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Characterization of *Lactobacillus zeae* as probiotic and starter culture for tamarillo fermented product

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Abstract

Probiotic was known as the microorganism that can improve human health. It can be in the form of supplements, probiotic food, or fermented food. Probiotic delivery as fermented food has several challenges. Bacteria must have characteristics as probiotics and must be able as a starter culture for fermentation. The characteristics of probiotics were safety, functionality, and high viability during manufacturing, storage, and transit in the gastrointestinal tract. Some fruit-based fermentations provide a protective environment for probiotics that can improve viability. This study aimed to isolate *Lactobacillus,* potentially a probiotic and a starter culture for tamarillo juice fermentation. The isolate was characterized for antibiotic susceptibility, haemolytic activity, lysozyme, acid and bile salt resistance, antimicrobial activity, bile salt hydrolase activity, and tamarillo's fermenting ability. *Lactobacillus plantarum* and *Lactobacillus acidophilus* were used as comparison bacteria in this study. The results indicated that *Lactobacillus zeae* could be isolated from tamarillo fruit. It was potential as a probiotic, which was safe and had functional properties. Then, it has high resistance to lysozyme, acid, and bile salt, with the percentage of survival successively 92.62, 89.93, and 96.75%. *L. zeae* can grow in tamarillo juice until the 30th hours.

Keywords: probiotic; tamarillo fruit; *Lactobacillus zeae*; fermentation.

Practical Aplication: *Lactobacillus zeae* can be used as a probiotic and starter culture for fruit-based fermentation beverages.

1 Introduction

When administered in adequate amounts, probiotics are live microorganisms that confer a health benefit on the host (Morelli & Capurso, 2012). Probiotics have anti-pathogenic activity, anti-diabetic activities, anti-obesity activity, anti-inflammatory activity, anti-cancer activity, angiogenic activity, anti-allergic activity, urogenital health care, and give effect on the brain and CNS (Kerry et al., 2018). *Lactobacillus* and *Bifidobacteria* are commonly used as probiotics because of having Generally Regarded as Safe (GRAS) status (O'Toole et al., 2017). Other genera are *Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Propionibacterium, Streptococcus, Peptostreptoccus, Saccharomyces, Enterococcus, Bacillus, Bacteroides* and *Akkermansia* (Kerry et al., 2018).

Probiotic can be isolated from the various environment such as the gastrointestinal tract (Nueno-Palop & Narbad, 2011; Kim et al., 2007), human/animal stool (Rubio et al., 2014; Pithva et al., 2014), human milk (Rajoka et al., 2017), fermented food (Nuraida, 2015; Swain et al., 2014), raw fruit/vegetables (Vitali et al., 2012) and marine sample (Das et al., 2016). Different habitats affect the characteristics of probiotics, although from the same species (Martino et al., 2016). Probiotics isolated from the gastrointestinal tract were recommended by the Food Drug Administration (FDA) and World Health Organization (WHO) for human purposes (Nueno-Palop & Narbad, 2011). Probiotics originating from the gastrointestinal tract are more resistant to extreme conditions and more attached to the human intestinal cell wall. However, isolates from the gastrointestinal tract are more at risk of resistance to antimicrobials (Fu et al., 2017) and may not be able to grow well in fruit or vegetables substrate. On the other hand, probiotics isolated from fruits or vegetables show high resistance in the gastrointestinal tract (Vitali et al., 2012), exhibit probiotic properties (Zielińska & Kolozyn-Krajewska, 2018) and may ensure better performance in fruits or vegetables fermentation (Di Cagno et al., 2011).

The current trend of fermentation products has begun to shift from dairy fermentation products to plant-based fermentation products (Granato et al., 2010; Prado et al., 2008; Ranadheera et al., 2017; Valero-Cases et al., 2020). Plant-based fermentation products are solutions for vegetarian consumers and lactose intolerance sufferers. Besides, fermentation of fruit and vegetables by lactic acid bacteria can improve the product's functional properties. Some bioactive compounds produced during fermentation are vitamins, bioactive peptides, organic acids, and fatty acids (Stanton et al., 2005). Fermentation can increase nutritional values, decrease anti-nutritional compounds, improve the bioavailability of bioactive compounds such as phenolic compounds (Septembre-Malaterre et al., 2018). These advantages lead to plant-based fermentation products that have a great opportunity to be developed into functional food.

