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PROBIOTIC PROPERTIES OF ANTIMICROBIAL-PRODUCING LACTIC ACID BACTERIA ISOLATED FROM DAIRY PRODUCTS AND RAW MILK OF SABAH (NORTHERN BORNEO), MALAYSIA

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ABSTRACT

This study aims to evaluate the probiotic properties of the antimicrobial-producing lactic acid bacteria (LAB) isolated from cow and goat milk, and various types of cheese. The isolated strains were biochemically characterized by sequence of tests namely Gram staining, catalase test and carbon fermentation. The agar well diffusion assay was performed by utilizing the *Listeria monocytogenes* ATCC 7644 and *Listeria monocytogenes* ATCC 13933 as the indicator microorganisms prior to biochemical and physiological tests to assess the beneficial properties of the strains. Results showed that 5 out of 20 isolated LAB strains were the antimicrobial-producer indicated by the formation of inhibition zones against the *L. monocytogenes* ATCC 7644 and *L. monocytogenes* ATCC 13933. All five strains were able to utilize glucose, and also tolerate various concentrations of NaCl and wide range of temperatures. Strains CCB1, GB3 and CB3 showed positive proteolytic activity, while CCB1, GB3 and CA1 were able to hydrolyse starch. Other than that, isolates CCB1, CB3 and CA1 showed ability to deconjugate bile salt in both aerobic and anaerobic conditions. Moreover, CCB1, GB3 and CA4 were susceptible to ampicillin, tetracycline, ceftriaxone, penicillin G and chloramphenicol. However, most of the strains were resistant to norfloxacin, amikacin, colistin sulphate, streptomycin and nalidixic acid. Lastly, all of the five isolates were tolerant to bile salt and phenol as no growth inhibition was observed. The newly isolated LAB strains with valuable features might offer an unfolded potency that are beneficial for applications in food industry.

Key words: Isolation, lactic acid bacteria, food industry, milk products

INTRODUCTION

The rising awareness among the consumers on probiotics is expected to drive the industry growth over the next few years. The application of probiotic products is important in maintaining one's health as well as to cure gut-associated diseases such as bowel disease, lactose intolerance, indigestion and diarrhea (Nazir *et al.,* 2018). Nowadays, probiotics are widely used as health food and medicines in Asia and Europe, which subsequently increases the demand of these probiotic sources. Lactic acid bacteria (LAB) such as *Lactobacillus, Streptococcus* and *Bifidobacterium* species that commonly found in digestive system of human act as the main bacteria used as probiotics. LAB are Gram-positive bacteria, non-spore forming, catalase negative, and widely present in nature. LAB refer to a large group

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of good bacteria that are selected as probiotic due to the ability to promote human gastrointestinal health. Today, a wide variety of fermented milk products including yogurt and cheese, take benefits of these LAB applications (Linares *et al.*, 2017). As LAB are recognized as safe (GRAS), where it is safe to be consumed by human without causing side effects, it has been widely applied in food industry due to the production of organic acids, such as lactic acid which results in lowered pH value (Gemechu, 2015) and also by producing antimicrobial substances known as bacteriocins (Perez *et al.,* 2014). Many bacteriocins are active against foodborne pathogens such as *Listeria monocytogenes, Escherichia coli, Campylobacter jejuni, Yersinia enterocolitica* and *Vibrio parahemolyticus* (Altuntas, 2013). In recent years, due to the applications as safe additives in food preservation, bacteriocin producing LAB have become significant. Bacteriocin possess a lot of potential applications such as extending the time for food preservation which could be beneficial for the food industry, treatment of pathogen disease, maintaining human health and cancer therapy (Yang *et al.,* 2014). Many studies have been reported on LAB isolation from dairy products, however, in Malaysia it is not yet been well studied especially in Sabah. Therefore, this study aimed to highlight the searching of potential antimicrobial-producing LAB from dairy products and raw milk of Sabah (Northern Borneo) for food applications.

