BIODIVERSITAS
Volume 23, Number 12, December 2022
Pages: 6379-6385

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231235

The screening of probiotic lactic acid bacteria from honey of stingless bee from West Sumatra, Indonesia and using as starter culture

SRI MELIA♥, SALAM N. ARITONANG, INDRI JULIYARSI, YULIANTI FITRI KURNIA, RUSDIMANSYAH, VIRA OKTAVIA HERNITA

Faculty of Animal Science, Universitas Andalas. Jl. Raya Unand, Limau Manis, Padang 25163, West Sumatra, Indonesia.
Tel./fax.: +62-751-71464, ♥email: srimelia75@ansci.unand.ac.id

Manuscript received: 5 September 2022. Revision accepted: 11 December 2022.

Abstract. *Melia S, Aritonang SN, Juliyarsi I, Kurnia YF, Rusdimansyah, Hernita VO. 2022. The screening of probiotic lactic acid bacteria from honey of stingless bee from West Sumatra, Indonesia and using as starter culture. Biodiversitas 23: 6379-6385.* This study aimed to isolate probiotic lactic acid bacteria (LAB) from stingless bee honey (Galo-galo) and apply it as a starter culture. This research used stingless bee honey (Galo-galo) from west Sumatra, Indonesia. There are 6 types of honey bees, namely *Tetragonula sarawakensis (TS), Tetragonula testaceitarsis (TT), Tetragonula minangkabau (MK), Tetrigona binghami (TB), Geniotrigona thoracica (TR)* and *Heterotrigona itama (IT).* The screening of probiotics was evaluated as the LAB isolates' ability to resist acid (pH 3), bile salts (0xgall 0.5%), and antimicrobial activity against pathogenic bacteria. Based on the probiotic selection, the TB1 isolate from *Tetrigona binghami* honey had an acid tolerance of 82.75%, bile salt tolerance of 94.44%, and the highest antimicrobial activity against pathogenic bacteria with more excellent resistance than antibiotics. The identification with 16S rRNA showed that the TB1 bacterial isolate had 100% similarity with *Lactobacillus plantarum* SN13TFurthermore, starter culture inoculated with 6% *L.plantarum* SN13T met the starter culture parameters, with a pH of 4.7, titratable acidity of 1.46%, and lactic acid bacteria counts of 9.0 x 10^9 CFU/mL. *L.plantarum* SN13T is a candidate probiotic bacteria isolated from the stingless bee honey (*Tetrigona binghami*). This bacteria has the potential to be developed as a starter for fermented milk processing in the food industry.

Keywords: Fermented milk, Galo-galo, *Lactobacillus plantarum* SN13T, probiotic, stingless bee

INTRODUCTION

In Indonesia, the stingless bee has vernacular names in each region, such as *Klanceng* from Java, *Teuweul* from Sunda, *Galo-galo* from Minang, West Sumatra, *Merang* or *Katappe* from Mamasa, West Sulawesi, and *Tannese* from Kaili, Central Sulawesi (Kahono et al. 2018). The stingless bee, known as the Getah lebah in Indonesia, has a high diversity of about 46 species distributed in several areas, such as Sumatra and Kalimantan (Engel et al. 2019). There are 18 stingless bee species were discovered at various beekeeping and natural sites in West Sumatra (Herwina et al. 2020). Then Herwina et al. (2021) explain that Heterotrigona itama is the most popular species for beekeeping, followed by *Geniotrigona thoracica* and *Tetragonula laeviceps*, while *T. minangkabau*, *T. fuscobalteata*, and *T. testaceitarsis* are the least popular. In contrast to honey bees, nearly all species of stingless bees could be transferred into hives, and they are unlikely to leave or run. Meliponiculture is well-established in many nations (Cortopassi-Laurino et al. 2006), and Indonesia has great potential to exploit these species sustainably and fully.

Stingless bees produce several components such as wax, propolis, honey, and pollen (Ajibola et al. 2012). Several studies have shown that stingless bee honey has potential therapeutic benefits in several contexts, including wound healing, diabetes mellitus, eye disease, hypertension, fertility defects, cancer, microbial infections, and irregular lipid profiles (Zulkhairi Amin et al. 2018). Rosli et al. (2020) explained in a previous study that *Homotrigona fimbriata* honey has the highest antibacterial activity against five bacteria: *Serratia marcescens, Escherichia coli, Bacillus subtilis, Alcaligenes faecalis,* and *Staphylococcus aureus.*

It has been reported that stingless bee honey contains various microorganisms, namely some eight bacterial species, 71 families, 155 genera, and 70 species with antimicrobial activity against pathogenic bacteria. One of the dominant lactic acid bacteria found in almost all species studied is *Lactobacillus malefermentans*. Thus, the presence of lactic acid bacteria enhanced the ability of bee honey to inhibit pathogenic bacteria without stinging can be expected to expand the probiotic capabilities in daily life (Rosli et al. 2020).

Probiotics are living bacteria or other types of microorganisms that, when consumed in enough amounts, impose a wide variety of health benefits on the creatures that consume them (Aarti et al. 2016). These bacteria have long been associated with dairy products. This is because some of the bacteria related to fermented dairy products also live in various locations on the human body, such as the mouth, gastrointestinal tract, etc. Most of the probiotic bacteria used in commercial products are lactic acid bacteria, namely species of the *Lactobacillus* and *Bifidobacterium* (Salminen et al. 2004). Gram-positive bacteria, known as lactic acid bacteria, can be found in the digestive tracts of humans and ruminants. They are naturally present in these systems (Aarti

et al. 2018). These microorganisms are essential in preserving the microbial flora's delicate equilibrium (Aarti et al. 2017; Kerry et al. 2018).

