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Isolation and Antimicrobial Activities of Lactic Acid Bacteria Originated From Indonesian Local Goat's Colostrum

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Abstract. The objectives of this study were to isolate lactic acid bacteria (LAB) from Indonesian local goat colostrum and to characterize their suitable properties for bacteriocin production. LAB was isolated from goat colostrum. The characterization of LAB was carried out based on the shape, colony dispersal, and catalase test. For antimicrobial activity, LAB was tested by a well diffusion method followed by an antimicrobial activity test against pathogenic bacteria *B. cereus*, *E.coli*, *S. aureus* and *S. thypimurium*. A total of 8 strains of LAB were successfully isolated from goat colostrum and coded CT1 to CT8. All the isolates were rod-shaped, single or paired colonies, negative catalase, and glucose fermenting LAB. The isolates consist of four *L. casei,* two *L. brevis or L. plantarum*, one *L. rhamnosus*, and one, *L. paracasei*. CT3 isolate has 84% similarity with *L. plantarum* and 14.3% with *L. brevis* 1while CT8 isolate is 71% similar to *L. brevis* 1 and 28.9% to *L. plantarum.* Purity evaluation showed that CT3 and CT8 were *L. plantarum*. Well difusion test showed that all LAB strains possess very solid resistances, with diameters over 17 mm, against *B. cereus*, *E.coli*, *S. aureus* and *S. thypimurium*. The average inhibitory resistance against *B. Cereus, E.coli, S.aureus and S.typhimurium was* was 17.68 mm, 19.38, 19.30 and 19.03 mm, respectively. LAB isolated from Indonesian local goat colostrum are potential candidates for bacteriocin-producing bacteria.
Keywords : colostrum, lactic acid bacteria, pathogen, antimicrobial activity

Abstrak. Tujuan dari penelitian ini adalah untuk mengisolasi bakteri asam laktat (BAL) dari kolostrum kambing lokal Indonesia dan untuk mengkarakterisasi sifat bakteriosin. BAL diisolasi dari kolostrum kambing. Karakterisasi BAL dilakukan berdasarkan uji bentuk, dispersi koloni, dan katalase. Untuk aktivitas antimikroba, BAL diuji dengan metode difusi sumur diikuti dengan uji aktivitas antimikroba terhadap bakteri patogen *B. cereus, E. coli, S. aureus* dan *S. thypimurium*. Sebanyak 8 spesies LAB berhasil diisolasi dari kolostrum kambing dan diberi kode CT1 ke CT8. Semua isolat berbentuk batang, koloni tunggal atau berpasangan, katalase negatif, dan mampu mefermentasi glukosa. Isolat terdiri dari empat *L. casei*, dua *L. brevis* atau *L. plantarum*, satu *L. rhamnosus*, dan satu, *L. paracasei*. Isolat CT3 memiliki 84% kemiripan dengan *L. plantarum* dan 14,3% dengan *L. Brevis*, sedangkan isolat CT8 adalah 71% mirip dengan *L. brevis* dan 28,9% dengan *L. plantarum*. Evaluasi kemurnian menunjukkan bahwa CT3 dan CT8 adalah *L. plantarum*. Uji difusi sumur menunjukkan bahwa semua strain LAB memiliki ketahanan sangat kuat, dengan diameter lebih dari 17 mm, terhadap *B. cereus, E. coli*, S. aureus dan *S. thypimurium*. Resistensi penghambatan rata-rata terhadap *B. Cereus, E. coli*, *S. aureus* dan *S. typhimurium* masing-masing adalah 17,68 mm, 19,38, 19,30 dan 19,03 mm. BAL yang diisolasi dari kolostrum kambing lokal Indonesia merupakan kandidat potensial untuk bakteri penghasil bakteriosin.
Kata kunci : kolostrum, bakteri asam laktat, patogen, aktivitas antimikroba

Introduction

Bacteriocin is a secondary metabolite produced by lactic acid bacteria (LAB), capable of inhibiting spoilage microbes or pathogens. It is a protein or protein complex that is synthesised in the ribosome, displaying the antimicrobial features. The majority of Gram-positive and negative bacteria produce either protein or polypeptide in the course of growth. The production of bacteriocin takes place in the ribosome during the primary phase of growth. *Lactobacillus* is one among the Gram positive bacteria known for producing bacteriocin. The use of LAB and its metabolite products for food preservation is generally recognised as safe (GRAS). The use of antimicrobial compounds as a natural preservative to hinder the development of pathogenic and/or spoilage bacteria has been proven to be an efficient method (Zacharof and Lovitt, 2012).

