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Isolation and Identification of Lactic Acid Bacteria from Different Fruits with Proteolytic Activity

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Abstract: A total of eight from thirty LAB isolated from fruits samples showed clear zone on modified de Man, Rogosa and Sharp (MRS)-$CaCO_3$ agar, catalase negative and Gram positive were considered as LAB. From six out of eight isolates was observed good proteolytic activity when tested on skim milk agar. The isolates were identified with species of morphology and biochemical analysis using API 50 CHL kit assay and genotype identification. It can be concluded that isolated LAB from different fruits sources have probiotic properties and proteolytic properties which could benefit consumers.

Keywords: Lactic Acid Bacteria, Proteolytic Activity, Probiotic Properties, Antimicrobial Activity

1. Introduction

Lactic acid bacteria (LAB) is a gram-positive, nonsporing, catalase-negative, devoid of cytochromes, of nonaerobic conditions but are aerotolerant, fastidious, acid-tolerant and strictly fermentation (Yelnetyy et al. 2014). This groups of bacteria is nonpathogenic and save to use with the status of General Recognize as Safe (GRAS), acid resistant, bile tolerant and produce antimicrobial substances, including organic acids and hydrogen peroxide and bacteriocins (biologically active protein) (Aween et al. 2012). Generally LAB widely distributed in nature and found as indigenous microflora in raw milk and fermented milk with spontaneous fermentation. LAB are used in the production of foods prepared by lactic fermentation such as dairy products, fermented vegetables, fermented meats, and sourdough bread (Moulay et al. 2013). The most important genera are *Lactobacillus*, *Lactococcus*, *Enterococcoccus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. *Bifidobacterium* shares certain physiological and biochemical properties with LAB and some common ecological niches such as the gastrointestinal tract.

LABs have a wide range of antimicrobial activities, among these activities, the production of lactic acid and acetic acid is obviously the most important. On the other hand, certain strains of LAB are known to produce bioactive molecules like ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity (Aween et al. 2012).

In general LAB used in fermentation of milk products are proteolytic of milk due to the instability of milk proteins. proteolytic system of LAB is important for growth of microorganisms and in nattive free amino acid of milk. Proteolytic system involved in casine utilization within LAB cell and give contribution to development of organoleptic properties of fermented milk products (Yamina et al. 2013).

The identification of lactic acid bacteria was established many years ago because of the need to determine the strains that can be used in the industry to characterize the properties and the marketing value of the strain, and above of that is to confirm the safety of the strain to be used in the food application or even in pharmaceutical application. Historically, the identification of LAB was by using phenotypic and chemicals methods. These methods are based on the activity of the LAB and the different carbohydrate fermentation, hetero or homo fermentation, gas production, motility and spore producing (Ashmaig et al. 2009).

Recently, the molecular biology has developed very fast and that had high impact on the microbiology world. Using

the gene sequencing is the most reliable method of identifying the bacteria. 16S rDNA is one of these methods and it has been used for many cases for the identification of the bacteria especially LAB. That shows the use of the 16S rDNA is good for almost lactic acid bacteria species. The molecular identification is based on the similarity with other sequences within the data base. Although it is very useful and simple method for the identification of genus and species of bacteria, it does not allow differentiation of subspecies (Aween et al. 2012). Therefore, the aim of this study was to determine the proteolytic activity of LABs isolates, to identify the LAB isolated from different fruits by API CHL50 and 16sRNA.

2. Materials and Methods

2.1. Fruits Samples

A total of three fruits samples (Banana, Grape and Apple) were collected from different sources of supermarket. Samples were kept at room temperature before analysis.