MATERIALS AND METHODS

Sample preparation and isolation of lactic acid bacteria

Various types of cheeses (cow cheese, goat cheese and mozzarella) were obtained from the Desa Dairy Farm in Mesilau, Kundasang, Sabah while the raw milk (cow and goat milk) samples were collected from Department of Veterinary Services, Keningau, Sabah. The sample was stored at 4°C until used. The milk samples were homogenized by gentle shaking. About 10 mL of milk sample and 4 g cheese sample were taken aseptically then, mixed with 90 mL de Man Rogosa Sharpe (MRS) and M17 broth, respectively and were incubated at 37°C for 24 h. After the incubation, 10-fold of serial dilution was performed. For the preparation of agar plates, the MRS and M17 agar were supplemented with 0.01% (w/v) sodium azide to inhibit the growth of Gram-negative bacteria. About 100 µL of diluted sample was spread on agar plates and incubated in anaerobic conditions at 37°C overnight. The higher dilution was used to perform the total counts. The colonies were chosen randomly and streak plating was performed two times to purify the strains on respective media either M17 or MRS media.

Morphological, biochemical and physiological tests

The morphology of the LAB was examined after the overnight incubation on MRS or M17 agar. The general procedures were used to carry out the Gram Staining, and the cell morphology was observed and examined by light microscopy. In catalase test, a drop of 3% (v/v) H_20_2 was spotted on the heavy colony of the LAB. A positive reaction will show immediate effervescence. To evaluate the ability of the strains to ferment carbon, nutrient agar with 1% (w/v) of glucose and 0.004% (w/v) bromocresol purple (Sigma, United Kingdom) (as a pH indicator) was prepared. Then, about 10 µL of the culture was dotted on the surface of the agar. The acid production can be indicated by the production of yellow zone around the culture after 24 h incubation at 37°C.

Identification of isolates by16S rRNA sequencing and phylogenetic analysis

Extraction of DNA and sequencing of the amplified fragments were carried out according to methods of Reysenbach *et al*. (2000). Briefly, the bacterial 16S rDNA, full-length 1.5 kb, was amplified using universal primers 27F and 1492R. The total reaction volume of 25 µL contained genomic DNA purified using in-house extraction method, 0.3 pmol of each primer, deoxynucleotides triphosphates (dNTPs, 400 µM each), 0.5U DNA Taq polymerase, supplied PCR buffer and deionised water. PCR was performed as follows: 1 cycle (94°C for 2 min) followed by; 25 cycles (98°C for 10 sec; 53°C for 30 sec; 68°C for 1 min) for annealing and extension of the amplified DNA. The PCR products were purified by standard method and directly sequenced with primers 785F and 907R using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The fragments of sequences were assembled and consensus sequences were compared with those deposited in the GenBank DNA database using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree based on 16S rRNA 137 genes was constructed to determine the closest bacterial species by using Neighbour Joining (Unrooted Tree) by NCBI Blast Tree Method using Molecular Evolutionary Genetics Analysis (MEGA) software version 10.0.5 (Tamura *et al*., 2004). Distances and clustering with the Neighbour-Joining method was determined using bootstrap values based on 1000 replications. *Bacillus subtilis* NCDO 1769 and *Escherichia coli* strain U 5/41 were

used as an outgroup organism, respectively that serves as a reference group in evolutionary relationships determination of the ingroup.

Effect of NaCl and temperatures on the growth of LAB

About 1% (v/v) of the bacterial cultures were inoculated into M17 or MRS broth containing different concentrations of NaCl (0.5, 1.5, 2.5, 3.5, 4.5%, w/v) supplemented with 0.004% (w/v) bromocresol purple and incubated at 37°C. In temperature study, the isolates were inoculated into M17 or MRS broth containing 0.004% (w/v) bromocresol purple and was incubated at different temperatures (-20, 28, 37 or 70°C). The growth was evaluated which was indicated by the colour changes from purple to yellow after 48 h of incubation according to the method by Abbasiliasi *et al*. (2012).

Effect of bile salts and phenol on the growth of LAB

MRS broth containing 0.3% (w/v) bile salts was inoculated with 1% (v/v) of overnight culture and incubated in horizontal shaker for 4 h at 37°C and agitated at 100 rpm. The culture was harvested every hour by taking about 1 mL of sample culture. Pour plate method was performed to determine the growth of bacteria. Survival ability percentages of the LAB were calculated by comparing the growth (CFU/mL) at every hour with the number of viable cells at 0 hr. Phenol tolerance test was examined by taking 100 µL of overnight culture into 100 mL of MRS broth containing different concentrations of phenol, which are 0.1, 0.3 and 0.5% (w/v). About 1 mL of the culture was taken at 0 hr and 24 hr to be used in pour plate method. The inhibition effect was determined by comparing the viable count (Log_{10} CFU/mL) of LAB at 0 hr and 24 hr.