The initial test for bacteriocin-producing LAB is antimicrobial inhibition spectrum which can be conducted using a spot test and well diffusion assay (Khay et al., 2013). Subsequent tests include growth ability on various growth media, resistance to a range of pH and temperatures (Zouhir et al., 2011), and resistance to enzymes and detergents. The concluding test is aimed at measuring the activity of bacteriocin (Rajaram et al., 2010).

Our preceding study established that LAB *L. rhamnosus* TW 2 and *L. plantarum* TW 14 was successfully isolated from goat's milk.. Both LAB possessed antimicrobials properties against several pathogens and spoilage bacteria in foods, capable of withstanding low pH (2.0-3.2) and adhered well in vitro (Setyawardani et al., 2011; Setyawardani et al., 2014). Previous studied reported that colostrum isolated has superior antimicrobial properties as bacteriocin candidates against pathogenic bacteria (Viswanathan, Preethi, Veilumuthu, Rajesh, and Suba, 2015). Colostrum was reported to have antimicrobial ability against *E. coli*, *E. aerogene*s, *K. pneumonieae*, *B. subtilis* and S. aureus.

This paper presents our findings primarily in obtaining isolates of LAB from Indonesian local goats colostrum and testing the antimicrobial activities of bacteria as a potential candidate to produce bacteriocin.

Materials and Methods

Isolation of LAB

Colostrum from local Indonesian goats was collected from farmers in Banyumas district, Central Java. One mL was taken and enriched with 100 mL liquid de Man Rogosa and Sharpe (MRS; Difco Laboratories, Michigan USA), then incubated at 37°C for 24 hours. A serial dilution was carried out, then 0.1 mL was spread on MRS Agar (MRSA) containing bromocresol purple in Petri dishes. Colonies of the LAB appeared as those surrounded by yellow zonesthat would be isolated and scratched on MRSA media. The experiment was conducted repeatedly so as to establish a uniform colony.

LAB characterization

Characterization of isolated LAB was carried out by re-culturing each isolate in MRS Broth (MRSB) for 24 hours. All isolates were initially tested for Gram staining, motility, and catalase reaction. LAB identification was based on morphology, physiology and biochemical characteristics (Muyanja et al., 2003). These preliminary tests were conducted to classify the isolates into thse genus.

Antimicrobial Activity LAB

Antimicrobial activities of LAB were deduced in accordance with the procedures introduced by Liasi et al. (2009). Four pathogenic bacterial cultures, i.e. *S. typhimurium* (ATCC 14028), *E. coli* (ATCC 8739), *B. cereus* (ATCC 13061), and *S. aureus* (ATCC. 25923) were refined as stock cultures. The culture was diluted to a concentration of 6 log CFU/ mL as an indicator bacteria. A total of 20µl bacterial culture was put into a Petri dish and mixed with 20 mL of Mueller Hinton Agar (Savadogo et al., 2004). The agar in the Petri dish was left to solid, then a hole with 5mm diameter hole was bored into it. A total of 50µl LAB cultures were put into the well andstored in a refrigerator for 60 mins. After an incubation at 37° C for 24 hours, a clear zone formed around the well was measured using a caliper from three different points and the average of the results was calculated. Each test was conducted in three replicates.

Identification of LAB with API CHL 50 test

One of the isolates was inoculated into 10 mL MRSB media then incubated at 37°C for 24 hours. LAB culture was centrifuged at 9800xg for 10minutesand the yielded pellet was separated. Pellets were placed in API CHL 50 medium with sterile pipettes and homogenized with a vortex. All the strips were coated with paraffin oil to provide an anaerobic environment then incubated at 37°C for 24 hours. After incubation,

the samples underwent an observation process to detect colour changes, and the observed results were analysed using APIWEB™ software.