Although plant-based fermented food products have great opportunities, there are various challenges. The challenge in

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developing plant-based products is choosing a starter culture and probiotic that can ferment the product and is stable during processing, storage, and passing through the human digestive tract. The most commonly used culture starters are *Lactobacillus plantarum* (Vitali et al., 2012) and *Lactobacillus pentosus* (Aponte et al., 2012). The best starter cultures were microorganisms originating from their natural habitat, otherwise known as autochthonous bacteria. Autochthonous bacteria from fruit and vegetable substrates are *Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus rossiae, Lactobacillus fermentum, Lactobacillus curvatus, Lactobacillus brevis, Lactobacillus paraplantarum, and Leuconostoc mesenteroides* (Cagno et al., 2013).

In this study, isolation of *Lactobacillus* from the tamarillo fruit was carried out. The obtained isolates are expected to have probiotic characteristics and the ability as a starter culture for fermented tamarillo products. Tamarillo fruit was chosen because this commodity still has low consumption but has a great opportunity to be developed as a functional fermented food. Tamarillo fruit contains many bioactive compounds, including anthocyanin, phenolic compounds, and hydroxycinnamoyl derivatives (Espin et al., 2016). In addition, Tamarillo fruit contains hydrocolloid compounds that act as prebiotics (Gannasin et al., 2015b). Prebiotics can increase the viability of probiotics during processing, storage, and transit in the digestive tract.

2 Material and methods

2.1 Fruit samples

Purple-red varieties of Tamarillo fruits (*Solanum betaceum)* were collected from the West Java Province of Indonesia. *Lactobacillus* was isolated from ripe tamarillo fruit. Ripe fruits have had a purple to red skin color with a soft texture.

2.2 Reference probiotics and pathogens

Lactobacillus plantarum and *Lactobacillus acidophilus* were used as probiotic references for this study and purchased from Microbiology Laboratory, School of Life Science and Technology, Institut Teknologi Bandung, Indonesia. The microorganisms used for the antimicrobial activity test were *Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 8939*, Candida albicans* ATCC 10231*, Salmonella typhi* ATCC 6539*,* and *Staphylococcus aureus* ATCC 6538.

2.3 Isolation of Lactobacillus

One gram of Tamarillo fruits (pulp & mucilage) was aseptically dissolved and homogenized in 9 mL of salt solution (NaCl 0.85%). The sample was diluted to 10^{-2} dilutions in sterile salt solutions. One mL of sample suspension from each dilution was taken to be inoculated into the De Man Rogosa Sharpe (MRS) agar medium, then incubated for 48 hours at 37 °C. Each colony that showed a different morphology was cultured again in MRS agar. Then, it was separated to be purified and identified.

2.4 Identification of isolate

Initial screening for species identification was carried out by observing colony morphology, cell morphology, Gram staining, and catalase test. Colonies that appear white, round, smooth, convex, and translucent were suspected as *Lactobacillus*. *Lactobacillus* were Gram-positive, rod-shaped, and catalase-negative. Identification was continued using the API CHL 50 kit (bioMérieux, France) and 16S rRNA sequencing analysis by Macrogen, Inc., Korea. The primer sequences used for PCR were 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3 and 1492 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'. The primer sequences used for sequencing were 785F 5' (GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'. Sequence results were aligned with NCBI database using BLAST algorithm. The phylogenetic tree was created using MEGA 11 to determine the closest bacterial species.

2.5 Characterization of isolates as probiotic candidates

a. Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out using the disc diffusion method (Clinical and Laboratory Standards Institute, 2012) with modification. Each disc paper contains erythromycin (15 μg) or tetracycline (30 μg) or gentamicin (10 μg) or chloramphenicol (30 μg). The bacteria were streaked onto De Man Rogosa Sharpe Agar (MRSA), then disc paper for each antibiotic was placed on the agar surface. The plates were incubated at 37 °C for 24 hours. The bacteria were considered sensitive if clear zones diameter more than 18 mm for erythromycin, more than 19 mm for tetracycline, more than 15 mm for gentamicin, and more than 23 mm for chloramphenicol.

b. Haemolytic activity

The Haemolytic activity test refers to the Pithva et al. (2014) with modifications. The bacteria were inoculated on the surface of the blood agar medium (Oxoid) supplemented with 5% human blood. Then, it was incubated at 37 °C for 24-48 hours. A positive test is indicated by forming clear zones (β-haemolysis) and green zone (α-haemolysis) around bacterial colonies. In contrast, a negative test is indicated by the absence of clear/ green zones around the colony.

