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In vitro evaluation of α-glucosidase inhibitor and antioxidant activity of *Lactobacillus* isolates and their antidiabetic potential

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ABSTRACT

Aims: This study aimed to evaluate antidiabetic potential of indigenous *Lactobacillus* isolates by measuring the ability of α-glucosidase inhibitory (AGI) and antioxidant activity. The mechanism of probiotics as antidiabetic can occur through the AGI and antioxidant activity of LAB, which is able to suppress oxidative stress that causes chronic inflammation and pancreatic β cell apoptosis, and then through the ability to produce exopolysaccharide (EPS) and short chain fatty acids (SCFA).
Methodology and results: MRS broth enriched with 10% glucose was selected as the growth medium for *Lactobacillus*. The growth medium was then centrifuged to obtain CFS and CFE was produced by extracting the medium with 96% ethanol as a solvent. The results showed that *Lactobacillus pentosus* MK42 had the highest AGI activity of 80.32 ± 2.20%. Antioxidant activity was not significantly different (P>0.05) among the tested *Lactobacillus* isolates. *Lactobacillus paracasei* RK41 produced the highest EPS (360.13 ± 50.01 mg/L), which was not significantly different (P>0.05) from *Lactobacillus plantarum*1 RB210. All *Lactobacillus* isolates were able to produce acetic acid, but not all were able to produce propionic and butyric acid. The highest propionic acid was produced by *L. plantarum*1 RB210 at 0.40 ± 0.31 mmol/L and the highest butyric acid was produced by *L. plantarum*1 MK2 at 0.22 ± 0.08 mmol/L.
Conclusion, significance and impact of study: The results show definitively that indigenous *Lactobacillus* isolates have considerable α-glucosidase inhibitor, antioxidant activity and the ability to produce of EPS and SCFA. This preliminary study suggests the use of indigenous *Lactobacillus* isolates which have the potential as antidiabetic agent, although the responsible compounds are unknown.

Keywords: Lactobacillus, AGI, antioxidant activity, exopolysaccharides, SCFA, antidiabetic

INTRODUCTION

Lactic acid bacteria (LAB) are commonly used in foods and have been shown to have health benefits. The role of LAB in contributing to health has been widely studied, especially to overcome gastrointestinal problems and diseases related to the digestive tract. However, it is now known that LAB can help to boost the immune system (Isolauri *et al.*, 2001; Bermudez-Brito *et al.*, 2012; Amenu, 2015), inhibit pathogenic microbial growth (Bermudez-Brito *et al.*, 2012; Shehata *et al.*, 2016), reduce cholesterol (Ooi and Liong, 2010; Amenu, 2015; Daliri and Lee, 2015; Shehata *et al.*, 2016), decrease distention or accumulation of gas in the stomach (Hsieh *et al.*, 2013) and prevent diabetes mellitus (DM) (Matsuzaki *et al.*, 1997; Harisa *et al.*, 2009; Hsieh *et al.*, 2013; Panwar *et al.*, 2014). The antidiabetic mechanism of probiotics can occur through the inhibitory activity of the α-glucosidase enzyme (Ramchandran and Shah, 2008), antioxidant activity of LAB which is able to suppress oxidative stress (Ren *et al.*, 2014; Wang *et al.*, 2017) and the ability to produce EPS (Ramchandran and Shah, 2009b; Dilna *et al.*, 2015) and SCFA (Estiasih *et al.*, 2012; Lau *et al.*, 2015).

During T2DM, abnormalities in glucose metabolism occur. These abnormalities cause postpandrial blood

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sugar level to increase. The main source of blood glucose is carbohydrates in food which are hydrolyzed by the enzymes α-glucosidase and α-amylase into glucose so that it can be absorbed by the small intestine. One mechanism of oral antidiabetic drugs is to competitively inhibit the action of the α-glucosidase enzyme, which plays a role in the breakdown of carbohydrates (Hanefeld and Schaper, 2008; Hardman and Limbird, 2012). Intestinal α-glucosidase inhibitors can inhibit/delay the rate of carbohydrate digestion/absorption in the small intestine, lowering postprandial blood glucose and insulin levels (Lebovitz, 1998; van de Laar *et al*., 2005).