16S rRNA gene sequences analysis

Genotypic identification was conducted by extracting encoded DNA 16S rRNA, followed by amplification and sequencing. DNA extraction was carried out by a Mini DNA Kit Qiagen. First, 1.5 mL of bacterial cell was centrifuged with 10,000 rpm speed at 4^{o}C for 5 mins then the supernatant was discarded. The remaining pellets were dissolved by adding 180µl ATL buffer and 20µl proteinase K, then the solution was vortexed. Incubation was done for an hour at 56°C to trigger the RNA, then 200 AF buffer was added, followed by a 10-minute incubation at 70°C to disable the RNA. A total of 200µl of ETOH 100% was added after spindown. Next, the sample was vortexed for 15 seconds, spindown, and centrifuged for 3 mins with 8000rpm speed at 4^{o}C for 3 mins. The fluid was discarded then added with 500µl AW2, and re-centrifugated with 10,000rpm speed at 4°C for 5 mins. A total of 25µl AE buffer was added and let sit for 5 mins prior to 10-minute re-centrifuge with 10,000rpm speed at 4^{o}C for 10 mins.

DNA Amplification with Polymerase Chain Reaction

The amplification of DNA samples was carried out with a 0.2 mL PCR tube added with 5µl master mix and 63F universal primer (5 'CAG GCC TAA CAC ATG CAA GTC 3') and 0.25µl 1387R primer (5 'GGG CGG WTG GTA CAA GGC 3 '). The 2.5µl genome extract was then added to ddH2O to obtain 50µl volume. The amplification stage was conducted using PCR PTC tool (MJ Research, Inc.) at the following conditions: pre-denaturation at 94 °C for 5 mins (30 cycles), denaturation at 94 °C for 30 seconds (30 cycles); annealing at 50 °C for 1 min (30 cycles); extension at 72°C for 2 mins (30 cycles); post-extension at 72^{o}C for 5 mins, and cooling at 25^{o}C for 10 mins. The PCR outcomes were stored at 4 °C and then tested with electrophoresis using 0.1% agarose electrophoresis in TAE at 100 volts for 30 mins. The results were evaluated under the UV light on Gel Doc, resulting in 1200 bp PCR product.

Sequencing DNA encoding 16S rRNA

The sequence was performed by 1st BASE then by BLAST according to the order of the nucleotide and they were matched with the available database GenBank (*www. ncbi. nlm.nih.gov*) to identify the tested species tested.

Statistical analysis

Cell morphology data, API and 16S rRNA gene sequences description analysis and for antimicrobial data using completely randomized design followed by DMRT test.

Results and Discussion

Lactic acid bacteria isolation from goat colostrum

Eight isolates were selected to determine morphological characteristics. Morphology characteristics of the eight isolates came out to be rod-shaped in various forms—paired, single, long, short and slender colonies. This result is slightly different from Setyawardani et al. (2011), reporting varied shapes including rod (26 isolates), short rod (1 isolate), spherical, oval (3 isolates) and spherical (3 isolates). Other features included Gram positive, negative catalase and non-spore forming. All isolates were rods, and possessed other characteristics of *Lactobacillus* (Lee et al., 2016).

Table 1. shows that all eight isolates are Gram-positive bacteria. This finding was in accordance with the preceding examination which stated that the predominant bacteria isolated from the goat milk of Etawah crossbreed were Gram-positive, negative catalase and non-spore forming bacteria (Setyawardani et al., 2011). Furthermore, all isolates were homo-fermentative and capable of fermenting glucose. Homo-fermentative LAB are suitable for milk fermentation because lactic acid is the sole metabolite produced during fermentation.

Table 1. Morphology and physiology characteristics of LAB isolated from goat colostrum

Characteristics	Isolates							
	CT1	CT2	CT3	CT4	CT5	CT6	CT7	CT8
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colony form	Pairs	Pairs, short	Pairs, short	Pairs, single, short	Pairs, single, long	Pairs, short	Pairs, long, slender	Pairs, long, slender
Gram	+	+	+	+	+	+	+	+
Spores	-	-	-	-	-	-	-	_
Catalase	-	-	-	-	-	-	-	_
Glucose fermentation	+	+	+	+	+	+	+	+
Fermentation type*	Ho	Ho	Ho	Ho	Ho	Ho	Ho	Ho

*Ho: homo-fermentation

Homo-fermentative LAB were predominant in goat's milk (Setyawardani et al., 2011) as well as in cow's milk (Abdullah and Osman, 2010).

