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ASIAN JOURNAL OF MICROBIOLOGY, BIOTECHNOLOGY AND ENVIRONMENTAL SCIENCES (VOL. 20, October Suppl., 2018) CONTENTS S1–S6 IDENTIFICATION OF TGFB1 RECEPTOR EXPRESSION IN ABNORMAL OVARY: THE CASE OF OVARIAN HYPOFUNCTION —NURUL ISNAINI, YUDIT OKTANELLA AND HERAWATI S7–S10 COMPARATIVE STUDY OF ACTIVITY OF GREEN TEA AND CHLOROHEXIDINE GLUCONATE MOUTHWASH ON STREPTOCOCCUS MUTANS – AN IN-VITRO STUDY —PREETHI PAVITHRA AND ASHA RAMESH S11–S15 FORTIFICATION OF MANGOSTEEN (GARCINIA MANGOSTANA) PERICARP FILTRATE IN TRIS-EGG YOLK- BASED DILUENT ON SPERM MOTILITY OF CHILLED BUCK SEMEN —Nurul Isnaini and Nuryadi, Eko Nugroho S16–S23 COLIFORM ANALYSIS FOR TRACKING BACTERIOLOGICAL CONTAMINATION PATHWAYS IN RURAL WATER SOURCES —STEPHEN T. ODONKOR AND KENNEDY K. ADDO S24–S32 ANALYSIS OF DISEASE RISK FACTORS OF EARLY CHILDHOOD CARIES (ECC) ON PRE-SCHOOL CHILDREN PSICOSOCIAL PROJECT REVIEW —HARUN ACHMAD, RASMIDAR SAMAD, HENDRASTUTI HANDAYANI, SRI RAMADHANY, MARDIANA ADAM, MARDIANA AND ANDAM DEWI SUCI S33-S42 RESEARCH OF THE IMPACT OF A VEGETABLE PROTEIN COMPOSITION ON THE FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF NATIONAL MEAT PRODUCTS —SHOLPAN AMANKELDYKYZY ABZHANOVA, ASSEL ASSANKANOVNA BULAMBAYEVA, BAGILA SHAHIMARDANOVNA DZHETPISBAEVA, MADINA

 The views expressed in various articles are those of the authors and not of the Editors of the Journal. Printed at Cambridge Printing Works, New Delhi-110 028. Phone : 9811860113 Article-21 Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (October Suppl.) : 2018 : S131-S139 © Global Science Publications ISSN-0972-3005 PROBIOTIC CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM RAW MILK (BUFFALO, COW, AND GOAT) FROM WEST SUMATERA, INDONESIA S. MELIA*, FERAWATI, YUHERMAN, JASWANDI, H. PURWANTO AND E. PURWATI Department of Animal Science, Andalas University, Padang, 25171, Indonesia (Recieved 10 March, 2018; accepted 15 May, 2018) Key words : Lactic Acid Bacteria (LAB), Probiotics, Raw milk, Bile salts, Antimicrobial activity Abstract – This study aims to evaluate the in vitro potential probiotic properties of lactic acid bacteria from raw milk. The lactic acid bacteria (LAB) used in this study were isolated from raw milk (buffalo, cow, and goat) sampled from different locations in West Sumatera, Indonesia. Isolates were Gram-stained and tested for catalase reaction. Probiotic properties of isolates were investigated. The selected strains were further characterized by tolerance to acidity pH 2, 0.3% of bile salt, and antibiotics susceptibility. Antimicrobial activity of the isolated strains against pathogenic bacteria was assessed using well diffusion method and hydrophobicity percentage was also examined. Finally, the selected

strains' potential as probiotic was identified by using 16S rRNA gene sequence analysis. The strains code BM 2.1 (buffalo milk), CM 1.1. (cow milk), and GM 1.1 (goat milk) were considered to be acid and bile tolerant and they exhibited antagonistic activity towards pathogenic bacteria. This research showed that the LAB from raw milks contained probiotic bacteria, which are capable of living in digestive tract and fighting against pathogenic bacteria. The BLAST results analysis had also identified Lactobacillus fermentum strain IMAU70167 (BM 2.1), Lactobacillus fermentum strain MTCC 25067 (CM 1.1) and Lactobacillus fermentum strain NCC2970 (GM 1.1). INTRODUCTION Nowadays, there has been an increasing trend of probiotic product consumption in developing countries. Researchers in food industries and research centres have thus given special attention on studies that identify new probiotic sources with better probiotic characteristics. Probiotics are defined as living microorganisms that produce beneficial effects for their hosts and improve balance for microflora and intestine (Tulumoglu et al., 2013). There are three types of probiotic bacteria: lactobacillus, Bifidobacterium, and Gram-positive cocci (Tulumoglu et al., 2013; Choudhary, 2015) and Lactic Acid Bacteria (LAB hence to forth) are the most commonly probiotic type of bacteria. LAB produce acid and prevent the growth of pathogenic bacteria by converting lactose into lactic acid (Simova et al., 2009). Several types of pathogen such as Staphylococcus aureus play an important role in food hygiene because they can produce toxin and cause food poisoning (Charlier et al., 2009). To treat such bacterial diseases, antibiotics have long been used. Abbreviations LAB: Lactic Acid Bacteria, BM: buffalo milk, CM: cow milk, GM: goat milk However, constant use of antibiotics can also cause microbial resistant. Probiotic bacteria have currently become an important part of human dietary because they offer a protection upon organisms through their bacteriostatic function, which is capable of defining the dominant bacterial community in the human digestive ecological system (Tulumoglu et al., 2013). In addition to being naturally produced in the alimentary canal (Colombo et al., 2010), these bacteria can also be isolated from various sources of milk and dairy products. Humans generally consume milks from cow, buffalo, and goat because of their complete nutrients. Milks squeezed from different types of animal commonly have similar nutritional substances but in different compositions. LAB are normally available in milks and posses the lactose-fermenting ability. In Elgadi et al.'sstudy (2008), two LAB (lactobacillus and streptococcus) were isolated and identified from goat, cow, and sheep milks. Lactobacillus plantarum was isolated from cow milk while Lb. Fermentum was found in goat and camel milks. Another study (Shafakatullah & Chandra, 2014) also discovered lactobacillus acidophilus, lactobacillus rhamnosus, and Bifidobacterium longum, that are probiotic potentials, which were isolated from buffalo milk in Karnataka, India. All these bacteria were resistant to acid, bile salts, and alkaline stability thereby enabling them to survive in the stomach and the alimentary canal. In the end, such survival capacity enable the LAB strains to reach small intestine and colon and create a microfloral balance in the intestine. According to Mittu and Girdhar (2015), LAB with probiotic activities (such as Lactobacillus plantarum and Lactobacillus paracasei) can be isolated from goat milk in order to produce medicinal effects, such as to prevent colon and colorectal cancers. Many studies have been done in identifying characteristics of LAB isolated from goat milk (Mittu and Girdhar, 2015; Sharma et al., 2013). These studies detected several different species of LAB, namely lactobacillus acidophilus, L.reutei, L. plantarum, L. casei, L. paracasei, L. bulgaricus, l. lcatis, Bifidobaterium bifidum, B. Longum, B.lactis, and streptococcus thermophiles. Sharma et al. (2013) had also found streptococcus thermophilus MN-ZLW-002 strain MN-ZLW-002 which was isolated from cow milk originated from Gwalior district of Madhya Pradesh, India. Probiotics discovered in such scientific studies are expected to meet three suggested criteria Sieladie et al. (2011) in order for the research to fulfil the growing market demand. First of all, the studied LAB probiotics must be able to survive in the alimentary canal. Second of all, they must persist in their host. At last, they must be proven safe for human consumption. To meet these criteria, current and future research should select probiotics with a strong resistance to antibiotics, acid tolerance, bile tolerance, antibacterial activity, antibiotic sensitivity and percentage of hydrophobicity. Accordingly, this research is intended to investigate probiotic characteristics of LAB isolated from raw milk against acid tolerance, bile tolerance, antibacterial activity, antibiotic sensitivity, and percentage of hydrophobicity. MATERIALS AND METHODS Collection of samples