Malays. Appl. Biol. (2018) 47(6): 105–112

ANTIBIOTIC SUSCEPTIBILITY, ANTIBACTERIAL ACTIVITY AND PROBIOTIC CHARACTERISATION OF ISOLATED *Lactobacillus brevis* STRAINS FROM *Heterotrigona itama* HONEY

NOR HAZWANI HASALI, AMIR IZZWAN ZAMRI, MOHD NIZAM LANI* and AIDILLA MUBARAK

School of Food Science and Technology, Universiti Malaysia Terengganu (UMT), 21030 Kuala Nerus, Terengganu, Malaysia
**E-mail: nizamlani@umt.edu.my*

Accepted 10 December 2018, Published online 31 December 2018

ABSTRACT

Heterotrigona itama (Family: Apidae, Tribe: Meliponini, Genus: Trigona) is one of the stingless bee in Meliponiculture in Malaysia. *H. itama* honey is reported to be a good reservoir for novel lactic acid bacteria (LAB) with probiotic properties. In this study, five *Lactobacillus brevis* strains (strain Ibr-42, strain 37901, strain ATCC 367, strain NJ42 and strain KLDS) were previously isolated and identified from *H. itama* honey obtained from local stingless beekeepers in the coastal areas in Kelantan and Terengganu, were evaluated for antibiotic resistance, antibacterial activity, resistance to low pH, tolerance to bile salts and haemolytic activity. The results indicated that all five strains of *L. brevis* were susceptible to chloramphenicol, ampicillin, erythromycin, and tetramycin, but resistant to kanamycin. In terms of antagonistic activity among *L. brevis*, it was found antagonistic activity was minimum. For antibacterial activities of these strains against selected foodborne pathogenic bacteria by well diffusion method, *L. brevis* strain NJ42 exhibited the highest inhibition (24 mm) against *Pseudomonas aeruginosa* ATCC 15442. All *Lactobacillus* strains from *H. itama* honey were able to survive in pH 2 and 0.3% (w/v) bile salts concentration that mimic the conditions in the gastrointestinal system. The inability of *L. brevis* strains to exhibit β-haemolytic activity showing that haemolysis is not the virulence factor for these strains. These findings proved the isolated *L. brevis* in *H. itama* honey could be used as potential probiotic, envisaging its potential as one of functional foods for food industry.

Key words: Antibiotic susceptibility, antibacterial probiotic, low pH, haemolytic

INTRODUCTION

Lactic acid bacteria (LAB) are regarded as a major group of probiotic bacteria which are non-pathogenic, acid tolerance, bile tolerance and produce antimicrobial substances (Mojgani *et al*., 2015). They are found to be an important reservoir within humans, insects and animals that confer important microorganisms for food industries (Vásquez *et al*., 2012). Besides, few reports claim that they also can improve nutritional, organoleptic and shelf-life characteristics in food products as well as the safety of food products. (Yang *et al.,* 2012; Parvez *et al*., 2006; MacFarlane and Cummings, 2002).

Most probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium* (Prasad *et al*., 1998). Probiotics can be classified as "friendly" microorganism that live in human colon and when administered in adequate amounts, these probiotics could confer health benefits on human (Saarela *et al.,* 2000). Probiotics have received great attention due to the ongoing trend of vegetarianism and meeting the demands of lactose intolerant individuals (Vasudha & Mishra, 2013), inhibit pathogens in the human intestinal tract, and lower the risk of gastro-intestinal diseases (Cross, 2002).

Probiotics also have been reported to have anti-mutagenic, anti-carcinogenic, hypocholesterolemic and anti-hypertensive effects. The term "probiotics" microorganism can be applied when they can survive and resist any adverse factors in the gastrointestinal tract like stomach acidity and bile salt in the duodenum when they pass through it (Iñiguez *et al.,* 2008). From the medical perspective, consumption of an adequate amount of live cells of certain

* To whom correspondence should be addressed.

probiotic *Lactobacilli* strains is believed to promote several beneficial physiological effects on human such as maintaining and balancing the healthy intestinal microbiota as well as reducing the risk of intestinal infection (Gardiner *et al.,* 2002; Tannock, 2004). The good probiotics organisms have several criteria to be beneficial to human health such as high acid tolerance to condition of digestive tract, production of antibacterial factors, improvement in the microbial balance and resistance to heat treatment (Gomes *et al.,* 2009). Up till now, reports on the probiotic characteristics from LAB isolated from *Heterogona itama* is scarce.

