Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com/sajb/

OPEN ACCESS

Biotechnology

Identification of Lactic Acid Bacteria from Papuan Red Fruit (*Pandanus conoideus* Lam.) with Potential as Probiotics and Antibacterials

Charis Amarantini[1*], Vinsa Cantya Prakasita[1], Viona Ch Salakory[2]

[1]Department Biology, Faculty of Biotechnology, Duta Wacana Christian University, Jl. Dr. Wahidin Sudirohusodo No.5-25, Kotabaru, Gondokusuman, Yogyakarta, Daerah Istimewa Yogyakarta 55224, Indonesia
[2]Undergraduate Program, Faculty of Biotechnology, Duta Wacana Christian University, Jl. Dr. Wahidin Sudirohusodo No.5-25, Kotabaru, Gondokusuman, Yogyakarta, Daerah Istimewa Yogyakarta 55224, Indonesia

DOI: 10.36347/sajb.2020.v08i09.006 | **Received:** 06.09.2020 | **Accepted:** 14.09.2020 | **Published:** 30.09.2020

***Corresponding author:** Charis Amarantini

Abstract **Original Research Article**

Lactic acid bacteria (LAB) are mostly explored for probiotic activities. These organisms improve the organoleptic characteristics of food, inhibit microbial activities, and consequently prevent possible diseases. The aim of this study, therefore, is to investigate organisms with possible inhibitory action against pathogenic bacteria. Besides, Papuan red fruits obtained from Sorong (West Papuan) and Timika (Papuan) were estimated to contain LAB, hence samples were selected and isolated using de Man Rogosa Sharpe selective medium equipped with 1% $CaCO_3$. The organisms were then analyzed based on acid sensitivity (pH 2, 3, and 4) and bile tolerance (0.3%; 0.5% and 1%). The antibacterial activity was evaluated using well diffusion method on the Muller Hilton Agar medium against pathogenic bacteria *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* BPE 122.4 CCA, and *Salmonella typhi* NCTC 786. The identity of LAB isolates was confirmed based on the API 50CHL test, and a total of 25 isolates were successfully selected. These were characterized based on the cellular properties of rod-shaped, Gram-positive, homofermentative, non-motile, catalase-negative, as well as the capacity to grow at 10ºC and 45ºC. Furthermore, five of the isolates (strains S1B1, S2B1, S1T2, S2T4, and S1T1) were identified as *Lactobacillus plantarum*, with the ability to survive at pH 2 and in 1% bile salts. The antibacterial activity of the S2T4 strain was strong against *S. aureus* ATCC 25923, *S. typhi* BPE 122.4 CCA, and *S. typhi* NCTC 786. Besides, LAB S2B1 significantly restrained the growth of *Salmonella typhi* BPE 122.4 CCA, while LAB S1T1 inhibited *S. aureus* ATCC 25923. Therefore, it is possible to further explore *Lactobacillus plantarum* strains S2T4, S2B1, and S1T1 for the inherent health and nutritional values.

Keywords: Lactic acid bacteria, *Pandanus conoideus* Lam., Probiotics, Antibacterial, *Lactobacillus plantarum*.

Copyright @ 2020: This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited

INTRODUCTION

Lactic acid bacteria (LAB) are known to play a crucial role in the preservation and fermented foods. These organisms have been applied as natural microflora or as starter culture introduced under controlled conditions [1]. According to Gutiérrez-Cortés *et al*. [2], LAB metabolites can improve the organoleptic characteristics of fermented food and inhibit the growth of microorganisms responsible for food spoilage. These bacteria are classified as probiotics, considering the positive impact on human health. Meanwhile, there are numerous advantages derived from the consumption, including to increase immunity, suppress pathogenic bacteria, balance intestinal microbiota, and reduce serum cholesterol [3].

According to Allen *et al*. [3], LAB is used as a probiotic, particularly due to the inherent resistance against acids and bile. This microorganism can produce antibacterial substances required to suppress pathogenic enteric bacteria growth. Besides, the antifungal property produced is known to extend the shelf life of food, hence the potential for application as a natural preservative in various edible products [4].