and bacteria strains Samples of raw buffalo, cow, and goat milks were randomly obtained from five locations in West Sumatera, Indonesia. These samples were collected in sterile bottles and kept at a low temperature (8- 10°C) during transport and prior to analysis. A total of 105 LAB isolated from raw milks and 12 isolates of LAB were further tested against acid tolerance, bile salt, and other probiotic properties. All cultures were isolated and sub-cultured in MRS broth (Merck, Germany) prior to use. The cultures were incubated at 37oC for 24 hours (anaerob condition) and purified by repeated streaking. By following Prescott et al. (2002), morphological, physiological, and biochemical tests of isolates were determined by the standard procedure of gram staining, catalase test, and gas production test. Colonies of catalase negative, Gram-positive rods, and cocci were consequently presumed to be LAB. Acid Tolerance Acid tolerance was determined with slight modifications in the methods used by Rashid and Hassanshahian (2014). The enrichment of MRS broth was used to asses pH tolerance. Cell overnight cultures were collected and inoculated respectively into 1 mL of pH 2 and pH 5.5 (control) MRS broth (Germany). The cultures were incubated at 37oC for 90 minutes. Culture turbidity was measured at 600 nm. The tests were performed three times. Resistance to Bile Salt Measuring the resistance to bile salt was conducted by following Zhang et al.'s (2016) methods, i.e. using 0.3% of bile salt concentration, but, unlike Zhang, this study applied 4 hours of incubation period. MRS broth without bile salts was used as a control medium and MRS broth containing 0.3% (w/v) bile salt was inoculated overnight with cultures of LAB. Growth in control environment (no bile) and tested cultures (0.3% bile) are observed after 4 hours at OD600. The tests were triplicated. Antibiotic Sensitivity Test By following Srinu et al.'s (2013) methods of testing antibiotic sensitivity, the antibiotic resistance of LAB was assessed using different antibiotics on MRS agar plates seeded with the tested probiotic organism. Antibiotic discs were positioned on the agar surface and incubated at 37oC for 24 hours. There were five types of antibiotics being tested, namely: amphycilin (10 μg), chloramphenicol (30 μg), erytromicin (15 μg), penicillin (10μg) and tetracycline (30 μg). The zone size (mm) of Probiotic Characterization of Lactic Acid Bacteria Isolated from Raw Milk (Buffallo, Cow) S133 interpretative chart for antibiotics was measured according to performance Standard for Antimicrobial Disk Susceptibility Tests as described by Prescott et al., 2002). All these experiments were performed three times. Antimicrobial Activities of the LAB Isolates By a slight modification in Yang et al.'s (2012) methods, cell-free supernatans (CFS) for antibacterial test were prepared by growing the LAB isolates in MRS broth at 37oC for 24 hours and centrifuged at 12,000 rpm, during 10 minutes. Antimicrobial activities from CFS of LAB isolates against the indicator organisms was determined by Yang et al.'s well diffusion method. Aliquots of CFS (50 µl) were placed in the well (6 mm diameter) cut in cooled soft nutrient agar plates previously seeded (0,2% v/v) with appropriate indicator strains. Plates were incubated in the growth condition of indicator microorganism allowing the formation of inhibition zone around the well after 24 hours. The inhibition zone diameter was measured and recorded in milimetre (mm). The indicator strains used in this study were Staphylococcus aureus ATCC 25923 and Escherechia coli O157: H7. All these experiments were triplicated. Hydrophobicity Percentage Test LAB cultures were grown in MRSB and incubated at 37oC in anaerobic jar in the incubator for 18-22 hours. LAB cells were harvested by centrifugation at 10.000 g for 5 minutes. The bacterial cells were then washed twice with PBS pH 7 and suspended