The Effect of Prebiotic Types on The Metabolism of Indigenous *Lactobacillus* Isolates

Pengaruh Jenis Prebiotik Terhadap Metabolisme Isolat Lactobacillus Indigenous

Ni Nyoman Puspawati[1,3*], Nyoman Semadi Antara[2], I Dewa Gde Mayun Permana[3], I Dewa Made Sukrama[4]

[1]Udayana University, Postgraduate Program, Study Program of Agricultural Science, PB Sudirman Street, 80232, Denpasar City, +6281385314130
[2]Udayana University, Faculty of Agricultural Technology, Study Program of Agricultural Industrial Technology, Kampus Unud Bukit Jimbaran, 80361, Badung City, 0361-701801
[3]Udayana University, Faculty of Agricultural Technology, Study Program of Food Technology, Kampus Unud Bukit Jimbaran, 80361, Badung City, 0361-701801
[4]Udayana University, Faculty of Medical, Study Program of Medical Education, PB Sudirman Street, 80232, Denpasar City, 0361-222510

Penulis Korespondensi: Ni Nyoman Puspawati; Email: puspawati@unud.ac.id

Abstrak

Prebiotik adalah komponen makanan yang tidak dapat dicerna di dalam tubuh dan mendorong pertumbuhan atau aktivitas mikroba usus yang bermanfaat seperti bakteri asam laktat (BAL). Namun, kemampuan *Lactobacillus* yang diisolasi untuk memanfaatkan prebiotik masih sedikit diketahui. Penelitian ini bertujuan untuk mengetahui pengaruh berbagai jenis prebiotik sebagai sumber karbon terhadap metabolisme isolat *Lactobacillus* indigenous dari teh kombucha, dadih (susu fermentasi tradisional) dan bambu yang digunakan untuk wadah dadih. Kemampuan isolat *Lactobacillus* dalam memetabolisme berbagai prebiotik sebagai sumber karbon dilakukan dengan menumbuhkan BAL dalam media MRSB dimana kandungan glukosa diganti dengan prebiotik yaitu fruktooligosakarida (FOS), galaktooligosakarida (GOS) dan inulin, kemudian penurunan kadar glukosa diukur setelah inkubasi 24 jam. Pengukuran total glukosa dilakukan dengan metode fenol sulfat. Hasil penelitian menunjukkan keseluruhan isolat *Lactobacillus* mengalami peningkatan jumlah sel selama inkubasi 24 jam yaitu dari 0,52 menjadi 1,26 log. Penurunan kadar glukosa berkisar antara 3,80 ± 0,44% sampai 91,33 ± 4,83%. Penurunan kadar glukosa tertinggi terjadi pada isolat *L. plantarum*1 RB210 yaitu 91,33 ± 4,83% dan tidak berbeda nyata (P>0,05) dengan *L. paracasei* RK41 dan *L. paracasei* RL2 yang ditumbuhkan pada media inulin. *L. plantarum*1 MK2 menunjukkan penurunan pH tertinggi yaitu 1,91 ± 0,08 dan tidak berbeda nyata (P>0,05) dengan *L. rhamnosus* MY2 pada media yang mengandung GOS. Hasil penelitian ini menunjukkan bahwa isolat *Lactobacillus* Indigenus secara definitif dapat menggunakan prebiotik sebagai sumber karbon untuk metabolismenya. Hasil penelitian ini memberikan dasar pengaruh penggunaan probiotik dan prebiotik untuk aplikasi sinbiotik sehingga dapat memodulasi mikrobiota usus.

Kata kunci: *Lactobacillus*, metabolisme, prebiotik, FOS, GOS, Inulin

INTRODUCTION

Probiotics are foods containing live microorganisms that actively promote health by improving the balance of intestinal flora when consumed alive in adequate amounts (Fuller, 1989). FAO and WHO (2001) define probiotics as live microorganisms (bacteria or yeasts) which when consumed or used in sufficient quantities can improve the health of those who consume them. Microbes commonly used as probiotics are lactic acid bacteria (LAB). Lactic acid bacteria that have potential as probiotics must be resistant to gastric acid and bile salts (Shortt, C. 1999).

Prebiotics are food ingredients that cannot be digested by the intestines that actively increase the growth and activity of microflora in the

digestive tract (colon) thereby improving the health and well-being of the host (Roberfroid, 2007). Prebiotics are mostly dietary fiber, such as oligosaccharides but not all dietary carbohydrates are prebiotics (Gibson *et al*., 2004). Three criteria for prebiotic classification, which are: 1) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; 2) fermentation by intestinal microflora; 3) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson *et al*., 2004). Carbohydrates in the colon are fermented to short chain fatty acids (SCFA) mainly acetate, propionate and butyrate (Cummings, 1995; Flint, 2006). Short chain fatty acids (SCFA) in the large intestine have very important roles including roles as nutrients for the colonic epithelium, as colonic modulators and intracellular pH, other functions related to ion transport, and as regulators of proliferation, differentiation, and gene expression (Wong *et al*., 2006).