Acidification activity

Acidification activity was determined by inoculating about 1% (v/v) of bacterial strain into 10 mL of skimmed milk solution. The pH was measured at 0, 24, 48 and 72 hr. The texture and smell of the milk were also recorded to evaluate the coagulation of milk.

Starch hydrolysis test and Proteolytic activity

Starch hydrolysis test was conducted by inoculating bacterial colony and streaking it on nutrient agar containing 2% (w/v) of soluble starch powder. After overnight incubation, a small amount of iodine as indicating agent was poured on the surface of agar plate. A clear zone observed indicates positive reaction. The proteolytic activity was determined by inoculating the culture on nutrient agar supplemented with 1% (w/v) of skim milk. The positive reaction (clear zones) was observed after 24 hr of incubation.

Bile salt deconjugation test

The bacterial colony was inoculated on the surface of MRS agar supplemented with 0.5% (w/v) taurodeoxycholate (TDC). The growth of strain was tested in both aerobic and anaerobic condition. The growth of colonies was observed after 24 hr of incubation. The positive result is shown by the presence of precipitated bile acid around colonies (opaque halo).

Antimicrobial activity test

The antimicrobial activity of the isolated lactic acid bacteria was determined by using the agar diffusion method based on Abbasiliasi *et al.* (2012). Briefly, the isolate was grown in respective media either M17 or MRS broth and incubated at 37°C overnight. The culture was centrifuged at 10,000 rpm at 20 min at 4°C. About 100 µL of supernatant was placed into 6 mm wells of brain heart infusion (BHI) agar plates that were previously seeded with 1% (v/v) of actively growing strain which is *Listeria monocytogenes* ATCC 7644 and *L. monocytogenes* ATCC 13933. The plate was incubated at 37°C for 24 hr and the antimicrobial activity was determined by measuring the diameter of inhibition zones (mm).

Antibiotic sensitivity testing

The susceptibility to antibiotic was determined by using the disc diffusion method according to Tagg *et al.* (1976). A single colony of LAB was inoculated into the respective broth (M17 or MRS broth) and incubated at 37°C for 24 hr. The bacteria were spread evenly on the surface of respective agar either M17 or MRS agar plate by using the sterile cotton wool swab dipped into the bacterial suspension. The antibiotics disc containing erythromycin (10 µg), ampicillin (25 µg), tetracycline (10 µg), ceftriaxone (30 µg), penicillin G (2 unit), chloramphenicol (30 µg), norfloxacin (10 µg), amikacin (30 µg), colistin sulphate (10 µg), streptomycin (10 µg) and nalidixic acid (30 µg) were gently placed on the surface of dried agar plates to ensure uniform contact between the disc and agar. The plate was incubated at 37°C overnight. The zones of growth inhibition around each of the antibiotic disc were measured (including the disc diameter) to the nearest millimetre. The isolates were classified as susceptible ($\geq$ 21 mm), intermediate (16–20 mm), or resistant ($\leq$ 15 mm) according to Abbasiliasi *et al.* (2012).

RESULTS

Twenty lactic acid bacteria (LAB) strains were successfully isolated from various dairy products, raw goat milk and raw cow milk. Among these strains, five isolates namely CCB1, GB3, CB3, CA1 and CA4 were able to secrete antimicrobial substances as confirmed by their inhibition activity against *Listeria monocytogenes* ATCC 7644 and *L. monocytogenes* ATCC 13933 (Table 1). Therefore, all these five strains were further characterized to evaluate their probiotic potential. To evaluate the probiotics potential of LAB, the tests involved were based on the parameters usually applied in food processing and human digestive system. Species identification was performed by 16S rRNA gene sequencing. Analysis shown that the isolates CA1 and CA4 were highly similar and clustered together. Further analysis with the Average Nucleotide Index (ANI) was performed to check the correlation among the nearly clustered to evaluate the similarity (data not shown). Based on the ANI study the CA1 and CA4 was 100% ANI similar. The other near cluster group was 98.91% [NR_044701 (*Lactobacillus alimentarius*)] and 99.31% [NR_028949 (*Lactobacillus mindensis*)]. Meanwhile, the strains GC3 and CC3 were highly similar and also clustered together. CCB1 was slightly different as compared to GC3 and CC3. All the three strains were clustered with *Enterococcus lactis* strain F8, BT159, KH17, and KC17 (Figure 1).