in 0.2- 0.4 at 600 nm (A0). Xylene was used to determine bacterial adhesion to hydrocarbon. Then, 3 mL LAB suspension was mixed by vortexing for 60 s with 1 mL xylene. After 2 hours at 37oC, the lower aqueous phase was taken out and measured O.D. at 600 nm (A1) (Meira et al. 2012). The tests were performed three times in duplicates. Identification Using 16S rRNA 1) Genomic DNA Extraction: By following Feliatra et al.'s (2015) methods, bacterial cell was grown in MRS broth for genomic DNA extraction and purification. DNA was extracted using Presto™ Mini gDNA Bacteria kit. The 16S rRNA gene was amplified using Polymerase chain reactions (PCR) with primers 24F(52- AGAGTTTGATCCTGGCT-32) and 1541R (52- AAGGAGGTGATCCAGCCGCA- 32). PCR were performed in 20?L reaction volumes, containing1×TaqMasterMix, 0,1 mM dNTPs, 0.20 µM forward primer, 0. 20 µM reverse primer, and 1- 100 ng ofgenomic DNA. Temperature cycling conditions for PCR were as follows: aninitial heating of 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 59.5 °C for 30 s, extension at 72°C for 1 min, 30 s, and ended with a 10-min final incubation at 72°C. (2) Electrophoresis: By slightly modifying Roslim, Nisa, and Herman's [18] procedures, PCR products were then migrated at 1.2% agarose gel in 1 X TBE buffer at 65 volts for 1 hour (30 minutes longer than. The gel was soaked in 5 µg/ml ethidiumbromide solution to stain the DNA and then the DNA bands were observed under UV lamp transillumination (WiseUV WUV-M20). Sequencing was performed to determine the precise order of nucleotides within a DNA molecule.. (3) PCR Purification, Sequencing, and Data Analysis: Thepurified PCRproductsderived from isolates were sequenced by a sequencing company (First BASEL aboratories, Malaysia) using primers 24F and 1541 R. A homology search for the 16S rRNA sequences was carried out with Nucleotid Sequence Data Library using the BLAST program(http:// www. ncbi.nlm.nih.gov/). A maximum likelihood test procedure was applied to phylogenetic analysis and to investigate new LAB. The neighbor-joining tree was boot strapped 1,000 times and used from MEGA software version 6.06 package.

Distance matrices for the aligned sequences were calculated using the two-parameter method of Kimura (Tamura et al., 2007). RESULT Acid Tolerance All LAB isolates were tested for their survival rates at low pH of 2 .0 at OD 600 nm. Results are presented in Table 1. Compared to all other strains, BM 4.2 (buffalo milk), CM 2.2 (cow milk) and GM 1.1 (goat milk) demonstrated better resistance to pH 2 .0 after 90 min, and their survival rate were at 96.76%, 74.25%, and 99.75% respectively. However, as shown in Table 1, LAB isolates from buffalo milk were generally resistant to pH 2, i.e. over 65%. LAB isolates from GM were more resistant to pH 2, i.e. higher than 70%. However, for isolates from cow milk, the tolerance above 70% was only indicated by isolate CM2.2. In addition to confirming Sieladie et al.'s (2011) findings in which almost all isolates had more than 50% survival rate in pH 2, this study also discovered an exception for isolate CM 1.2 survival rate at 43.81%. Resistance to Bile Salt Acid- tolerance bacterial strains in this study were further tested for their tolerance to 0.3% bile salts at OD 600 nm. The test showed that probiotics is useful and capable of surviving in a number sufficiently enough to influence the intestine metabolism. The isolates demonstrated different degrees of tolerance to 0.3% bile salt (Table 2). After 4 hours of incubation, GM 1.1 (goat milk) were proven to be mostly resistant (70,81%) to bile salt and isolate BM 1.1 (buffalo milk), CM 1.2 (cow milk), and GM 2.1 (goat milk) were more sensitive to bile salt. Antibiotic Sensitivity All isolates of LAB were subjected to antibiotic susceptibility test using five different antibiotics (amphycilin (10 μg), chloramphenicol (30 μg), erytromicin (15 μg), penicillin (10μg) and