2.2. Isolation of Lactic Acid Bacteria (LAB)

Approximately 10 gram of sample was added to 90 ml of sterile peptone water (0.1% w/v) and homogenized in the stomacher (Stomacher® 400 Circular Seward). Appropriate dilutions were spread plated on de Man, Rogosa and Sharpe (MRS) agar (Oxoid CM0361) plates containing 0.8% calcium carbonate. Plates were incubated anaerobically in anaerobic jar with Anero*Gen* ™ (Oxoid) at 37°C for 48 h. Each of the isolates was tested for catalase activity by placing a drop of 4% hydrogen peroxide solution on the cells. Immediate formation of bubbles indicated the presence of catalase in the cells. Only those isolates which was catalase-negative was Gram-stained and the morphology was observed using Nikon microscope (Nikon Eclipse 80i) and streaked on MRS agar to obtain pure isolates. All bacterial strains used in this study were maintained in 15% glycerol stock and stored at -20°C. Prior to beginning the experiments, each bacterial strain was sub-cultured at least three times (1%, v/v) in MRS broth (Oxoid CM0359) at 37°C under anaerobic condition at 24 h intervals (Kheadr 2006).

2.3. Detection of Protein Hydrolysis

2.3.1. Preparation of Skim Milk Agar and Cultures

Skim milk agar was made as follows: 25 g of skim milk (Oxoid LP0031) was reconstituted with 250 ml of distilled water. The mixture was stirred thoroughly and autoclaved at 110°C for 10 min. Likewise, 500 ml of 2.5% agar solution was sterilized at 121°C for 15 min. For plating, skim milk and agar solutions were held in a water bath at 50°C and then the skim milk was poured into the agar bottle and mixed thoroughly. The skim milk agar was poured quickly into plates (Pailin et al. 2001).

2.3.2. Measurements of Proteolytic Activity (PA)

To detect protein hydrolysis, the selected LAB were inoculated on skim milk agar plates and were incubated at 37°C for 48 h in an anaerobic jar followed by cooling in a refrigerator (4°C) for 3 d. Protein hydrolysis was observed by the production of clear halos surrounding isolated colonies. Duplicate trials were conducted and all results were averaged and reported as diameter in mm. LAB that show good proteolytic activity (PA ≥ 6 mm) were used for future studies (Pailin et al. 2001).

2.4. Probiotic Properties of LAB Isolates

For the determination of probiotic properties of LAB isolates these major selection criteria were resistance to low pH, tolerance against bile salt and the antibacterial activity.

2.4.1. Bile Tolerance

LAB isolates were inoculated into MRS broth and MRS broth containing 0.3% of bile (Sigma), incubated at 37°C (Gilliland 1990). Growth of LAB was monitored hourly for 4 h by measuring absorbance at 560 nm using spectrophotometer (BioTek, USA) and spread plated on MRS agar incubated at 37°C for 24 h, anaerobically. Each test was carried out in triplicate.

2.4.2. Tolerance to Acidic pH Values

LAB isolates were grown in MRS broth at 37°C overnight, then sub-cultured into fresh MRS broth and incubated for another 24 h. The cultures were centrifuged at 5000 rpm for 10 min at 4°C (Eppendorf, centrifuge 5804 R). The pellets were washed in sterile phosphate-buffer saline (PBS) pH 7.2 and re-suspended in PBS. PBS was modified to pH 2, 3, and 4 with 1 M HCl. Each LAB isolates were inoculated into the pH adjusted PBS at ratio 1:100 (µl). Growth of LAB was monitored hourly for 4 h by measuring absorbance at 560 nm using spectrophotometer (BioTek, USA) and spread plated on MRS agar incubated at 37°C for 24 h, anaerobically (Gilliland 1990). Each test was carried out in triplicate.

2.4.3. Antibacterial Activity of LAB Isolates Against Target Bacteria by Dual Agar Overlay Method

Antibacterial activity of LAB isolates was determined against target bacteria (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579 using dual agar overlay method as described by Aween et al. (2012). The LAB1 and LAB2 were inoculated in spot on MRS agar plates and grown at 37°C for 24 h in anaerobic jars. The plates were overlaid with 15 ml of nutrient agar containing the target bacteria 10^6 cells per ml. After 24 h of aerobic incubation at 30°C the diameter of inhibition zone was measured. Accordingly; isolates, which gave an inhibition zone bigger than 1 mm, were determined to have antimicrobial activity. Each test was carried out in triplicate.