Several studies have successfully obtained LAB strains from various soured beverage ingredients, including yogurt and traditional fermented foods, specifically tape, growol and gatot [5]. The microorganism yield is naturally dominant in dairy products, whole grains, meat, fish, fruit, juices drinks, pickled vegetables, and sourdough batter. Meanwhile, numerous reports are indicating the minute quantities present in all plant materials, while abundant amounts have been observed in decaying varieties, especially rotting fruits. The species isolated from various plant sources include *Lactobacillus plantarum*, *L. brevis*, *L.*

coryniformis, L. casei, L. curvatus, L. fermentum [6, 7]. Furthermore, LAB is obtainable from a wide range of vegetable-based fermented products, encompassing flowers, and fruits [8].

The red fruit (*Pandanus conoideus* Lam.) is an endemic plant in Papua province, Indonesia and New Guinea [9]. Moreover, the inulin content in pedicel extract, a byproduct of red fruit oil production, is known to increase *Lactobacillus casei* growth. This also creates a more acidic environment and inhibits the development of enteric bacteria [10]. Meanwhile, LAB obtained from endemic plants and dominant in red fruit have good explorative potentials, especially as biocontrol and probiotic agents [8]. The aim of this research, therefore, is to isolation and identification LAB with probiotic and antibacterial characteristics, from Papuan red fruit (*Pandanus conoideus* Lam.).

MATERIAL AND METHODS

Red Fruit Samples

Samples of red fruit were obtained from Timika (Papua Province) and Sorong (West Papua Province) was sliced into 3 parts. Therefore, the pulp sections were collected from each piece and reserved for 72 hours, to ensure the spontaneous fermentation required for lactic acid bacteria growth.

Isolation and selection of Lactic Acid Bacteria

A total of 25 g of red fruit were inoculated into 225 mL de Mann Rogosa Sharpe (MRS, Oxoid) liquid medium and incubated at 37°C for 48 hours. Furthermore, step dilution was carried out by inoculation through the pour plate method into the MRS agar medium completed with 1% $CaCO_3$ (Merck), before incubating at 37°C for 48 hours. The bacteria were then selected based on phenotypic properties through Gram stain, catalase, gas production, motility test, and survival at 10°C or 45°C [11, 12].

Probiotic Selection

LAB potential as a probiotic candidate was selected through a tolerance test for acidic pH and bile salts, along with antibacterial assessment.

Acid pH tolerance test

The LAB isolates were grown in 5 ml of liquid MRS medium for 48 hours, and the cells were harvested by centrifugation at 13,500 rpm for 15 minutes. Furthermore, the cell pellets were washed twice with Phosphate Buffer Saline (PBS, Merck) and dissolved in 100 µl of PBS solution. These globules were then inoculated into a liquid MRS medium adjusted to pH 2, 3, and 4 for 4 hours at 37°C. Subsequently, the respective culture growth results were determined by growing on MRS agar using the streak plate method. This was followed by incubation at 37°C for 24 to 48 hours [13].

Bile salts tolerance test

Approximately, 1ml of LAB culture aged 48 hours was inoculated into 5 ml liquid MRS medium containing bile salts (Oxoid) with concentrations of 0.3%, 0.5%, and 1%. Furthermore, growth tests were carried out after 4 hours incubation at 37°C by inoculating on the MRS agar medium using streak plate method and incubated at 37°C for 24 to 48 hours [13].

Potential Test as Antibacterial

Solution preparation of Cell-Free Culture Supernatant (CFCS)

The LAB cultures were obtained by ose aseptically, inoculated in 5 mL of liquid MRS medium, and incubated at 37°C for 18 hours. Besides, the CFCS solution was harvested by centrifugation at 13,500 rpm for 15 minutes. Meanwhile, the supernatant was neutralized at pH 6.5 with 1 N NaOH solution and refrigerated before being used for antibacterial assessment [14].

Pathogenic bacteria strain preparation

The pathogenic bacteria used were Gram-positive, specifically *Staphylococcus aureus* ATCC 25923. However, *Salmonella typhi* 122.4 CCA [15] and *Salmonella typhi* NCTC 786 obtained from PT. Biofarma were equally used. These bacterial cultures were obtained by ose aseptically and inoculated into liquid BHI medium at 37°C for 18 hours before applying antibacterial analysis.