The main group of prebiotics commonly used in food ingredients or supplements comes from two chemical groups, namely inulin-type fructans (ITF) and galacto-oligosaccharides (GOS). These have repeatedly demonstrated the capacity to selectively stimulate the growth of bifidobacteria and, in some cases, lactobacilli leading to significant changes in gut microbiota composition (Salminen and Wright, 2004). Commercial oligosaccharides commonly used as prebiotics are inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Inulin, FoS and GOS are not substrates for hydrolytic enzymes in the upper gastrointestinal tract (Macfartan *et al*., 2008; Gropper *et al*., 2009). Inulin and FOS are fructans that have a specific structure (bond β(2-1)) that cannot be hydrolyzed by human digestive enzymes, and these compounds will reach the large intestine and be fermented by microbes (Frank and Anne, 2000; Asto *et al*., 2019). According to Zhang, *et al*. (2007), in general, *Lactobacillus* species have the ability to ferment most of the prebiotics. The utilization of prebiotic oligosaccharides by probiotic bacteria depends on the species (Manning *et al*., 2004). Several studies have shown that the addition of prebiotics to LAB growth medium can increase the proliferation of *Bifidobacterium* spp. cells (Bouhnik, 1999; Shin *et al*., 2000; Moro *et al.*, 2002). The effect of prebiotic on gut health is associated with the capability of gut beneficial microbes to utilize prebiotics as a carbon source, which results in the selective growth of these microbes, the decline of the gastrointestinal pH, and production of short chain fatty acids (Zhu *et al*., 2021). However, there is limited knowledge on the lactic acid bacteria isolated from kombucha tea, dadih and bamboo to consume prebiotics. Therefore, this study aims to determine the ability of *Lactobacillus* isolates to use prebiotics as a carbon source in their metabolic processes.

METHODS

Media and Reagents

The materials used in this study were de Man Rogosa (MRS) broth (Oxoid), de Man Rogosa (MRS) agar (Oxoid), NaCl (Merck), 90% ethanol (Merck), calcium carbonate (Merck), aquadest, fructooligosaccharide/ FOS (Fibrulose® F97,

Cosucra), galactooligosaccharide/ GOS (FocusHerb LLC), inulin (Now Foods), barium chloride (Merck), sulfuric acid (Merck).

Probiotic Cultures

The probiotic cultures used in this study were 13 indigenous LAB isolates from kombucha tea, dadih (traditional fermented milk), and bamboo of dadih container (Table 1). The probiotic strains were preserved in 20% glycerol and stored at -80 ^{o}C. The cultures belong to the collection of the Food Microbiology Laboratory, Faculty of Agricultural Technology, Udayana University, Indonesia.

Table 1. Type and sources of *Lactobacillus* isolates

Type of *Lactobacillus* isolates	Sources
L. plantarum 1 RB210	kombucha tea
L. pentosus MK42	kombucha tea
L. pentosus MS21	kombucha tea
L. pentosus MB23	kombucha tea
L. paracasei RK41	kombucha tea
L. plantarum 1 RN9	dadih
L. plantarum 1 MA1	dadih
L. plantarum 1 ML7	dadih
L. rhamnosus MY2	dadih
L. plantarum 1 RJ1	bamboo of dadih container
L. plantarum 1 MK2	bamboo of dadih container
L. paracasei RL2	bamboo of dadih container
L. pentosus RG5	bamboo of dadih container

Preparation culture

LAB isolates from kombucha tea were previously stored using the immobilization method [26] using beads in cryotube tubes containing a mixture of culture and 20% glycerol and stored at -80 °C in the freezer. Before use, the isolate must be refreshed by taking 2 to 4 beads using a loop needle into a tube containing 4.5 ml of MRSB and incubating at 37 °C for 24 h. The growth of bacteria on MRSB can be seen from the change in the media to become cloudy and ready for use.

Confirmation test

Gram stain

Gram stain which refers to (Harrigan and Mc Chance, 1998). Sterile water was dripped on an object glass and smeared 1 ose of isolate from the *Lactobacillus* culture that had been refreshed, then fixed over a Bunsen fire. Furthermore, as much as 1 drop of crystal violet dye was given and allowed to stand for 1 minute. Rinse with water and dry with a tissue. The next step is to give 1 drop of Lugol's liquid and let it sit for 2 minutes. Rinse and dry with a tissue. The object glass is then dripped with 95% alcohol acetone, and allowed to stand for 10 to 20 seconds. The object glass is then rinsed with water and dried with a tissue. Finally, a glass object dripped with safranin dye and let stand for a few minutes. Bacterial cells on the prepared preparations were then observed using a microscope with a magnification of 1000x. Negative bacterial cells belonging to the Gram positive group are cells that have a purple color and

those belonging to the Gram group are cells that have a pink color.