The ability of isolates to grow at different concentrations of NaCl varied among strains. All the five strains were able to grow in MRS medium in the presence of NaCl up to 6.5% (w/v) but were inhibited at 10% (w/v). In temperature study, CCB1, GB3 and CB3 showed growth up to 70°C, while CA1 and CA4 preferred low temperature (-20°C) to grow (Table 2). The percentage of bacterial survivability in the presence of bile salt as shown in Figure 2. All five strains achieved the percentage of survival greater than 90% after 1 hr incubation and continued to increase until 4 hr of incubation indicating their tolerance towards bile salts. In addition, all of the selected strains were resistant to phenol at concentrations of 0.1, 0.3 and 0.5% (w/v) (Table 3) and were able to acidity skim milk with pH reduction ranging between 4.6 to 5.1 (Table 4). Isolates CCB1, GB3 and CA1 were able to hydrolyse starch. Results also showed that most of the strains showed positive reaction in proteolytic activity with the exception of CA1 and CA4. For the bile salt deconjugation test, it was identified that CCB1 and CB3 were able to deconjugate TDC in aerobic and anaerobic conditions. No harmful effect of atmospheric oxygen was observed, as both strains were able to grow at aerobic condition. GB3 was only capable to grow in anaerobic condition while CA1 and CA4 did not grow in both conditions. Most of the strains were susceptible to ampicillin, tetracycline, ceftriaxone, penicillin G and chloramphenicol but resistant to erythromycin, norfloxacin, amikacin, colistin sulphate, streptomycin and nalidixic acid (Table 5).

DISCUSSION

Lactic acid bacteria (LAB) are well known microorganism commonly found in dairy products especially in milk. In a study conducted by Masood *et al.* (2011), they revealed LAB found in goat milk possess the medicinal properties such as the ability to prevent colon and colorectal cancer. Alnakip *et al.* (2016) isolated several types of LAB and reported that the most predominant genus of LAB from the raw milk source is *Enteroccocus* sp. In this study, all the selected five isolates were able to secrete antimicrobial substance. The ability of the isolated strains to produce bacteriocin-like

Table 1. Morphological, biochemical characteristics and antimicrobial activity of LAB isolates

Characteristic	Cow cheese	Mozzarella	Goat cheese	Cow milk	Goat milk
No. of LAB isolates	3	1	1	8	7
No. of isolates showing antimicrobial activity against *L. monocytogenes* ATCC 7644/ ATCC13932	1 (CCB1)	0	0	3 (CA1, CA4, CB3)	1 (GB3)
Cell morphology	Cocci	Bacilli	Cocci	Cocci (CA1, CA4) Rod (CB3)	Cocci
Gram stain reaction	+	+	+	+	+
Catalase activity	–	–	–	–	–
Glucose fermentation	+	+	+	+	+

Note: Positive reaction (+), negative reaction (–).

