurt which is beneficial for health. Probiotic foods and drinks have health benefits, such as helping the digestive system (lactase enzymes, stimulating the function of the intestinal wall) in the absorption of nutrients, inhibits and kills pathogenic bacteria in the digestive tract such as *E. coli*, *S. aureus*, *S. typhimurium*, *V cholerae*, and *M. tuberculosi*, prevent constipation, as an anti-cancer, reduce blood cholesterol, prevent Lactose intolerant, increase the body's immune response [5] dan as a therapy for TB (Tuberculosis) [6].

Several previous studies reported that LAB is often found in various types of milk products and various kinds of fermented milk products, such as from Sumbawa horse milk [7], fresh goat milk [8], goat milk hybrid (Etawa) that obtained from the Center for Artificial Insemination, Singosari, Indonesia [9], fermented buffalo milk typical of West Sumatra [10], commercial yoghurt [11] and toddler formula milk [12]. Based on this description, it is necessary to conduct preliminary research on the isolation of LAB as the development of future probiotics that can be applied to food products as a prevention against several diseases. Therefore, the identification of bacteria was carried out based on the catalase test and Gram staining of Etawa goat's milk in the Kopelma Village area, Darussalam District, Banda Aceh City, Aceh Province.

2. Materials and methods
Etawa Goat Milk is obtained from dairy goat breeders in Kopelma Village, Darussalam District, Banda Aceh City, Aceh Province (Figure 1). Fresh goat milk is taken directly from 10 different goats with 50 mL each and packaged in a high density polyethylene (HDPE) plastic container. Goat milk samples were brought in to the laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University for initial identification of the LAB types that contained in goat milk based on microscopic characteristics, morphological observations, catalase test and gram stain.

![Google earth map of the Etawa goat farm location (red mark).](image-url)
2.1. Tools and materials
The instruments used are Microscope (Olympus), Shaker Incubator (Barnstead MAXQ 7000), Micropipette (Eppendorf), Analytical Scales (Mettler Toledo), Autoclave (Hirayama), Oven (Memmert), Water Bath (Memmert), Laminar Air Flow, Erlenmeyer, Reaction Tubes, Beaker Glass, Bunsen and Ose Needles. The materials used are goat milk samples, MRSA (Oxoid) media, BHIB (Oxoid) media, aquadest, 70% alcohol and disinfectant.

2.2. Making of media BHIB (Brain Heart Infusion Broth)
The procedure for making BHIB media is by mixing 3 Grams of BHIB media powder and 100 mL of distilled water in an erlenmayer. Stir until homogeneous and sterilized in an autoclave at 121 °C for 15 minutes. Incubated for 24 hours at 37 °C. This was done to prove that the BHIB media was sterile before inoculation [13].

2.3. Making of media MRSA (de Man, Rogosa, Sharpe Agar)
MRSA media are made by dissolving as much as 68.2 Grams of MRSB powder in 1 liter of distilled water. The media was heated and homogenized using a magnetic stirer, then sterilized in an autoclave at 121 °C for 15 minutes. Incubated for 24 hours at 37 °C. This was done to prove that MRSA media was sterile before inoculation [14].

2.4. Catalase test
The catalase test was carried out by dropping 3% hydrogen peroxide (H₂O₂) on a clean glass slide. Milk isolate is rubbed on a glass slide that has been dripped with H₂O₂. The suspension was slowly mixed using a Ose, the positive result is indicated by the formation of air bubbles [15].

2.5. Gram staining
When bacteria are stained with a primary dye (crystal violet), the bacteria will absorb the dye or will release the dye. After rinsing with alcohol and safranin, bacteria will absorb dyes [16]. Subsequently observed under a microscope, the Gram-positive test indicated that the bacterial cells would be colored purple, while the Gram-negative bacterial cells would be pink.