Morphological observation

Observation of bacterial morphology refers to (Harrigan and Mc Chance, 1998). The preparations obtained during Gram staining were then observed using a microscope with 1000x magnification. Lactic acid bacteria used in this study have the shape of a rod, both long and short rods.

Catalase test

The catalase test conducted in this study refers to (Harrigan and Mc Chance, 1998). The catalase test was carried out by applying 1 ose of isolate on a glass slide and given 2 drops of 3% H_2O_2 solution. A positive reaction was indicated by the formation of gas bubbles while a negative reaction was indicated by the absence of gas bubbles in the preparation. Lactic acid bacteria have negative catalase properties.

Growth of *Lactobacillus* isolates on a medium containing prebiotics

The growth ability of *Lactobacillus* isolates on various carbon sources was carried out by growing LAB on MRS-based media by replacing glucose with oligosaccharides (Nuraida *et al*., 2011; Hu *et al*., 2012). Prebiotics as carbon sources used in this study were FOS, GOS and inulin. As much as 1% of *Lactobacillus* isolates were grown in 10 ml of MRSB media containing 5% prebiotics, then incubated at 37ºC for 24 h. Bacterial growth was determined based on optical density at a wavelength of 620 nm using a spectrophotometer. McFarland standard solution was prepared as a reference standard for the total amount of LAB. The McFarland standard is on a scale numbered from 0.5 to 10, which describes the specific concentration of bacteria per ml. McFarland standard was prepared by mixing 1% w/v $BaCl_2$ solution with 1% w/v H_2SO_4 according to the standard scale made. Then the McFarland standard was read for absorbance at 625 nm. Measurement of total LAB was carried out by taking *Lactobacillus* culture after incubation for 24 h, then vortex until homogeneous and then reading the absorbance at 625 nm. Total BAL is determined by referring to the McFarland standard value.

Decrease in glucose levels in the growth medium

Measurement of total glucose was carried out using the phenol sulfate method (Dubois *et al*., 1956). The total sugar test was carried out at 0 and 24 hours after incubation. The standard used is glucose with a concentration of 2, 4, 6, 8, 10, 12 ppm. Measurement of glucose standards and samples of LAB growth medium was carried out by taking 1 mL of standard solution each and adding 1 mL of 5% phenol, shaking and adding 5 mL of concentrated sulfuric acid solution rapidly by pouring perpendicular to the surface of the solution. The solution was allowed to stand for 10 minutes, shaken and then placed in a bath filled with warm water for 15 minutes. Absorbance measurements were carried out using a UV-Visible spectrophotometer at a wavelength of 490 nm.

Changes in the pH of the growth medium in different types of prebiotics

The pH value of the growth medium at 0 h and 24 h of incubation was measured using a pH meter. The culture sample was placed in a beaker then immersed in a previously calibrated pH meter.

The samples were then homogenized and the pH value was measured 3 times. The pH values were averaged and the standard deviation was calculated.

Data analysis

The average and standard deviation of the data obtained were calculated. The data were statistically processed using One Way ANOVA at a confidence level of 95% using SPSS version 22.0. Differences between *Lactobacillus* isolates were declared significant if the P value < 0.05. If there was a significant difference, then it was continued with Duncan's test.

RESULTS

Confirmation test

The results of Gram staining showed that all isolates had Gram-positive properties as shown in Table 2.

The results of the confirmation test showed that the cell shape of the *Lactobacillus* isolate was typically rod shape, catalase negative. *Lactobacillus* isolates had milky white colonies with round, smooth and nontransparent colonies with a size of about 1 mm in diameter. Lactic acid bacteria (LAB) are a group of bacteria that have Gram positive characteristics, are bacilli or cocci shaped, and do not have spores (Axelsson, 2004).

Table 2. *Lactobacillus* isolate confirmation test results

***Lactobacillus* isolates**	**Cell shape**	**Catalase**	**Gram**
L. plantarum 1 RN9	Bacilli	Negative	Positive
L. plantarum 1 MA1	Bacilli	Negative	Positive
L. plantarum 1 RB210	Bacilli	Negative	Positive
L. plantarum 1 ML7	Bacilli	Negative	Positive
L. plantarum 1 RJ1	Bacilli	Negative	Positive
L. plantarum 1 MK2	Bacilli	Negative	Positive
L. paracasei RL2	Bacilli	Negative	Positive
L. pentosus RG5	Bacilli	Negative	Positive
L. pentosus MK42	Bacilli	Negative	Positive
L. pentosus MS21	Bacilli	Negative	Positive
L. pentosus MB23	Bacilli	Negative	Positive
L. rhamnosus MY2	Bacilli	Negative	Positive
L. paracasei RK41	Bacilli	Negative	Positive

According to Salminen *et al*. (2004), LAB generally has a bacillus or cocci shape. In addition, differences in cell wall structure in bacteria also affect the results of Gram staining.