tetracycline (30 μg)). Table 3 presented the results obtained from antibiotic susceptibility test of bacteria. From this table, 67% of LAB isolates were resistant to amphycilin. This result confirm Zhang et al.'s (2016) research findings in which 50% of the strains showed tolerance to amphycilin. Antimicrobial Activities of the LAB Isolates All isolates of LAB showed good antimicrobial activity (Table 4). All strains of LAB were able to inhibit growth of Staphylococcus aureus ATCC 25923 and Escherechia coli O157 : H7. LAB isolates used in Table 1. Survival in pH 2.0 - OD 600 value Raw Milk LAB isolates OD at 600 nm (90 minutes) Control pH 2.0 of % survival Buffalo Milk BM 1.1. BM 2.1 BM 3.2 BM 4.2 0.669 ± 0.011 0.832 ± 0.009 0.713 ± 0.000 0.648 ±0.036 0.467 ± 0.002 0.544 ± 0.010 0.464 ± 0.001 0.627 ± 0.012 69.81 65.33 65.08 96.76 Cow Milk CM 1.1 CM 1.2 CM 2.1. CM 2.2 0.647 ± 0.002 0.444 ± 0.001 0.705 ± 0.003 0.649 ± 0.009 0.421 ± 0.008 0.195 ± 0.012 0.487 ± 0.019 0.482 ± 0.005 65.12 43.81 69.06 74.25 Goat Milk GM 1.1 GM 2.1 GM 3.1 GM 4.2 0.598 ± 0.004 0.728 ± 0.027 0.836 ± 0.021 0.650 ± 0.005 0.597 ± 0.023 0.562 ± 0.010 0.59 ± 0.006 0.498 ± 0.012 99.75 77.25 70.56 76.53 Table 2. Tolerance against 0.3% bile - OD 600 value Raw Milk LAB isolates OD at 600 nm (4 hours) Control 0.3% bile of % survival Buffalo Milk BM 1.1. BM 2.1 BM 3.2 BM 4.2 1.245 ± 0.006 1.284 ± 0.010 1.425 ± 0.018 1.307 ± 0.011 0.400 ± 0.002 0.591 ± 0.040 0.699 ± 0.117 0.729 ± 0.066 32.23 46.18 49.41 56.13 Cow Milk CM 1.1 CM 1.2 CM 2.1. CM 2.2 1.136 ± 0,017 0.768 ± 0,02 1.137 ± 0,048 1.179 ± 0,009 0.474 ± 0.015 0.260 ± 0.019 0.544 ± 0.023 0.536 ± 0.011 41.96 34.62 47.85 45.34 Goat Milk GM 1.1 GM 2.1 GM 3.1 GM 4.2 1.086 ± 0,023 1.352 ± 0,024 1.214 ± 0,003 1.287 ± 0,044 0.769 ± 0.093 0.509 ± 0.004 0.693 ± 0.047 0.532 ± 0.005 70.81 37.49 57.08 42.21 Table 3. LAB showing sensitivity/resistant with different antibiotics Raw Milk BAL Isolates Antibiotics Ampicillin Chloramphenicol

Erythromycin Penicillin Tetracycline Buffalo Milk BM 2.1 BM 3.2 BM 4.2 R R I R I R R I R S R R R R S I R I Goat Milk GM 1.1 S S GM 3.1 S I GM 4.2 R R R S R R I R R R R S R R S R S I R I R R R In brackets R = resistant, S = Sensitive, I = Intermediate (Cockerill, 2010) this study had shown good antagonistic activity against different foodborne pathogens with various degrees of inhibition zone. All LAB in this study can inhibit the growth of E.coli and S.aureus. LAB isolated from milk generally have antimicrobial activity against S. aureus (with the inhibition zone diameter of 12.00 to 21.33 mm) which was higher than against E. coli (with the inhibition zone diameter 6.33 to 26.33), with the exception in isolate GM 1.1 (raw goat milk) that had the highest inhibition zone diameter against S.aureus, i.e. 26.33 mm. Table 4. Antibacterial activity of LAB against various pathogenic bacteria Raw Milk LAB Isolates S. aureus Pathogen inhibition E. coli O157:H7 Buffalo Milk BM 2.1. BM 3.2 BM 4.2 21.33 ± 0.58 21.33 ± 1.15 20.33 ± 0.58 10.66 ± 1.15 12.33 ± 0.58 14.67 ± 1.15 Cow Milk CM 1.1 CM 2.1. CM 2.2 19.00 ± 0.00 12.00 ± 0.02 20.67 ± 1.15 12.33 ± 0.58 12.50 ± 0.01 10.33 ± 0.57 Goat Milk GM 1.1 GM 3.1 GM 4.2 18.67 ± 1.15 18.33 ± 0.58 15.00 ± 0.25 26.33 ± 1.53 6.33 ± 0.58 12.00 ± 0.58 Hydrophobicity Percentage ofLAB Isolates Table 5 showed that isolates GM 1.1 (goat milk) had high hydrophobicity (65.31%), followed by isolate CM 1.1 (cow milk) with hydrophobicity scored at 37.62%, which categorized it into medium hydrophobicity. Consequently, these two types of isolates can be promoted as the probiotic bacteria candidates. Hidrophobicity of isolate GM 1.1 was higher as compared to Al Atya et al.'s findings (2015). In this study, the hydrophobicity percentage of Enterococcus faecalis ranged between 34 to 47%. Nevertheless, this level was lower when compared to Meira et al.'s findings (2012). Meira et al. isolated LAB from Brazilian regional