Recently, several strains of LABs were isolated from honey that possessed antibacterial activity against both Gram-negative and Gram-positive pathogenic bacteria (Lee *et al.,* 2008; Aween *et al.,* 2012; Ibarguren *et al.,* 2010; Mohd Hasali *et al.,* 2015) and fungi (Bulgasem *et al*., 2016, Bulgasem *et al*., 2017). Interest in LAB from stingless bee honey has increased as limited studies have reported on their potential as natural food preservative for extending shelf life. To the best our knowledge, no study has yet investigated the effect of probiotic *Lactobacillus* isolated from *H. itama* honey as most of studies focused on health benefits from Tualang and Manuka honey (Roowi *et al*., 2012). Therefore, the purpose of this study is to identify probiotic characteristics of five *Lactobacillus brevis* isolated from *H. itama* honey according to their antibacterial activity, antibiotic resistance, antagonistic study, low pH resistance, bile tolerance test and haemolytic activity.

MATERIALS AND METHODS

Probiotics strain of *Lactobacillus brevis* strains

Five *L. brevis* strains were obtained from study done by Mohd Hasali *et al.* (2015). Originally, these strains were previously isolated from *H. itama honey* from various local honey beekeeper in Kelantan and Terengganu, located at the East Coast of Peninsular Malaysia. Before use, *L. brevis* strains were subcultured onto agar plate of de Man, Rogosa and Sharpe (MRS) (Merck, Germany). The cultures were maintained on slant agar supplemented with 20% glycerol and kept in the freezer at 4°C and -20°C.

Susceptibility of *Lactobacillus brevis* against antibiotics

The strains isolated from *H. itama* honey were screened for their antibiotic susceptibility by disk diffusion method using antibiotic disc. The strains were grown in MRS broth incubated at 37°C for 18 h. The culture suspensions were swabbed onto MRS agar plates. Antibiotic discs (Oxoid) consisted of chloramphenicol (30 µg), ampicillin (10 µg), erythromycin (15 µg), tetramycin (30 µg), and kanamycin (30 µg) were placed on MRS agar plates and incubated at 37°C for 48 h. The inhibition zone surrounds the antibiotic disk was measured using a caliper. The assay was performed in triplicate.

Antagonistic activity of *L. brevis* against other *L. brevis* strain using overlay method

The *Lactobacillus* strains were tested for antagonistic activity against each other in order to evaluate the ability of each strain of *L. brevis* to inhibit other *L. brevis* strain. This is very important in the development of multi starter cultures for food products. The fresh 24 h cultures of indicator organism was spotted onto MRS agar plates (first layer) and incubated for 48 h in CO_2 incubator. After incubation, the fresh molten MRS agar was poured and over-layered on the top of the first layer with the producer strain. The culture plates were placed in CO_2 incubator under anaerobic condition. The ability of producer organism to inhibit the indicator organism was measured via inhibition zone surrounding the indicator strain colonies (El Soda *et al.,* 2003).

Antibacterial activity of LAB isolates against food borne pathogens

Five pathogenic bacteria (*Bacillus cereus* ATCC 11778*, Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 25923, *Salmonella* Thyphi (clinical strain from local hospital) and *Pseudomonas aeruginosa* ATCC 15442*)* were cultured in Mueller-Hinton broth (Merck, Germany) at 37°C for 18 h and swabbed onto Mueller-Hinton agar (Merck, Germany) using sterile cotton swab. The 24 h cultures of LAB were sub-cultured into MRS broth and incubated for 24 h in CO_2 incubator. Then, the cell-free supernatant was obtained from centrifugation of the isolated LAB at 12 000 rpm for 10 min at -4°C. The plate agar containing the pathogen was punched in to make agar well (5 mm of diameter) using a sterile stainless steel borer. The agar wells were filled with 70 µl of each *L. brevis* cell-free supernatant with the concentration of 100 µg/ml per well and the culture plates were incubated at 30°C overnight. Commercial antibiotic discs consisted of chloramphenicol (30 µg), ampicillin (10 µg), erythromycin (15 µg), tetramycin (30 µg), and kanamycin (30 µg) were used as positive control. The antimicrobial activity was expressed as the diameter of the inhibition zone (mm) around the well (Boyanova *et al.,* 2005). Zones of inhibition more than 10 mm were regarded as positive (Georgieva *et al*., 2015).

Resistance of *L. brevis* strains to low pH

The resistance of *L. brevis* strains to low pH were evaluated based on the ability of this micro-organism to survive at pH 2 according to the method described by Thirabunyanon *et al.* (2009). One ml from each overnight pure strains was mixed with 9 ml of MRS broth that had already adjusted to pH 2 and pH 7.2 (control) and incubated at 37°C for 3 h incubation period. The viable population of *L. brevis* strains (log_{10} CFU/ml) were determined using serial dilution method by spreading on MRS agar and placed in CO_2 incubator at 37°C for 48 h. The experiment was performed in triplicates and calculated in form of bacterial cell counts (log_{10} CFU/ml).