The results of the confirmation test showed that the cell shape of the *Lactobacillus* isolate was typically rod shape, catalase negative. *Lactobacillus* isolates had milky white colonies with round, smooth and nontransparent colonies with a size of about 1 mm in diameter. Lactic acid bacteria (LAB) are a group of bacteria that have Gram positive characteristics, are bacilli or cocci shaped, and do not have spores (Axelsson, 2004). According to Salminen *et al*. (2004), LAB generally has a bacillus or cocci shape. In addition,

differences in cell wall structure in bacteria also affect the results of Gram staining.

Growth of *Lactobacillus* isolates on a medium containing prebiotics

All *Lactobacillus* isolates showed an increase in the number of cells during 24 hours of incubation. The growth of *Lactobacillus* isolates was followed by a downward trend in the pH of the growth medium. Changes in total LAB after 24 h of incubation on various carbon sources can be seen in Table 3.

Table 3. Changes in total LAB (log cfu/ml cycles) with various carbon sources after 24 h incubation

Lactobacillus isolate	Changes in total LAB (log cfu/ml cycles)		
	FOS	GOS	Inulin
L. plantarum 1 RN9	0.52 ± 0.00^{h}	1.21 ± 0.12^{ab}	0.52 ± 0.00^{h}
L. plantarum 1 MA1	0.52 ± 0.00^{h}	1.06 ± 0.09^{cd}	0.52 ± 0.00^{h}
L. plantarum 1 RB210	1.18 ± 0.00^{abc}	1.00 ± 0.00^{d}	1.06 ± 0.09^{cd}
L. plantarum 1 ML7	0.52 ± 0.00^{h}	1.00 ± 0.00^{d}	0.52 ± 0.00^{h}
L. plantarum 1 RJ1	0.52 ± 0.00^{h}	1.12 ± 0.00^{abcd}	0.52 ± 0.00^{h}
L. plantarum 1 MK2	0.70 ± 0.00^{fg}	1.12 ± 0.00^{abcd}	0.52 ± 0.00^{h}
L. paracasei RL2	1.26 ± 0.06^{a}	1.15 ± 0.04^{abc}	1.12 ± 0.00^{abcd}
L. pentosus RG5	0.70 ± 0.00^{fg}	1.09 ± 0.12^{bcd}	0.52 ± 0.00^{h}
L. pentosus MK42	0.70 ± 0.00^{fg}	1.09 ± 0.12^{bcd}	0.52 ± 0.00^{h}
L. pentosus MS21	0.61 ± 0.12^{gh}	1.06 ± 0.09^{cd}	0.52 ± 0.00^{h}
L. pentosus MB23	0.61 ± 0.12^{gh}	1.09 ± 0.12^{bcd}	0.52 ± 0.09^{ef}
L. rhamnosus MY2	0.52 ± 0.00^{h}	1.12 ± 0.00^{abcd}	0.52 ± 0.00^{h}
L. paracasei RK41	0.70 ± 0.09^{a}	1.12 ± 0.00^{abc}	0.52 ± 0.09^{cd}

Note: the same letter behind the average value in the same column indicates a non-significant difference (P>0.05)

Based on statistical analysis of interactions between treatments, the type of isolate and type of prebiotics had a very significant effect (P<0.01) on changes in the growth of *Lactobacillus* isolates (Table 3). Initial total LAB ranged from 3.0 x 10^8 cfu/ml to 4.5 x 10^8 cfu/ml and after 24 h incubation increased to 1.5 x 10^9 cfu/ml until 6.5 x 10^9 cfu/ml or increased about 0.52 to 1.26 log cycles. The highest LAB growth occurred in *L. paracasei* RL2 which was grown in medium containing FOS and not significantly different (P>0.05) from *L. paracasei* RK41, L. plantarum1 RB210 in FOS medium, *L. plantarum*1 RN9, *L. plantarum*1 RJ1, *L. plantarum*1 MK2, *L. paracasei* RL2, *L. rhamnosus* MY2, *L. paracasei* RK41 in GOS medium and *L. paracasei* RL2 isolates in inulin medium.

