Assessment of Potential Probiotic Lactic Acid Bacteria from Tempe and Tape

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Abstract. Probiotics are living organisms with many beneficial effects on the health of host if consumed in sufficient quantities. These beneficial effects have been initiating efforts directed towards exploring strains Lactic Acid Bacteria (LAB) as probiotic from fermented food such as tempe and tape. Although several groups of microorganism from fermented foods such as tempe and tape were reported, the potential LAB derived from those food sources is limited. The aim of this study was to assess probiotic candidate from LAB strains isolated from tempe and tape based on in vitro analysis. A total of 30 LAB isolates were tested for probiotic properties including tolerance to bile salt and acid, antimicrobial activity, simulated gastric juice (SGJ) and simulated intestinal juice (SIJ), physiology and enzymatic properties. The results showed eight bacterial isolates (Pediococcus pentosaceus Su-ls13, P. pentosaceus Su-ls14, Enterococcus faecalis Su-ls15, P. pentosaceus Su-ls16, P. pentosaceus Su-ls21, P. pentosaceus Su-ls22, P. pentosaceus Su-ls24 and Lactobacillus plantarum Su-ls29) fulfilled the criteria as probiotic candidates, including the capability of producing antimicrobial activity by inhibiting the growth of 12 pathogenic bacteria, have survivability under the condition of (or high tolerance to) low pH, and being exposed to bile salt, simulated gastric juice and simulated intestinal juice. All isolates were able to grow at NaCl 3-6.5%, 30-45°C and produce phytase. In addition, six isolates (Su-ls13, Su-ls14, Su-ls15, Su-ls16, Su-ls21, Su-ls22) were able to produce protease and two isolates (Su-ls22, Su-ls24) were able to produce amylase.

1. Introduction
Lactic acid bacteria (LAB) are a group of gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates [1]. LAB play a significant role in fermented foods and produce antimicrobial metabolite compounds such as lactic acid, bacteriocins, and hydrogen peroxide [2]. Some fermented food such tempe (mold fermented soybean) and tape (alcoholic fermented steamed glutinous rice or cassava) use a starter culture, however microorganisms from the environment may contaminate the ferments and grow during the fermentations. Rhizopus oligosporus is main starter culture for tempe fermentation however yeasts and lactic acid bacteria (LAB) are also detected in tempe [3]. In tape fermentation, LAB contributes to the development of flavor [4].
Traditional fermented foods can be used as potential sources of probiotics as they commonly contain LAB, including species of Lactobacillus, Pediococcus, Enterococcus, Weissella and Leuconostoc [4]. Over the past two decades, a large number of LAB isolated from various fermented food systems in different parts of the world, have been studied for their probiotic potential and ability to produce industrially important substances [5]. Some strains of LAB can be considered as probiotic bacteria. Probiotics can be defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host. Many studies have demonstrated the efficacy of probiotics to offer a proper alternative to the use of antibiotics in the treatment of enteric infection or to reduce the symptoms of antibiotic-associated diarrhea [2].

Probiotic and other functional properties are strain dependent and all probiotic strains are unique and different; therefore, their properties and characteristics need to be well defined. Several criteria have to be met in selecting probiotic strains, including acid and bile tolerance, survival through the gastrointestinal tract, ability to adhere to intestinal surfaces, antimicrobial activity against potentially pathogenic bacteria, and good technological properties [4]. Although several groups of microorganism from fermented foods such as tempe and tape were reported, the potential probiotic LAB derived from those food sources is limited. This study was aimed to assess the probiotic potential of LAB from tape and tempe which may exert beneficial effects for mankind.