c. Lysozyme resistance

This method was adopted from Zago et al. (2011). Sample preparation was carried out by inoculating bacteria in 10 mL of MRS broth and incubated for 24 hours at 30 °C. The bacterial culture was centrifuged to obtain a bacterial suspension; the pellets were washed 2 times using a sterile phosphate buffer solution (0.1 M, pH 7, 0), and then added 2 mL of sterile salt solution (0.85% NaCl). The test was carried out by inoculating a 10% bacterial suspension into a sterile electrolyte solution (0.22 g/L $CaCl_2$, 6.2 g/L NaCl, 2.2 g/L KCl, and 1.2 g/L $NaHCO_3$) which had been added 100 mg/l Lysozyme (Sigma-Aldrich). The samples were incubated at 37 °C, and cell counts were counted at 0, 30, and 120 minutes using the total plate count

method. The bacterial cells in the treatment without lysozyme were used as controls.

d. Acid resistance

The method was adopted from Shehata et al. (2016) with modification. The isolate was activated by inoculating in MRS broth for 20 hours at 37 °C. The bacteria were washed with sterile salt solution (0.85%) 2 times and then diluted 1/10 with a sterile salt solution. One mL of bacterial suspension was applied to 5 mL of gastric fluid simulation containing 3 g/l pepsin in a 0.5% (w/v) sterile salt solution with a pH 2 (using concentrated HCl). The test solution was shaken for 10 seconds and incubated at 37 °C for 3 hours. The number of cells was counted at 0, 1, 2, and 3 hours using the total plate count method (using MRS agar, incubation at 37 °C for 72 hours).

e. Bile salt resistance

The method was adopted from Shehata et al. (2016). The bacteria were first activated by inoculating in MRS broth for 20 hours at 37 °C. The bacterial culture in MRS broth is separated from the medium by centrifugation (3400 g, 10 min). The bacterial cells were washed twice using sterile salt solutions (0.85% NaCl), administration of a 10 mL physiological solution, centrifugation (3400 g, 10 min), and supernatant removal. The bacterial suspension is made by adding 10 mL of MRS broth to the washed bacterial cells. 1% of the bacterial suspension was inoculated on MRS broth, which was added with 3% bile salts (w/v) (Himedia) and then incubated for 3 hours at 37 °C. The bacterial population is counted every hour, starting at the 0^{th}, 1^{st}, $2^{nd,}$ and 3^{rd} hours. The control is made by inoculating the bacterial suspension on MRS broth without bile salts.

f. Antimicrobial activity

Antimicrobial activity was carried out using the disc diffusion method, and the bacteria were grown in MRS broth at 37 °C for 24 hours for the activation. The activation process was carried out twice. Bacterial culture in the liquid media was centrifuged (3400 g, 10 min), then separated between cell biomass and its supernatant. Supernatants were used for antibacterial testing. Disc paper was dipped in the supernatant, then stored on Mueller-Hinton Agar (MHA) surface. The test bacteria had been inoculated previously. The plates were incubated for 24 hours at 30 °C for *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli,* and 24 °C for *Candida albicans*. Positive results were indicated by the formation of clear zones around the disc paper.

g. Bile Salt Hydrolase activity

The qualitative analysis procedure refers to Shehata et al. (2016). The bacterial isolates were inoculated into wells on MRS agar media containing 0.5% (w/v) sodium taurodeoxycholate hydrate (TDCA; Sigma, USA) and 0.037% (w/v) calcium chloride and then incubated at 37°C for 72 hours. The positive results are indicated by the formation of the zone of precipitation around the well.

2.6 Fermentation in tamarillo fruit juice

a. Inoculum preparation

The inoculum was prepared by reactivation culture twice in MRS Broth for 24 hours at 37 °C and under anaerobic conditions. The density of culture was 10^6-10^7 cfu/mL in the final concentration after added to the fermentation substrate.

b. Fermentation

Pulp and mucilage from red-purple tamarillo were used as the substrate for fermentation. The tamarillo juice was made using a juice extractor and then diluted with sterile distilled water in a 1:1 ratio (6,5°Brix). The juice was then added with 2% (w/v) sucrose (9,8 °Brix). The juice was pasteurized for 20 min at 80 °C. After the temperature down, inoculum was added to the tamarillo juice and fermented for 36 hours.

c. Fermented tamarillo juice analysis

Bacterial population (cfu/mL) and pH changes were determined during fermentation (36 h) in 6 h intervals. The bacterial population was determined using the total plate count method (cfu/mL) in MRS agar. The pH was determined using a pH meter (Peak S-610L, USA).

2.7 Statistical analysis

Values that are presented are mean and standard deviation from the triplicate analysis. Characterization results between *Lactobacillus* species were compared using One-way ANOVA ($p < 0.05$) and followed up with Tukey's Multiple Comparison Test ($p < 0.05$). The statistical analysis was performed using SPSS version 25.0.