Decrease in glucose levels in the growth medium

During incubation, *Lactobacillus* isolates will use a carbon source for their metabolic processes. This causes the breakdown of carbohydrates into simple sugars. Along with this there will be a decrease in the glucose level of the medium because it is used by microbes for growth. The decrease in glucose levels of *Lactobacillus* isolate growth medium with various prebiotics after 24 h incubation can be seen in Table 4.

Table 4. Decreased glucose level (%) of *Lactobacillus* isolate growth medium with various prebiotics after 24 h incubation

Lactobacillus isolate	Decreased glucose level (%)		
	FOS	GOS	Inulin
L. plantarum 1 RN9	7.61 ± 0.78^{rst}	51.08 ± 0.44^{de}	18.07 ± 2.37^{mn}
L. plantarum 1 MA1	7.33 ± 1.16^{rst}	44.85 ± 2.16^{fg}	30.92 ± 5.12^{ijk}
L. plantarum 1 RB210	83.40 ± 1.36^{c}	34.43 ± 0.61^{ij}	91.33 ± 4.83 ^{a}
L. plantarum 1 ML7	9.64 ± 0.67^{qrs}	49.23 ± 0.08^{ef}	14.84 ± 4.04^{mnop}
L. plantarum 1 RJ1	18.13 ± 0.95^{mn}	48.47 ± 0.28^{ef}	3.80 ± 0.44^{t}
L. plantarum 1 MK2	13.91 ± 1.04^{nopq}	30.38 ± 0.31^{jk}	7.17 ± 0.99^{rst}
L. paracasei RL2	83.15 ± 2.91^{c}	35.37 ± 4.94^{i}	87.76 ± 0.85^{abc}
L. pentosus RG5	7.99 ± 4.89^{rst}	19.63 ± 0.99^{lm}	28.20 ± 0.68^{k}
L. pentosus MK42	17.81 ± 0.36^{mn}	25.93 ± 0.24^{k}	5.43 ± 0.61st
L. pentosus MS21	11.43 ± 0.51^{opqr}	35.61 ± 1.54^{hi}	16.19 ± 0.67^{mno}
L. pentosus MB23	10.67 ± 1.41^{pqr}	17.99 ± 1.74^{mn}	29.34 ± 1.85^{k}
L. rhamnosus MY2	18.26 ± 3.40^{mn}	43.91 ± 0.98^{g}	10.11 ± 3.36^{pqrs}
L. paracasei RK41	85.79 ± 2.83^{bc}	16.34 ± 1.86^{mno}	89.45 ± 2.47^{ab}

Note: the same letter behind the average value in the same column indicates a non-significant difference (P>0.05)

Based on statistical analysis, the interaction between treatments, isolate type and prebiotic type had a very significant effect (P<0.01) on changes in glucose levels of growth media (Table 4). The decrease in glucose levels ranged from 3.80 ± 0.44% to 91.33 ± 4.83%. The highest decrease in glucose levels occurred in *L. plantarum*1 RB210 isolate, which was 91.33 ± 4.83% and was not significantly different from *L. paracasei* RK41 and *L. paracasei* RL2, respectively 89.45 ± 2.47% and 87.76 ± 0.85% with inulin growth medium. The decrease in glucose levels after 24 hours of incubation was closely related to the growth of LAB and a decrease in the pH of the growth medium. The more active the LAB isolate, the higher the growth rate, the greater the number of cells and carbon sources that can be metabolized/broken down and the lower the pH of the medium because one of the LAB metabolites is acid which causes a decrease in pH.

Changes in the pH of the growth medium in different types of prebiotics

Based on statistical analysis, the interaction between treatments and types of prebiotics had a significant effect (P<0.05) on changes in pH, while the type of isolate had no significant effect (Table 5). The decrease in pH of the growth medium of *Lactobacillus* isolates on various carbon sources after 24 hours incubation can be seen in Table 5. *Lactobacillus* growth medium had an initial pH ranging from 5.97 to 6.62 and after incubation there was a decrease in pH to 4.23 to 6.14. *L. plantarum*1 MK2 showed the highest decrease in pH, namely 1.91 ± 0.08 of the medium being 4.23 and was not significantly different from *L. rhamnosus* MY2 in the medium containing GOS.