Several studies have shown that LAB has α-glucosidase inhibitory activity. EPS, which is a compound produced by LAB, is thought to play a role in inhibiting the action of α-glucosidase (Ramchandran and Shah, 2008; Chen *et al.*, 2014). In addition, some research results also showed that LAB as a probiotic has antioxidant activity. Free radicals can cause oxidative stress. The results of Zhang and Zhang's (2013) study stated that LAB can prevent or reduce the effects of type 2 diabetes by reducing pancreatic oxidative stress, which causes chronic inflammation and pancreatic beta cell apoptosis. Lin and Chang (2000) stated that *Lactobacilli* have the ability to ward off free radicals *in vitro*. This study aimed to evaluate the ability of α-glucosidase inhibitory (AGI) and antioxidant activity of indigenous *Lactobacillus* isolates and its potential as antidiabetic *in vitro*.

MATERIALS AND 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, p-nitrophenyl α-D-glucopyranoside (p-NPG) (Sigma-Aldrich N1377), α-D-glucosidase from *Saccharomyces cereviciae* (Sigma-Aldrich G5003), fructooligosaccharide/FOS (Fibrulose F97), galactooligosaccharide/GOS (FocusHerb LLC), inulin (Now Foods), potassium dihydrogen phosphate (Merck), calcium hydrogen phosphate (Merck), bovine serum albumin (BSA), pharmaceutical agar, acarbose (Glucobay®Bayer Indonesia), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (Merck) and PVDF membrane filter (Merck Millipore).

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). *Lactobacillus* isolates used in this study were identified physiologically, morphologically and biochemically by API CHL 50 (data not shown). The probiotic strains were preserved in 20% glycerol and stored at –80 °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 of cell-free supernatant (CFS) and cell-free intracellular extract (CFE)

All *Lactobacillus* isolates were inoculated in MRSB and incubated at 37 °C for 24 h. After incubation, 1 mL of culture was inoculated in 100 mL of MRSB with the addition of 10% glucose and incubated at 37 °C for 36 h. The cultures were then used for preparing cell-free supernatant (CFS) and cell-free intracellular extract (CFE). To obtain CFS, 50 mL of the culture was centrifuged at 5000 rpm for 30 min at 4 °C. The supernatant was then collected by separating from the cell pellet. The supernatant was adjusted to pH 7.4 with NaOH and then filtered using a 0.22 µm PVDF membrane filter to get CFS. All of the CFS were stored at –20 °C until later use (Zeng *et al*., 2016).

To prepare CFE, 100 mL of ethanol was added to 50 mL culture in a volume ratio of 2:1, followed by shaking in a shaker for 4 h. The mixture was then centrifuged at 5000 rpm for 30 min at 4 °C to obtain the supernatant and cell pellet. The supernatant was evaporated using a rotary evaporator at a temperature of 40 °C to obtain an ethanol extract of *Lactobacillus* isolate (Bajpai *et al*., 2016). The ethanol extract was stored at –20 °C until later use.

Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was done according to Sancheti *et al.* (2009) and Farida *et al.* (2017). The reaction mixture, which consisted of 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL p-NPG 0.5 mM (Sigma-Aldrich N1377), 10 µL sample (S) in concentration 500 µg/mL and 25 µL of 0.04 U/mL α-glucosidase enzyme was added into a microplate reader. The control sample (S0) did not use α-glucosidase enzyme. Blank (B) consisted of 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of p-NPG 0.5 mM substrate and 25 µL of 0.04 U/mL α-glucosidase enzyme. The control blank (B0) did not use α-glucosidase enzyme. The reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 100 µL of 0.2 M sodium carbonate (Sigma-Aldrich). Then, the absorbance was measured at a

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wavelength of 410 nm. The positive control used was the anti-diabetic drug acarbose (Glucobay®). The concentration of acarbose solution used was 10 µg/mL made from 10 mg of Glucobay® tablets dissolved in 100 mL of distilled water and 2 N HCl (1:1) to obtain a concentration of 100 ppm. Acarbose solution of 100 ppm was then diluted to obtain a concentration of 10 ppm. The solution was then centrifuged and as much as 10 µL of the supernatant was taken and added to the reaction mixture as in the sample. Inhibition reaction of α-glucosidase can be seen in Table 2.

Table 2: Inhibition reaction of α-glucosidase.

Reaction mix	S (µL)	S0 (µL)	B (µL)	B0 (µL)
Sample (CFE or CFS)	10	10	-	-
Phosphate buffer	50	50	50	50
PNPG substrate	25	25	25	25
α-glucosidase enzyme	25	-	25	-
Incubation at 37 °C for 30 min				
Natrium carbonate	100	100	100	100

Note: S = Sample; S0 = Sample control; B = Blank; B0 = Blank control.
Percent inhibition of the α-glucosidase enzyme was calculated by the formula below:
α-glucosidase inhibitory activity (%) = [K – (A1 – A0)/K] × 100%
Where, K = Blank absorbance (B1) minus blank control (B0); A0 = Absorbance sample control; A1 = Absorbance sample.