There were eight out of 20 isolates of LAB from goat colostrum that displayed good inhibitory activity. All eight isolates referred to as the genus *Lactobacillus*, characterised as a group of Gram-positive, non-spores, negative catalase and capable of fermenting glucose. The identification process through API CHL 50 test revealed that two isolates were *L rhamnosus*and five isolates were *L plantarum* (Table 2). The form and colony dispersion of *Lactobacillus* for a number of isolates were similar specifically among CT2, CT3, and CT5. CT4 and CT5 isolates showed colonies that were paired, single and long rod in appearance. However, all CT1 isolates were in pairs. Similar patterns of colony spread were also present in LAB isolated from a traditionally produced milk product (Abd El Gawad et al., 2010). LAB isolates predominated by *Lactobacillus* are readily adaptable to acidic conditions— a very vital feature for the fermentation process. Both homo-fermentative and hetero-fermentative *Lactobacillus* are capable of withstanding pH 2.0; 2.5 and 3.2 (Soomro and Masud, 2007; Setyawardani et al., 2011).

All isolates tested with API CHL 50 were part of the genus *Lactobacillus* (Table 2.). The test result shows that the isolates consist of 50% *L. casei,* 25% *L. brevis /L.plantarum*, 12.5% *L. rhamnosus*, and 12.5% *L. paracasei*. Isolate CT3 had significant similarities; 84% to *L. plantarum* and 14.3% to *L. brevis* 1. However, isolate CT8 has substantial similarities to *L. brevis* 1 (71%), and *L. plantarum* (28.9%). The purity of two isolates, CT3 and CT8, was established by molecular identification through 16S rRNA amplification. The next stage was sequencing and phylogenetic tree (Table 3).

The results (Figure 1) indicated that both isolates belong to the species *L. plantarum*. Phylogenic analysis showed that CT3 isolate was closely related to *L. plantarum* KCCM200656 strain and CT8 isolate to *L. plantarum* A223 strain. Analysis of 16S rRNA sequence showed that CT3 isolate and *L. plantarum* IMAU 40170 have a kinship value of 0.005. CT8 isolates and *L. plantarum* AZZ3 strain have a kinship value of 0.005, whereas with *L. plantarum* N8 strain is with a value of 0.0025. It is evidenced that both isolates (CT3 and CT8) are *L. plantarum* species. *L. plantarum* bacteria are characterized by the rod-shaped, gram-positive, negative catalase, homofermentative or partially hetero-fermentative and capability of fermenting glucose. These characteristics are included in the probiotic groups such as *L. plantarum* TW4 (Setyawardani et al., 2011).

Table 2. LAB identification using API CHL 50 test

Isolates code	Identification	Similarity (%)
CT1	*Lactobacillus paracasei*	100
CT2	*Lactobacillus rhamnosus*	99,9
CT3	*Lactobacillus plantarum*	84.2
	Lactobacillus brevis1	14.3
CT4	*Lactobacillus casei*	99,9
CT5	*Lactobacillus casei*	99,9
CT6	*Lactobacillus casei*	100
CT7	*Lactobacillus casei*	99,9
CT8	*Lactobacillus brevis1*	71
	Lactobacillus plantarum	28.9

Table 3. Analysis of gen 16S rRNA

Isolates	Homologous LAB species	Query coverage (%)	Maximum Identity (%)	Access Code
CT3	*Lactobacillus plantarum* KCCM200656	100	100	MF992228.1
	Lactobacillus plantarum P1	100	100	CP023174.1
	Lactobacillus plantarum IMAU 40170	100	100	MF678771.1
	Lactobacillus plantarum TEP12	100	100	MF632300
CT8	*Lactobacillus plantarum* A223	100	100	KY55681.1
	Lactobacillus plantarum BGDP2	100	100	CP023174.1
	Lactobacillus plantarum N.8	100	100	MF583018.1
	Lactobacillus plantarum CAU8434	100	100	MF583018.1
	Lactobacillus plantarum CAU4398	100	100	MF582772.1

Table 4. Inhibitory effects on several pathogenic bacteria (mm)

Isolates code	Pathogenic Bacteria			
	B.cereus	*E.coli*	*S.aureus*	*S.typhimurium*
CT1	17.13 ± 0.18^{bcd}	19.84 ± 0.47^{c}	19.30 ± 0.42^{cd}	19.78 ± 0.02^{c}
CT2	18.23 ± 0.03^{a}	20.53 ± 0.00^{c}	19.84 ± 0.05^{ab}	19.43 ± 0.03^{d}
CT3	18.99 ± 0.00^{ab}	20.53 ± 0.03^{ab}	19.44 ± 0.33^{bcd}	20.10 ± 0.00^{ab}
CT4	18.12 ± 0.82^{a}	19.62 ± 0.59^{c}	19.49 ± 0.08^{bcd}	19.10 ± 0.14^{e}
CT5	16.53 ± 0.12^{d}	14.17 ± 0.01^{d}	19.10 ± 0.11^{d}	14.23 ± 0.03^{f}
CT6	17.51 ± 0.09^{abc}	20.10±0.14bc	19.67 ± 0.07^{abc}	19.55±0.07d
CT7	17.55 ± 0.03^{cd}	19.94 ± 0.12^{a}	17.43 ± 0.17^{c}	19.80 ± 0.07^{b}
CT8	17.41 ± 0.58^{abcd}	20.29 ± 0.01^{bc}	20.14 ± 0.05^{a}	20.25 ± 0.03^{a}