Table 5. Decreased pH of *Lactobacillus* isolate growth medium with various types of prebiotics after 24 h incubation

Isolat *Lactobacillus*	Decreased of pH		
	FOS	GOS	Inulin
L. plantarum 1 RN9	0.35 ± 0.21 ^{ghijkl}	1.24 ± 0.25 ^{abcdefg}	0.05 ± 0.01 ^{l}
L. plantarum 1 MA1	0.74 ± 0.88 ^{cdefghijkl}	1.23 ± 0.10 ^{abcdefg}	0.66 ± 0.37 ^{efghijkl}
L. plantarum 1 RB210	1.21 ± 0.80 ^{abcdefgh}	1.04 ± 0.00 ^{abcdefghij}	1.28 ± 0.08 ^{abcdef}
L. plantarum 1 ML7	0.69 ± 0.57 ^{defghijkl}	1.28 ± 0.25^{abcdef}	0.24 ± 0.26 ^{jkl}
L. plantarum 1 RJ1	0.93 ± 0.08 ^{bcdefghijk}	1.60 ± 0.15^{abcd}	0.12 ± 0.06 ^{kl}
L. plantarum 1 MK2	0.41 ± 0.21 ^{fghijkl}	1.91 ± 0.08^{a}	0.32 ± 0.34 ^{hijkl}
L. paracasei RL2	1.05 ± 0.22 ^{abcdefghij}	0.78 ± 0.13 ^{cdefghijkl}	1.24 ± 0.05 ^{abcdefg}
L. pentosus RG5	0.69 ± 0.28 ^{efghijkl}	1.20 ± 0.25 ^{abcdefgh}	0.28 ± 0.26 ^{ijkl}
L. pentosus MK42	0.77 ± 0.30 ^{cdefghijkl}	1.61 ± 0.23^{abc}	0.39 ± 0.21 ^{fghijkl}
L. pentosus MS21	0.44 ± 0.09 ^{fghijkl}	1.40 ± 0.85^{abcde}	0.25 ± 0.01 ^{ijkl}
L. pentosus MB23	0.81 ± 0.60 ^{cdefghijkl}	1.16 ± 0.47 ^{abcdefghi}	1.00 ± 0.57 ^{bcdefghijk}
L. rhamnosus MY2	0.96 ± 0.78 ^{bcdefghijk}	1.78 ± 0.26^{ab}	0.40 ± 0.28 ^{fghijkl}
L. paracasei RK41	0.87 ± 0.28 ^{cdefghijkl}	0.69 ± 0.06 ^{defghijkl}	1.41 ± 0.07^{abcde}

Note: the same letter behind the average value in the same column indicates a non-significant difference (P>0.05)

DISCUSSION

The growth of *Lactobacillus* strains is strongly influenced by many factors, one of which is the source of nutrients. Some types of carbohydrates that are not able to be broken down by digestive enzymes are FOS, GOS and inulin or commonly known as prebiotics. Components of FOS, GOS or inulin can only be partially digested by humans. Other parts that cannot be digested are used as a food source for other beneficial bacteria such as *Bifidobacteria* and *Lactobacillus*. The addition of carbohydrates FOS, GOS and inulin as a substitute for glucose in LAB growth medium aims to see the ability of LAB to use the carbon source for its growth. Production of short chain fatty acids (SCFA) is the result of carbohydrate metabolism by LAB, especially carbohydrates that cannot be broken down by digestive enzymes (Cummings, 1995; Flint, 2006). Short chain fatty acids (SCFA), especially acetate, propionate, butyrate and lactate, contribute towards energy metabolism of the large gut mucosa and colonic cell growth, and are also metabolised systemically by host tissues such as the liver, muscle and brain (Steer *et al*., 2000).

Based on the results of the study, it was found that all *Lactobacillus* isolates were able to grow and used FOS, GOS and inulin carbon sources for their growth (Table 3). This can be seen from the increase in the number of LAB cells after 24 hours of incubation increased to 1.5 x 10^9 cfu/ml until 6.5 x 10^9 cfu/ml. The increase in the number of LAB indicated that the isolate was able to grow and use prebiotics as a carbon source. In general, prebiotics have a low molecular weight except for inulin. Galactooligosaccharides (GOS) have a carbon chain or degree of polymerization (DP) ranging from 2-7, FOS has a DP range from 2-9 and inulin has a DP range from 2-60. Carbohydrates with long molecular chains are metabolized more slowly than short ones. Polysaccharides can function as prebiotics in the distal colon than oligosaccharides because oligosaccharides are broken down more quickly in the proximal colon than polysaccharides (Rastall, 2004).

In general, among the three types of prebiotics FOS, GOS and inulin, the growth of LAB isolates with GOS media showed the highest increase compared to FOS and inulin. The average increase in growth in GOS medium ranged from 1 log cfu/ml cycle to 1.21 log cfu/ml cycles (Table 3). These results are in accordance with the results of research by (Nuraida *et al*., 2011) which showed the highest LAB growth in medium with GOS as a carbon source. This indicates that LAB isolates are able to produce the enzyme β-galactosidase which plays a role in the breakdown of GOS (Nuraida *et al*., 2011). The growth of LAB in FOS and inulin medium was slightly lower than GOS, namely the lowest growth was 0.52 log cfu/ml cycles except for some isolates with growth above 1 log cfu/ml cycle in both FOS and inulin, i.e., *L. plantarum*1 RB210, *L. paracasei* RL2 and *L. paracasei* RK41 (Table 3) and this was also confirmed by a decrease in the pH of the medium (Table 5).