The bacteria obtained was used as a starter culture in milk processing. Starter cultures are microorganisms that are employed in the production of dairy products such as yogurt and cheese. The microbes chosen for this purpose are intended to produce the required results in the fermented milk processing final product. When the starter culture is put into milk, the features of the fermented milk produced are more controlled.

Based on this background, a study was conducted to select and identify lactic acid bacteria molecularly using the 16S rRNA method from West Sumatra, Indonesian stingless bee honey, and its use as a starter culture. Furthermore, this research is expected to add to the collection of probiotic bacteria, particularly those from stingless honey bees, which are commonly consumed for their health benefits and can be developed as a fermented milk starter for the food industry.

MATERIALS AND METHODS

Sampling

Total six types of honey from stingless bee species from West Sumatra, Indonesia, such as *Tetragonula sarawakensis*, *Heterotrigona itama*, *Tetragonula testaceitarsis*, *Tetragonula minangkabau*, *Geniotrigona thoracica*, *Tetrigona binghami* were collected from stingless beekeeping at the Faculty of Animal Science, Andalas University, Padang, Indonesia. All honey samples were filtered and stored in bottles at room temperature for further analysis.

Procedures

Isolation of lactic acid bacteria.

One mL of honey sample was added to 9 mL of De Man Rogosa and Sharpe (MRS) broth into On MRS agar (Neogen, USA), serial dilutions were made to a concentration of 10^{-8}, and the samples were incubated anaerobically for 48 hrs at 37ºC. A single colony was selected randomly for other probiotic selection (Klingberg et al. 2005).

Probiotic characterization

Test for acid tolerance

Total of 0.1 mL of LAB isolate was added to 10 mL of pH 3.0 MRS broth, and the mixture was then incubated for 90 min. After incubation, serial plating of the dilution on MRS agar was used to determine how many live bacteria were present. By comparing LAB isolates on MRS agar (NEOGEN, USA) for cells that survived after incubation at pH 3.0 for 90 min, LAB viability to acid was estimated. After 0 and 90 min of incubation, the survival rate (%) on MRS agar was calculated using the plate count method (Klingberg et al. 2005). Each determination was made in duplicate. The formula (N/N0) x 100% was used to calculate the survival rate (%) by comparing the number of bacteria (N) after incubation to the number of surviving bacteria (N0).

Test for bile salts tolerance

It was carried out in acid-resistant conditions for viability testing of LAB against bile salts (pH 3.0, 90 min). It was inoculated into 10 mL of MRS broth with and without 0.3% oxgall bile salt (Sigma, USA) and cultured for 8 hrs using as little as 0.1 mL of the LAB isolate that had been incubated for 24 hrs at 37ºC. After 0 and 5 hrs of incubation, the rate of survival (%) on MRS agar was calculated using the plate count method Klingberg et al. (2005). The formula (N/N0) x 100% was used to calculate the survival rate (%) by comparing the number of bacteria (N) after incubation to the number of surviving bacteria (N0).

Antimicrobial Activity test

Based on Davoodabadi et al. (2015) using the diffusion method, the antimicrobial activity of the LAB isolate against a number of enteropathogenic bacteria was evaluated. *Listeria monocytogenes* VTO, *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Salmonella*, *Propionibacterium acnes*, *Pseudomonas* sp., *Klebsiella* sp., *Acinetobacter baumannii*, and *Escherichia coli*. LAB isolates were cultured overnight at 37ºC for 24 hrs. Using centrifugation, cell-free culture supernatant (CFCS) was produced (10,000 g, 10 min). A 100 mL of CFCS was added to an 8 mm diameter, perforated well on a Muller Hinton agar plate, and incubated there for 18-24 hrs at 37°C. A clean zone formed around the well provided was considered an indicator of antimicrobial action, and its diameter was determined.

Molecular characterization using 16S rRNA gene sequencing

The genomic DNA of bacteria was extracted using the PrestoTM Mini gDNA bacteria kit (GBB100 Geneaid). The isolated DNA was amplified at using 24F: S′ AGA GTT TGA TGG CT 3′ and 1541R: S′ AAG GAG GTG ATC CCG CA 3′. The PCR procedure required 50 µl of water, bacteria DNA, and DreamTaq DNA polymerase (Thermo Scientific). We began with a 3-minute pre-PCR. Total 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 min 30 seconds. The further 10 min of post-PCR were conducted at 72°C. The PCR results were loaded onto 1% agarose gel containing 5 g/mL of ethidium bromide. The electrophoresis was done for 45 min in a 1X TBE (Tris Borate EDTA) buffer. The bands were then seen on a UV transilluminator (Vilber Lourmat) and recorded with an Olympus SP 500-UZ digital camera with a UV filter (Feliatra et al. 2019). Furthermore, using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.html), the sequencing results were compared to known sequences in the GenBank database.