Tolerance of *L. brevis* strains to Bile Salts

The method described by Thirabunyanon *et al.* (2009) was used to determine the tolerance of *Lactobacillus* strains to bile salts condition. One ml from each overnight pure strain was inoculated in 9 ml of MRS broth supplemented with 0.3% of bile salts (treatment culture) and MRS broth only (control culture) and incubated for 4 h of incubation period. The viable strains (log_{10} CFU/ml) were determined using serial dilution method by spreading on MRS agar and placed in CO_2 incubator at 37°C for 48 h. The experiment was performed in triplicates and the survivor of bacterial cell counts were determined as log_{10} CFU/ml.

The haemolytic activities

The haemolytic activity of *L. brevis* strains was determined according to Maragkoudakis *et al.* (2009). All strains were separately grown in MRS broth at 37°C for 24 h. Then, the single colony of each strain was streaked onto Columbia agar base (Oxoid, UK) plates incorporated with 5% (v/v) sheep blood. The plates were incubated anaerobically at 37°C for 48 h. Then, the clear zones and the colour of haemolysis around the growth colonies were observed. The experiment was performed in triplicates. The plates were observed for signs of β-haemolysis (clear zones around colonies), α-haemolysis (green-hued zones around colonies) or γ-haemolysis (no zones around colonies).

RESULTS AND DISCUSSION

Susceptibility of *L. brevis* strains against antibiotics

The results obtained for antibiotic resistance of the five *L. brevis* strains isolated from *H. itama* honey is shown in Table 1. The results indicated that all strains of *L. brevis* were susceptible to chloramphenicol, ampicillin, erythromycin and tetramycin but resistant to kanamycin. Results showed that *L. brevis* NJ42 was found to be the most susceptible compared to other *L. brevis* strains towards erythromycin and chloramphenicol with inhibition zone of 30.0 ± 0.0 mm and 29.00±0.0 mm as shown in Figure 1, respectively. These result also was accordance to Mourad and Nour-Eddine, (2006) which found that most strains of *Lactobacillus* sp. were susceptible to most of antibiotics that belonged to the major classes of antibiotics used in human clinical therapy. Resistance against kanamycin had been observed more frequently among *Lactobacillus* sp. (Zhou *et al.,* 2005). These result also similar to the study done by Argyri *et al*. (2013) which reported that the majority of LAB isolates including *Leuconostoc* strains, *L. plantarum* strains, *L. pentosus* strains, *L. paracasei* subs. *paracasei* strains and *L. rhamnosus* GG were characterized to be resistant to kanamycin. As *L. brevis* strains only resistant to kanamycin and susceptible to most of antibiotics, it is suggested that *L. brevis* is a good potential as probiotic for food industry.

Antagonistic activity of *L. brevis* with other *L. brevis* strains

The *L. brevis* strains were also tested for antagonistic activity against each other. The ability of producer strain of *L. brevis* to inhibit indicator strain of *L. brevis* was observed and summarized in

Table 1. Susceptibility of *L. brevis* strains against antibiotics determined by well diffusion method

Antibiotics	Diameter of inhibition zone (mm)*				
	L. brevis strain Ibr-42	*L. brevis* strain 37901	*L. brevis* ATTC 367	*L. brevis* strain NJ42	*L. brevis* strain KLDS
Ampicillin	26.3 ± 0.3	23.3 ± 0.3	24.8 ± 0.3	30.8 ± 0.3	24.3 ± 0.3
Erythromycin	23.4 ± 0.5	23.0 ± 0.0	24.8 ± 0.3	30.0 ± 0.0	24.3 ± 0.3
Chloramphenicol	24.0 ± 0.0	25.2 ± 0.2	27.3 ± 0.3	29.0 ± 0.0	29.2 ± 0.2
Tetracycline	25.2 ± 0.2	17.0 ± 0.0	19.3 ± 0.3	15.9 ± 0.1	19.8 ± 0.3
Kanamycin	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
Control (distilled water)	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition

*Each result was tabulated from the mean of three independent experiments.

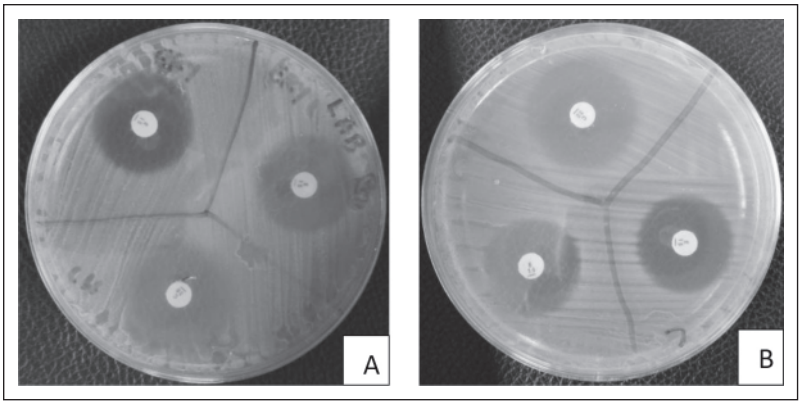

Fig. 1. MRS plates showing inhibition zone of *Lb. brevis* strain NJ42 against erythromycin (A) and chloramphenicol (B).