3 Results and discussion

3.1 Isolation and identification

From 8 samples of tamarillo fruit (taken from different gardens and different harvest times), 22 isolates were obtained. One of the them showed the characteristics of *Lactobacillus*. The code of the isolate obtained was TLB-2. It was Gram-positive rod, as shown in Figure 1a. The others were Gram-positive coccus bacteria, Gram-negative coccus bacteria and yeast.

TLB-2 was identified using the API CHL 50 kit based on the carbohydrate fermentation pattern. The identification results showed that the isolates were presumed as *Lactobacillus casei,* but the % identity was relatively low. Re-identification is done using 16s rRNA sequencing analysis. The results showed that the TLB-2 was *Lactobacillus zeae* (99% homology), as shown in Figure 1b. *Lactobacillus zeae* was a reclassified bacterial strain of *Lactobacillus casei* subsp. *casei* (ATCC 393) and *Lactobacillus rhamnosus* (ATCC 15820) (Dicks et al., 1996).

L. zea was isolated from raw tamarillo fruit. It can be autochthonous bacteria that have potential as a starter culture for tamarillo fermentation. Based on other research, *L. zeae*

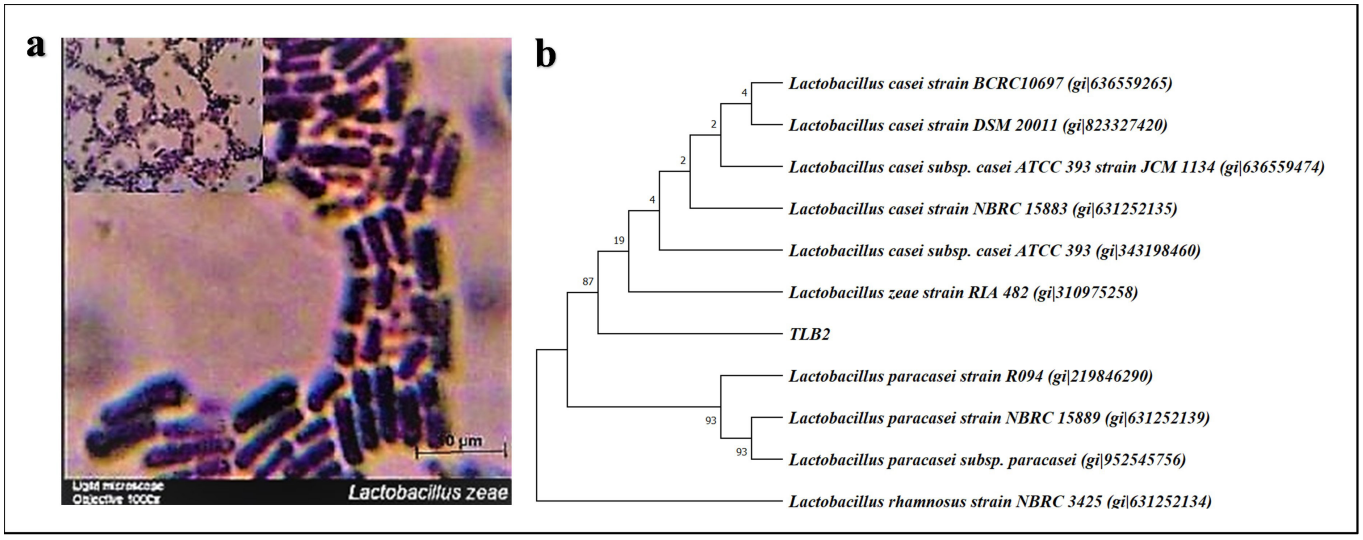

Figure 1. (a) Morphology of isolate from raw tamarillo fruits (1000x magnification using a light microscope), (b) Phylogenetic tree of isolate that has 99% homology with *Lactobacillus zeae.*

has been found in the middle stage of Chinese sauerkraut fermentation (Xiong et al., 2012). *L. zeae* was found in raw milk (Výrostková et al., 2020), dairy products (Švec et al., 2005) and animal intestine (Wang et al., 2011).

3.2 Characterization probiotic

a. Antibiotic susceptibility testing

Probiotics should not be resistant to antibiotics because they can potentially transfer this resistance gene to other bacteria in the environment, especially pathogenic bacteria (Ashraf & Shah, 2011). Based on the research, *L. zeae, L. plantarum,* and *L. acidophilus* were sensitive to erythromycin, gentamicin, tetracycline, and chloramphenicol. The inhibition zone of the three bacteria is still below the sensitivity limit set by Clinical and Laboratory Standards Institute (2012), as in Table 1. The results indicate that *L. zeae* from tamarillo fruit was safe and can be used as probiotic.