Glucose levels in the medium resulting from the breakdown of carbohydrates during 24 h of incubation there was a decrease indicating that there was an overhaul and use of sugar by LAB for its metabolism (Table 4). This shows that prebiotics as a source of carbon contained in the media can be used for the growth of LAB. The highest decrease in glucose levels occurred in media with inulin as a carbon source. However, not all *Lactobacillus* isolates showed the highest decrease in glucose levels. There were 3 *Lactobacillus* isolates that showed a fairly high decrease in glucose levels, i.e., *L. plantarum*1 RB210, *L. paracasei* RL2 and *L. paracasei* RK41 both with inulin and FOS media. This shows the potential of prebiotics derived from selective fermentation by *Lactobacillus*. Acording to statement of (Steer, 2000) which state that the prebiotic potential stems from their selective fermentation by *Bifidobacterium* spp. and, to a lesser extent, by *Lactobacillus* spp. in the colonic microflora. The ability of LAB to metabolize is countered by the presence of the inulase enzyme produced by LAB. inulase enzyme is an enzyme that can hydrolyze inulin (Nakamura *et al*., 1995). FOS medium is capable of being broken down in the presence of the β-fructosidase enzyme, where this enzyme is inductive. Enzymes will be produced when the appropriate substrate, namely FOS, is in the LAB growth environment (Nuraida *et al*., 2011).

The decrease in pH and the growth of LAB are interrelated. Lactic acid bacteria are bacteria capable of producing lactic acid as the main metabolite of their metabolism in addition to other organic acids such as acetic, propionic and butyric acids. The acid produced during LAB metabolism causes a decrease in the pH of the medium. The production of SCFA results in a hostile environment for pathogens such as *E. coli*, *Campylobacter* and *Salmonella spp* (Steer *et al*., 2000).

CONCLUSION

All *Lactobacillus* isolates were able to use prebiotics as a carbon source in the metabolic process. The highest decrease in glucose levels occurred in *L. plantarum*1 RB210 isolate, which was 91.33 ± 4.83% and was not significantly different (P>0.05) with *L. paracasei* RK41 and *L.*

paracasei RL2 with inulin growth medium. *L. plantarum*1 MK2 showed the highest decrease in pH after incubation, namely 1.91 ± 0.08 and was not significantly different from *L. rhamnosus* MY2 in the medium containing GOS. The results of this study indicate that the isolates of *Lactobacillus* indigenus can definitively use prebiotics as a carbon source for their metabolism. The results of this study provide a basis for the effect of using probiotics and prebiotics for synbiotic applications so that they can modulate the gut microbiota.

REFERENCES

Asto, E., Mendez, L., Rodriguez-Prado, M., Cune, J., Espadaler, J. and Farran-Codina, A. 2019. Effect of the degree of polymerization of fructans on ex vivo fermented human gut microbiome. Nutritients. 11: 1-14.

Axelsson, L. 2004. *Lactic Acid Bacteria: Classification and Physiology*. *In*: Salminen, S., and A. Wright, editor. *Lactic Acid Bacteria: Microbiological and Functional Aspect*. 3rd edition. Marcel Dekker Inc., New York. Page 1-66. https://doi.org/10.1201/9780824752033.ch1

Bouhnik, Y., Kvahedi, Achou, L., Attar, A., Salfat, J., Pochart, P., Marteau, P. and Flourie, B. 1999. Short chain fructooligosaccharides administration dose dependently increase faecal bifidobacteria in healthy human. J. Nutr. 129: 113-116.

Cummings, J.H. 1995. Short chain fatty acids. In Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology. pp. 101–130. GR Gibson and GT Macfarlane, editors. Boca Raton, FL: CRC Press. 51.

Dubois, M., Gilles, K., Hamilton, J., Roberts, P and Smith, F. 1956. Colorimetric method for determining sugars and related substance. Analytical chemistry. 28:350-354.

FAO/WHO. 2001. Evaluation of Health and Nutritional Properties of Powder Milk and live Lactic Acid Bacteria. Food and Agriculture Organization of The United Nations and World Health Organization Report (FAO/WHO Experts' Report). http://www.fao.org/es/ESN/Probio/Probio htm.

Flint, H.J. 2006. Prokaryote diversity in the human GI tract. In Prokaryotic Diversity: Mechanisms and Significance, Society for General Microbiology Symposium no. 66, Warwick April 2006, pp. 65–90. N Logan, H Lappin-Scott and P Oyston, editors. Cambridge, MA: Cambridge University Press

Franck, and Anne, M.E. 2000. Inulin dan Oligofruktosa. Dalam: Glen Gibson dan Fiona Angus (eds). *Prebiotics and Probiotics*. LRFA Limited, United Kingdom.