Antibacterial activity test

The potential of LAB as an antibacterial was tested using the CFCS solution based on the well-diffusion agar method [14, 16, 17]. Besides, an 8 mm well was created in Mueller Hinton agar (MHA, Oxoid) medium, then indicator bacteria swab was performed using sterile cotton on the surface. Therefore, each well was filled with 100 µl of CFCS solution and allowed to cool, to permit a more rapid diffusion, before the petri dishes were incubated at 37°C for 24 hours. Furthermore, antibacterial activities were determined based on the assessment of clear zone (mm) formed around the well, where bacterial growth was reduced to a well diameter (8 mm) [18, 19].

Identification of Lactic Acid Bacteria

The LAB isolates with probiotic and antibacterial potential were identified using 50 CHL Analytical Profile Index (API) (BioMerieux) and grown in MRS agar medium for 48 hours. These cultures were obtained aseptically and inoculated into ampoules containing 50 CHL API medium, with turbidity equalized to the 2 McFarland. Furthermore, the specimen was inoculated into a microtube strip at an API 50CHL kit and incubated for 48 hours according to a predetermined procedure [20]. Also, the respective identity was determined using an API web software and

based on the fermentation profile of 49 types of carbohydrates contained in the kit.

RESULTS AND DISCUSSION

Isolation and Selection of Lactic Acid Bacteria from Red Fruit

Figure 1 depicts the growth of LAB colonies in the MRS agar medium equipped with $CaCO_3$. This is characterized by the formation of a clear zone, due to a reaction between the chemical and lactic acid compounds where dissolved calcium lactate (Ca-lactate) is produced [21]. The colonies with clear zones are then selected through phenotypic tests to determine the bacteria main character. Table 1 illustrates 25 LAB isolates obtained in the form of rods, Gram-positive, homofermentative, non-motile, and catalase-negative cells, grown at 10ºC and 45ºC, according to the digestive tract temperature range. These are the predominant bacteria traits [22], used in the selection of potential probiotics.

Fig-1: The isolation results of lactic acid bacteria from Papua red fruit (*Pandanus conoideus* Lam.) on the MRS agar medium equipped with $CACO_3$

According to Table 1, the 25 selected LAB isolates were grown in homofermentative conditions at 10ºC and 45ºC. This means there was no gas production during the growth phase. These results make it possible to determine the potential to isolate technology in food industry applications [2]. Based on the tolerance test, 24 lived on 0.3% while 18 out of the total survived with 1% bile salts. Moreover, 8 lived in acidic environments of up to pH 2. The probiotic microbe requires strength to survive against various extreme conditions in the human digestive tract, including acidic pH and bile salts [23]. These characteristics are important for the organism to reach the digestive tract alive, adhere to the mucosal layer and other components of the extracellular matrix. Also, the formation of a fast-microbial community triggers a reduction in pH and competition for adhesion with pathogenic bacteria, therefore preventing colonization [13]. The ability to fight stomach acid is considered as the main condition during screening because these bacteria pass through the stomach before digestion. Moreover, gastric acid in the human body has a pH of 3 with a digestion time between 1-3 hours. Therefore, the tolerant LAB is assumed to be a potential probiotic candidate [24].

Table-1: Characteristics of LAB isolates from Papuan red fruit (*Pandanus conoideus* Lam.), a potential probiotic candidate

No	Isolate	Acid pH tolerance test			Bile salts tolerance test (%)			Temperature test (^{0}C)		Gas Production
		2	3	4	0,3	0,5	1	10	45	
1	S1A1	-	-	+	+	-	-	+	+	Homofermentative
2	S1A2	-	-	-	+	-	-	+	+	Homofermentative
3	S1A3	-	+	+	+	+	+	+	+	Homofermentative
4	S1A4	-	-	-	+	+	+	+	+	Homofermentative
5	S1T1	-	+	+	+	+	+	+	+	Homofermentative
6	S1T2	+	+	+	+	+	+	+	+	Homofermentative
7	S1T3	+	+	+	+	+	+	+	+	Homofermentative
8	S1T4	+	+	+	+	+	+	+	+	Homofermentative
9	S1B1	+	+	+	+	+	+	+	+	Homofermentative
10	S1B2	-	+	+	+	+	+	+	+	Homofermentative
11	S1B3	-	-	+	+	+	+	+	+	Homofermentative
12	S2A1	-	-	+	-	-	-	+	+	Homofermentative