Table 2. Antagonistic test of *L. brevis* strains isolated from *H. itama* honey against other *L. brevis* as measured by inhibition zone

L. brevis	Diameter of clear zone (mm)*				
	L. brevis strain lbr-42	*L. brevis* strain 37901	*L. brevis* ATTC 367	*L. brevis* strain NJ42	*L. brevis* strain KLDS
L. brevis strain lbr-42		2.8±0.28	3.85±0.21	2.9±0.14	3.9±0.14
L. brevis strain 37901	2.9±0.14		3.9±0.14	3.1±0.14	3.95±0.07
L. brevis ATCC 367	2.8±0.28	2.9±0.14		3.2±0.28	3.9±0.21
L. brevis strain NJ42	2.85±0.21	2.8±0.28	3.75±0.35		3.85±0.21
L. brevis strain KLDS	2.85±0.21	2.85±0.21	3.8±0.28	3.0±0.0	

*Each result was tabulated from the mean of three independent experiments.

Table 2. Although the inhibition zones were small, ranging from 2.8 mm to 3.95 mm, the results confirms that each *Lb. brevis* can inhibit other *L. brevis*. This characteristic confirms that all *L. brevis* strains were able to produce bacteriocin, which was active against the lactic acid bacteria itself (El Soda *et al.,* 2003). As the inhibition against other LAB was small, this characteristic is important in the development of food products that require multiple starter cultures, in order to reduce the inhibition against each other.

Antibacterial activity of *L. brevis* strains against food-borne pathogens

The antibacterial activity of *L. brevis* strains against foodborne pathogens (*B. cereus, S. typhi, P. aeruginosa, E. coli* and *S. aureus*) is shown in Table 3. Five strains of *L. brevis* strains exhibit inhibition zone ranging from 11 mm to 24 mm against tested foodborne pathogenic bacteria. The highest inhibition was found to be *L. brevis* strain NJ42 against *P. aeruginosa* with 24 mm as shown in Figure 2 compared to commercial antibiotics (kanamycin, chloramphenicol, tetracycline and erythromycin) with inhibition zones of 19.5 mm, 15.0 mm, 13.0 mm and 10.0 mm, respectively. For inhibition of *B. cereus*, *L. brevis* strain KLDS and NJ42 showed the lowest inhibition with 11.3 mm, whereas commercial antibiotics showed greater inhibition than all *L. brevis* strains against *B. cereus*. Previous study by Mohd Hasali *et al*. (2015) reported that *L. brevis* exhibited a good antibacterial activity against *S. epidermis, P. aeruginosa* and *L. monocytogenes* with inhibition zones of 32 mm, 16 mm and 18 mm, respectively.

In the present study, *P. aeruginosa* was found to be resistant to ampicillin, which is classified as broad spectrum anti-pseudomonal antibiotics. *Pseudomonas* infection can be treated with a combination of an anti-pseudomonal beta-lactam and aminoglycoside. *P. aeruginosa* resistance towards antibiotics was reported by Akpaka *et al.* (2002) who found *P. aeruginosa* to be the second most common isolate among patients with over 30% of the isolates being resistant to the standard anti-pseudomonal antibiotics. Interestingly, *L. brevis* strain NJ42 showed greater inhibition against *P. aeruginosa* than available commercial antibiotics (kanamycin, chloramphenicol, tetracycline and erythromycin).

LAB are known as natural antibacterial agents that have ability to produce various metabolites such as bacteriocins, hydrogen peroxide and organic acid (Lawalata *et al.,* 2011). The production of

Table 3. Antibacterial activity of isolated *Lb. brevis* from H. itama honey against pathogenic bacteria

Reference cultures of ATCC Bacteria	Zone of inhibition (mm)				
	Bacillus cereus ATCC 11778	*Salmonella typhi*	*Pseudomonas aeruginosa* ATCC 15442	*Escherichia coli* ATCC 11775	*Staphylococcus aureus* ATCC 25923
L. brevis strain Ibr-42	12.3 ± 1.1	15.00 ± 0.0	17.0 ± 1.7	15.7 ± 1.1	15.0 ± 1.0
L. brevis strain 37901	12.3 ± 2.0	12.67 ± 0.5	16.7 ± 0.5	18.7 ± 0.5	14.0 ± 0.0
L. brevis ATCC 367	13.7 ± 0.5	13.00 ± 1.7	17.67±0.5	18.3 ± 0.5	12.3 ± 0.5
L. brevis strain NJ42	11.3 ± 0.5	14.33 ± 0.5	24.00±0.0	19.3 ± 0.5	13.0 ± 0.0
L. brevis strain KLDS	11.3 ± 1.5	16.33 ± 0.5	17.33±0.5	19.3 ± 0.5	12.3 ± 0.5
Ampicillin	13.5 ± 0.7	33.0 ± 0.0	No inhibition	18.0 ± 0.0	38.5 ± 0.7
Erythromycin	34 ± 0.0	24.0 ± 0.0	10.0 ± 0.0	11.0 ± 0.0	27.0 ± 0.0
Chloramphenicol	27.0 ± 0.0	34.0 ± 0.0	15±0.0	32.0 ± 0.0	23.0 ± 0.0
Tetracycline	35.5 ± 0.7	32.0 ± 0.0	13±0.0	34.5 ± 0.7	37.0 ± 0.0
Kanamycin	25.0 ± 0.0	24.0 ± 0.0	19.5±0.7	24.5 ± 0.7	24.0 ± 0.0
Control (distilled water)	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition

*Each result was tabulated from the mean of three independent experiments.

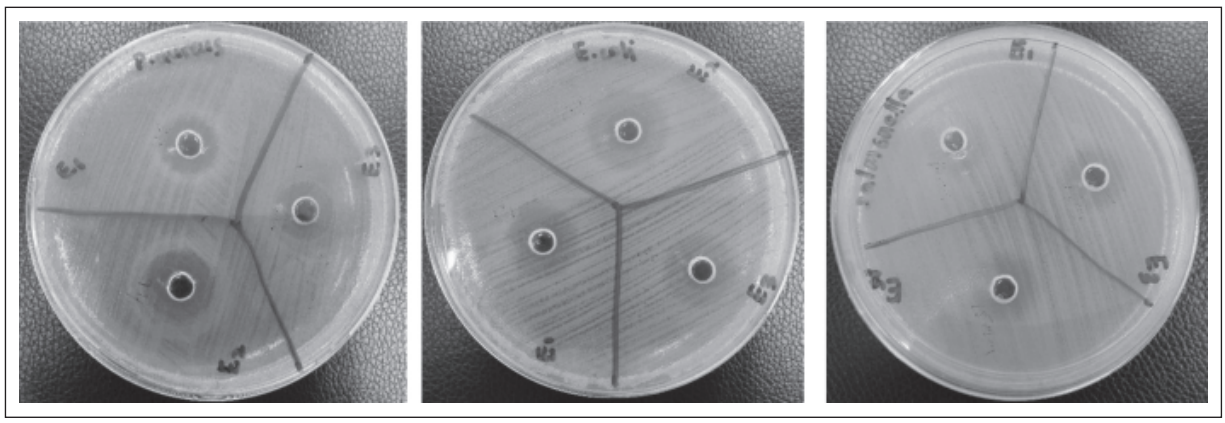

Fig. 2. Inhibition zone of antibiotic resistant of *L. brevis* strain NJ42 against *P. aeruginosa*, *E. coli* and *S. typhi*.

antibacterial compounds is the most important functional properties used to characterize good probiotics (FAO & WHO, 2006). These results showed that *L. brevis* strains exhibit antibacterial properties that can be used as natural preservative, especially in dairy food products.

Resistance of *L. brevis* strains to low pH

Survival of *Lactobacillus* strains to low pH condition is one of the important criteria for probiotics strains (Cakir, 2003). Before reaching the small intestinal tract, the microorganisms must pass through the highly acidic stomach conditions where the pH can be as low as 1 to 2 in order to exert their optimal beneficial probiotic effects in the human host (Dunne *et al.,* 2001). The results of tolerance to acidic pH is shown in Figure 3. All *L. brevis* strains were able to survive in pH 2 during 3 h of incubation, which represented time taken during the digestion of food in stomach. The result was calculated based on viable cell count (CFU/mL) that *L. brevis* strains were able to survive in pH 2 and maintain the bacterial population between 5-6 $\log_{10}$ CFU/ml during 3 h incubation. The viability of *Lactobacillus* strains in pH 2 decreased slightly (about 2 to 3 $\log_{10}$ reduction) compared to control group of MRS agar without acidic treatment (pH 7) but the survival rate was more than 50%, which was considered as intrinsically tolerant to gastric transit site (Ashraf & Smith, 2016). Our finding was supported by Argyri *et al*. (2013), who reported that nine strains of *L. plantarum, L. pentosus* and *L. paracasei* subsp. *paracasei* showed very high resistance to low pH with final populations exceeding 8 $\log_{10}$ CFU/ml whereas another 12 strains showed high resistance to low pH with final populations were within 6-8 $\log_{10}$ CFU/ml during 3 h incubation.