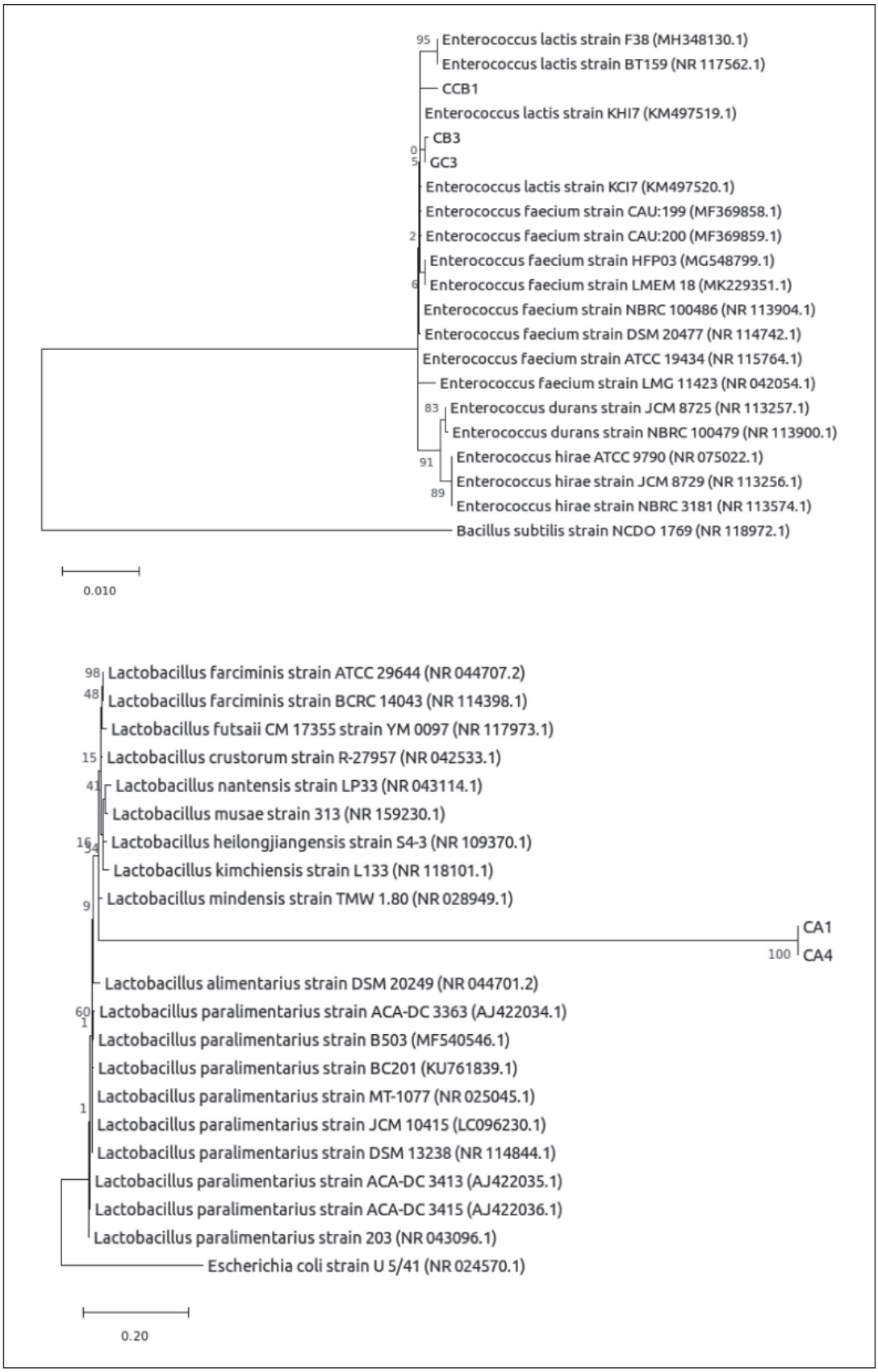

Fig. 1. Phylogenetic tree of five isolates and related taxa based on partial 16S rDNA sequences cut-off 1363bp (CCB1, GB3 and CB3) and 1521bp (CA1 and CA4). The phylogenetic tree was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (MEGA X 10.). The numbers at the nodes are books trap confidence levels from 1000 replicates. *Escherichia coli* strain U 5/41 and *Bacillus subtilis* NCDO 1769 were used as outgroup organism, respectively. (A) CCB1, GB3, CB3; (B) CA1 and CA4.

Table 2. Growth of antimicrobial-producing LAB to NaCl and temperatures

Strain	Characteristics			
	Range of NaCl (%, w/v)		Range of temperature (°C)	
	Minimum	Maximum	Minimum	Maximum
CCB1	0.5	6.5	28	70
GB3	0.5	4.0	37	70
CB3	0.5	4.0	28	70
CA1	0.5	6.5	-20	37
CA4	0.5	6.5	-20	37