ovine cheese and indicated hydrophobicity level for Lactobacillus brevis as high as 88% while the hydrophobicity of Lb. Casei SM-G was the lowest, i.e. 15.2%. Table 5. Hydrophobicity percentage of LAB isolates Raw Milk LAB Isolates Hydrophobicity (%) Buffalo Milk BM 2.1 BM 3.2 BM 4.2 34.57 24.81 21.00 Cow Milk CM 1.1 CM 2.1. CM 2.2 37.62 5.50 16.27 Goat Milk GM 1.1 GM 3.1 GM 4.2 65.31 20.00 20.93 Identification Using 16S rRNA In this study's selection of probiotics, three types of LAB were evidently potential as the next probiotics, namely isolates BM 2.1 (buffalo milk), CM 1.1 (cow milk), and GM 1.1. (goat milk). To ensure a more accurate result, molecular identification was conducted using 16S rRNA method. The 16SrRNA genes from extracted DNA of bacterial colonies were amplified with polymerase chain reaction using lactobacilli genus primers (24F and 1541R). The BLAST results analysis had then revealed Lactobacillus fermentum strain IMAU70167 (BM 2.1), Lactobacillus fermentum strain MTCC 25067 (CM 1.1), and Lactobacillus fermentum strain NCC2970 (GM 1.1). Reference Sequences Used in Phylogenetic Analysis Based on the likelihood test procedure, the result of phylogenetic analysis can be seen from Table 6. The following bacterial 16S rRNA gene sequences were tested as out-groups in phylogenetic analysis: a c c e s s i o n n u m b e r G Q 1 3 1 2 8 2 . 1 (L a c t o b a c i l l u s fermentum strain IMAU70167) for BM 2.1, AP017973.1 (Lactobacillus fermentum strain MTCC 25067) for CM 1.1, and CP017151.1 (Lactobacillus fermentum strain NCC2970) for GM 1.1 (Table 6). The phylogenetic trees of lactobacillus spp based on 16S rRNA gene sequences from raw milk (BM 2.1, CM 1.1. and GM 1,1,) can be seen respectively in Figure 1, 2, and

3. The trees were constructed with the neighbour-joining method, which were analysed by means of MEGA software version 6.06. Genetic distances were calculated using the Kimura's two- parameter model. Fig. 1. Phylogenetic tree of Lactobacillus spp based on 16S rRNA gene sequences from buffalo milk (BM 2. Fig. 2. Phylogenetic tree of Lactobacillus spp based on 16S rRNA gene sequences from cow milk (CM 1.1) Fig. 3. Phylogenetic tree of Lactobacillus spp based on 16S rRNA gene sequences from goat milk (GM 1.1) Table 6. Bacterialphylotypesoriginatingfrom raw milk Sample Code Description Query cover Identity Accession BM 2.1 Lactobacillus fermentum strain IMAU70167 16S ribosomal 100% 99% GQ131282.1 RNA gene, partial sequence CM 1.1. Lactobacillus fermentum DNA, complete genome, strain: 100% 100% AP017973.1 MTCC 25067 GM 1.1 Lactobacillus fermentum strain NCC2970, complete genome 100% 100% CP017151.1 DISCUSSION Acid Tolerance In order to have probiotic effects in intestinal tract, LAB must have the ability to survive to go through gastrointestinal tract (GIT). Therefore, probiotic bacteria's resistance to gastric acid environment is prerequisite for survival and function in intestinal tract. Depending on the specific individual's diet, the pH of human gastric environment varies from 1.5 to 3.0 and LAB still survived in these ph levels (Solieri et al., 2014). While Sieladie et al. (2011) found out that lactobacillus plantarum isolated from cow milk in Western Highlands of Cameroon had the potential to become probiotics with antimicrobial and lowering-cholesterol properties, Bassyouni et al. (2012) discovered LAB strains (Lactobacillus acidophillus, L.casei and L. lactis) isolated from dairy products in Egypt, which have probiotic potentials for their resistance to low pH. The more recent study (Zhang et al., 2016) also found that LAB strain Lactobacilli, isolated from traditional Tibetan sugar, a raw yak milk, has the potential as probiotics for its resistance to pH 2.0 and 3.0. Resistance to Bile Salt Since bile salts or oxgall (i.e. a natural dried bovine bile component containing both conjugated and unconjugated bile salts) are surface-active chemicals which are produced in the liver by the catabolism of cholesterol, they form bile acid that consists of chenodeoxycholic acid, cholic acid, deoxycholic acid, and other minor components secreted from spleen into the duodenum of small intestine (Corzo & Gililand, 1999). Even though concentration of cholic acid in intestinal tracts varies from 0.03% to 0. 