Resistance of *L. brevis* strains to high bile salts

One of the important characteristic of probiotic is the ability of microorganism to withstand high bile salts concentration that mimic the human digestive system. This study screened five *L. brevis*

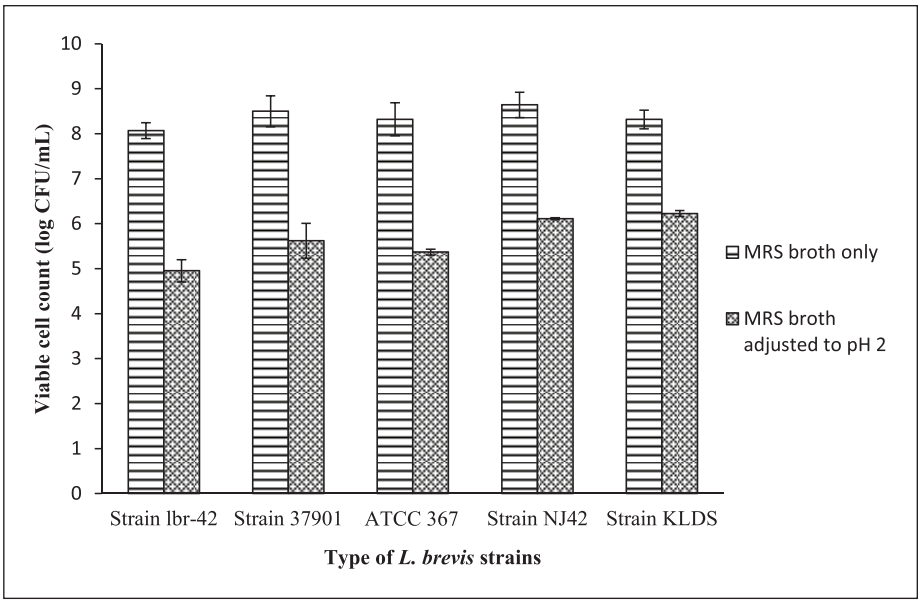

Fig. 3. Resistance of *L. brevis* strains to low pH after 3 h incubation (Error bars indicate standard deviation from three replications).

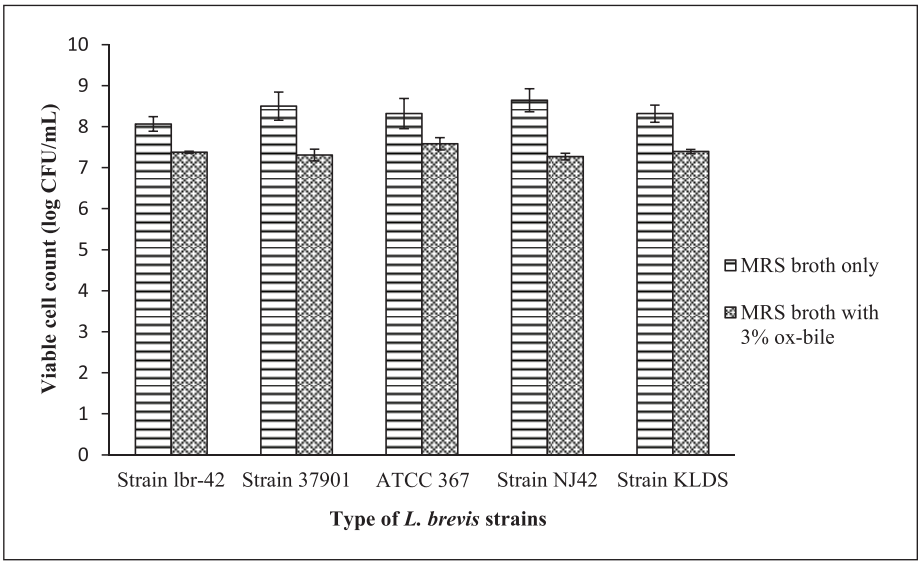

Fig. 4. Resistance of *L. brevis* strains to bile salts (3% ox-bile) after 4 h exposure (Error bars indicate standard deviation from three replications).

strains to tolerate high bile salt condition (approximately 0.3% (w/v) concentration of bile salt) as suggested by Mourad and Nour-Eddine (2006). As displayed in Figure 4, all *L. brevis* strains were highly resistant to 0.3% of bile salt environment during 4 h incubation period, with growth above $\log_{10}$ 7 (CFU/ml). Gilliland *et al.* (1984) reported that survival rate of *Lactobacillus* strains towards bile salts differed depending on the sources of the strains. The resistance of bile salts also reported by Abriouel *et al.* (2012) who reported that all the LAB isolated from fermented olive were able to grow and survive in high bile salt concentration.

The haemolytic activities

Five strains of *L. brevis* were tested on their ability to induce haemolysis *in vitro* when grown on the sheep blood agar. Non-haemolytic activity is considered as a safety prerequisite for a probiotic to be use in food products. Haemolysis can be defined as a known virulence factor among pathogenic microorganism. This test is important to indicate that *L. brevis* did not have haemolytic activity, therefore lack of this virulence factor. The result indicated that all *L. brevis* strains did not exhibited β-haemolytic activity when grown in blood agar contained 5% of sheep blood. Only *L.*

brevis strain lbr-42 exhibited α-haemolytic (partial haemolysis) while the others were γ-haemolytic, which can be classified as non-haemolysis strains. Our results were supported by Maragkoudakis *et al.* (2010) who reported that food containing *Lactobacillus* strains was rarely to have bacteria that possesses haemolysis activity.