2. Materials and Methods

2.1. Bacterial strains and growth conditions
LAB isolated from tempe originally from Bali were used, including: Lactobacillus fermentum Su-ls1, Weissella paramesenteroides Su-ls2, Lactobacillus plantarum Su-ls3, Lactobacillus plantarum Su-ls4, Lactobacillus plantarum Su-ls5, Weissella paramesenteroides Su-ls6, Enterococcus faecalis Su-ls7, Enterococcus faecalis Su-ls8, Enterococcus faecium Su-ls9, Lactobacillus plantarum Su-ls10, Pediococcus pentosaceus Su-ls11, Enterococcus faecium Su-ls12, Pediococcus pentosaceus Su-ls13, Pediococcus pentosaceus Su-ls14, Enterococcus faecalis Su-ls15, Pediococcus pentosaceus Su-ls16 and LAB isolated from tape: Lactobacillus vini Su-ls18, Leuconostoc mesenteroides Su-ls19, Weissella paramesenteroides Su-ls20, Pediococcus pentosaceus Su-ls21, Pediococcus pentosaceus Su-ls22, Lactobacillus fermentum Su-ls23, Pediococcus pentosaceus Su-ls24, Lactobacillus fermentum Su-ls25, Lactobacillus fermentum Su-ls26, Lactobacillus fermentum Su-ls27, Lactobacillus kunkeei Su-ls28, Lactobacillus plantarum Su-ls29, Lactobacillus plantarum Su-ls30, Leuconostoc mesenteroides Su-ls31. These bacteria were cultivated in de man, rogosa and sharpe (MRS) broth performed at 37°C for 24 h. Pathogenic bacteria (Escherichia coli, Bacillus cereus, Lysteria monocytogenes, Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae, Aeromonas hydrophilla, Edwardsiella ictaluri, Aeromonas sobria, Plesiomonas shigelloides and Klebsiella pneumonia) were cultivated in brain heart infusion (BHI) broth at 37°C for 24 h.

2.2. Bile tolerance
LAB strains were grown at 37°C for 24 h in MRS broth without bile. Approximately 5 μL of the LAB culture broth was spotted onto MRS agar containing bile salt (oxgall) at concentrations of 0.1%, 0.2%, 0.3%, and 0.4%, respectively. Bacterial growth in bile was determined after incubation at 37°C for 48 h [6].

2.3. Acid tolerance
LAB strains tested that were resistant to bile salts were subsequently underwent acid tolerance tests. The LAB grown in 0.5 mL MRS broth at 37°C for 24 hour were collected by centrifugation at 5400 rpm for 15 min under room temperature. Cell pellet was washed with 1 mL of phosphate-buffered saline (PBS) vortex in few seconds and collected by centrifugation at 5400 rpm for 15 min under room temperature. The pellet cell resuspended with 1 mL sterile PBS with the pH values ranged from 2-2.5 (adjusted using 5 M HCl) to achieve 10⁶ cfu/mL. PBS was prepared by dissolving NaCl (9 g/L), Na₂HPO₄.2H₂O (9 g/L)
and KH$_2$PO$_4$ (1.5 g/L) in distilled water. The tubes were incubated at 37°C for 4 hours. The viable cell was determined by spotted the cell suspension onto Glucose-Yeast Extract-Peptone (GYP) agar incubated at 37°C for 48 h. Bacterial growth was then observed [6,7].

2.4. Antimicrobial test

Agar spot test method was used for detection of antimicrobial activity. The certain LAB passed previous test were tested antimicrobial test. Overnight cultures of LAB (5 µL) were spotted on the surface of 20 mL GYP agar plates (containing 1.8% agar) and incubated for 24 hours at 37°C to allow colonies to develop. Overnight culture of indicator pathogens P. aeruginosa 2%, K. pneumonia 2%, B. cereus 2%, E. ictaluri 5%, E. coli 5%, A. sobria 5%, L. monocytogenes 5%, A. hydrophilla 5%, S. aureus 5%, and P. shigelloides 5% were inoculated into 5 mL of soft Mueller Hinton (MH) agar (containing 0.7% agar) and S. agalactiae 5%, S. enterica 5% inoculated into 5 mL of soft BHI agar (containing 0.7% agar) were poured over the plate which the LAB was grown, respectively. The plates were incubated at 37°C for 24 hours. At the end of the incubation, inhibition zone diameter surrounding the spotted isolates was measured [8,9].

2.5. Simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) resistance analysis

SGJ which was prepared by suspending 3 mg/mL pepsin in sterile saline solution (0.85% NaCl, w/v) was adjusted to pH 2.5. SGJ was inoculated with active LAB cultures (incubated at 37°C for 24 h in MRS Broth at an inoculums concentration of 1% (v/v) and incubated at 37°C for 4 h [7].