2.5. Identification of LAB Isolates

2.5.1. Phenotypic Identification of LAB Isolates Using API 50 CHL Kit Assay

Two isolates were identified by API 50 CHL (API system,

BioMérieux, France) assay. Purified LAB were cultivated in 20 ml MRS broth incubated at 30°C over night, after which the culture was washed and resuspended into API®50 CHL medium (Bio- Merieux®SA 69280, France) The turbidity of the suspension was determined by the McFarland method according to the instructions provided by the manufacturer. Cell suspension was applied into API 50 CHL strip wells and paraffin oil was pipetted to each well to create anaerobic condition. The strips were incubated at 30°C. The results were read after 24h and verified after 48 h. Fermentation of carbohydrates in the carbohydrate medium was indicated by a yellow colour except for esculine (dark brown). Colour reactions were score against a chart provided by the manufacture (Conter. et al. 2005). The results were analyzed with API WEB (Bio-Merieux).

2.5.2. Genotypic Identification of LAB Isolates Using 16s rDNA and Two Specific Primers

Genomic identification of the two strains of LAB was determined following the method described by Jarvis and Hoffman (2004).

i. DNA extraction of LAB isolates

Total genomic DNA was extracted from an overnight culture in 20 ml MRS broth at 30°C using Master Pure™ Gram positive DNA Purification Kit (USA). One ml of overnight culture was centrifuged 11500 rpm for 10 min at 25°C (Eppendorf centrifuge 5804 R) and the pallet was collected. To the pallet 150 µl of TE buffer was added and incubated at 37°C overnight. 1 µl of proteinase K (50 µg/µl, Sigma) was mixed to 150 µl of gram positive lysis solution and then added to TE buffered mixture and mixed thoroughly. The sample was incubated at 65-70°C for 15 min and vortexed every 5 min, followed by placing in ice for 5 min. 175 µl of MPC protein precipitation reagent were added to each sample, vortexed and centrifuged at 13000 rpm for 10 min at 4°C (Eppendorf centrifuge 5804 R). The supernatants were transferred to new tubes and the pellets were discarded. 1 µl of RNase II (5 µg/µl) was added to each sample and mixed thoroughly. The samples were incubated at 37°C for 30 min; 500 µl of isopropanol was added to the supernatant, centrifuged at 4°C for 10 min at 13000 rpm (Eppendorf centrifuge 5804 R). Isopropanol was removed using an eppendorf pipette without dislodging the DNA pellet. The pellets were rinsed with 200 µl ethanol 70% and centrifuged at 5000 rpm for 2 min at room temperature. The ethanol was removed carefully and the DNA was resuspended with 35 µl of deionized water and kept at -20°C for further study.

ii. Amplification of DNA in polymerase chain reaction (PCR)

Purified DNA of each sample was processed to the PCR using Fail Safe™ Pre Mix Kit Epicentre® (an Illumina® company). The genomics DNA were amplified using 16S rRNA gene universal bacterial primer for all. The universal primer was used as external standard in PCR reaction. A set two specific primers (Lbp11 and LMM) for *L. plantarum* and *L. mesenteroides*, respectively were also used to monitor all the incidence of *L. plantarum* and *L. mesenteroides*. All primers sets were purchased and commercially synthesized from 1st BASE, Selangor, Malaysia. The sequence of the primers used in this study is shown in Table 1. The reaction mixture (23.25 µl) consisted of (10 µl of deionized water, 12.5 µl of Fail Safe™ PCR 2xpreMixA, 0.5 µl of each primer, 0.25 µl of Fail Safe™ PCR enzyme Mix and 1 µl of DNA extraction). All the reaction mixtures were amplified in a thermocycler PCR system (Eppendorf, Hamburg, Germany).