The sensitivity to antimicrobials was different for each species and strain in the genus *Lactobacillus. Lactobacillus* had genes resistant to erythromycin, tetracycline, and gentamicin (Danielsen & Wind, 2003; Guidone et al., 2014; Ouoba et al., 2008; Zago et al., 2011). Meanwhile, other research shows that the *Lactobacillus* strain obtained are sensitive to tetracycline, erythromycin, and chloramphenicol (Gotteland et al., 2014). *L. zea* from raw milk was resistant to erythromycin, clindamycin, and ampicilin (Výrostková et al., 2020). The resistance of a bacterium to antimicrobial substances is a specific strain (Danielsen & Wind, 2003).

b. Haemolysis test

Probiotics must not be pathogenic. One of the safety analysis parameters is the haemolysis test. Haemolytic activity is usually shown by pathogenic bacteria capable of producing toxin compounds that damage red blood cells. Probiotics should test negative for this test (Byakika et al., 2019). Based on the results, *L. zeae, L. plantarum,* and *L. acidophilus* showed negative results for the haemolysis test, indicated by the absence of a clear zone around the bacterial colony. The absence of haemolytic activity is also called type γ-haemolysis. Species in the genus *Lactobacillus* generally do not show haemolytic activity, but some do show α-haemolytic activity. *L. pentosus* shows α-haemolytic activity (Argyri et al., 2013).

c. Lysozyme resistance

Lysozymes are antimicrobial enzymes found in saliva (Rada et al., 2010). This enzyme can inhibit microbial growth because it can hydrolysis the β- (1,4) N-acetylglucosamine and N-acetylmuramic bonds in bacterial cell wall components. Resistance to lysozyme is an important criterion for probiotics because probiotics must survive until colonization in the intestine and provide health effects for the host.

L. zeae showed a high resistance to 100 mg/L lysozyme (92.62%) under simulated conditions of saliva, which *L. plantarum* and *L. acidophilus* were 93.57 and 89.96%, respectively. Based on statistical analysis, there was no difference in the average reduction of the bacterial population between the three bacteria during 120 minutes of incubation, as shown in Table 2. The resistance of the genus *Lactobacillus* to lysozyme varies widely. In one strain of *L. plantarum,* the resistance to lysozyme varied between 24 -99.97% (Zago et al., 2011). *L. plantarum* strain has resistance to lysozyme more than 85%, with a variation of 0.1-1.25 log reduction and an incubation time of 10 minutes (Shekh et al., 2016).

d. Acid resistance

Probiotics must survive the extreme conditions of gastric juices. Low pH and the presence of hydrolytic enzymes can kill

microbes. The resistance of bacteria to gastric juices was simulated by inoculating the bacteria into a solution containing pepsin and HCl (pH 2) then incubated for 3 hours. The viable cells per hour were observed. The results showed that *L. zeae* had a better survival percentage (89.93%) than *L. plantarum* (88.74%) and *L. acidophilus* (88.14%), as shown in Table 3. Other research showed that the resistance of lactic acid bacteria to acids varies between 68-88.3%, with the percentage of survival reaching 85% owned by *Lactobacillus lactis* (Shehata et al., 2016). *Lactobacillus* is resistant to acids because it could control the cytoplasm pH by pumping H^+ out of the cell through a proton pump using ATP (Kullen & Klaenhammer, 1999) or it could produce exopolysaccharide components (Caggianiello et al., 2016).

e. Bile salt resistance

Bile salts are bile acid conjugates toxic to bacteria (Ridlon et al., 2016). Resistance to bile salts is one of the microbial selectors that can live in the digestive tract. *L. zea, L, plantarum,* and *L. acidophilus* had a fairly high resistance to bile salts, with % survival being 96.75, 94.63, and 95.94%, respectively. Based on Table 4, the decrease in bacterial population was less than 1 log during 3 hours incubation. Other research (Vera-Pingitore et al., 2016) showed that the decrease in the number of bacterial cells for 4 hours of incubation on media containing bile salts ranged from 0.63 to 4.93 logs. The bacteria neutralize the toxic properties of bile salts by deconjugating bile salts to produce bile acid conjugates (cholic acid/ chenodeoxycholic acid) and amino acids (glycine/ taurine) (Ridlon et al., 2016).

f. Antimicrobial activity

Probiotics were useful in suppressing the growth of pathogenic bacteria in the digestive tract. The ability of probiotics to suppress the growth of pathogenic bacteria occurs due to production of antimicrobial substances. The ability to produce antimicrobial substances is one of the functional criteria possessed by probiotics.