The production of a starter culture

As much as 20% of skim milk and 0.05% of CMC (Carboxy Methyl Cellulose) were added to the Erlenmeyer, along with 100 mL of distilled water, and stirred using a magnetic stirrer for 5 min at a speed of 1000 rpm during the manufacture of fermented milk (Pato et al. 2019). The homogeneous solution was then sterilized at 105ºC for 10

min and cooled to 37ºC. The LAB isolates of as much as 3%, 4%, 5%, and 6% were entered into the skim milk and incubated at 37ºC for 15 hrs.

pH testing and titratable acidity

A digital pH meter measured the pH of yogurt samples between 17°C and 20°C (Hanna Instruments). The titratable acidity (lactic acid,%) was measured after mixing yogurt samples, mixing distilled water of 10 mL, and titrating with 0.1 N NaOH that used a 0.5% phenolphthalein indicator. The total dry matter of yogurt and milk samples was then determined gravimetrically (Čakmakči et al. 2012).

Calculation of total lactic acid bacteria

A total of 1 mL of material was mixed with 9 mL of sterile peptone diluent (10% w/v). The appropriate dilutions were then poured in duplicate onto MRS medium plates. Samples were incubated for 48 hrs at 37ºC (Othman et al. 2012).

Data analysis

All studies were performed in triplicate, one-way analysis of variance (ANOVA) was used to evaluate significant differences, SPSS software was used to run Duncan's multiple range tests.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria

Six different types of honey from stingless bee species were used to isolate and identify potentially probiotic lactic acid bacteria. They are *Tetragonula sarawakensis* which produced six isolates (TS1-TS6), *Heterotrigona itama*, which had four isolates (IT1-IT4), *Tetragonula testaceitarsis* which yielded four isolates (TT1-TT4), *Tetragonula minangkabau* which produced three isolates (MK1-MK3), *Geniotrigona thoracica* (TR1-TR3) and *Tetrigona binghami* (TB1-TB3). The total isolates of lactic acid bacteria from stingless bee honey were continued for testing the characteristics of probiotic candidates that consisted of 23 isolates (Figure 1). All isolates were Gram-positive, catalase-negative, and cream-colored were homo fermentative and heterofermentative.

It follows the opinion Salminen et al. (2004) that lactic acid bacteria are Gram-positive, rod-shaped or round in shape, facultatively anaerobic, non-sporulating, and create lactic acid as the primary result of carbohydrate fermentation (glucose). Added by Aritonang et al. (2017) and Aarti et al. (2018), lactic acid bacteria (LAB) are Gram-positive and naturally exist in the digestive tract of humans and ruminants.

After testing the tolerance of LAB isolates to bile salts, it was found that 4 LAB isolates had resistance to bile salts of 0.3% above 50%, namely isolates TS2, TS6, TB1, and TR1 (Figure 2). Furthermore, the four isolates were tested for antimicrobial activity against pathogenic bacteria, namely *Listeria monocytogenes* VTO, *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Salmonella*, *Propionibacterium acnes*, *Pseudomonas*, *Klebsiella*, *Acinetobacter baumannii*, and *Escherichia coli* (Table 1). All of these pathogenic bacteria were compared with Kanamycin and Amphicylin antibiotics. Isolates TS2 and TB1 had antimicrobial activity against the above pathogenic bacteria.

Based on the tolerance test to acid pH (pH 3) for 90 min, 0.3% bile salt for 5 hrs, and antibacterial activity against pathogenic bacteria, the TB1 isolate with the highest activity and potential as a probiotic candidate was obtained. The results of isolate TB1 16S rRNA gene amplification via polymerase chain reaction were obtained (Figure 3).