Pancreatin or SIJ resistance test was prepared by dissolving bile salt (0.3%) and pancreatin (1 mg/mL) in sterile saline solution adjusted to pH 8.0. SIJ was inoculated with active LAB cultures at an inoculum size of 1% (v/v) and incubated at 37°C for 6 h. The viable cell population was determined before and after incubation on GYP agar plates by the spread plate method [7].

2.6. The physiology and enzymatic properties

LAB isolates were subjected to gram staining, catalase test, growth curve, and growth at various temperature, pH, and salt concentration. Catalase activity was detected on cells grown on MRS agar by placing a drop of hydrogen peroxide solution on the cells. Growth at temperatures (5°C, 30°C, 45°C, and 60°C), at pH (3, 4, and 6), and at various concentrations of NaCl (3%, 6.5%, and 10%) were tested using MRS broth medium. For the growth curve, 1% of overnight LAB culture inoculated into 100 mL of GYP broth then homogenized with vortex. The growth of LAB was measured every 2 hours for 26 hours using UV-Vis spectrophotometry at $\lambda$ 600 nm [10].

The selected LAB strains were checked for presence of enzyme (i.e., amylase, protease, and phytase) activities according to Kim et al. [11] and Taheri et al. [12] with modifications. To detect the amylase, protease, and phytase activities, active LAB cultures were spot-inoculated onto relevant medium and then incubated for 48 h at 37°C. Amylase activity was examined using a GYP medium (without glucose) consisted of 1% starch. Protease activity was examined using NA medium consisted of 2% skim milk and phytase activity was examined using modified MRSA medium (without K$_2$HPO$_4$) containing 1% sodium phytate. After incubation, the halo zone surrounding each colony was measured with a caliper. The size of the halo zones surrounding the colonies gave an approximate indication of extracellular enzyme level.

3. Result and Discussion

Thirty isolates observed in this study were able to grow in the absence of bile as well as in the presence of 0.1 and 0.2% bile while only ten isolates grew on 0.3% bile, however two of those isolates (Su-Ls22, Su-Ls24) could not tolerate the higher concentration of bile (0.4%) (table 1). Two isolates (Su-Ls19, Su-Ls20) were not continued for analysis because have no antimicrobial activity (based on preliminary
antimicrobial tests, data not shown). Buntin et al. [6] suggested that bile salt concentration of 0.3% is considered as critical and high enough to screen for resistant strains. Therefore, the eight LAB isolates resistance to 0.3% bile were chosen to do the next analysis. Millet et al. [2] emphasized that bile salt tolerance is considered one of the most important attributes required by LAB to survive in the duodenum and the upper small intestine. Their ability to hydrolyze bile salt by bile salt hydrolase enzyme (BSH) are able to reduce this detergent effect. This particular enzyme decreases bile solubility and weakening its detergent effect thus minimizing its bactericidal effect on strains.

Among eight 0.3% bile resistance bacterial isolates, all isolates were able to survive in acidic condition (pH 2.5) and one isolate could not survive in pH 2.0 for four hours (table 2). Tolerance to acidity (pH 2.0-2.5) is considered as a key functional requirement for probiotics, which enables them to survive during passage through the gastrointestinal tract [7]. Based on Argyri et al. [13] the pH value 2.5 used in this study for the selection of potential probiotic strains is very selective even though it is not the most common pH value in the human stomach, therefore it assures the selection of very acid-tolerant strain. The survival ability of lactobacilli at pH 2.5 was also reported. However, variability in acidic response was obtained among the tested strains, indicating that resistance to low pH is a strain-specific property [7].