Results are expressed as mean ± standard deviation (n= 3). Mean with common superscript (a-f) in column are significantly different *(P<0.05).*

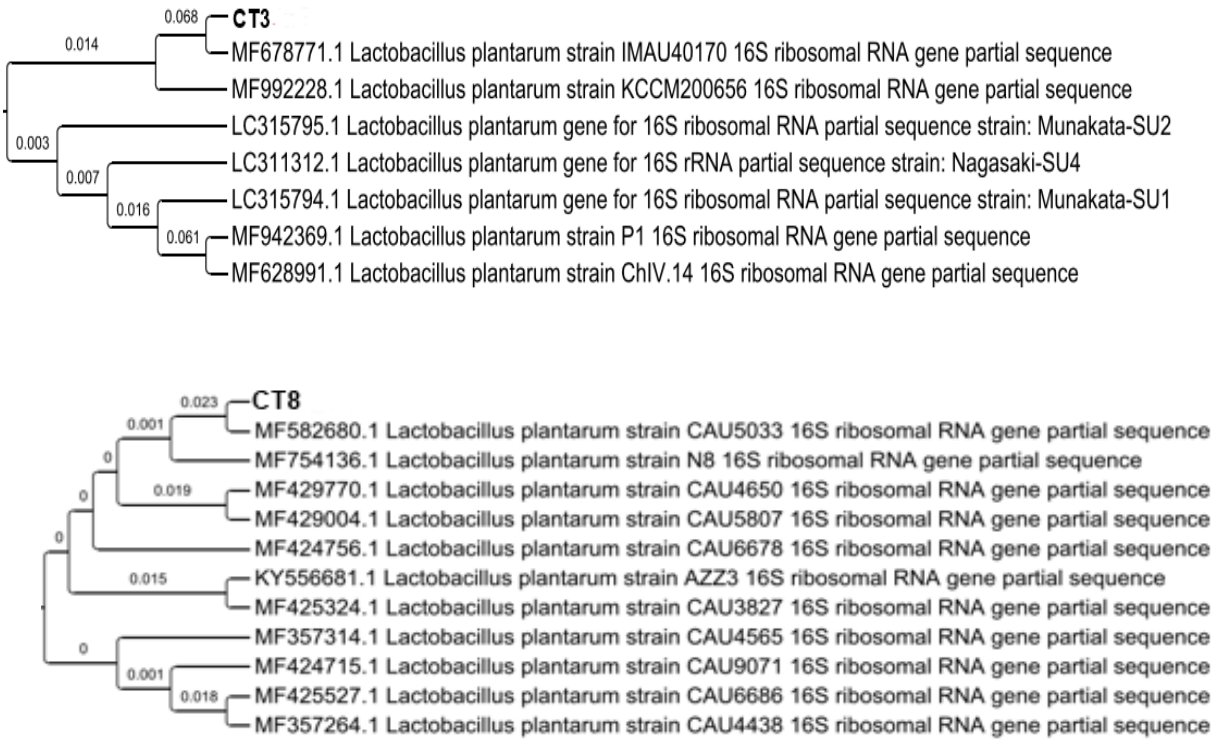

Figure 1. The phylogenic tree of *Lactobacillus plantarum* based on DNA sequence of 16S rRNA encoding using MEGA 6 program and physiological tree using Tregraph 2.

Antimicrobial Activity Isolate Origin of Goat Colostrum

The ability of LAB isolates to repel pathogenic bacteria and prevent food spoilage was discovered by measuring the antimicrobial activity using bacteria indicator. Table 4 illustrates the inhibitory activity arising from all eight isolates.