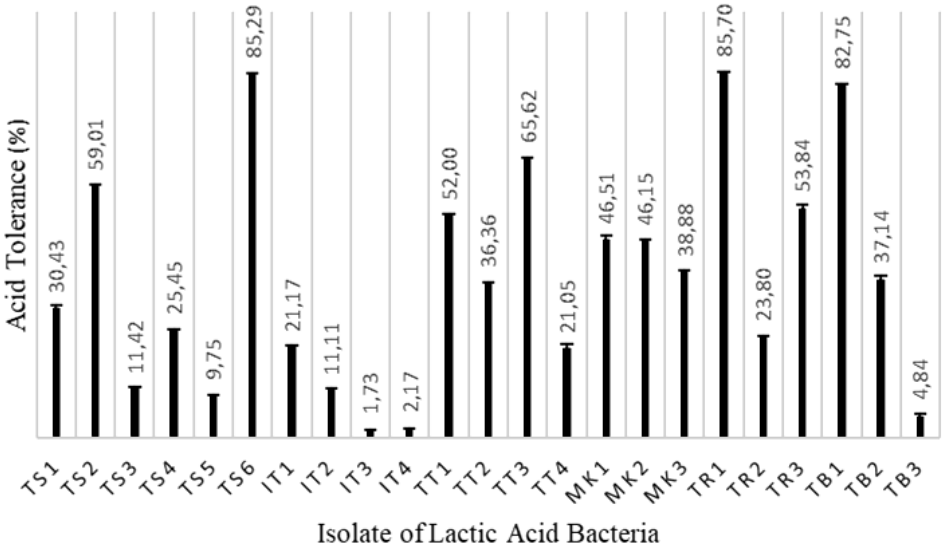

Figure 1. Acid tolerance of lactic acid bacteria isolated from stingless bee

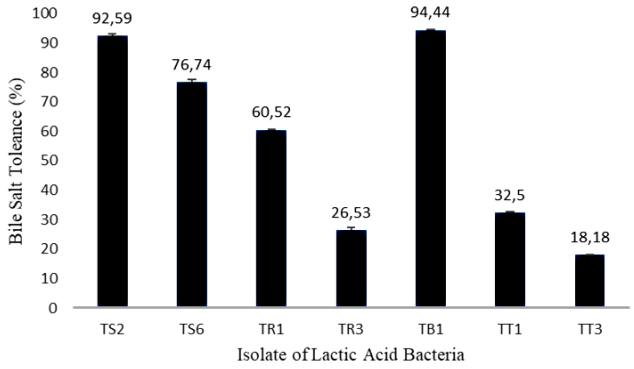

Figure 2. Bile salt tolerance of lactic acid bacteria from stingless bee

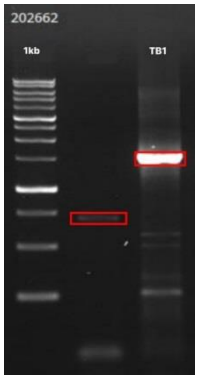

Figure 3. The amplification of the ribosomal RNA gene by PCR with the primers l1492R and 27F (M = 1 kB DNA Ladder)

Table 1. Antimicrobial activity of LAB isolates from stingless bee

Sample	Zona bening (mm)									
	LM-VTO	**LM**	**LA**	**SA**	**SN**	**PA**	**PS**	**KL**	**AB**	**EC**
TS2	9.7	8.9	12.4	8.8	19.7	9.3	13.1	14	19.3	18.7
TS6	11.8	8.7	0	6.2	25.1	9.9	10.9	12.7	15.4	18.3
TB1	18.9	11.1	9.3	10.2	26.9	10.2	13.2	12.7	12.9	10.5
TR1	15.1	7.6	10.5	0	25.3	0	8	18.3	16.8	9.9
Kanamycin	7.3	6.9	5.5	19.1	20.1	17.5	0	7.5	13.0	16.2
Ampicillin	0	5.95	0	0	17.1	0	8.6	10	0	0

Note: LM VTO: *Listeria monocytogenes* VTO, LM: *Listeria monocytogenes*, LA: *Listeria innocua*, SA: *Staphylococcus aureus*, SN: *Salmonella*, PA: *Propionibacterium acnes*, PS: *Pseudomonas*, KL: *Klebsiella*, AB: *Acinetobacter baumannii*,EC: *Escherichia coli*

Tabel 2. pH, titratable acidity, and total lactic acid bacteria (LAB) starter culture of *L. plantarum* SN13T

***L. plantarum* SN13T**	**pH**	**TTA (%)**	**Total LAB (10^9 CFU/mL)**
3%	$5.38^{c} \pm 0.22$	$1.17^{a} \pm 0.31$	$3.0^{a} \pm 0.15$
4%	$5.37^{c} \pm 0.17$	$1.28^{a} \pm 0.11$	$5.2^{b} \pm 0.10$
5%	$5.22^{b} \pm 0.35$	$1.34^{b} \pm 0.29$	$7.2^{c} \pm 0.32$
6%	$4.78^{a} \pm 0.10$	$1.46^{c} \pm 0.18$	$9.0^{d} \pm 0.20$

The pH value of starter culture of *L. plantarum* SN13T ranges from 4.78 to 5.38. Adding *L. plantarum*, SN13T decreased the pH of fermented milk significantly (P<0.01). The pH of the starter culture decreases to 4.78 when the presence of *L. plantarum* SN13T (6%) increases. The decrease in pH is possible because *L. plantarum* SN13T increases the synthesis of lactic acid, decreasing the pH value. According to Table 2, the titratable acidity of the starter culture ranged from 1.17 to 1.46 percent. Adding up to 6% *L. plantarum* SN13T significantly enhanced the starter culture's titratable acidity (P<0.01). Titratable acidity is the quantity of lactic acid produced during fermentation due to LAB's breakdown of lactose. The acidity of fermented milk is induced by acid-forming bacteria, such as *L. plantarum* SN13T. These bacteria can convert lactose into lactic acid. Adding *L. plantarum* SN13T increased the titratable acidity of fermented skim milk because the higher the concentration of LAB, the greater the activity of lactic acid bacteria in creating organic acids during fermentation, leading to an increase in titratable acidity. The titratable acidity of starter culture of *L. plantarum* SN13T ranged from 3.0 to 9.0 10^9 CFU/mL, as shown in Table 2. Adding up to 6% *L. plantarum* SN13T raised the total lactic acid bacteria in starter culture considerably (P<0.01). The quantity of LAB populations in starter culture measures the product's microbiological quality. Lactic acid bacteria are bacteria that can ferment glucose into lactic acid.

Discussion

The primary host conditions that a probiotic strain must survive to exert its probiotic impact on the host are stomach acidity and bile component concentration in the proximal intestinal (Ketema et al. 2009). Probiotics must survive the really acidic stomach in order to reach the intestines and provide suitable living conditions (Yadav Nisha et al. 2013). Therefore, evaluating the tolerance of probiotic lactic acid bacteria isolates is necessary to stimulate gastric juice.