Table 1. *Primers Sequences Used in This study for Amplification of DNA.*

Target Organisms	Targeted gene	Primer sequence	Size (bp)	References
All bacteria	16S rRNA gene	forward: (5'AGAGTTTGATCCTGGCTC-3') reverse: (5'-CGGGAACGTATTCAC-CG-3')	450	Magnusson *et al.,* (2003)
L. plantarum 1	Lbp11 gene	forward: (5'AATTGAGGCA GCTGGCCA3') Reverse: (5'GATTACGGGAGTCCAAGC3')	319	Massi *et al.,* (2004)
L. mesenteroides	LMM gene	forward: (5'CCGTTACCCCTAAATTTTGAC3') Reverse: (5'GACCAAATACAATAGGTTGCG3')	1, 15	Moschetti *et al.,* (2000)

iii. Gel electrophoresis

The PCR products amplification from universal and specific bacterial primer were analyzed for expected size. Two µl of each amplification mixture were subjected to electrophoresis in 1.5% (w/v) agarose gels in 0.5 x TEA buffer for 45 min and 110 V. DNA molecular mass marker (250 to 10000 bp) molecular ladders from 1st Base, Malaysia was used as standard. After electrophoresis the gels were stained in ethidium bromide and after washing the gels were visualized and photographed with UV transilluminator (Bio-Rad Laboratories, Segrate, Italy). The partial 16S rDNA, Lbp11 and LMM primers sequences were determined by 1st Base, Malaysia and sequences were compared with databases (Gen- Bank).

2.6. Statistical Analysis

The results were presented as mean ± standard deviations of triplicate determinations and were statistically analyzed by two-way analysis of variance (ANOVA) using (Minitab, Inc.) version 15 (Germany), $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Isolation of LAB

A total of eight from thirty LAB isolated from fruits showed clear zone on modified MRS-$CaCO_3$ agar, catalase

negative and Gram positive and were considered as LAB (Figure 1 and Table 2).

Table 2. *Phenotypic Characteristics of LAB Isolated from Fruits.*

Sample	LAB Codes	Catalase reaction	Gram reaction	Cell morphology
Banana	Bn1	-	+	Rod
(Bn)	Bn2	-	+	Rod
	Gr1	-	+	Short rod
Grape	Gr2	-	+	Short rod, cluster
(Gr)	Gr3	-	+	Short rod
	Gr4	-	+	Short rod
	A1	-	+	Short rod, cluster
Apple (A)	A2	-	+	Short rod, cluster

(+) positive, (-) negative reactions

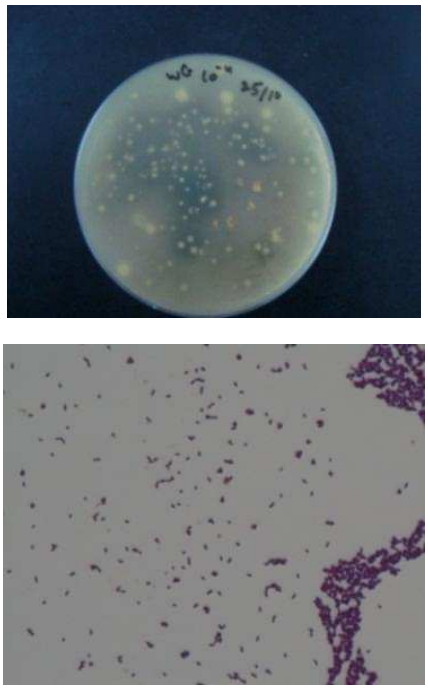

Figure 1. *LAB isolates producing clear zone on modified MRS-$CaCO_3$ agar and Gram positive of LAB.*

3.2. Proteolytic Activity (PA) of LAB Isolates

Good proteolytic activity was observed from six out of eight isolates when tested on skim milk agar. The clear halos surrounding the colonies were greater than 6 mm (Table 3) indicative of good proteolytic activity.

Table 3. *Proteolytic activity of LAB isolated on skim milk agar [a].*

Code of LABs	Diameter of clear zone (mm)
Control milk	0
Gr1	3 ± 0.21
Gr2	10 ± 0.11
Gr3	6 ± 0.09
Gr4	8 ± 0.67
Bn1	7 ± 0.42
Bn2	9 ± 0.33
A1	7 ± 0.08
A2	2 ± 0.22

[a]Results are mean values of triplicate determinations ± sd.