| Table 1. The bile salts resistance of LAB origin tape and temper observation based on bacterial growth. |
|-----------------------------------------------|
| **No** | **Strain** | **Concentration of bile salt (Oxgall)** |
|       |           |                                | **0.1%** | **0.2%** | **0.3%** | **0.4%** |
| 1     | *L. fermentum* Su-ls1 | + | + | - | - |
| 2     | *W. paramesenteroides* Su-ls2 | + | + | - | - |
| 3     | *L. plantarum* Su-ls3 | + | + | - | - |
| 4     | *L. plantarum* Su-ls4 | + | + | - | - |
| 5     | *L. plantarum* Su-ls5 | + | + | - | - |
| 6     | *W. paramesenteroides* Su-ls6 | + | + | - | - |
| 7     | *E. faecalis* Su-ks7 | + | + | - | - |
| 8     | *E. faecalis* Su-ks8 | + | + | - | - |
| 9     | *E. faecium* Su-ks9 | + | + | - | - |
| 10    | *L. plantarum* Su-ks10 | + | + | - | - |
| 11    | *P. pentosaceus* Su-ks11 | + | + | - | - |
| 12    | *E. faecium* Su-ks12 | + | + | - | - |
| 13    | *P. pentosaceus* Su-ks13 | + | + | + | + |
| 14    | *P. pentosaceus* Su-ks14 | + | + | + | + |
| 15    | *E. faecalis* Su-ks15 | + | + | + | + |
|       | **No** | **Strain** | **Concentration of bile salt (Oxgall)** |
|       |           |                                | **0.1%** | **0.2%** | **0.3%** | **0.4%** |
| 16    | *P. pentosaceus* Su-ks16 | + | + | + | + |
| 17    | *L. vini* Su-ks18 | + | + | + | + |
| 18    | *L. mesenteroides* Su-ks19 | + | + | + | + |
| 19    | *W. paramesenteroides* Su-ks20 | + | + | + | + |
| 20    | *P. pentosaceus* Su-ks21 | + | + | + | + |
| 21    | *P. pentosaceus* Su-ks22 | + | + | + | + |
| 22    | *L. fermentum* Su-ks23 | + | + | + | + |
| 23    | *P. pentosaceus* Su-ks24 | + | + | + | + |
| 24    | *L. fermentum* Su-ks25 | + | + | + | + |
| 25    | *L. fermentum* Su-ks26 | + | + | + | + |
| 26    | *L. fermentum* Su-ks27 | + | + | + | + |
| 27    | *L. kunkeei* Su-ks28 | + | + | + | + |
| 28    | *L. plantarum* Su-ks29 | + | + | + | + |
| 29    | *L. plantarum* Su-ks30 | + | + | + | + |
| 30    | *L. mesenteroides* Su-ks31 | + | + | + | + |

(+) no growth was observed

**Table 2. The acid resistance of selected bile-resistant LAB based on bacterial growth.**

| **No.** | **Strain** | **pH 2.5** | **pH 2.0** |
|---------|------------|------------|-----------|
|         |            | **0 h** | **4 h** | **0 h** | **4 h** |
| 1       | *P. pentosaceus* Su-ks13 | + | + | + | + |
| 2       | *P. pentosaceus* Su-ks14 | + | + | + | - |
| 3       | *E. faecalis* Su-ks15 | + | + | + | + |
| 4       | *P. pentosaceus* Su-ks16 | + | + | + | + |
| 5       | *P. pentosaceus* Su-ks21 | + | + | + | + |
| 6       | *P. pentosaceus* Su-ks22 | + | + | + | + |
| 7       | *P. pentosaceus* Su-ks24 | + | + | + | + |
| 8       | *L. plantarum* Su-ks29 | + | + | + | + |

(+) no growth was observed

Acid and bile tolerance properties are two fundamental properties that indicate the ability of probiotic microorganism to survive the passage though the upper gastrointestinal tract, particularly acidic condition in the stomach and the presence of bile in the small intestine [6]. Those properties are important for selection of the probiotic candidate, since the probiotic strains have to possess the ability to overcome the extremely low pH and the deterrent effect of bile salts for leading to the site of action in a viable physiological state [10]. Thus, only eight tested LAB strains with acid and bile tolerance are
suggested to survive in the gastrointestinal tract. Its reflected the phenomenon of bacterial strain-dependent in response to bile and acid tolerance [14].

Tolerance to stomach and intestinal conditions is an important trait for probiotic bacteria in terms of their performance to survive, grow, and exert action in the gut [14]. After 4 hours exposure to SGJ at pH 2.5, this study showed slightly decreased population of LAB compared to initial number of population but still survived well (table 3, figure 1A). Concerning on the survivability in the SIJ at pH 8.0, all tested isolates were survived well after exposure to SIJ for 6 h (table 3, figure 1B) suggesting a potential recuperation of the initial levels during the passage of the small intestine. However, the susceptibility or resistance of probiotic cultures to bile is species dependent as well as strain specific as mentioned [2].