The antimicrobial activity of the source of goat colostrum is included in the genus *Lactobacillus*, with eight isolates bearing all antimicrobial properties of four pathogenic bacteria (Table 4.). All isolates have inhibitory activities against *B. cereus* bacteria, with diameter of inhibition from 16.53 to 18.99 mm. CT3 showed the highest inhibitory activity.The inhibitory diameter of *E. coli* bacteria ranged from 14.17 to 20.53 mm, with highest inhibition was obtained from CT2 and CT3 isolates. The isolate inhibition diameter of *S. aureus* was within the range of 17.43 - 20.14 mm, while *S. thypimurium* was 14.23 - 20.25 mm. The mean diameter of resistance obtained from goat colostrum isolate was considerably higher than that of goat milk i.e. *S. typhimurium* (12.6 - 19.9 mm), *E. coli* (11.3 - 21.4 mm), *B. cereus* (7.5 - 19.9 mm), and *S. aureus* (13.13 - 14.77 mm) (Setyawardani et al., 2014).

Antimicrobial activity of *Lactobacillus* is due to the presence of metabolite products such as lactic acid. This acid contributes to lower pH which repels pathogenic bacteria. Some strains that could inhibit *S. aureus* include *L. rhamnosus* TW2, TW3, and TW32; *L. plantarum* TW 4; and *L. plantarum* TW14 (Setyawardani et al., 2014).

The greatest inhibitions of the eight isolates tested were that of *E. coli* (20.53 mm); *S. aureus* (20.14 mm) and *S. typhim*urium (20.25 mm). These isolates potentially developed to a stage of producing bacteriocin as a natural preservative. The initial test of bacteriocin produced by LAB can be conducted with an antimicrobial inhibition spectrum with the use of a spot test or well diffusion assay (Khay et al., 2013).

Supplementary tests can be carried out using various growth media, resistance to pH and temperature (Zouhir et al., 2011), resistance to enzymes and detergents, and by measuring the activity of bacteriocin (Rajaram et al., 2010) .

Results and Discussion

Morphology and physiology characteristics of LAB from goat colostrum

Colostrum is a potential source of superior LAB. Earlier studies reported that goat colostrum contains proteins, vitamins, minerals, enzymes, antimicrobial peptides and immunoglobulins (Argüello et al., 2006; Górová et al., 2011). LAB are classified to be morphological as regards the forms and colonies, and physiologically based on the catalase test. LAB was isolated to attain a single colony identified with its specific properties. A total of 20 isolates of the LAB have been successfully detached from goat colostrum. Morphological characteristics of LAB isolates were identified through the Gram staining technique. Eight out of 20 isolates were selected after testing the antimicrobial activities on several pathogenic bacteria. The selected isolates were rod-shaped and included in the genus *Lactobacillus* according to Von-Wright and Axelsson (2012) .

The initial study reported that LAB from colostrum comprised of *L. casei* (27%), *L. delbrukii* (43%) and *L. fermentum* (30%) (Viswanathan et al., 2015). Two isolates of *L. rhamnosus* and five isolates of *L. plantarum* were successfully extracted from goat colostrum (Setyawardani et al., 2011). *L. paracasei*, which is usually found in the human intestinal tract, is closely related to *L. casei* and *L. plantarum*. *L. paracasei* has also been extracted from plant materials (e.g., wine, pickle, silage, and kimchi) and natural habitats such as raw and fermented dairy products (especially cheese) and plant material (e.g., wine, pickle, silage, and kimchi) (Toh et al., 2013).

All isolates from goat colostrum appear to be rod-shaped. *Lactobacillus* is a genus that prevails in warm climatic conditions (Cueto et al., 2007). LAB is characterised as Gram-positive and negative catalase (Von-Wright and Axelsson, 2012). Setyawardani et al. (2011) reported that 27 out of 33 isolates from Indonesia's local goat milk were also rod-shaped.

An identification process carried out with API CHL 50 test (Biomerieoux), followed by analysis using APIWEB^{TM} revealed that all eight isolates were proficient at fermenting sugar. Four *L. casei* isolates are capable of fermenting 26 varieties of sugar including ribose, galactose, glucose, fructose, mannose, sorbose, inositol, sorbitol, N-acetylglucosamine, amygdalin, esculin, salicin, cellubiocene, and trehalose. In contrast, *L. plantarum* isolates are capable of fermenting 22 varieties of sugar.