13	S2A2	-	-	-	+	+	-	+	+	Homofermentative
14	S2A3	-	-	-	+	+	+	+	+	Homofermentative
15	S2A4	-	+	+	+	+	-	+	+	Homofermentative
16	S2T1	-	-	+	+	+	+	+	+	Homofermentative
17	S2T2	+	+	+	+	+	+	+	+	Homofermentative
18	S2T3	+	+	+	+	+	+	+	+	Homofermentative
19	S2T4	+	+	+	+	+	+	+	+	Homofermentative
20	S2T5	-	-	-	+	+	-	+	+	Homofermentative
21	S2T6	-	-	-	+	+	-	+	+	Homofermentative
22	S2B1	+	+	+	+	+	+	+	+	Homofermentative
23	S2B2	-	-	-	+	+	+	+	+	Homofermentative
24	S2B3	-	-	-	+	+	+	+	+	Homofermentative
25	S2B4	-	-	-	+	+	+	+	+	Homofermentative

Description: (+) LAB growth; (-) LAB not growth

Determination of Lactic Acid Antibacterial Activity based on agar well diffusion method

The Agar well diffusion method was used to determine the antibacterial activity produced by LAB isolates as potential probiotic candidates. This ability was tested against three pathogenic organisms, including *S. aureus* ATCC 25923 representing Gram-positive bacteria, while *S. typhi* BPE 122.4 CCA and *Salmonella typhi* NCTC 786 as Gram-negative. The results in table 2 showed a different inhibition spectrum for each bacterium. According to a report by [18], the activity against the pathogenic bacteria is grouped into 3 categories, including weak (0-3mm), moderate (3-6mm), and strong (> 6mm) activity.

Table-2: Antibacterial activity of LAB isolates from Papua red fruit (*Pandanus conoideus* Lam.) against pathogenic bacteria based on agar well diffusion method.

Isolate	Inhibition zone (mm) against indicator bacteria		
	***Salmonella typhi* BPE 122.4 CCA**	***Salmonella typhi* NCTC 786**	***Staphylococcus aureus* ATCC 25923**
S1A1	2.00±0.00	2.33±0.58	2.33±0.58
S1A2	2.33±0.57	3.67±0.58	2.00±0.00
S1A3	2.33±0.58	1.33±0.58	3.33±0.58
S1A4	2.00±1.00	4.00±1.00	2.67±0.57
S1T1	2.33±0.57	5.53±0.57	6.67±0.57*
S1T2	3.00±0.00	1.67±0.57	2.33±0.57
S1T3	2.33±0.58	1.33±0.57	1.67±0.57
S1T4	2.67±0.58	1.00±0.00	3.33±0.57
S1B1	3.00±0.00	2.67±0.57	2.33±0.57
S1B2	1.00±0.00	1.67±0.57	2.67±0.57
S1B3	2.00±1.00	3.00±1.00	2.00±1.00
S2A1	2.00±0.00	2.33±0.57	1.33±0.57
S2A2	3.00±1.00	2.67±0.57	1.00±0.00
S2A3	2.33±0.57	2.67±0.57	2.33±0.57
S2A4	1.67±0.57	2.67±0.57	1.33±0.57
S2T1	2.00±0.00	3.67±0.57	2.00±1.00
S2T2	2.67±0.57	1.00±0.00	1.00±0.00
S2T3	2.33±0.57	3.00±1.00	2.67±0.57
S2T4	6.33±0.57*	6.67±0.57*	6.00±0.00*
S2T5	1.67±0.57	2.67±0.57	1.33±0.57
S2T6	2.33±0.57	1.67±0.57	2.00±0.00
S2B1	6.33±0.57*	2.67±1.15	0
S2B2	3.00±0.00	2.67±0.57	3.33±0.57
S2B3	2.00±1.00	2.33±0.57	2.67±0.57
S2B4	3.33±0.57	2.67±0.57	3.67±1.15