It has been shown that probiotic bacteria can endure a pH 3 environment for 2.5 hrs. Because instabilities made it difficult for bacteria to survive in the stomach during transit, resistance to low pH was an essential selection requirement for probiotic strains (Wu et al. 2011). There were 23 isolates of lactic acid bacteria from 6 species of stingless bee honey that were tested for resistance to acidic conditions (pH3) for 90 min (Figure 1). Of the 23 LAB isolates, there were 7 isolates that had resistance to pH 3 above 50%, namely TS2 59.01%, TS6 85.29%, TT1 52.00%, TT3 65.62%, TR1 85.70%, TR3 53.84% and TB1 82.75%. So almost 70% of these LAB isolates have resistance to acid conditions below

50%. According to Ketema et al. (2009), 44.4% of LAB isolates isolated from *Wakalim* (fermented beef sausage) were resistant to pH 3 for 3 hrs. Ramadhanti et al. (2021) found that *Lactobacillus fermentum* has resistance to pH 3. Furthermore Pratama et al. (2021) and Shafakatullah and Chandra (2014) respectively found *Lactobacillus brevis* and *lactobacillus rhamnosus* are resistant to pH 3. Added by Abiad et al. (2022), the lactobacilli genus from Anbaris-traditional Lebanese fermented dairy products is resistant to pH 3 and 5. Later Zhang et al. (2020), in their research, found *L. plantarum* LP049 had viability at pH 3, reaching 92 ± 4.2%.

The decrease in a bacterial population at low pH (pH 3) is caused by the influence of hydrochloric acid that affects cell biomolecules, DNA, proteins, and fatty acids. Probiotic LAB is capable of traversing the human gastrointestinal tract, as well as the saliva (pH 6.5-7.5), upper stomach (pH 4.0-6.5), lower stomach (pH 1.5-4.0), and intestinal (pH 4.0-7.0 (Fallingborg 1999). Consequently, it gives the product beneficial health effects. This will enable the bacteria to thrive, proliferate, and produce the essential positive effects in the digestive tract, recreating the lactic acid microflora responsible for the body's immune system (Usman and Hosono 1999). In addition to surviving acidic conditions in the stomach, probiotics must also be capable of living in intestine bile salts to exert their therapeutic effects (Yang et al. 2020), because bile salts and pancreatin can contribute to adverse conditions in the small intestine. Probiotics must be able to adapt to high concentrations of bile salts (Mulaw et al. 2019).

Based on the LAB isolates resistance to gastric acid conditions (pH 3) above 50%, seven isolates continued for resistance testing to 0.3% bile salts, namely TS1, TS6, TR1, TR3, TB1, TT1, and TT3 (Figure 2). The respective values are 92.59%, 76.74%, 60.52%, 26.53%, 94.44%, 32.5% and 18.18%. In a previous study Pratama et al. (2021), LAB isolates with resistance to bile salts of 0.3% and 0.5% were 60.5% and 34.9%, respectively. Furthermore Yang et al. (2020) explained that *L. rhamnosus* CG, *P. pentosaceus* SC28, and *L. brevis* KU15151 had tolerance to 0.3% bile salt, 101.83%, 100.05%, and 97.96% respectively. These results were almost the same as research Mulaw et al. (2019), which isolated LAB from traditional Euthopia food and had a bile salt resistance of 0.3% above 90%. Likewise Zhang et al. (2020). *L. plantarum* LP049 showed strong tolerance to bile salts (0.25%) with a survival rate of 93.3 ± 2, followed by *L. brevis* LB0112 (90 ± 4.3%).

TB1 isolates had the highest inhibition value than TS2 isolates against pathogenic bacteria except for *L. innocua* (LA), *Klebsiella* (KL), and *Acinetobacter baumannii* (AB). Furthermore, when compared with the control, namely the antibiotic kanamycin, TB1 had higher activity against pathogenic bacteria except for *Staphylococcus aureus* (SA) and *Acinetobacter baumannii* (AB). In contrast, compared to ampicillin, isolate TB1 had better activity than all pathogenic bacteria (Table 1). Antimicrobial activity varied among LAB isolates against pathogenic bacteria. *Lactobacilli* are Gram-positive, non-spore-forming, fermenting various sugars into organic acids. Some strains of *lactobacilli* produce lactic acid. Low pH values affect the growth of other bacteria (Zhang et al. 2020). According to a prior study, the production of H_2O_2 during metabolic activities reduces the growth of pathogenic microorganisms significantly (Ilavenil et al. 2016). Certain lactobacilli are responsible for producing compounds like bacteriocins, which prevent the growth of bacteria of pathogens and are widely utilized in the food preservation industry (Zhao et al. 2016). In addition to this, a research Melia et al. (2017) reported that *L. fermentum* isolated from buffalo milk could inhibit the growth of *L. monocytogenes*. Further added by Melia et al. (2019), the lactic acid bacterium *Pediococcus acidilactici* BK01 isolated from *Bekasam* had inhibitory power against *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*. Then in the research of Aritonang et al. (2022), *Lactobacillus casei* strain HDS-01, isolated from Dadih, was able to inhibit *Escherichia coli* O157:H7, *L. monocytogenes*, and *S. aureus*.

Based on molecular identification with 16S rRNA gene sequencing and according to phylogenetic trees created from analyses of sequences of 16S rRNA genes, it was obtained that the TB1 isolate had a 100% match with *Lactobacillus plantarum* SN13T. The strain of *Lactobacillus plantarum* SN13T, isolated from banana leaves, can potentially change the build of the gut microbes and improve liver function in patients with mild liver disease (Higashikawa et al. 2020).

According to Costa et al. (2016), organic acids produced by lactose fermentation by lactic acid bacteria are converted to glucose and galactose, resulting in a reduction in pH. Moreover, according to Dianasaril et al. (2018), a high concentration of lactic acid will lower the pH, resulting in a sour flavor in fermented milk. The value of fermentation increases the titrated acidity, resulting in a reduction in pH. Lactic acid bacteria will utilize the existing carbohydrates to produce lactic acid, resulting in a fall in pH and an increase in acidity.