**Table 3.** Viable count of selected LAB in simulated gastric and intestinal juice test.

| No. | Strain         | SGJ (log cfu/mL) | SIJ (log cfu/mL) |
|-----|----------------|------------------|------------------|
|     |                | 0 h   | 4 h   | 0 h   | 4 h   |
| 1   | *P. pentosaceus* Su-ls13 | 8.48  | 4.86  | 8.30  | 8.13  |
| 2   | *P. pentosaceus* Su-ls14 | 8.51  | 6.09  | 8.69  | 8.54  |
| 3   | *E. faecalis* Su-ls15   | 8.24  | 4.85  | 7.39  | 8.58  |
| 4   | *P. pentosaceus* Su-ls16 | 7.88  | 4.85  | 7.91  | 7.84  |
| 5   | *P. pentosaceus* Su-ls21 | 8.31  | 4.77  | 7.50  | 7.23  |
| 6   | *P. pentosaceus* Su-ls22 | 8.07  | 4.39  | 7.50  | 7.55  |
| 7   | *P. pentosaceus* Su-ls24 | 8.00  | 4.17  | 8.12  | 8.06  |
| 8   | *L. plantarum* Su-ls29  | 9.07  | 5.34  | 9.00  | 8.93  |

**Figure 1.** Survival of LAB strains in simulated gastric (A) and intestinal juice (B).

The results of spot-on-lawn test performed in this study showed that all isolates presented a wide range of inhibitory effects. All lactobacilli inhibited the growth of Escherichia coli, Bacillus cereus, Lysteria monocytogenes, Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Edwardsiella ictaluri, Aeromonas sobria, Plesiomonas shigelloides, Klebsiella pneumonia, Aeromonas hydrophilla, Streptococcus agalactiae and the strongest antimicrobial effect was shown by *L. plantarum* SU-LS29 while antimicrobial effect of other lactobacilli were varies (table 4, figure 2). The inhibitory properties of LABs are commonly formed by producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances, and bacteriocins. Bacteriocins may enhance the survival of LAB in complex ecological systems. It is more important with respect to probiotics may inhibit growth pathogenic microorganisms by secreted products, and not merely an effect of acidic pH [6,8]. Besides the various essential characteristics, the probiotic should exhibit health benefits with functional properties. Probiotics should present their antimicrobial actions particularly to the pathogens in the GI system [15]. Probiotics affect the host animal by improving its intestinal balance and mode of action is related to the competition for attachment sites (competitive exclusion). The probiotics attach to the intestinal mucosa
and block the attachment of pathogenic bacteria by forming a physical barrier [16] and control intestinal pathogens by production of antibacterial compounds [17].

**Figure 2.** Antimicrobial activity of selected LAB against *Bacillus cereus*, TE: Tetracycline 30 µg, GM: Gentamicin 10 µg.

**Table 4.** Diameter of inhibition (mm) of selected LAB against pathogenic bacteria.

| Strain          | EC  | BC  | LM  | SE  | PA  | SA  | SAg | AH  | EI  | PS  | KP  | AS  |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *P. pentosaceus* Su-Is13 | 13.0 | 20.0 | 16.5 | 16.0 | 7.5 | 15.0 | 15.0 | 15.0 | 20.0 | 19.00 | 6.0 | 20.0 |
| *P. pentosaceus* Su-Is14 | 13.0 | 19.5 | 18.0 | 17.0 | 8.0 | 16.0 | 15.0 | 16.5 | 20.0 | 17.00 | 7.0 | 20.0 |
| *E. faecalis* Su-ls15 | 10.0 | 13.0 | 14.0 | 10.0 | 5.0 | 10.0 | 10.0 | 10.0 | 15.5 | 14.00 | 6.0 | 15.5 |
| *P. pentosaceus* Su-Is16 | 13.0 | 19.0 | 19.0 | 17.0 | 9.0 | 16.0 | 15.0 | 18.5 | 21.0 | 19.00 | 7.0 | 21.0 |
| *P. pentosaceus* Su-Is21 | 14.0 | 22.5 | 18.0 | 15.0 | 9.0 | 19.0 | 14.0 | 16.0 | 21.0 | 14.00 | 8.0 | 21.0 |
| *P. pentosaceus* Su-Is22 | 14.0 | 24.0 | 19.5 | 18.0 | 11.0 | 20.0 | 17.0 | 19.0 | 22.0 | 16.00 | 8.0 | 22.0 |
| *P. pentosaceus* Su-Is24 | 14.0 | 22.5 | 19.5 | 17.0 | 10.0 | 19.5 | 16.0 | 17.0 | 21.0 | 17.50 | 7.5 | 21.0 |
| *L. plantarum* Su-ls29 | **19.0** | **25.0** | **24.0** | **24.0** | **14.0** | **26.0** | **22.0** | **26.0** | **26.0** | **22.00** | **14.0** | **26.0** |
| Tetracycline 30 µg | 17.0 | 25.0 | 22.0 | 19.0 | 0   | 15.0 | 11.0 | 22.0 | 15.0 | 11.00 | 18.0 | 15.0 |
| Gentamicin 10 µg  | 11.0 | 21.0 | 19.0 | 13.0 | 12.0 | 15.0 | 16.0 | 15.0 | 16.0 | 14.00 | 10.0 | 16.0 |