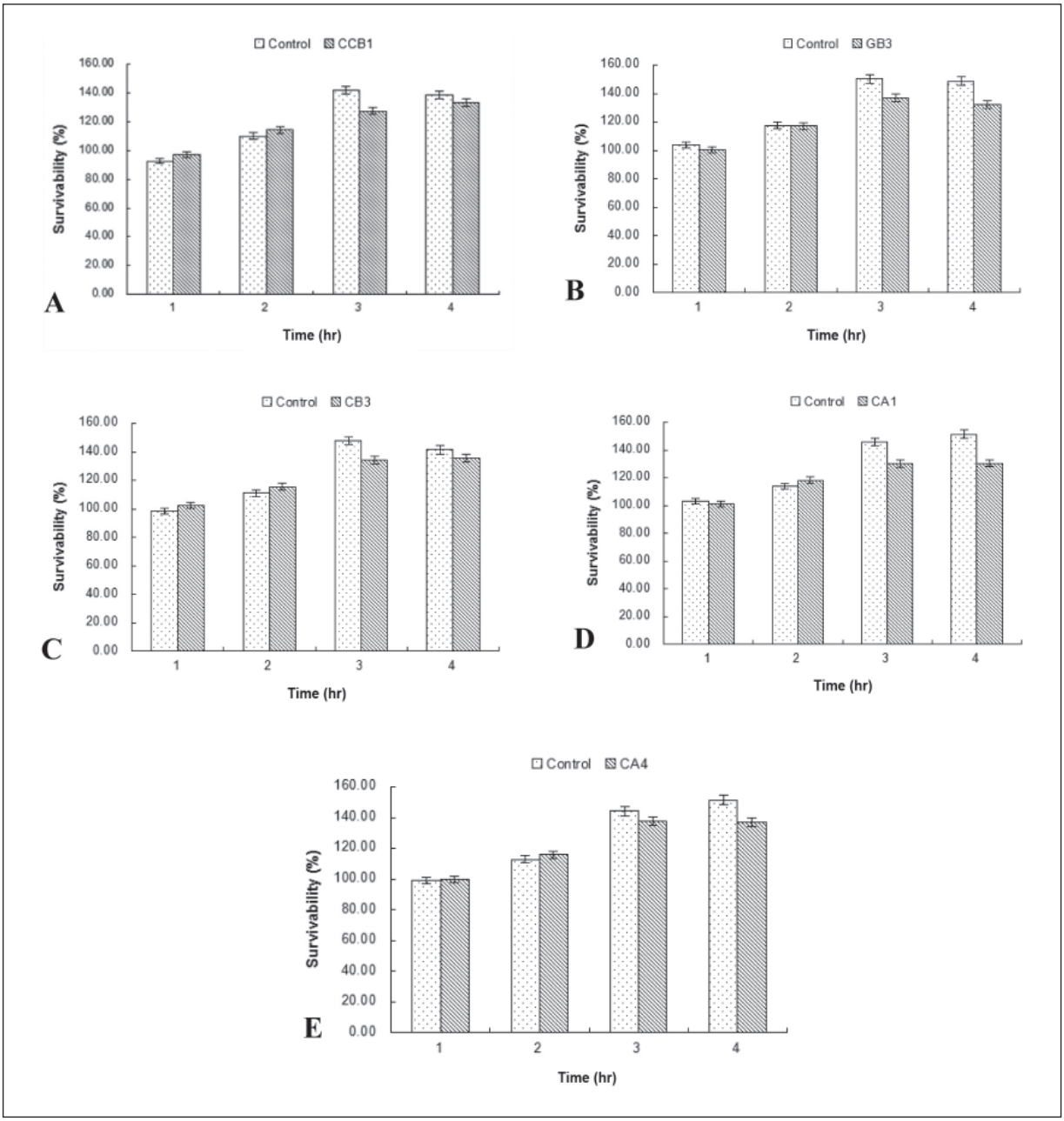

Fig. 2. Tolerance of selected five strains to bile salts. (A) CCB1; (B) GB3; (C) CB3; (D) CA1; (E) CA4; Control: MRS medium.

Table 3. Effect of phenol on the growth of antimicrobial-producing LAB

Strain	MRS + % (w/v) of phenol	Viable count (Log_{10} CFU/mL)		Growth inhibition*
		Time		
		T_0	T_{24}	
CCB1	0	9.62 ± 0.10	10.26 ± 0.74	-0.64
	0.1	9.49 ± 0.14	9.59 ± 0.03	-0.10
	0.3	9.41 ± 0.07	9.83 ± 0.18	-0.42
	0.5	9.46 ± 0.04	9.77 ± 0.08	-0.31
GB3	0	9.56 ± 0.02	9.66 ± 0.04	-0.11
	0.1	9.67 ± 0.14	10.23 ± 0.05	-0.56
	0.3	9.36 ± 0.03	9.57 ± 0.14	-0.21
	0.5	9.5 ± 0.08	9.61 ± 0.11	-0.11
CB3	0	9.87 ± 0.03	9.89 ± 0.03	-0.03
	0.1	9.64 ± 0.02	10.42 ± 0.06	-0.8
	0.3	9.37 ± 0.01	9.63 ± 0.3	-0.26
	0.5	9.54 ± 0.13	9.60 ± 0.08	-0.06
CA1	0	9.63 ± 0.04	9.70 ± 0.01	-0.07
	0.1	10.36 ± 0.05	10.44 ± 0.03	-0.1
	0.3	9.38 ± 0.08	9.41 ± 0.04	-0.03
	0.5	9.37 ± 0.07	9.67 ± 0.22	-0.30
CA4	0	9.57 ± 0.03	9.63 ± 0.03	-0.07
	0.1	9.38 ± 0.05	9.96 ± 0.09	-0.58
	0.3	9.64 ± 0.05	9.96 ± 0.04	-0.04
	0.5	9.40 ± 0.08	9.79 ± 0.10	-0.38

Note: Inhibition* = Average CFU/mL (T_{24}) – Average CFU/mL (T_0); Negative sign indicating no growth inhibition occur.