The pH of the research results is lower than that of fermented whey products with *Pediococcus acidilactici* (Melia et al. 2021) but higher than the study of Susmiati et al. (2022), namely pH 3.57 - 4.23, where fermented milk *L. pentosus* HBUAS53657 was made from buffalo milk. On the other hand, the pH in this study is almost the same as that of yogurt using *Lactobacillus plantarum* strain 1.1623, which is 4.85-5.51 (Riftyan et al. 2022). According to Wu et al. (2011), lactic acid bacterial fermentation produces organic acids that can reduce pH. This is supported by the opinion Shahein et al. (2022) that lactic acid bacteria culture from fermented milk converts lactose to lactic acid.

In fermented skim milk, an increased quantity of lactic acid bacteria causes a fall in pH and a rise in titratable acidity. According to the opinion Sebastian et al. (2018), there is a correlation between the decrease in pH and the increase in titratable acidity during fermentation. A rise in the number of lactic acid bacteria increased titratable acidity, which produced a reduction in pH. The total LAB results from this investigation are nearly identical (Melia et al. 2022). According to Najgebauer-Lejko (2014), the effect of probiotics on health is realized when there are between 10^6 and 10^9 CFU/mL of lactic acid bacteria.

In conclusion, based on the probiotic selection from 23 LAB isolates, isolate TB1 from *Tetrigona binghami* honey

had an acid tolerance of 82.75%, bile salt tolerance of 94.44%, and the highest antimicrobial activity against pathogenic bacteria with more excellent resistance than antibiotics. So it can be said that the TB1 isolate had the potential as a probiotic candidate which is then identified with 16S rRNA. The results of identification with 16S rRNA showed that the TB1 isolate had 100% similarity with *Lactobacillus plantarum* SN13T. Furthermore, fermented milk produced by inoculating 6% *L.plantarum* SN13T with a pH of 4.7, titratable acidity of 1.46%, and lactic acid bacteria counts of 9.0 x 10^9 CFU/mL met the criteria for probiotic fermented milk.

ACKNOWLEDGEMENTS

This study was made available thanks to funding from the Cluster publications to professors program at the Institutions of Research and Community Service at Universitas Andalas, Padang, Indonesia (Contract No. T/14/UN.16.17/PP.Pangan-PTU-KRP2GB-Unand/2022).

REFERENCES

Aarti C, Khusro A, Arasu MV, Agastian P, Al-Dhabi NA. 2016. Biological potency and characterization of antibacterial substances produced by *Lactobacillus pentosus* isolated from Hentak, a fermented fish product of North-East India. SpringerPlus 5 (1): 1743. DOI: 10.1186/s40064-016-3452-2.

Aarti C, Khusro A, Varghese R, Arasu M.V, Agastian P, Al-Dhabi N.A, Ilavenil S, Choi KC. 2017. In vitro studies on probiotic and antioxidant properties of *Lactobacillus brevis* strain LAP2 isolated from Hentak, a fermented fish product of North-East India. LWT - Food Sci Technol 86: 438-446. DOI: 10.1016/j.lwt.2017.07.055.

Aarti C, Khusro A, Varghese R, Arasu MV, Agastian P, Al-Dhabi N.A, Ilavenil S, Choi KC. 2018. In vitro investigation on probiotic, anti-Candida, and antibiofilm properties of *Lactobacillus pentosus* strain LAP1. Arch Oral Biol 89: 99-106. DOI: 10.1016/j.archoralbio.2018.02.014.

Abiad MG, Ajjour C, Kassaify Z, Iskandar CF, Hamzeh R, Hassan HF. 2022. Preliminary physiological, phenotypic, and probiotic characterization of *Lactobacillus strains* isolated from Anbaris-traditional Lebanese fermented dairy product. Intl J Food Prop 25: 1266-1278. DOI: 10.1080/10942912.2022.2077363.

Ajibola A, Chamunorwa JP, Erlwanger KH. 2012. Nutraceutical values of natural honey and its contribution to human health and wealth. Nutr Metab 9: 1-12. DOI: 10.1186/1743-7075-9-61.

Aritonang SN, Roza E, Rossi E, Purwati E, Husmaini. 2017. Isolation and identification of Lactic Acid Bacteria from okara and evaluation of their potential as candidate probiotics. Pak J Nutr 168: 618-628. DOI: 103923/pjn2017618628.

Aritonang SN, Roza E, Yetmaneli, Sandra A, Rizqan. 2022. Characterization of lactic acid bacteria from buffalo dairy product (Dadiah) as potential probiotics. Biodiversitas 23: 4418-4423. DOI: 10.13057/biodiv/d230906.

Čakmakči S, Četin B, Turgut T, Gürses M, Erdoğan A. 2012. Probiotic properties, sensory qualities, and storage stability of probiotic banana yogurts. Turk J Vet Anim Sci 36 (3): 232-237. DOI: 10.3906/vet-1007-2.

Cortopassi-Laurino M, Imperatriz-Fonseca V, Roubik D, Dollin A, Heard T, Anguilar I, Venturieri G, Eardley C, Nogueira-Neto P. 2006. Global meliponiculture : challenges and opportunities. Apidologie 37 (2): 275-292. DOI: 10.1051/apido:2006027.

Costa MP, Da Frasao BDS, Lima BRCDC, Rodrigues BL, Junior CAC. 2016. Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation. Talanta 152: 162-170. DOI: 10.1016/j.talanta.2016.01.061.