Description: *strong inhibition against indicator bacteria

According to Table 2 the LAB isolates S1T1 have stronger inhibition against Gram-positive indicator (*S. aureus* ATCC 25923) than Gram-negative (*S. typhi* BPE 122.4 CCA and *S. typhi* NCTC 786), while strain S2B1 has the reverse effect. However, the S2T4 variant had strong inhibition with broad-spectrum against all the microbes investigated. Furthermore, some of the organisms produce antibacterial effects and prevent the growth of pathogenic bacteria, therefore protecting the host's defense against infections in the intestinal lumen [13]. The LAB is more profitable compared to other microorganisms, due to the antibacterial substances produced [25]. This characteristic is important for assessing the potential as a probiotic and producer of antimicrobial compounds [26]. Besides, tolerance to acidic pH and bile salts, with the ability to inhibit pathogenic bacteria are of benefit to human health.

Identification of Lactic Acid Bacteria Isolates

Furthermore, five LAB isolates tolerant of acidic pH and 1% bile salts, with strong inhibition against pathogenic bacteria were selected and identified using 50 CHL Analytical Profile Index (API) (BioMérieux). These include S1B1, S2B1, S1T2, S2T4, and S1T1. Based on the Gram staining results in Figure 2, the Gram-positive characteristics of long rod cells were identified and arranged in groups or chains. These features are initial indications for the classification of isolates suspected to be *Lactobacillus*.

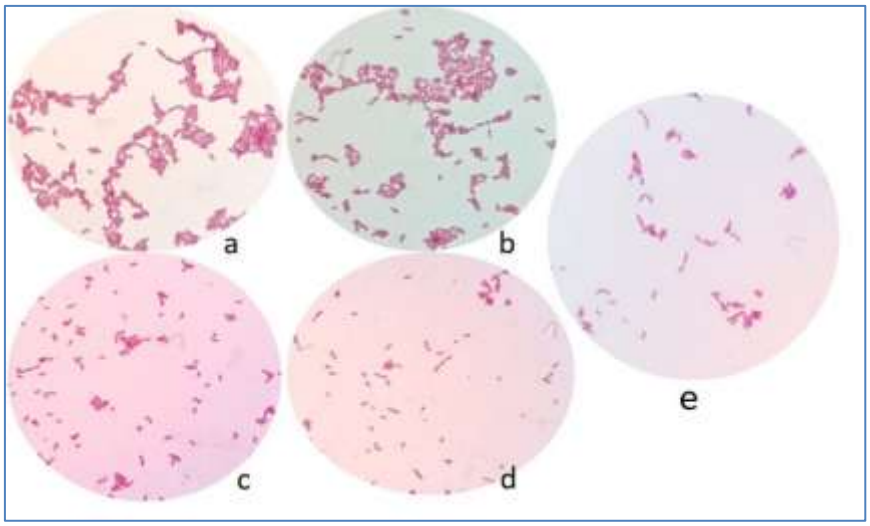

Fig-2: Cell morphology of lactic acid bacterial isolate from Papua red fruit, as potential probiotic candidate, based on Gram staining (a = strain S1B1; b = strain S2B1; c = strain S1T2; d = strain S2T4; e = strain S1T1)

Table 4 shows the results of the LAB isolates identification test performed using the API 50 CHL kit. Also, S1B1, S2B1, S1T2, S2T4, and S1T1 strains were able to ferment 23 carbon sources. These include L-arabinose, ribose, galactose, glucose, fructose, mannose, mannitol, sorbitol, methyl-D-monoxide, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, β-gentiobiose, and D-turanose. Besides, data confirmation was performed using API web software, where 99.9% of the discovered isolates were similar to *Lactobacillus plantarum* 1.