Based on Table 5, the supernatant of *L. zeae, L. plantarum, and L. acidophilus* showed inhibition against *B. subtilis, E. coli, S. typhi, S. aureus* and *C. albicans*. *L. plantarum* had the highest inhibition zone against *B. subtilis, S. typhi,* and *S. aureus*, while *L. zeae* has the highest inhibition zone against *E. coli*. Among lactobacilli, *L. plantarum* was known as species that produce the highest antimicrobial compounds: organic acid, diacetyl, bacteriocin (plantaricins), hydrogen peroxide, and antimicrobial peptides (Danilova et al., 2019). The antimicrobial activity of *Lactobacillus* can result from the accumulation of organic acids affecting the cytoplasmic acidification with a subsequence of energy production and accumulation of acid anion dissociating to toxic level (Hu et al., 2019). In addition, bacteriocin can cause cell death due to membrane leakage after the bacteriocin binds to the receptor. (Giani et al., 2019).

g. Bile Salt Hydrolase Activity

Bile Salt Hydrolase (BSH) is an enzyme that can hydrolyses bile salt conjugates. The activity of the BSH enzyme is related both to the resistance of bacteria in the digestive tract and to inhibit the fat emulsification process in the digestive tract (Begley et al.,

Table 1. Antibiotic susceptibility testing of *Lactobacillus* spp. against 4 different antibiotics.

Bacteria	Inhibition zone (mm)			
	Erythromycin	Gentamicin	Tetracycline	Chloramphenicol
L. zeae	33,43 ± 1,25 **(S)**	26,73 ± 0,29 **(S)**	29,07 ± 2,06 **(S)**	35,97 ± 2,22 **(S)**
L. plantarum	21,63 ± 0,81 **(S)**	17,30 ± 0,63 **(S)**	19,60 ± 1,08 **(S)**	26,67 ± 1,61 **(S)**
L. acidophilus	31,00 ± 2,17 **(S)**	27,43 ± 1,00 **(S)**	22,98 ± 2,25 **(S)**	32,00 ± 1,37 **(S)**

(S) Sensitive to erythromycin (clear zone > 18 mm); Gentamicin (clear zone > 15 mm); Tetracycline (clear zone > 19 mm); Chloramphenicol (clear zone > 23 mm), (CLSI, 2012).

Table 2. Survival of *Lactobacillus* spp. in the presence of 100 mg/L lysozyme for 120 minutes incubation at 37 °C.

Bacteria	Mean of viable count (log_{10} cfu/mL)			% Survival
	Time exposure (minutes)			
	0	30	120	
L. zeae	10,67[a] ± 0,577	10,12[a] ± 0,332	9,61[a] ± 0,201	92,62
L. plantarum	10,52[a] ± 0,827	10,26[a] ± 0,679	9,39[a] ± 0,208	93,57
L. acidophilus	10,67[a] ± 0,577	9,74[a] ± 0,090	9,41[a] ± 0,343	89,96

[a]Indicates mean of viable count similarity in the same column.

Table 3. Survival of *Lactobacillus* spp. during 3 hours exposure in simulated gastric juice at 37 °C.

Bacteria	Mean of Viable Count (log_{10} cfu/mL)				% Survival
	Time exposure (hours)				
	0	1	2	3	
L. zeae	13,01[a] ± 0,024	12,93[a] ± 0,033	12,17[a] ± 0,450	11,70[a] ± 0,373	89,93
L. plantarum	13,14[a] ± 0,121	12,95[a] ± 0,082	12,24[a] ± 0,505	11,66[a] ± 0,154	88,74
L. acidophilus	12,98[a] ± 0,029	12,89[a] ± 0,048	12,17[a] ± 0,536	11,44[a] ± 0,127	88,14

[a]Indicates mean of viable count similarity in the same column.

Table 4. Survival of *Lactobacillus* spp. in MRS broth supplemented with 0,3% bile salts during 3 hours exposure.

Bacteria	Mean of Viable count (log_{10} cfu/mL) Time of exposure (hours)				% Survival
	0	1	2	3	
L. zeae	12,71[a] ± 0,380	12,35 [a] ± 0,059	12,20[a] ± 0,156	11,90 [a] ± 0,031	96,75
L. plantarum	12,86 [a] ± 0,569	12,61 [a] ± 0,336	12,28 [a] ± 0,149	12,16 [a] ± 0,112	94,63
L. acidophilus	12,67 [a] ± 0,091	12,42 [a] ± 0,068	12,25 [a] ± 0,073	12,15 [a] ± 0,069	95,94

[a]Indicates mean of viable count similarity in the same column.