Table 4. Milk acidification activity of CCB1, GB3, CB3, CA1 and CA4

Strain	Incubation time (hr)	pH	Characteristics		
			Curd appearance	Aroma	Colour
CCB1	0	6.65	Runny	Flat	White
	24	4.59	Coagulated	Foreign	Cream-white
	48	4.04	Coagulated	Rancid	Cream-white
	72	3.87	Coagulated	Rancid	Cream-white
GB3	0	6.52	Runny	Flat	White
	24	4.38	Coagulated	Foreign	Cream-white
	48	3.94	Coagulated	Rancid	Cream-white
	72	3.78	Coagulated	Rancid	Cream-white
CB3	0	6.77	Runny	Flat	White
	24	4.67	Coagulated	Foreign	Cream-white
	48	3.94	Coagulated	Rancid	Cream-white
	72	3.81	Coagulated	Rancid	Cream-white
CA1	0	6.75	Runny	Flat	White
	24	4.54	Coagulated	Foreign	Cream-white
	48	4.34	Coagulated	Rancid	Cream-white
	72	4.18	Coagulated	Rancid	Cream-white
CA4	0	6.37	Runny	Flat	White
	24	5.14	Coagulated	Foreign	Cream-white
	48	4.83	Coagulated	Rancid	Cream-white
	72	4.60	Coagulated	Rancid	Cream-white

Table 5. Antibiotic sensitivity testing of antimicrobial-producing LAB

Antibiotic	Disc content	Inhibition zone diameter (mm)				
		CCB1	GB3	CB3	CA1	CA4
Ampicillin	25 µg	27.75±0.35 (S)	36.75±0.35 (S)	37.50±0.71 (S)	26.25±1.06 (S)	34.0±1.41 (S)
Amikacin	30 µg	0 (R)	7.75±0.35 (R)	7.25±0.35 (R)	0 (R)	0 (R)
Ceftriaxone	30 µg	29.0±1.41 (S)	23.0±0.71 (S)	20.75±1.06 (S)	23.75±1.06 (S)	32.25±1.06 (S)
Chloramphenicol	30 µg	36.75±0.35 (S)	21.25±0.35 (S)	20.75±1.06 (S)	19.50±0.71 (I)	25.5±1.06
Colistin sulphate	10 µg	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Erythromycin	10 µg	8.25±0.35 (R)	9.50±0.35 (R)	11.0±1.06 (S)	10.75±1.06 (R)	0 (R)
Nalidixic acid	30 µg	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Norfloxacin	10 µg	13.25±0.35 (R)	14.75±1.06 (I)	8.0±1.41 (R)	0 (R)	0 (R)
Penicillin G	2 units	30.0±0.71 (S)	23.75±0.35 (S)	22.75±1.06 (S)	20.75±0.35 (I)	30.75±0.35 (S)
Streptomycin	10 µg	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Tetracycline	10 µg	0 (R)	30.0±0.35 (S)	21.75±0.35 (S)	14.75±0.35 (R)	0 (R)

Note: Results of zone of inhibition are triplicate and expressed as Mean±S.D. Resistance (R) ≤15 mm; Intermediate (I) 16–20 mm; Susceptible (S) ≥21 mm.

inhibitory substances (BLIS) is very significant since bacteriocin plays the role to inhibit the growth of pathogens that can cause food spoilage (Mittu & Girdhar, 2015). Many studies had been reported on the potential application of bacteriocin especially in the food industry.

Most of our strains are not tolerant in medium with more than 6.5% (w/v) of NaCl. This result is in line with the study conducted by Khedid *et al.* (2009), whereby the range of LAB growth is in between 2 to 6.5% (w/v) and mostly inhibited at 10% (w/v) of NaCl concentration. Tolerance of NaCl is important since some of the processes in food production involve exposure to high salt content and this subsequently will affect their food nutrient content (Ni *et al.,* 2010). Some LAB are categorized either as thermophilic or mesophilic bacteria due to their ability to grow in different temperatures. The present study showed that CCB1, GB3 and CB3 are in a group of thermophilic bacteria since they are able to grow in 70°C, while CA1 and CA4 could be considered to be psychrophilic bacteria as they are able to grow below 15°C.