Antioxidant activity of *Lactobacillus* Isolates

DPPH scavenging activity assay was performed using spectrophotometry, as described by Ng *et al*. (2020) with some modifications. Antioxidant activity analysis was carried out using DPPH by looking at the ability of the sample to reduce free radicals. As much as 500 µL of the sample (CFS or CFE) was inserted into the microplate. The positive control used was ascorbic acid. The sample and ascorbic acid were added with 500 µL of DPPH 125 µM (Sigma-Aldrich D9132), while the negative control only contained 1000 µL of ethanol (Merck, USA). Blank was prepared by mixing 500 µL of ethanol and 500 µL of DPPH. The mixture was homogenized and incubated at 37 °C for 30 min in a dark room. The resulting absorption was measured at a wavelength of 517 nm (Genesys 15s UV-Vis Spectrophotometer). The percentage of free radical scavenging activity was calculated using the equation:

Antioxidant activity (%) = [(A – B)/A] × 100%

Where, A = corrected blank absorbance; B = corrected sample absorbance.

Production of exopolysaccharides

Total of exopolysaccharides (EPS) was determined using the gravimetric method (Smitinont *et al.*, 1999; Alp and Aslim, 2010). The LAB cultures were inoculated in MRSB media containing 2% sucrose and incubated at 30 °C for 2-3 days. The growth medium was then heated at 100 °C for 10 min and then cooled. Furthermore, trichloroacetic acid solution with a concentration of 85% was added to the medium as much as 17% (v/v). Cell pellet and protein were separated by centrifugation at 5000 rpm for 20 min and then the supernatant was taken. The EPS in the supernatant was further precipitated by adding five times the volume of cold ethanol 95% (v/v). The mixture was left overnight and then centrifuged at 5000 rpm for 20 min at 4 °C. Then, the EPS precipitate was dried at 60 °C and the weight was measured.

Production of short chain fatty acids (SCFA)

The ability of *Lactobacillus* isolates to produce SCFA was carried out by growing LAB in MRSB medium in which glucose content was replaced with oligosaccharides (Zhang *et al.*, 2007). As much as 1% of *Lactobacillus* isolates were grown in 10 mL of MRSB medium containing 5% oligosaccharides, i.e., inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS) and incubated at 37 °C for 24 h. After 24 h, the bacterial culture was then centrifuged and the organic acid profile, including SCFA was measured on the supernatant using gas chromatography (GC). Chromatographic analysis was performed using an Agilent System 6890 N GC equipped with a flame ionization detector (FID) and an automatic liquid sampler N10149 (Agilent, USA). GC column (BP21, Shimadzu), with a length of 25 m, an inner diameter of 0.53 mm and coated with a film of 0.50 m thickness. The carrier gas used was helium (He) at a temperature of 240 °C, flow control mode at a pressure of 18.9 kPa with a total flow of 102.1 mL/min, column flow 3.2 mL/min, linear velocity 28.4 cm/sec, the corresponding flow of 3.0 mL/min and a split ratio of 1:30. The initial oven temperature was at 110 °C maintained for 3 min. Then, the temperature reached 160 °C at a rate of 9°C/min and was maintained for 15 min. FID and injection port temperature was 250 °C. The hydrogen and airflow rates were 40 and 400 mL/min, respectively. The sample volume injected for GC analysis was 0.2 µL and the run time for each analysis was 20.56 min. The concentration of each SCFA (acetic, propionic, butyric) was carried out by comparing the area with a standard curve. The ratio of SCFA was calculated based on the ratio area of each SCFA to the total area. The test was repeated independently twice for each sample. The mean value and standard deviation were calculated from each replication.

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 $P<0.05$. If there was a significant difference, then it was continued with Duncan's test.