Fuller, R. 1989. Probiotics in Man and Animals. Journal of Applied Bacteriology 66:365-378.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A. and Roberfroid, M.B. 2004. Dietary modulation of the human colonic microbiota: updateing the concept of prebiotics. Nutrition Research Rewievs. 17: 259-275. DOI: 10.1079/NRR200479

Gropper, S.S., Smith, J.L. and Groff, J.R. 2009. Advanced nutrition and human metabolism. Wadsworth Publishing, USA.

Harrigan, W.F. and Mc Chance M.E. 1998. Laboratory Methods in Food Microbiology 3rd edition. Academic Press, Inc., New York.

Hu, J. L., Nie, S. P., Min, F. F. and Xie, M. Y. 2012. Polysaccharide from Seeds of Plantago asiatica L. Increases Short Chain Fatty Acid Production and Fecal Moisture along with Lowering pH in Mouse Colon. Journal of Agricultural and Food Chemistry. 60:11525-11532. https://doi.org/10.1021/jf302169u

Macfartan, G.T., Steed, H. and Macfartan, S. 2008. Bacterial metabolism and health related effect of galactooligosaccharides and other prebiotics. Journal of Application Microbilogy. 104: 305-344.

Manning, T.S., Rastall, R. and Gibson, G. 2004. Prebiotics and lactic acid bacteria. Dalam Salminen S, Wright AV, Ouwehand A (Eds). Lactic acid bacteria: Microbiology and functional aspects. 3th edition, Revised and expanded. P. 419-430. Marcel Dekker, Inc., New York.

Moro, G., Minoli, L., Mosca, M., Farano, S., Jelinek, J., Stahl, B. and Boehm, G. 2002. Dosage-related bifidogenic effects of galacto and fructo-oligosaccharides in formula-fed

term infant. J. Pediatric Gastroenterol Nutr. 34: 291-295.

Nakamura, T., Ogata, Y., Shitasa, A., Nakamura, A. and Ohta, K. 1995. Continuous production of fructose syrups from inulin by immobilized inulase from Aspergillus niger mutan 817. J. of Fermentation and Bioeng. 80(2): 164-169.

Nuraida, L., Mardiana, N. R., Faridah, D.N., Hana. 2011. Metabolisme Prebiotik oleh kandidat Probiotik isolate ASI sebagai dasar Pengembangan Produk Sinbiotik. Jurnal Teknologi dan Industri Pangan. XXII (2): 156-163.

Rastall, R.A. 2004. Bacteria in the Gut: Friends and Foes and How to Alter the Balance. The Journal of Nutrition. 134(8): 2022S-2026S. https://doi.org/10.1093/jn/134.8.2022S

Roberfroid, M. B. 2007. Inulin-type fructans: functional food ingredients. *The Journal of Nutrition* 137(11): 2493S-2502S. https://doi.org/10.1093/jn/137.11.2493S.

Salminen, S. dan Wright, A. V. 2004. Lactic Acid Bacteria: Microbiology and Functional Aspects. 2nd edition. Revised and Expanded. Marcel Dekker, inc., New York.

Shin, S.H., Lee, J.h., Pestika, J.J. and Ustunol, Z. 2000. Growth and viability of commercial Bifidobacterium spp in skim milk containing oligosaccharides and inulin. J. Food Sci. 65(5):884-887

Shortt, C. 1999. The Probiotic Century: Historical and Current Perspectives. Review Trends Food Science and Tech. 10:411-417. http://doi.org/10.1016/s0924-2244(00)00035-2

Steer, T., Carpenter, H., Tuohy, K. and Gibson, G.R. 2000. Perspectives on the role of the human gut microbiota and its modulation by pro-and prebiotics. Nutrition Research Rewievs. 13: 229-254.

Wong, J. M. W., de Souza, R., Kendall, C. W. C., Emam, A. and Jenkins, D. J. A. 2006. Colonic Health: Fermentation and Short Chain Fatty Acids. Journal Clinical Gastroenterol. 40(3):235–243. https://doi.org/10.1097/00004836-200603000-00015

Zhang, F., Hang, X., Fan, X., Li, G. and Yang, H. 2007. Selection and optimization procedure of synbiotic for cholesterol removal. *Anaerobe* 13: 185-192. https://doi.org/10.1016/j.anaerobe.2007.06.001

Zhu, Y., Yue, S., Li, R., Qiu, S., Xu, Z., Wu, Y., Yao, J., Zuo, Y., Li, K. and Li, Y. 2021. Prebiotics inulin metabolism by lactic acid bacteria from young rabbits. Frontiers in veterinary science. 8: 1-12.