LAB can be considered as probiotic only if it has the characteristics including the ability to overcome the effect of bile salt and low environmental pH of gastrointestinal tract especially in human. In our study, the survivability of the five bacterial strains was in increasing pattern before the growth rate was reduced after 3 hr of incubation (Figure 2). Bile salt plays a crucial role in terms of mechanism defence of intestinal tract. Hence, the bile tolerance of lactic acid bacteria is considered beneficial. Davati *et al*. (2015) reported that several isolates such as *L. casei, E. durans, L. casei* and *P. pentosaceus* isolated from raw camel milk exhibited high tolerance to bile salt 0.4% (w/v) after being exposed for 6 hr. Moreover, all of the isolated strains are resistant to phenol at concentration of 0.1, 0.3 and 0.5% (w/v). Present study is in line with the study carried out by Mannan *et al.* (2017) who found that LAB isolated from buffalo milk were tolerate to 0.1, 0.3 and 0.4% (w/v) of phenol. Phenol is known as a toxic metabolite produced during the process of digestion in intestinal tract (Rowland *et al.,* 2018). Hence, probiotic bacteria should be able to tolerate the phenol inside gastrointestinal tract of a host.

The ability in coagulating milk demonstrates the potential of LAB strains for application as a starter culture in the production of fermented products. All of the five isolated strains showed decrease in pH during the 72 hr of incubation. The decrease in pH of skim milk revealed that these bacterial strains were able to act as the starter culture in milk fermentation due to their ability to produce acid, which is important in flavour enhancement; improve milk texture by producing exopolysaccharides and as preservative agents (Widyastuti & Febrisiantosa, 2014). Additionally, the combination functions of antimicrobial substances such as organic acids with bacteriocins may be effective in preventing and inhibiting the growth of foodborne pathogen especially in fermented foods (Altuntas, 2013).

The hydrolysis of starch is the results of amylase enzyme activity. The ability of LAB to produce amylase is important as its potential could be applied in the food industry. Our results showed that most of the strain were positive in proteolytic activity (except CA1 and CA4). Similar study conducted by Donkor *et al*. (2007), who found that *L. acidophilus, L. casei* and *S. thermophilus* isolated from the dairy source also exhibited positive proteolytic activity. Bile salt deconjugation test is useful in order to understand the relationship

between deconjugation of bile acid by probiotic strain and antibiotic susceptibility when there is a deconjugated bile acids present (He *et al.,* 2012). In our study, 3 out of 5 strains were susceptible to ampicillin, tetracycline, ceftriaxone, penicillin G and chloramphenicol, but resistant to erythromycin, norfloxacin, amikacin, colistin sulphate, streptomycin and nalidixic acid. Gueimonde *et al.* (2013) stated that antibiotic resistant strain could transmit the resistance gene to the pathogenic microorganism inside gut microbiota. Moreover, probiotics also have the potential of carrying resistant genes that may be transferred to the other organisms as well as to the other LAB species (Sengupta & Ghosh, 2015). The antibiotic resistance gene is transferred vertically between generations and increasing the survivability of resistant microbes (Tsang, 2017). Study conducted by Thumu and Halami (2012) stated that LAB, mostly from *Enterococcus* and *Lactobacillus* isolated from fermented food products have the resistance genes (tetM, tetL, tetO and tetK) for antibiotics namely erythromycin and tetracycline. Isolate CCB1 and GB3 also were highly resistant to collistion sulphate, streptomycin and nalidixic acid. In contrast with the study conducted by Shazali *et al.* (2014), the resistance of the LAB against streptomycin and nalidixic acid was highly significant.

CONCLUSION

Five antimicrobial-producing LAB coded as CCB1, GB3, CB3, CA1 and CA4 were successfully isolated from the local dairy products and raw milk. All these strains showed an inhibitory effect towards *L. monocytogenes* ATCC7644 and *L. monocytogenes* ATCC13932, also possessing probiotic properties and therefore possibly could be applied in food industry. In future, more intensive *in vitro* and *in vivo* assessments need to be carried out such as adhesion to the intestinal mucosa and development of effective delivery system of probiotics to the gastrointestinal tract.

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