Table-4: Identification of lactic acid bacterial isolates from Papuan red fruit selected as probiotic candidates using Analytical Profile Index (API) 50 CHL (BioMérieux)

No	Carbon sources	Isolate				
		S1B1	**S1T2**	**S2B1**	**S2T4**	**S1T1**
0	Control	-	-	-	-	-
1	Glycerol	-	-	-	-	-
2	Erythritol	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-
4	L-Arabinose	+	+	-	+	+
5	Ribose	+	+	+	+	+
6	D-Xylose	-	-	-	-	-
7	L-Xylose	-	-	-	-	-
8	Adonitol	-	-	-	-	-
9	B-Methyl-D-Xyloxide	-	-	-	-	-
10	Galactose	+	+	+	+	+
11	Glucose	+	+	+	+	+
12	Fructose	+	+	+	+	+
13	Mannose	+	+	+	+	+
14	Sorbose	-	-	-	-	-
15	Rhamnose	-	-	-	-	-
16	Dulcitol	-	-	-	-	-
17	Inocitol	-	-	-	-	-
18	Mannitol	+	+	+	+	+
19	Sorbitol	+	+	+	+	+
20	Methyl-D-Mannoxide	+	+	+	+	+
21	Methyl-D-Glucoxide	-	-	-	-	-
22	N-Acetyl-Glucosamine	+	+	+	+	+
23	Amygdalin	+	+	+	+	+
24	Arbutin	+	+	+	+	+
25	Esculin	+	+	+	+	+
26	Salicin	+	+	+	+	+
27	Cellobiose	+	+	+	+	+
28	Maltose	+	+	+	+	+
29	Lactose	+	+	+	+	+
30	Melibiose	+	+	+	+	+
31	Sucrose	+	+	+	+	+
32	Trehalose	+	+	+	+	+
33	Inulin	-	-	-	-	-
34	Melezitose	+	+	+	+	+
35	Raffinose	+	+	+	+	+
36	Starch	-	-	-	-	-
37	Glycogen	-	-	-	-	-
38	Xylitol	-	-	-	-	-
39	β-Gentiobiose	+	+	+	+	+
40	D-Turanose	+	+	+	+	+
41	D-Lyxose	-	-	-	-	-
42	D-Tagatose	-	-	-	-	-
43	D-Fucose	-	-	-	-	-
44	L-Fucose	-	-	-	-	-
45	D-Arabitol	-	-	-	-	-
46	L-Arabitol	-	-	-	-	-
47	Gluconat	-	-	-	-	-
48	2-Keto-Gluconat	-	-	-	-	-
49	5-Keto-Gluconat	-	-	-	-	-
Identification Results		*L. plantarum 1*	*L. plantarum 1*	*L. plantarum 1*	*L. plantarum 1*	*L. plantarum 1*
Similarity Index		99,9%	99,9%	99,9%	99,9%	99,9%

The LAB isolate identification proves *L. plantarum* to be naturally present in plant materials and also its fermentation products [27, 28]. Furthermore, the prebiotic inulin content in red fruits is also considered a factor supporting LAB growth. Murtiningrum *et al*. [10] conducted an *in vitro* evaluation, and the results showed the ability for inulin present in the pedicel extract to supports *Lactobacillus casei* development.

The *Lactobacillus plantarum* strain S2T4 grows under acidic pH conditions and are also tolerant to bile salts. These microorganisms have strong inhibitory power with varying spectra against *S. aureus* ATCC 25923, *S. typhi* BPE 122.4 CCA, and *S. typhi* NCTC 786 indicator bacteria. Furthermore, the strain S1T1 had stronger inhibition against Gram-positive species (*S. aureus* ATCC 259230) than the Gram-negative. However, the inverse was reported for the S2B1 strain and was estimated to significantly inhibit only *S. typhi* BPE 122.4 CCA, while the other two strains (S1B1, S1T2) had a moderate effect, and similar results have been reported in other studies. Among the *Lactobacillus* members, *L. plantarum* species are known as functional probiotics and have been identified in many fermented, probiotic, and naturally processed foods [29]. Hu *et al*. [19] isolated three species of antimicrobial producing *L. plantarum* (strains P1; M7, and S11), with the capacity to inhibit some indicator bacteria, including *S. aureus* ATCC 12600, *E. coli* ATCC 35128, and *Salmonella* ASI.1174. A research conducted by Gutiérrez-Cortés *et al*. [2] recognized the presence of four *Lactobacillus* (*L. casei, L. brevis, L. paracasei, and L. plantarum*) and *Pediococcus acidilactici* species with antagonistic properties against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 7644. The five isolates of *L. plantarum* (S1B1, S2B1, S1T2, S2T4, and S1T1) are essential for further research on the intrinsic potentials as probiotic candidates for use as antibacterial and preservative agents.