3. Result and discussion
Lactic acid bacteria (LAB) which were isolated from goat’s milk obtained 10 isolates base on negative catalase test and positive Gram staining. LAB isolates were isolated using MRSA media and characterized by morphology in each colony including colony shape, colony edge shape, colony size and colony color. Based on the microscopic test, it was explained that all colonies had similar characteristics, namely having a round shape, convex surface shape, 1-2 mm diameter and cream color. With these characteristics, according to the research of Holdeman, Moore and Cato [17] LAB isolates that have been incubated and grown on selective media are Lactobacillus LAB colonies. All isolates were then subjected to a gram staining test and a catalase test. The LAB was included in the category of Gram-positive staining and the catalase test was negative [18].

The results of the LAB identification test for Etawa Goat Milk isolates based on the catalase test, Gram staining and morphology refer to the Bergey's Manual of Determinative Bacteriology 9th [18]. Based on Table 1, it explains that the results of microscopic observations with Gram staining obtained purple milk isolate (Gram positive) and negative catalase test. It is suspected that milk isolates 1-10 are classified as LAB, this is base on the result of Gram staining is positive. As well as Yousef and Carlstrom’s statement [19] that LAB is Gram positive, spherical and catalase negative.
Table 1. The results of the LAB identification test for Etawa Goat Milk isolates were based on the catalase test, gram staining and morphology.

| No | Code          | Catalase | Gram | Morphology |
|----|---------------|----------|------|------------|
| 1  | Milk Isolate 1| -        | +    | Bacilli    |
| 2  | Milk Isolate 2| -        | +    | Bacilli    |
| 3  | Milk Isolate 3| -        | +    | Bacilli    |
| 4  | Milk Isolate 4| -        | +    | Bacilli    |
| 5  | Milk Isolate 5| -        | +    | Bacilli    |
| 6  | Milk Isolate 6| -        | +    | Bacilli    |
| 7  | Milk Isolate 7| -        | +    | Bacilli    |
| 8  | Milk Isolate 8| -        | +    | Bacilli    |
| 9  | Milk Isolate 9| -        | +    | Bacilli    |
| 10 | Milk Isolate 10| -        | +    | Bacilli    |

In the catalase test, all isolates did not produce gas bubbles after being dripped with \( \text{H}_2\text{O}_2 \) solution. This indicated that all isolates were catalase negative. This characteristic is as expected, because it is one of the characteristics of LAB as reported by Suskovic [20] that BAL is a group of bacteria that does not have the catalase enzyme. Some examples of bacteria that are catalase negative are Streptococcus, Leuconostoc, Lactobacillus and Clostridium [21].

![LAB isolates in the milk samples were purple (gram positive) from the bacilli cells.](image)

Observation of Gram stain showed that all isolates were Gram positive and bacilli cell shape. Figure 2 is an example of the morphology of Gram-positive milk isolates marked with purple cells. Based on the results of Gram staining, LAB isolates had bacilli morphology with a chain arrangement and it is suspected that this isolate is a candidate for the Lactobacillus genus.

Observation of the morphological characteristics of bacterial colonies is necessary to facilitate the identification of bacterial species. This is in accordance with Lay's statement [22], that based on the morphological characteristics of bacterial colonies and pure cultures, the identification process of the types of microorganisms can be carried out, but to obtain perfect identification results it must be followed by a biochemical test. According to Djide and Kadir [23] microscopic diagnosis is only
presumptive. Therefore it needs a conclusive diagnosis, such as biochemical properties which are important factors that must be carried out for the next stage.

4. Conclusion
In the Etawa Goat Milk bacterial isolate, there were Gram-positive bacteria and negative catalase tests. Based on the microscopic test, it was explained that all colonies had similar characteristics, that having a round shape, convex surface shape, 1-2 mm diameter and cream color. In the catalase test, all isolates did not produce gas bubbles after dripped with H2O2 solution. This indicated that all isolates were catalase negative. The morphological results of the milk isolates showed that all isolates were gram positive, marked by purple cells and bacilli cell. Based on the Gram stain and the shape of the bacilli cells, it was suspected that milk isolates are candidates for the genus Lactobacillus. Microscopic diagnosis is only a provisional presumption. Therefore it needs a conclusive diagnosis, such as biochemical properties which are important factors that must be carried out for the next stage.