Table 5. Antimicrobial activities of cell-free supernatants *Lactobacillus* spp. against various pathogens.

Bacterial Supernatant	Inhibition Zone (mm)				
	B. subtilis	*E.coli*	*S. typhi*	*S. aureus*	*C. albicans*
L. zeae	6,95[b] ± 0,20	8,20[b] ± 0,18	6,67[a] ± 0,25	6,63[a] ± 0,16	6,10[a] ± 0,13
L. plantarum	7,15[b] ± 0,49	6,13[a] ± 0,08	8,13[b] ± 0,26	8,43[b] ± 0,45	6,33[a] ± 0,58
L. acidophilus	6,10[a] ± 0,05	6,23[a] ± 0,14	7,28[ab] ± 0,55	7,58[ab] ± 0,59	6,37[a] ± 0,64

[a-b] Means within the row with different superscript letters are significantly different (P<0.05).

2006). Bacteria with BSH activity are good probiotic candidates to be developed as functional foods with cholesterol-lowering effects.

L. zeae, L. plantarum, and L. acidophilus showed positive results for BSH enzyme activity, which was indicated by the formation of precipitates around the wells where the bacteria were inoculated in Figure 2. *L. zeae* was an isolate from tamarillo fruit, where its habitat does not contain bile salts. These results were proved that the production of the BSH enzyme does not correlate with the bacterial habitat. This finding follows the research of Shehata et al. (2016), which showed positive results of BSH enzyme activity from 6 isolates apart from the digestive tract. Several species of bacteria produce this enzyme in the digestive tract, such as *Lactobacillus* sp.*, Bifidobacterium longum, Clostridium perfringens, and Bacteroides fragilis* ssp. *fragilis* (Corzo & Gilliland, 1999).

3.3 Fermentation in tamarillo juice

In developing fermented probiotic beverages, probiotics should act as a starter culture. Starter culture must be able to grow on a fermentation substrate. Not all microorganisms can be used as a starter culture for fruit juice fermentation due to inappropriate pH or lack of nutrients. In this study, *Lactobacillus* was grown in a tamarillo juice. The results showed that *L. zeae, L. plantarum,* and *L. acidophilus* were able to grow in Tamarillo Juice. Bacterial growth is characterized by an increase in bacterial population and a decrease in pH due to the accumulation of bacterial metabolites, such as lactic acid, as shown in Figure 3. These results indicate that the tamarillo juice contains nutrients for the growth of *Lactobacillus*. In addition, pH substrate supports the growth of *Lactobacillus*. Tamarillo juice has a high protein, starch, and food fiber content (Gannasin et al., 2015a).

Although all three *Lactobacillus* are capable of growing in the tamarillo juice, the growth rate for each species was different. In the first 6 hours of fermentation, there was an increase in the bacterial population of *L. acidophilus* and *L. plantarum*. However, the bacterial population of *L. zeae* was a decrease. The decrease