CONCLUSIONS

The lactic acid bacteria strains S1B1, S2B1, S1T2, S2T4, and S1T1 isolated from Papuan red fruit were identified as *Lactobacillus plantarum* and determined to survive at pH 2 and bile salt 1%. These isolates have the potential for further development as probiotic candidates. Specifically, the S2T4 strain has a strong inhibitory capacity against *S. aureus* ATCC 25923, *S. typhi* BPE 122.4 CCA, and *S. typhi* NCTC 786, while S2B1 and S1T1 significantly hinder the growth of *Salmonella typhi* BPE 122.4 CCA and *S. aureus* ATCC 25923, respectively. Besides, three isolates, including S2T4, S2B1, and S1T1 also have the potential for further advancement as biopreservatives.

ACKNOWLEDGEMENTS

Our gratitude goes to the Faculty of Biotechnology Universitas Kristen Duta Wacana which is funding this research in 2020 with a project number 118/D.01/Bio/2020.

REFERENCES

1. Yang E, Fan L, Jiang Y, Doucette C, Fillmore S. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express. 2012; 2: 48-60.
2. Gutiérrez CC, Suárez H, Buitrago G, Moreno CD. Isolation and evaluation of the antagonist activity of lactic acid bacteria in raw cow milk. Agronomía Colombiana. 2017; 35(3): 357-364.
3. Allen SJ, Martinez EG, Gregorio GV, Dans LF. *Probiotics for treating acute infectious diarrhoea*. John Wiley & Sons Ltd. UK; 2011.
4. Mokoena, MP. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and application against uropathogens: a mini-review. Molecules. 2017; 22(8): 1-13.
5. [Tham CSC, Peh KK, Bhat R, Liong MT. Probiotic properties of *Bifidobacteria* and *Lactobacilli* isolated from local dairy products. Annals of Microbiology. 2012; 62(3):1079-1087.
6. Kandler, O. Current taxonomy of *Lactobacilli*. Industrial Microbiology. 1984; 25: 9-123.
7. Saguibo JD, Mercado MA, Maldia ST, Jimeno BT, Perez MTM, Calapardo MR, Elegado FB. Identification and characterization of lactic acid bacteria isolated from some medicinal and/or edible Philippine plants. Food Research. 2019; 3(6): 698-712.
8. Dinoto A, Rosyidah A, Susilo ARPS, Julistiono H. Isolation, identification and antimicrobial activities of lactic acid bacteria from fruits of wild plants in Tambrauw Forest, West Papua, Indonesia. Biodiversitas. 2020; 21(7): 3391-3397.
9. Rohman A, Man YBC, Riyanto S. Authentication analysis of red fruit (*Pandanus conoideus* Lam.) oil using FTIR spectroscopy in combination with chemometrics. Phytochemical Analysis. 2011; 22(5): 462-467.
10. Murtiningrum, Suryadarma P, Suryani A, Manguwidjaja D. Identification of inulin profile from red fruit (*Pandanus conoideus*) pedicel extract using LC-MS and it's in vitro prebiotic activity test. International Journal Advanced Research. 7(11): 344-351.
11. Sneath PHA, Mair NS, Sharpe ME, Holt JG. *Bergey's manual of systematic bacteriology*,

vol. 2. in Garrity, GM (Ed.), Williams JG, Wilkins (Co.). Baltimore Md; 2009.