References

[1] Haenlein G F W 2004 Goat milk in human nutrition Small Rumin. Res. 51 155–63
[2] Gonzalez B Arca P Mayo B and Suárez J E 1994 Detection, purification, and partial characterization of plantaricin C, a bacteriocin produced by a Lactobacillus plantarum strain of dairy origin. Appl. Environ. Microbiol. 60 2158–63
[3] Navarro L Zarazaga M Aenz J S Ruiz-Larrea F and Torres C 2000 Bacteriocin production by lactic acid bacteria isolated from Rioja red wines J. Appl. Microbiol. 88 44–51
[4] Lahtinen S Ouwehand A C Salminen S and von Wright A 2011 Lactic acid bacteria: microbiological and functional aspects (Crc Press)
[5] Deegan L H Cotter P D Hill C and Ross P 2006 Bacteriocins: biological tools for bio-preservation and shelf-life extension Int. dairy J. 16 1058–71
[6] Mading M and Adyana N W D 2014 Nutritional and immunization status as determinant of pneumonia incident in children under five in East Nusa Tenggara Province Bul. Penelit. Sist. Kesehat. 17 20920
[7] Sujaya I N Aryantini N P D Nursini N W Cakrawati C I D Juliasari N L M E Dwipayanti N M U and Ramona Y 2012 Ekspolisisakarida dari Lactobacillus sp. Isolat Susu Kuda Sumbawa dan Potensinya sebagai Prebiotik J. Vet. Juni 13 136–44
[8] Ernawati E 2010 Isolasi dan identifikasi bakteri asam laktat pada susu kambing segar
[9] Fitria I N and Ardyati T 2014 Skrining Bakteri Asam Laktat asal Susu Kambing Peranakan Etawa sebagai Penghasil Bakteriosin Bioher. J. Trop. Biol. 2 164–8
[10] Trisna W N 2012 Identifikasi Molekuler dan Pengaruh Pemberian Probiotik Bakteri Asam Laktat (Bal) Asal Dadih dari Kabupaten Sijunjung Terhadap Kadar Kolesterol Daging Pada Itik Pitalah Sumber Daya Genetik Sumatera Barat Artik. Progr. Pascasarj. Univ. Andalas, Padang 32
[11] Nuryadi M M Istiqomah T Faizah R Uabidillah S and Mahmudi Z 2013 Isolasi dan identifikasibakteri asam laktat asal youghurt J. Univ. Jember 1 1–11
[12] Indriyati A S 2009 Isolasi dan karakterisasi bakteri asam laktat (BAL) dari susu formula balita yang berpotensi menghasilkan substansi antimikroba.[Skripsi] Yogyakarta UIN Sunan Kalijaga
[13] Atlas R M 2010 Handbook of microbiological media (CRC press)
[14] De Man J C Rogosa deM and Sharpe M E 1960 A medium for the cultivation of lactobacilli J. Appl. Bacteriol. 23 130–5
[15] Alfonso-Prieto M, Biarnés X Vidossich P and Rovira C 2009 The molecular mechanism of the catalase reaction J. Am. Chem. Soc. 131 11751–61
[16] Creager J Black J G and Davison V E 1990 Laboratory manual: Microbiology principles and applications (Prentice Hall)
[17] Holdeman L V Moore W E C and Cato E P 1977 Anaerobe laboratory manual
[18] Bergey D H, Holt J G and Krieg P 1994 Bergey’s manual of determinative bacteriology. 1994 Williams Wilkins, Balt. MD, USA
[19] Yousef A E and Carlstrom C 2003 Food microbiology: a laboratory manual (John Wiley & Sons)
[20] Šušković J Kos B, Goreta J and Matošić S 2001 Role of lactic acid bacteria and bifidobacteria in synbiotic effect Fo. Tech., Biotech.. 39 227–35
[21] Dwidjoseputro D 1978 Pengantar mikologi (Penerbit Alumni)
[22] Lay B W 1994 Analisis mikroba di laboratorium PT. Raja Graf. Persada. Jakarta 168
[23] Djide M N and Kadir S 2003 Mikrobiologi Farmasi Terapan Univ. Hasanuddin, Makassar