3.3. Probiotic Properties of LAB Isolates

The probiotic potential of the LAB isolates was determined by growing the bacteria in MRS broth with 0.3% bile, pH 2.0 to 4.0 and antibacterial activity. All isolates grew in 0.3% bile after 4 h incubation at 37°C; similar growth pattern was also observed in the absence of bile (Table 4). However, significant increase ($p \leq 0.5$) LAB-Gr2 and LAB-Bn2 was observed in MRS with 3% bile better than growth without bile (Table 6). The PH-stressed LAB-Gr2 and LAB-Bn2 cells were able to tolerate PH 2.0 to 4.0 but grow better between pH 3 and 4 (Table 5 and 6). It was observed that LAB-Gr2 able to tolerate low pH stressed than LAB-Bn2. The both LAB isolates showed to inhibit the test organisms included in this study though they vary in zone of inhibition diameter (Figure 2). The results showed that the highest inhibitory activity of isolate LAB-Gr2 was demonstrated against *S. typhimurium* (9.7 mm) and lowest zone of inhibition was (3.3 mm) against *B. cereu*. The highest diameter of inhibition zone of isolate LAB-Bn2 the was showed against *S. typhimurium* (7.1 mm) and lowest zone (4.2 mm) against *B. cereus* after 24 hour incubation.

Table 4. *Growth of LAB Strains in MRS Broth with 0.3% of Bile Incubated at 37°C[a].*

LAB isolates	Media	Time (h)			
		1	2	3	4
LAB-Gr2	With bile	0.119±0.12	0.122±0.25	0.122±0.11	0.123±0.33
	Without bile	0.119±0.17	0.122±0.10	0.122±0.37	0.123±0.26
LAB-Bn2	With bile	0.142±0.32	0.142±0.76	0.143±0.15	0.143±0.68
	Without bile	0.141±0.40	0.142±0.12	0.143±0.41	0.143±0.13

[a] Growth was monitored at OD $_{560}$ nm.

Table 5. *Survival of pH-stressed LAB-Gr2 in MRS Incubated at 37°C [abc].*

pH-stressed time (h)	pH		
	2.0	3.0	4.0
1	0.3251±0.65	0.5238±0.22	0.9382±0.21
2	0.3290±0.32	0.5231±0.16	0.9330±0.39
3	0.3320±0.12	0.5205±0.81	0.9278±0.11
4	0.3328±0.17	0.5202±0.10	0.9253±0.96

[a] Growth LAB was monitored at OD $_{560}$ nm after 24 h incubation at 37°C
[b] pH of MRS broth was adjusted with 1 M HCl.
[c] Initial concentration of LAB cell was 0.300 measured at OD $_{560}$ nm.

Table 6. *Survival of pH-stressed LAB-Bn2 in MRS Incubated at 37°C [abc].*

pH -stressed time (h)	pH		
	2.0	3.0	4.0
1	0.2200±0.43	0.3240±0.33	0.4320±0.76
2	0.2250±0.56	0.3210±0.49	0.4290±0.10
3	0.2290±0.21	0.3150±0.18	0.4251±0.54
4	0.2298±0.12	0.3120±0.98	0.4224±0.67

[a] Growth LAB was monitored at OD $_{560}$ nm after 24 h incubation at 37°C.
[b] pH of MRS broth was adjusted with 1 M HCl.
[c] Initial concentration of LAB cell was 0.210 measured at OD $_{560}$ nm.