12. Pramono H, Suciati P, Andriyono S. Isolation of lactic acid bacteria that produce protease and bacteriocin-like substance from mud crab (*Scylla sp.*) Digestive Tract. Ilmu Kelautan. 2015; 20(1): 33-7.
13. Shi T, Nishiyama K, Nakamata K, Aryantini NPD, Mikumo D, Oda Y, Yamamoto Y, Mukai T, Sujaya IN, Urashima T, Fukuda K. Isolation of potential probiotic *Lactobacillus rhamnosus* strains from traditional fermented mare milk produced in Sumbawa Island of Indonesia. Bioscience, Biotechnology, and Biochemistry. 2012; 76: 1897-1903.
14. Baloch MN, Siddiqi R, Zehra S, Zia M. Characterization of lactocin lc-09 produced by *Lactobacillus acidophilus*. International Journal of Current Research. 2015; 7: 13471-13476.
15. Amarantini C, Sembiring L, Kushadiwijaya H, Asmara W. Identification and characterization of *Salmonella typhi* isolates from South-west Sumba District, East Nusa Tenggara based on 16S rRNA gene sequences. Biodiversitas. 2011; 12: 1-6.
16. Klayraung S, Okonogi S. Antibacterial and Antioxidant Activities of Acid and Bile Resistant Strains of Lactobacillus fermentum Isolated from Miang. Brazilian Journal of Microbiology. 2009; 40(4): 757-766.
17. Fernandez B, Le Lay C, Jean J, Fliss I. Growth, acid production and bacteriocin production by probiotic candidates under simulated colonic conditions. Journal of Applied Microbiology. 2013; 114: 877-885.
18. Pan X, Chen T, Wu H, Tang, Zhao Z. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control. 2009; 20: 598-602.
19. Hu CH, Ren QL, Zhou Y, Ye CB. Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy food. Food Science & Nutrition. 2019; 7(6): 199-2005.
20. Martinez RC, Wachsman M, Torres NI, Leblanc JG, Todorov SD, Franco BD. Biochemical, antimicrobial and molecular characterization of a noncytotoxic bacteriocin produced by *Lactobacillus plantarum* ST71KS. Food Microbiol. 2013; 34(2):376-381.
21. Dewi SS, Herlisa A. Viabilitas bakteri asam laktat asal ASI terhadap pH asam lambung dan garam empedu. Seminar Penelitian LPPM UNIMUS ISBN: 978-602-18809-0-6. 2012; 97-102.
22. Lahtinen S, Ouwehand AC, Salminen S, Wright AV. *Lactic acid bacteria: microbiological and functional aspects 4th Ed*. 1:2-14. CRC Press: Taylor and Francis Group; 2012.
23. Makinen K, Berger B, Bel-Rhlid R, Ananta E. Science and technology for the mastership of probiotic applications in food products. Journal of Biotechnology. 2012; 162(4): 356-365.
24. Qian Y, Long X, Pan Y, Li G, Zhao X. Isolation and identification of lactic acid bacteria (*Lactobacillus plantarum* YS2) from yak yogurt and its probiotic properties. Biomedical Research. 2018; 29(4): 815-820.
25. [25] Nigam A, Kumar A, Madhusudan HV, Bhola N. In-vitro screening of antibacterial activity of lactic acid bacteria against common enteric pathogens. Journal of Biomedical Sciences. 2012; 1(4): 2.
26. Amarantini C, Satwika D, Budiarso TY, Yunita ER, Laheba EA. Screening of antimicrobial-producing lactic acid bacteria isolated from traditional fish fermentation against pathogenic bacteria. Journal of Physics. Conference Series 1397 2019; 012045.
27. Todorov SD, Franco B. *Lactobacillus plantarum*: characterization of the species and application in food production. Food Review International. 2010; 26(3): 205-229.
28. Khemariya P, Singh S, Jaiswal N, Chaurasia SNS. Isolation and identification of *Lactobacillus plantarum* from vegetable samples. Food Biotechnology. 2016; 30(1): 49-62.
29. Guidone A, Zotta T, Ross RP, Stanton C, Rea MC, Parente E, Ricciardi A. Functional properties of *Lactobacillus plantarum* strains: a multivariate screening study. Food Science and Technology. 2014, 56(1): 69-76.