EC: *Escherichia coli*, BC: *Bacillus cereus*, LM: *Lysteria monocytogenes*, SE: *Salmonella enterica*, PA: *Pseudomonas aeruginosa*, SA: *Staphylococcus aureus*, EI: *Edwardsiella ictaluri*, AS: *Aeromonas sobria*, PS: *Plesiomonas shigelloides*, KP: *Klebsiella pneumonia*, AH: *Aeromonas hydrophilia*, SAg: *Streptococcus agalactiae*

The determination of the growth phase was important to form the growth curve in order to analyze the speed of which the bacteria reached the exponential phase and the bacteria generation time [18]. The fast growing characteristics of LAB and their metabolic activity have been the key in most applications including food production, agricultural industry, and probiotics [19]. The isolates’ growth was measured to determine biomass in the growth phases of each bacterial isolate. The growth curves formed in the observation period of 26 hours showed that isolates had varied patterns. Based on the initial growth phase or lag phase during the counting of colony numbers, it was showed that the probiotic candidates Su-Is13, Su-Is14, Su-Is16, Su-Is21, Su-Is22, Su-Is24 and Su-Is29 could reach high mass in their exponential phase compared to Su-Is15 isolate (figure 3A).
The probiotic candidates were characterized by classical methods. All the isolates were Gram positive and three isolates have positive catalase (Su-ls21, Su-ls22, Su-ls24). Based on physiological tests, the isolates showed good growth at 37°C and 45°C whereas no growth was observed at 5°C, indicating their mesophilic character. The lactobacilli were able to tolerate salt concentration of 6.5%, but were unable to grow at 10% NaCl, indicating their mesophilic character. The lactobacilli were able to tolerate salt concentration of 6.5%, but were unable to grow at 10% NaCl, indicating their mesophilic character. The lactobacilli were able to tolerate salt concentration of 6.5%, but were unable to grow at 10% NaCl, indicating their mesophilic character.

The size of the halo zones surrounding the colonies gave an approximate indication of extracellular enzyme levels. All bacterial isolates displayed different protease, amylase, and phytase activities (figure 3B). These results were similar to those reported by Kim et al. [11]. These exogenous enzymes will help the host’s endogenous enzymes in hydrolyzing nutrients such as breaking down long chains found in carbohydrates and protein. Breaking down complex molecules into simpler molecules will make the process of digesting and absorbing digestive tract easier. Its production of dietary enzymes will promote animal growth [11]. Probiotics are able to produce several exogenous enzymes to digest feed may increasing the nutritional value food/feed.

4. Conclusions
The six isolates of P. pentosaceus (Su-ls13, Su-ls14, Su-ls16, Su-ls21, Su-ls22, Su-ls24), E. faecalis Su-ls15, and L. plantarum Su-ls29 origin from tempe and tape were considered as potential candidates of probiotics. Those isolates demonstrate high tolerance to 0.3% bile salt and acid (pH 2.5), simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) and inhibited 12 pathogenic bacteria, while some strains producing dietary enzyme.

Acknowledgment
Authors thank to Research Center for Biology - LIPI for supporting the research funding.

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