3%, cholic acid can affect and cause leakage to the cell membrane structure. Therefore, LAB strains' ability to be resistant against bile and cholic acids influence becomes the most important criteria for determining their probiotic potential. Antibiotic Sensitivity Sensitivity to antibiotics is the most important factor in safety evaluation of probiotics. Antibiotics resistance is a potential risk of probiotic application. While this study showed that 33% of LAB isolates were resistant to chloramphenicol, de Almeida Junior et al.' s research (2015) proved that 96% of isolates were sensitive to chloramphenicol. There were only two isolates (CM 2.2 and GM 4.2) that were resistant to all antibiotics tests. Almost all isolates were resistant to erythromysin and there were only two isolates (BM 2.1 and CM 2.2) that showed intermediate sensitivity. LAB strains exhibited resistance to different antibiotics discs probably due to their natural and intrinsic resistance. Additionally, it might be also due to the cell wall structure and membrane impermeability of LAB strains (Sieladie et al., 2011). Srinu et al. (2013) indicated in their study that lactic acid species were resistant

to all the antibiotics tested (Ampicillin, Nalidixic acid, Ciprofloxacin, Co-Trimoxazole, Gentamicin, and Cefpodoxime). In relation to this, de Almeida Júnior et al. (2015) argued that since antibiotic penicillin had been widely used in clinical practices over a long period of time, tolerance for this antibiotics is a widespread problem. This very study showed there were only 30% of LAB isolates that were sensitive to penicillin, much higher as compared to Zhang et al.'s (2016) findings. LAB isolates originated from goat milk were more resistant to tetracycline as compared to isolates from buffalo and cow milks. Antimicrobial Activities of the LAB Isolates Antimicrobial activity is one of the most crucial properties of a functional probiotics. The antimicrobial spectrum of nine isolates of LAB from raw milk against some pathogenic bacteria were demonstrated by agar well diffusion method. Ramasamy et al.'s (2012) study showed that LAB isolated from Malaysian food and milk product also indicated antimicrobial activity against E .coli and S .aureus. LAB had the ability to inhibit E.coli growth by producing an inhibition zone diameter between 8.8 to 12.5 mm (well size included) and Lactobacillus sp showed inhibiting activity against S.aureus, which was indicated by the creation of inhibition zone diameter between 6.2 to 13.2 mm. Also, Bassyouni et al. (2012) described that LAB isolates can effectively inhibit the growth of E.coli and S.aureus. Unlike Akalu et al's findings (2017), LAB isolated from fermented Shamita and Kocho from Arat-Killo and Markato sites in Addis Ababa were proven unable to inhibit the growth of S.aureus but capable of inhibiting the growth of E. coli (inhibition zone 8.5 to 17.5 mm). Vasiee et al.'s (2014) argued that, by using S.aureus and E.coli as the indicator bacteria, LAB might cause gastroenteritis because these two bacteria are occasionally found in foodborne microorganisms. Vasiee et al.'s research showed that LAB isolated from tarkhineh (traditional fermented food produced from a mixture of spontaneously fermented butter milk and wheat flour in Iran), namely Lactobacillus plantarum and Lactobacillus fermentum, were potential to become probiotics because they could inhibit the growth of S.aureus and E.coli. Organic acid and hydrogen peroxide produced by lactobacilli were reported to have been able to inhibit the growth of