Davoodabadi A, Soltan Dallal MM, Rahimi Foroushani A, Douraghi M, Sharifi Yazdi M, Kazem, Amin Harati F. 2015. Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Anaerobe 34: 53-58. DOI: 10.1016/j.anaerobe.2015.04.014.

Dianasaril U, Malaka R, Maruddin F. 2018. The Values of pH, Lactid Acid and Color of the Fermented Milk Added with Red Dragon (*Hylocereu Polyrhizus*) at Various Incubation Period. J Sains Teknologi 18 (3): 213-218.

Engel MS, Kahono S, Peggie D. 2019. A key to the genera and subgenera of stingless bees in Indonesia (*Hymenoptera*: *Apidae*). Treubia 45: 65-84. DOI: 10.14203/treubia.v45i0.3687.

Fallingborg J. 1999. Intraluminal pH of the human gastrointestinal tract. Dan Med Bul. 46 (3):183-96.

Feliatra F, Hamdani R, Lukystyowati I, Nurachmi I. 2019. Sensitivity of heterotrophic bacteria in the low-salinity water areas and estuary in siak district toward pathogenic bacteria in fish. Intl J Microbiol 2019. DOI: 10.1155/2019/7456410.

Herwina H, Salmah S, Jasmi, Yaherwandi, Mairawita, Janra MN, Rusdimansyah, Christy BY, Sari DA, Putri G. 2020. West Sumatran Stingless Bees (Hymenoptera: Apidae: Meliponini): What can be told from its Local Distribution. IOP Conf Ser: Earth Environ Sci 757: 012084. DOI:10.1088/1755-1315/757/1/012084.

Herwina H, Salmah S, Janra MN, Mairawita, Nurdin J, Jasmi, Yaherwandi, Rusdimansyah, Sari DA. 2021. Stingless bee-keeping (Hymenoptera: Apidae: Meliponini) and Its Potency for Other Related-Ventures in West Sumatra. J Phys: Conf Ser 1940: 012073. DOI: 10.1088/1742-6596/1940/1/012073.

Higashikawa F, Danshiitsoodol N, Kanno K, Ishida R, Tazuma S, Sugiyama M. 2020. *Lactobacillus plantarum* SN13T Cells Improve Hepatic Dysfunction And Fecal Microbiota: A Randomized Pilot Study. Arch Clin Biomed Res 04 (06): 605-625. DOI: 10.26502/acbr.50170129.

Ilavenil S, Vijayakumar M, Kim DH, Valan Arasu M, Park HS, Ravikumar S, Choi KC. 2016. Assessment of probiotic, antifungal and cholesterol lowering properties of *Pediococcus pentosaceus* KCC-23 isolated from Italian ryegrass. J Sci Food Agric 96 (2): 593-601. DOI: 10.1002/jsfa.7128.

Kerry GR, Patra JK, Gouda S, Park Y, Shin HS, Das G. 2018. Benefaction of probiotics for human health: A review. J Food Drug Anal 26 (3): 927-939. DOI: 10.1016/j.jfda.2018.01.002.

Ketema B, Mehari T, Ashenafi M. 2009. In-vitro probiotic potential of lactic acid bacteria isolated from "wakalim", a traditional Ethiopian fermented beef sausage. Ethiop J Health Sci 10 (1): 21-27.

Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB. 2005. Identification of potential probiotic starter cultures for *Scandinavian*-type fermented sausages. Intl J Food Microbiol 105 (3): 419-431. DOI: 10.1016/j.ijfoodmicro.2005.03.020.

Melia S, Juliyarsi I, Fitri Kurnia Y, Endra Pratama Y, Alzahra H. 2021. Examination of Titratable Acidity, pH, Total Lactic Acid Bacteria and Sensory Properties in Whey Fermented with Probiotic *Pediococcus acidilactic* BK01. Adv Anim Vet Sci 10 (1): 114-119. DOI: 10.17582/journal.aavs/2022/10.1.114.119.

Melia S, Juliyarsi I, Kurnia YF. 2022. Physicochemical properties, sensory characteristics, and antioxidant activity of the goat milk yogurt probiotic *Pediococcus acidilactici* BK01 on the addition of red ginger (*Zingiber officinale var. rubrum rhizoma*). Vet World 15 (3): 757-764. DOI: 10.14202/vetworld.2022.757-764.

Melia S, Purwati E, Kurnia YF, Pratama DR. 2019. Antimicrobial potential of *Pediococcus acidilactici* from Bekasam, fermentation of sepat rawa fish (*Tricopodus trichopterus*) from Banyuasin, South Sumatra, Indonesia. Biodiversitas 20 (12): 3532-3538. DOI: 10.13057/biodiv/d201210.

Melia S, Purwati E, Yuherman, Jaswandi, Aritonang SN, Silaen M. 2017. Characterization of the antimicrobial activity of lactic acid bacteria isolated from buffalo milk in west sumatera (Indonesia) against *Listeria monocytogenes*. Pak J Nutr 16 (8): 645-650. DOI: 10.3923/pjn.2017.645.650.

Mulaw G, Tessema TS, Muleta D, Tesfaye A. 2019. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. Intl J Microbiol 2019. DOI: 10.1155/2019/7179514.

Najgebauer-Lejko D. 2014. Effect of green tea supplementation on the microbiological, antioxidant, and sensory properties of probiotic milks. Dairy Sci Technol 94 (4): 327-339. DOI: 10.1007/s13594-014-0165-6.