DNA 16S rRNA sequencing with BLAST

The two selected isolates had a 100% proximity to reference bacteria *L. plantarum* from the Genes Bank (*www. ncbi. nlm.nih.gov*). Homologous sequence ≥ 97 equal to 70% hybridisation was used to determine a group of isolates in the same species. Therefore, CT3 and CT8 isolates belong to *L. plantarum* species. A phylogenic analysis was conducted to figure out the phylogenic closeness of both isolates. Results of the 16S rRNA sequence revealed that CT3 and CT8 isolates had a phylogenic closeness to some of the reference bacteria. The former was relatively close to *L. plantarum* KCCM200656, *L. plantarum* P1, and *L. plantarum* IMAU 40170.

Reference bacteria for CT8 isolate was *L. plantarum* A223, which was considerably close to *L. plantarum* BGDP2; *L. plantarum* N.8, *L. plantarum* CAU8434, and *L. plantarum* CAU4398. The features of *L. plantarum* species include micro-aerophilic, hetero-fermentative, rod-shaped, and single or short chain form. The species is customarily recognized as safe and can be isolated from meat, fish, fruits, vegetables, milk and cereal products. *L. plantarum* is also

utilised as a starter culture in fermentation products (Todorov and Franco, 2010) and as probiotics (Setyawardani et al., 2014).

Antimicrobial activities

The preservative ability of LAB is derived from the resulting metabolites including organic acids, hydrogen peroxide, diacetyl, reuterin, antifungal peptide and bacteriocin (Stoyanova et al., 2012; Ghanbari et al., 2013). It can, therefore, be applied as a preservative agent in the food industry. Bacteriocin is non-toxic, sensitive to protease, stable in a broad range of pH and temperatures, and efficient against gram-positive bacteria. The crude extract of bacteriocin from *L. fermentum* bacteria displayed strong activity against *S. aureus*, *L. innocua, E. coli*, and *S. cholerae* (Heredia-Castro et al., 2015).

The eight isolates possess antimicrobial activity against four pathogenic bacteria where inhibitory ability against *E.coli* ranged from 14.17 to 20.53 mm. Isolates CT2 and CT3 displayed the highest inhibitory ability. This ability against *S. aureus* was within the range of 17.43 - 20.14 mm, and *S. thypimurium* was within 14.23 - 20.25 mm. The antimicrobial activity of crude bacteriocin produced by the LAB from goat colostrum is higher than that from goat's milk. LAB isolated from goat colostrum potentially produce bacteriocin due to the higher immunity and antimicrobial properties compared to that of from goat milk. The antimicrobial activity of LAB is an essential physiological characteristic to reduce or inhibit pathogenic and spoilage bacteria in foods. It can also can be utilized to prevent or minimize pathogenic bacteria in the gut.

Goat colostrum contains nutrients such as proteins, vitamins, minerals, enzymes, antimicrobial peptides and immunoglobulins (Argüello et al., 2006; Yang et al., 2009; Górová et al., 2011). LAB in goat colostrum includes genus *Lactobacillus* such as *L. casei* (27%), *L. delbrukii* (43%) and *L. fermentum* (30%). All bacteria possess antimicrobial properties to inhibit *E. coli*, *E. aerogenes, K. pneumonia, B. subtilis* and *S. aureus* (Viswanathan et al., 2015).

The tested eight isolates showed an inhibitory activity against Gram-positive and negative pathogenic bacteria. One of proposed inhibitory mechanisms is through the role of undissociated organic acids produced by LAB that will be dissociated in the bacterial cytoplasm. This will lead to a reduction in intracellular pH or an accumulation of ionized acids capable of killing pathogenic bacteria. Antimicrobial properties are also resulted from the low pH (Park et al., 2005; De Keersmaecker et al., 2006; Von-Wright and Axelsson, 2012).

Conclusions

To sum up, this study obtains eight *Lactobacillus* isolates from goat colostrum characterized with the rod-shaped, Gram-positive, negative catalase, non-spore forming, and capable of fermenting glucose. The eight isolates identified using API CHL 50 test were *L. casei* (50%), *L. brevis*/*L. plantarum* (25%), *L. rhamnosus* (12.5%), and *L. paracasei* (12.5%). Molecular identification using 16S rRNA revealed two isolates (CT3 and CT8) to be *L. plantarum*. All eight isolates had antimicrobial properties against four pathogenic bacteria namely *B. cereus*, *E.coli, S. aureus* and *S. thypimurium*, with a very strong resistance of more than 17 mm in diameter.

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