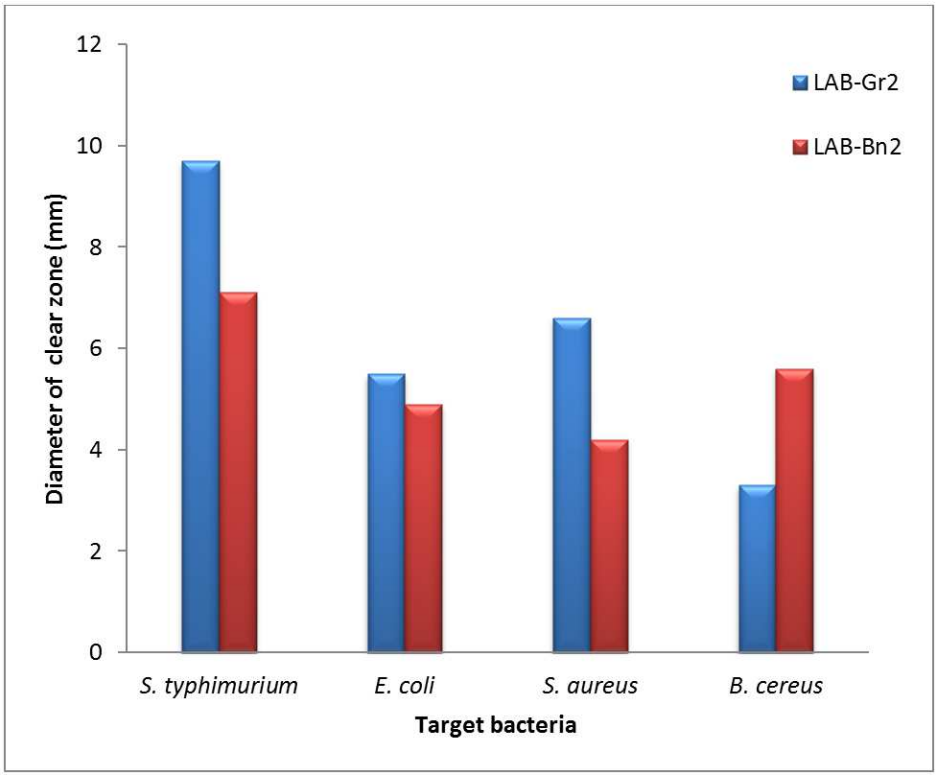

Figure 2. *Growth inhibition zone of LAB isolates against pathogenic bacteria by dual agar overlay method after 24 h incubated at 30°C.*

3.4. Identification of LAB Isolates

3.4.1. Phenotypic Identification of LAB Isolates Using API 50 CHL Kit Assay

Results from API 50 CH test kits and API web identified the two LAB isolates (LAB-Gr2) from grape as *L. plantarum*1 with similarity 99.9 % while (LAB-Bn2) from banana as *L. pentosus* 82.0% (Table 8). There was variation in the utilization of carbohydrates sources of the API CHL 50 systems by LAB-Gr2 and LAB-Bn2 isolates (Table 7).

Table 7. *Carbohydrates fermentation by lactic acid bacteria isolates using API 50 CHL kit*[a].

Carbon source	LAB	
	Gr2	Bn2
Control	-	-
(1) Glycerol	-	-
(2) Erythritol	-	-
(3) D-Arabinose	-	-
(4) L-Arabinose	+	+
(5) Ribose	+	+
(6) D-Xylose	+	+
(7) L-Xylose	-	-
(8) Adonitol	-	-
(9) β-Methyl-xyloside	-	-
(10) Galactose	+	+
(11) D-Glucose	+	+
(12) D-Fructose	+	+
(13) D-Mannose	+	+
(14) L-Sorbose	-	-
(15) Rhamnose	-	+
(16) Dulcitol	+	-
(17) Inositol	-	-
(18) Mannitol	+	+
(19) Sorbitol	+	+
(20)α-Methyl-D- mannoside	+	-
(21) α-Methyl-D-Glucoside	-	+
(22) *N*-Acetyl glucosamine	+	+
(23) Amygdaline	+	+
(24) Arbutine	+	+
(25) Esculine	+	+
(26) Salicine	+	+
(27) Cellobiose	+	+
(28) Maltose	+	+
(29) Lactose	+	+
(30) Melibiose	+	+
(31) Saccharose	+	+
(32) Trehalose	+	+
(33) Inulin	-	-
(34) Melezitose	+	+
(35) D-Raffinose	+	+
(36) Amidon	-	-
(37) Glycogène	-	-
(38) Xylitol	-	-
(39) β-Gentiobiose	+	+
(40) D-Turanose	+	+
(41) D-Lyxose	-	-
(42) D-Tagatose	+	+
(43) D-Fucose	-	-
(44) L-Fucose	-	-
(45) D-Arabitol	-	-
(46) L-Arabitol	-	-
(47) Gluconate	+	+
(48) 2 Ceto-gluconate	-	-
(49) 5 Ceto-gluconate	-	-