Othman SFC, Aziah AAN, Ahmad R. 2012. Effects of jackfruit puree supplementation on lactic acid bacteria *(Lactobacillus acidophilus* FTDC 1295) in terms of viability and chemical compositions of dadih. Intl Food Res J 19 (4): 1687-1697.

Pato U, Yusuf Y, Nainggolan YP. 2019. Effect of *Lactobacillus casei* subsp. *casei* R-68 isolated from dadih on the procarcinogenic enzyme activity and fecal microflora count of rats challenged with pathogenic bacteria. Intl J Adv Sci Eng Inform Technol 9 (5): 1656-1662. DOI: 10.18517/ijaseit.9.5.8812.

Pratama YE, Jamsari, Yuherman, Melia S, Purwati E. 2021. Molecular identification of lactic acid bacteria from " budu " of West Sumatera, Indonesia as a potential probiotic Molecular identification of lactic acid bacteria from " Budu" of West Sumatera, Indonesia, as a potential probiotic. AACL Bioflux 12 (5): 2837-2844.

Ramadhanti N, Melia S, Hellyward J, Purwati E. 2021. Characteristics of lactic acid bacteria isolated from palm sugar from West Sumatra, Indonesia and their potential as a probiotic. Biodiversitas 22 (5): 2610-2616. DOI: 10.13057/biodiv/d220520.

Riftyan E, Rossi E, Efendi R, Marcellina Z. 2022. The Producing of Fermented Milk as an Application of Alternative Halal Culture Medium for the Growth of *Lactobacillus plantarum* TMW 1.1623 and *Streptococcus thermophilus*. In IOP Conf Ser: Earth Environ Sci 1059 (1): 012052. DOI: 10.1088/1755-1315/1059/1/012052.

Rosli FN, Hazemi MHF, Akbar MA, Basir S, Kassim H and Bunawan H. 2020. Stingless bee honey: Evaluating its antibacterial activity and bacterial diversity. Insect 11: 500. DOI:10.3390/insects11080500.

Salminen S, Atte von W, Arthur O. 2004. Lactic Acid Bacteria : Microbiological and Functional Aspects (Second). Marcel Dekker.

Sebastian A, Barus T, Mulyono N, Yanti. 2018. Effects of fermentation and sterilization on quality of soybean milk. Intl Food Res J 25 (6): 2428-2434.

Shafakatullah N, Chandra M. 2014. Screening of Raw Buffalo's Milk from Karnataka for Potential Probiotic Strains. Res J Recent Sci 3 (9): 73-78.

Shahein MR, Atwaa ESH, Elkot WF, Hijazy HHA, Kassab RB, Alblihed MA, Elmahallawy EK. 2022. The Impact of Date Syrup on the Physicochemical, Microbiological, and Sensory Properties, and Antioxidant Activity of Bio-Fermented Camel Milk. Fermentation 8 (5): 1-13. DOI: 10.3390/fermentation8050192.

Susmiati, Melia S, Purwati E, Alzahra H. 2022. Physicochemical and microbiological fermented buffalo milk produced by probiotic *Lactiplantibacillus pentosus* HBUAS53657 and sweet orange juice (*Citrus nobilis*). Biodiversitas 23 (8): 4329-4335. DOI: 10.13057/biodiv/d230858.

Usman, Hosono A. 1999. Bile tolerance, taurocholate deconjugation, and binding of cholesterol by *Lactobacillus gasseri* strains. J Dairy Sci 82 (2): 243-248. DOI: 10.3168/jds.S0022-0302 (99)75229-X.

Wu SC, Su YS, Cheng HY. 2011. Antioxidant properties of *Lactobacillus*-fermented and non-fermented *Graptopetalum paraguayense* E. Walther at different stages of maturity. Food Chem 129 (3): 804-809. DOI: 10.1016/j.foodchem.2011.05.025.

Yadav Nisha R, Milind B, Imran A. 2013. Probiotic delivery systems: applications, challenges and prospective. Intl Res J Pharm 4 (4): 1-9. DOI: 10.7897/2230-8407.04401.

Yang SJ, Kim K, Kim TY, Paik H. 2020. Probiotic Properties and Antioxidant Activities of *Pediococcus pentosaceus* SC28 and *Levilactobacillus brevis* KU15151 in Fermented Black Gamju. Foods 9: 1154. DOI: 10.3390/foods9091154.

Zhang X, Ali Esmail G, Fahad Alzeer A, Valan Arasu M, Vijayaraghavan P, Choon Choi K, Abdullah Al-Dhabi N. 2020. Probiotic characteristics of *Lactobacillus* strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens. Saudi J Biol Sci 27 (12): 3505-3513. DOI: 10.1016/j.sjbs.2020.10.022.

Zhao S, Han J, Bie X, Lu Z, Zhang C, Lv F. 2016. Purification and Characterization of Plantaricin JLA-9: A Novel Bacteriocin against *Bacillus* spp. Produced by *Lactobacillus plantarum* JLA-9 from Suan-Tsai, a Traditional Chinese Fermented Cabbage. J Agric Food Chem 64 (13): 2754-2764. DOI: 10.1021/acs.jafc.5b05717.

Zulkhairi Amin FA, Sabri S, Mohammad SM, Ismail M, Chan KW, Ismail N, Norhaizan ME, Zawawi N. 2018. Therapeutic properties of stingless bee honey in comparison with european bee honey. Adv Pharmacol Sci 2018. DOI: 10.1155/2018/6179596.