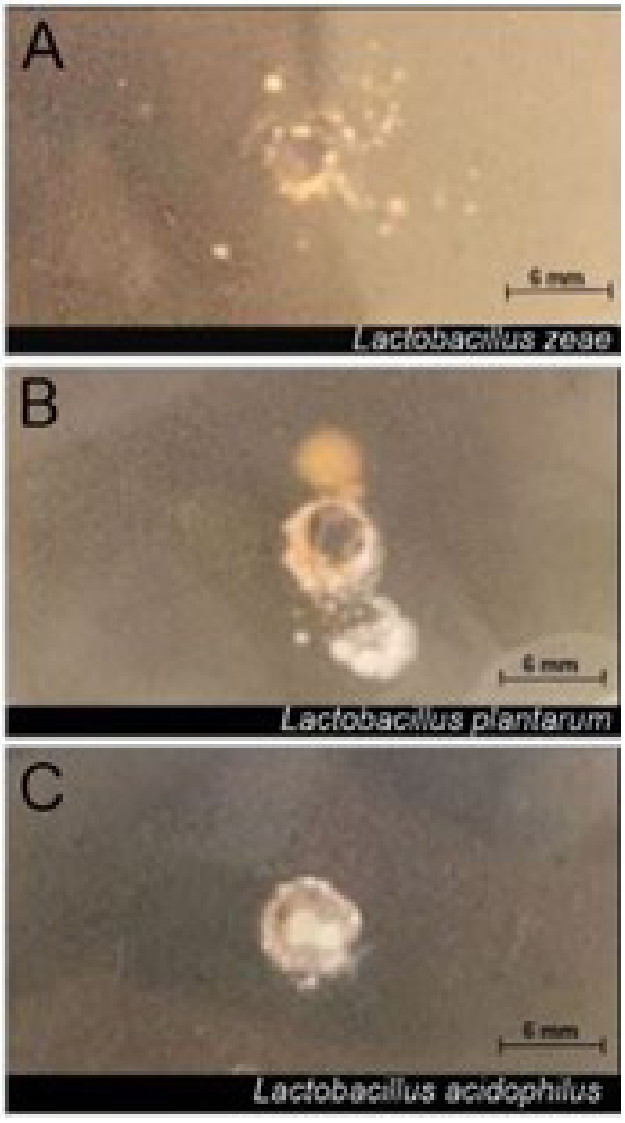

Figure 2. Bile salt hydrolase activity of *Lactobacillus* spp.; (A) *L. acidophilus*, (B) *L. plantarum*, (C) *L. zeae.*

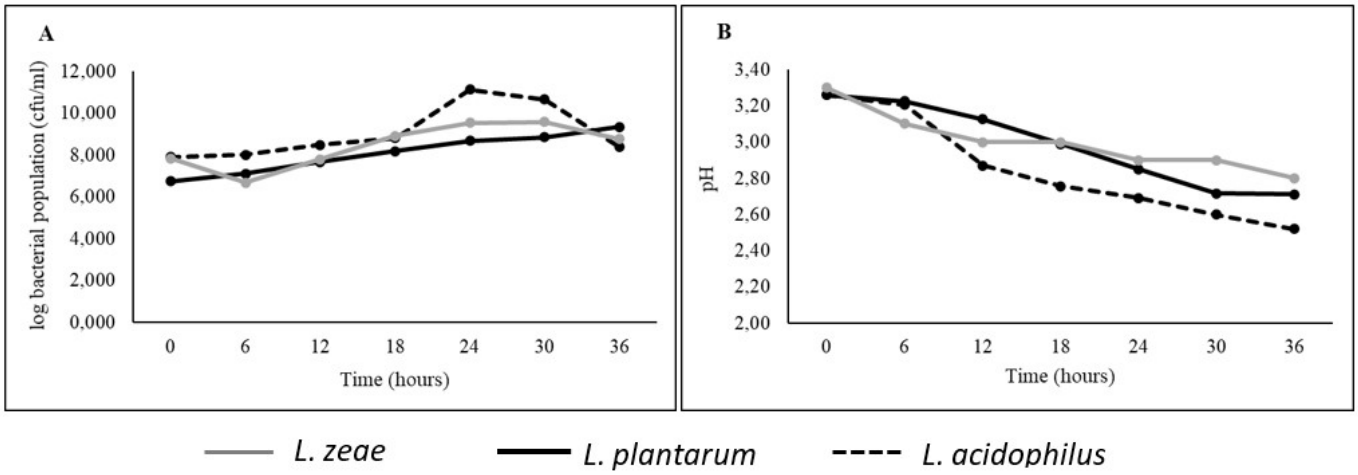

Figure 3. (A) Growth curve of *Lactobacillus* spp. in tamarillo fruit juice (added 2% sucrose) for 36 hours; (B) Changes in pH during the fermentation by *Lactobacillus* spp.

of *L. zea* cells in the early stages is due to the bacterial cells adapting to the adaptation phase's environmental conditions. In the adaptation phase, the bacterial cell produces the enzymes needed in the breakdown of the substrate and adapts to the substrate's external conditions like pH, temperature, oxygen content, etc.

L. zea was grown until the 30th hour, *L. acidophilus* was grown until the 24th hour, and *L. plantarum* continued to grow until the end of the fermentation period. This finding shows that *L. plantarum* more acid-tolerant than *L. zeae*. This result is in accordance with Xiong et al. (2012), which showed that *L. plantarum* persisted until the end of the sauerkraut fermentation period, while *L. zea* survived a short time. The factors affecting probiotic resistance in fruit substrates are species/strain, culture preparation method, product composition, temperature and storage time, the fiber in the product, oxygen content, and type of packaging (Nualkaekul & Charalampopoulos, 2011).

4 Conclusions

Lactobacillus zeae isolated from tamarillo fruit has probiotic characteristics similar to *L. plantarum* and *L. acidophilus*, which has high resistance to digestive tract conditions (lysozyme, low pH, bile salt), sensitive to antibiotics, does not cause haemolysis, has bile-salt-hydrolase- enzyme activity, and produces antimicrobial compounds. In addition, *L. zeae* can be used as a starter culture to produce fermented food from tamarillo fruit juice, although its growth is not as good as *L. plantarum*.

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