[a] (+) fermented, (-) not fermented

Table 8. *Phenotypic and Genotypic Identification of LAB Isolates.*

Code of LAB	Source	Identification	Similarity Index %
LAB-Gr2	Grapes	*L. plantarum* 1	99.9
LAB-Bn2	Banana	*L. pentosus*	82.0

3.4.2. Genotypic Identification of LAB Isolates Using 16s rDNA and Two Specific Primers

The genotype identification of DNA using universal primer and two specific primers (Lbp11 and LMM) showed clear bands of isolates (Figure 3 and 4) with approximate molecular weight 1500 bp and similarity (99%) for (LAB-Gr2) *L. plantarum*1 and (95%) for (LAB-Bn2) *L. mesenteroides* (Table 9). The sequences of the isolates (LAB-Gr2) *L. plantarum*1 and (LAB-Bn2) *L. mesenteroides* were determined and deposited in the Gene Bank database under accession number EU600918.1 and EU419606.1, respectively.

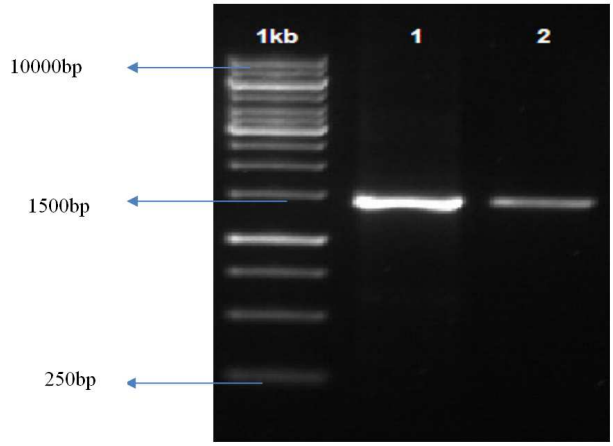

Figure 3. *The DNA Bands of LABs on the 1.5 % Agarose Gel Using Universal Primers16S. S: (5-AGAGTTTGATCCTGGCTC-3) and 16S. R: (5-CGGGAACGTATTCACCG-3), Lane 1kb: DNA Ladder, Lane 1: LAB-Gr2 and Lane 2: LAB-Bn2.*

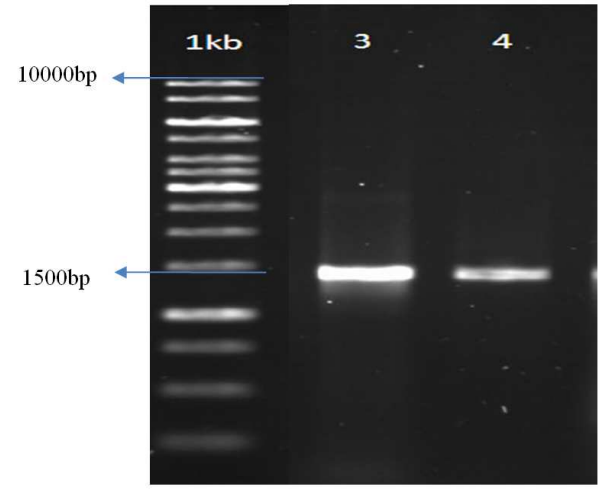

Figure 4. *The DNA Bands of LABs on the 1.5 % Agarose Gel using Specific Primers of L. plantarum LbP11F: (5'AATTGAGGCAGCTGGCCA3') and Lbp11 R:(5'GATTACGGGAGTCCAAGC3'); L. mesenteroides LMMF:(5'CCGTTACCCCTAAATTTTGAC3')andLMR:(5'GACCAAATACAA TAGGTTGCG3'), Lane 1kb: DNA Ladder, Lane 3: LAB-Gr2 and Lane 4: LAB-Bn2.*