CONCLUSION

Five *L. brevis* strains isolated from *H. itama* honey were resistant to kanamycin and had good anti-bacterial activities against food-borne pathogens. These strains could be considered as appropriate probiotic candidates due to their resistance against low pH (pH 2) and 0.3% bile salts concentration as well as did not exhibited β-haemolysis activity. They also could be used as adjunct cultures to improve the quality and nutritional value of dairy products.

ACKNOWLEDGMENT

Authors thank the Ministry of Higher Education, Malaysia who had financially funded this project through the FRGS research project Vot No. 59329 and the scholarship of MyBrain awarded to the first author.

REFERENCES

Abriouel, H., Benomar, N., Cobo, A., Caballero, N., Fernández Fuentes, M.A., Pèrez-Pulido, R. & Gálvez, A. 2012. Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. *Food Microbiology*, **32(2)**: 308-316

Akpaka, P.E., Sue-Ho & Bodonaik, N.C. 2002. Frequency of occurrence and antibiotic resistance in bacteria isolated from patients in the Intensive Care Unit. *West Indian Medicine Journal*, **51 (Suppl 3):** 17.

Argyri, A.A., Zoumpopoulou, G., Karatzas, K.A., Tsakalidou, E., Nychas, G.J., Panagou, E.Z. & Tassou, C.C. 2013. Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests. *Food Microbiology*, **33**: 282-291

Ashraf, R. & Smith, S.C. 2016. Commercial lactic acid bacteria and probiotic strains-tolerance to bile, pepsin and antibiotics. *International Food Research Journal,* **23(2)**: 777-789.

Aween, M.M., Hassan, Z., Muhialdin, B.J., Eljamel, Y.A., Al-Mabrok, A.S.W. & Lani, M.N. 2012. Antibacterial activity of *Lactobacillus acidophilus* strains isolated from honey marketed in Malaysia against selected multiple antibiotic resistant (MAR) Gram positive Bacteria. *Journal of Food Science*, **77(7**): M364-371.

Boyanova, L., Gergova, G., Nikolov, R., Derejian, S., Lazarova, E., Katsarov, N. & Krastev, Z. 2005. Activity of Bulgarian propolis against 94 *Helicobacter pylori* strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods. *Journal of Medical Microbiology,* **54(5)**: 481-483.

Bulgasem, B.Y., Hassan, Z., Huda-Faujan, N., Omar, R.H.A., Lani, M.N. & Alshelmani, M.I. 2017. Effect of pH, heat treatment and enzymes on the antifungal activity of lactic acid bacteria against *Candida* species. *Malaysian Journal of Microbiology*, **13(3)**: 195-202.

Bulgasem, B.Y., Lani, M.N., Hassan, Z., Wan Yusoff, W.M. & Fnaish, S.G. 2016. Antifungal activity of lactic acid bacteria strains isolated from natural honey against pathogenic *Candida* species. *Mycobiology*, **44(4)**: 302-309.

Cakir, I. 2003. Determination of some probiotic properties on *Lactobacilli* and *Bifidobacteria. PhD Thesis*, Ankara University, Turkey.

Cross, M.L. 2002. Microbes versus microbes: Immune signals generated by probiotic *lactobacilli* and their role in protection against microbial pathogens. *FEMS Immunology and Medical Microbiology,* **34(4)**: 245-253.

Dunne, C., Mahony, L.O., Murphy, L., Thornton, G., Morrissey, D., Halloran, S.O. & Collins, J.K. 2001. *In vitro* selection criteria for probiotic bacteria of human origin/: Correlation with *in vivo* findings. *American Society for Clinical Nutrition*, **73(2)**: 386-392.

El Soda, M., Ahmed, N., Omran, N., Osman, G. & Morsi, A. 2003. Isolation, identification and selection of lactic acid bacteria cultures for cheesemaking. *Emirates Journal of Food and Agriculture*, **15(2)**: 51-71.

FAO & WHO. 2006. Probiotics in food. *Food and Nutrition Paper*, **85**: 71.

Gardiner, G.E., Heinemann, C., Baroja, M.L., Bruce, A.W., Beuerman, D. & Reid, G. 2002. Oral administration of the probiotic combination *Lactobacillus rhamnosus* GR-1 and *Lacto-bacillus fermentum* RC-14 for human intestinal applications. *International Dairy Journal,* **12**: 191-196.

Gilliland, S.E., Staley, T.E. & Bush, L.J. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *Journal of Dairy Science*, **67**: 3045-3051.

Gomes, D.C.A., Buriti, F.C.A., De Souza, C.H.B., Faria, J.A.F. & Saad, S.M.I. 2009. Probiotic cheese: Health benefits, technological and

stability aspects. *Trends Food Science Technology*, **20(8)**: 344-354.

Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A., Danguleva, A., Ivanova, G., Karapetkov, N., Rumyan, N. & Karaivanova, E. 2015. Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifodobacterium* spp. intended for use as starter and probiotic cultures. *Biotechnology & Biotechnological Equipment,* **29(1)**: 84-91.

Ibarguren, C., Raya, R.R., Apella, M.C. & Audisio, M.C. 2010. *Enterococcus faecium* isolated from honey synthesized bacteriocin-like substances active against different *Listeria monocytogenes* strains. *Journal of Microbiology,* **48(1)**: 44-52.

Iñiguez-Palomares, C., Perez-Morales, R. & Acedo-Felix, E. 2008. Evaluation of probiotic properties in *Lactobacillus* isolated from small intestine of Piglets. *Revista Latinoamericana de Microbiologia*, **49(3–4)**: 46-54.

Lawalata, H.J., Sembiring, L. & Rahayu, E.S. 2011. Molecular identification of lactic acid bacteria producing antimicrobial agents from Bakasang, An indonesian traditional fermented fish product. *Indonesian Journal of Biotechnology*, **16(2)**: 93-99.

Lee, H., Churey, J.J. & Worobo, R.W. 2008. Antimicrobial activity of bacterial isolates from different floral sources of honey. *International Journal of Food Microbiology*, **126(1–2)**: 240-244.

Macfarlane, G.T. & Cummings, J.H. 2002. Probiotics, infection and immunity. *Current Opinion in Infectious Diseases*, **15**: 501-506.

Maragkoudakis, P.A., Mountzouris, K.C., Rosu, C., Zoumpopoulou, G., Papadimitriou, K., Dalaka, E. & Tsakalidou, E. 2010. Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats – a preliminary study. *International Journal of Food Microbiology,* **141**: 109-116.

Maragkoudakis, P.A., Papadelli, M., Georgalaki, M., Panayotopoulou, E.G., Martinez-Gonzalez, B., Mentis, A.F. & Tsakalidou, E. 2009. *In vitro* and *in vivo* safety evaluation of the bacteriocin producer *Streptococcus macedonicus* ACA-DC 198. *International Journal of Food Microbiology*, **133(1–2)**: 141-147.

Mohd Hasali, N.H., Zamri, A.I., Lani, M.N., Mubarak, A. & Suhaili, Z. 2015. Identification of lactic acid bacteria from Meliponine honey and their antimicrobial activity against pathogenic bacteria. *American-Eurasian Journal of Sustainable Agriculture,* **9(6)**: 1-6.

Mojgani, N., Hussaini, F. & Vaseji, N. 2015. Characterization of indigenous *Lactobacillus* strains for probiotic properties. *Jundishapur Journal of Microbiology*, **8(2)**: e17523.

Mourad, K. & Nour-Eddine, K. 2006. *In vitro* preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. *International Journal of Probiotics and Prebiotics,* **1(1)**: 27-32.

Parvez, S., Malik, K.A., Kang, S.A. & Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology,* **100**: 1171-1185.

Prasad, J., Gill, H., Smart, J. & Gopal, P.K. 1998. Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. *International Dairy Journal*, **8**: 993-1002.

Roowi, S., Muhamad, S.A., Sipon, H., Jaafar, M.F., Daud, M.N.H. & Othman, R. 2012. Asid fenolik bebas dalam madu *H. itama*. *Buletin Teknologi MARDI*, **2**: 145-147.

Saarela, M., Mogensen, G., Fondén, R., Mättö, J. & Mattila-Sandholm, T. 2000. Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology,* **84(3)**: 197-215.

Siamansouri, M., Mozaffari, S. & Alikhani, F. 2013. Bacteriocins and lactic acid bacteria. *Journal of Biology and Today's World*, **2**: 227-234.

Tannock, G.W. 2004. Mini reviews: A special fondness for *Lactobacilli*. *American Society for Microbiology,* **70(6)**: 3189-3194.

Thirabunyanon, M., Boonprasom, P. & Niamsup, P. 2009. Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnology Letters,* **31(4)**: 571-576.

Vasudha, S. & Mishra, H.N. 2013. Non dairy probiotic beverages. *International Food Research Journal*, **20(1)**: 7-15.

Vásquez, A., Forsgren, E., Fries, I., Paxton, R.J., Flaberg, E., Szekely, L. & Olofsson, T.C. (2012) Symbionts as major modulators of insect health: Lactic acid bacteria and honeybees. *PLoS ONE*, **7(7)**: 10.1371/annotation/3ac2b867-c013-4504-9e06-bebf3fa039d1.

Yang, E., Fan, L., Jiang, Y., Doucette, C. & Fillmore, S. 2012. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express,* **2(1)**: 48.

Zhou, J.S., Pillidge, C.J., Gopal, P.K. & Gill, H.S. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *International Journal of Food Microbiology*, **98(2)**: 211-217.