gram- positive and gram -negative bacteria while bacteriocin was highly influential on gram-positive bacteria. According to Srinu et al. (2013), Lactobacillus delbrueckii sub spp. bulgaricus 281, Lactobacillus casei 297 and Lactobacillus fermentum 141 inhibited the growth of all the pathogenic bacteria and they could also prevent the growth of E.coli ATCC (American type culture collection centre), Pseudomonas aeruginosa, Salmonella paratyphi, Staphylococcus aureus. There were also slight differences in antagonistic activity of LAB's on tested pathogenic organisms due to the production of antimicrobial compounds to a varying degree. The increase in the production of lactic acid with time has been attributed to lowered pH which permits the growth of LAB. The antimicrobial effect of lactic acid is due to undissociated form of acid, which penetrates the membrane and liberates hydrogenion in the neutral cytoplasm thus leading to inhibition of vital cell functions. LAB strains used in this study had exhibited good antibacterial activity against the food bornepathogens tested. Hydrophobicity Percentage of LAB Isolates Sánchez-Ortiz(2015) suggested that xylene was used because bacterial adhesion to this solven reflected the hydrophobic or hydrophilic nature of the cell surface. Values under 30% (<30%) were considered as "Low" and values between 30 and 60% (?30%, <60%) were referred to as "medium". Mean while, uppervalues60%(e"60%) were considered as "high". Strainswithlow adhesiontop- xylene (<30%) were discarded from the list of potentialprobiotics. According to Ramasamy et al. (2012), colonisation of probiotic strains in gastrointestinal tract will prevent their immediate elimination by peristalsis and provide a competitive advantage over pathogens. Schilinger et al. (2005) then added that adherence to intestinal mucous is among the in vitro test that is habitually proposed in order to assess the probiotic possibility of a bacterial strain. Attachment of probiotic strains to the epithelial cells and intestinal mucosal is prerequisite for the intestine colonization as it influences the time of bacteria reaction in the intestines and the functional activity of bacteria. CONCLUSION This study has concluded that there were several LAB isolated from raw milk (buffalo, cow, and goat milks) that have strong potentials to become probiotics. These strains were capable of growing and surviving in the pH 2 and 0,3% bile salt condition. They could also inhibit the growth of Staphylococcus aureus ATCC 25923 and Escherechia coli O157 : H7, andresistant to several types of antibiotics with the hydrophobicity percentage between 30%-65% (medium hydrophobicity). The selected strains' potential as probiotics was then identified by using 16S rRNA gene sequence analysis. The strains code BM 2.1 (buffalo milk), CM 1.1. (cow milk), and GM 1.1 (goat milk) were considered as probiotic bacteria. The BLAST results analysis had finally revealed those Lactobacillus fermentum strain IMAU70167 (BM 2.1) from buffalo milk, Lactobacillus fermentum strain MTCC 25067 (CM 1.1) from cow milk, and Lactobacillus fermentum strain NCC2970 (GM 1.1) from goat milk. ACKNOWLEDGMENT The financial support from Direktorat Riset dan Pengabdian Masyarakat Direktorat Jenderal Penguatan Riset dan Pengembangan, Kementrian Riset, Teknologi, dan Pendidikan Tinggi (Contract No: 059/SP2H/LT/ DRPM/IV/2017) was greatly appreciated. A special appreciation is also given to the Rector of Universitas Andalas, Chairman of LPPM, Dean of the Faculty of Animal Science, Head of Laboratory Animal Product Technology, and Head of laboratory of Animal Biotechnology. REFERENCES Akalu, N., Assefa, F. and Dessalegn, A. 2017. In vitro evaluation of lactic acid bacteria isolated from traditional fermented Shamita and Kocho for their desirable characteristics as probiotics. Afr J Biotechnol. 16 : 594-606. 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