Table 9. *Genotypic and Genotypic Identification of LAB Isolates.*

Code of LAB	Source	Identification	Similarity Index %	Accession No.
LAB-Gr2	Grapes	*L. plantarum*	99	EU600918.1
LAB-Bn2	Banana	*L. mesenteroides*	95	EU419606.1

4. Discussion

Lactic acid bacteria are known to have proteolytic activity that hydrolyses protein to produce peptides with bioactivity. In this study the results indicated that the LAB isolates from different fruits have good proteolytic activity when tested on skim milk agar. Similar results reported by Yelntty et al. (2014) where indicated that the isolates which estimated as LAB were continued determined for their proteolytic activity by growing the selected isolates in skim milk media. Skim milk media were inoculated with the selected isolates and incubated at 37°C for 24-48 hours, after incubation clear zone was measured. Every isolate producing clear zone in the skim milk media indicated that this isolate could degrade protein or had proteolytic activity. In all, proteolytic LAB presumably hydrolyzes the protein-lipid complex, after which protein released probably gets further hydrolysed into smaller peptides due to the proteolytic action of LAB as well as the lactic acid produced by them. these hydrolysed protein presumably exhibit biofunctional activity like antioxidant properties and antibacterial properties (Jini et al. 2011).

The probiotic properties of the LAB isolates would be useful in the dairy manufacturing industry. They could beneficially affect the consumer by providing effective and security dietary source of antioxidants. Additionally, LAB have some probiotic functions, such as adjusting the balance of intestinal flora, reducing serum cholesterol, inhibiting and reducing the risk of tumors, and revitalizing the immune system among others (Leroy and Vuyst 2004). However, this study observed that the species of lactobacillus form non-dairy sources have the probiotic properties. Theses Isolates of the present study have almost similar antimicrobial capability. The results showed that the LAB-Gr2 and LAB-Bn2 were able to inhibit activity against *S. typhimurium* (9.7 and 7.1 mm), respectively. Also these isolates were capacity to inhibit growing *E. coli* (5.5 and 4.9 mm), respectively. In the study of Osuntoki et al. (2008) *Lactobacillus* spp. isolated from fermented dairy products showed antibacterial activity against some clinically important pathogens such as Enterotoxigenic *E. coli* (4.2 mm), *S. typhi*murium (4.3 mm) and *Listeria monocytogenes* (5.0 mm). Isolates of the present study have better antimicrobial capability than *Lactobacillus* spp. This observation further supported the results reported by Osuntoki and Korie (2010) that lactobacilli from non-dairy food sources may serve as a delivery vehicle for probiotic lactobacilli and provide antimicrobial activity from non-dairy source. Probiotic LABs namely, *L. acidophilus*, *B. longum*, *L. fermentum* and *L. sake* (Kullisaar et al. 2002; Amanatidou et al. 2001) had been shown able to decrease the risk of accumulation of ROS.

Antimicrobial activity is an important mechanism to exert

probiotic properties, and considerable research has focus on the production of antimicrobial substances from lactobacilli in last decade (Bao et al. 2010). Probiotics such as *Lactobacillus* spp. are reported to have inhibitory activity against common human pathogens. They are able to produce antimicrobial substances such as bacteriocins which have great potential to be used in therapeutics and as food bio-preservatives (Chowdhury et al. 2012). Probiotic fermented dairy products provide a healthy functional food for health properties. Many previous researches mentioned that, milk fermented by selected culture of LAB has high biochemical activity and antioxidant activity (Kullisaar et al. 2003; Villani et al. 2005). Among lactic acid bacteria, species of *Lactobacillus* have attracted a lot of attention for their potential probiotic effects in human health. *Lactobacillus* spp are important members of the healthy human microbiota (Naaber et al. 1998).

5. Conclusion

The results of this study concluded that the isolation and identification of the isolate of LAB from different fruits had shown good proteolytic activity and probiotic properties. Therefore, these results suggest that the *L. plantarum* and *L. mesenteroides* great potential to be used in therapeutics and as food bio-preservatives.

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