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RESULTS AND DISCUSSION

Alpha-glucosidase inhibition (AGI) assay

α-glucosidase (EC 3.2.1.20) is an enzyme located at the border of the small intestinal septum that acts on the α-1,4 linkage to catalyze the terminal hydrolysis of non-reducing glucose residues on various substrates and produces α-D-glucose (Gao *et al.*, 2007). Testing of AGI activity was carried out to determine the ability of LAB metabolites to inhibit glucose hydrolysis reactions on p-NPG substrates. The results of testing the inhibitory activity of α-glucosidase enzyme of *Lactobacillus* strain can be seen in Figure 1. Based on the test results on CFE and CFS of LAB, it was found that all *Lactobacillus* isolates had AGI activity. Based on the results, the AGI of CFE and CFS showed a significant difference (P<0.05) between all *Lactobacillus* isolates tested and a very significant difference (P<0.01) against control. The AGI of CFE ranged from 56.36 ± 3.76% to 80.32 ± 2.20%, while the activity of CFS ranged from 4.43 ± 1.13% to 18.47 ± 9.09%. The AGI for positive control acarbose (10 ppm) in this study was 98.50 ± 0.55%, which was comparable to Glucobay's AGI of 98.92 ± 0.07% (Farida *et al.*, 2017). CFE of *Lactobacillus pentosus* MK42 had the highest AGI activity of 80.32 ± 2.20% and was not significantly different from isolates of *Lactobacillus plantarum*1 ML7, *Lactobacillus plantarum*1 MA1, *Lactobacillus plantarum*1 RJ1 and *Lactobacillus plantarum*1 RN9. On the other hand, the AGI of CFS ranged from 4.43 ± 1.13% to 18.47 ± 9.09%. CFS of *Lactobacillus pentosus* MB23 had the highest AGI activity of 18.47 ± 9.09%, which was significantly different between other isolates and the control acarbose. The CFE of LAB obtained from the extraction with ethanol solvent had a greater inhibitory activity than the CFS of LAB. This is in line with the results of Bajpai *et al.* (2016), which tested the AGI activity of *Lactobacillus sakei* 111 of ethanol extract with an activity of 60.69% and the results of Farida *et al.* (2017) where 13 isolates of *Lactobacillus* tested had AGI activity ranging from 54.01 ± 1.25% to 75.22 ± 1.07%. The inhibitory activity of α-glucosidase on CFS in this study is also in line with the results of Chen *et al.* (2014) on CFS of several LAB strains, which showed quite low activity, ranging from 3.42 ± 0.14% to 29.57 ± 1.38%. The results of previous studies showed that AGI of LAB metabolites is very beneficial in the regulation of glycemia (Panwar *et al.*, 2014; Muganga *et al.*, 2015; Zeng *et al.*, 2016). The AGI of LAB is thought to originate from several components of compounds, such as EPS (Ramchandran and Shah, 2009a), proteins or bioactive peptides (BAPs) (Lacroix and Li-Chan, 2013; Zeng *et al.*, 2016; Kinariwala *et al.*, 2019) and homogenistic acids (Nguyen *et al.*, 2017).

The results of Muganga *et al.* (2015) showed that the AGI activity of several *Lactobacillus* strains grown in skimmed milk ranged from 5.3 ± 0.7% to 32.9 ± 1%. According to Ramchandran and Shah (2009a), this activity is thought to be caused by the hydrolysis of milk protein that has been hydrolyzed by protease bacteria, as well as EPS produced during milk fermentation. Although the mechanism of AGI by hydrolyzate protein is unknown, non-saccharide components are thought to have this activity by binding to the active site of the enzyme via hydrophobic interactions (Lacroix and Li-Chan, 2013).

Antioxidant activity

The DPPH method is a commonly used antioxidant activity test. The radical capture ability of DPPH was used as an indicator to evaluate the antioxidant activity of LAB. *Lactobacillus* metabolites tested for antioxidant activity were in the forms of CFE and CFS. CFE are components in LAB cells that are released by breaking down LAB cell walls both physically (ultrasonication) and chemically (extraction with solvents). CFS are components produced by LAB and released into the growth medium. The antioxidant activity of indigenous LAB can be seen in Figure 2.

Figure 2 shows the antioxidant activity of CFE and CFS of *Lactobacillus* isolates. Based on the results, the ability of CFE to capture free radicals showed no significant difference (P>0.05) between the tested *Lactobacillus* isolates and significant effect (P<0.01) compared to ascorbic acid control. Similarly, the ability of CFS to capture free radicals showed no significant difference (P>0.05) among the tested *Lactobacillus* isolates but had a significant effect (P<0.01) when compared to the ascorbic acid control. From the test results, it is known that *Lactobacillus* CFE has the ability to capture DPPH radicals ranging from 80.33 ± 0.55% to 82.75 ± 2.91%, while CFS has the ability to capture DPPH radicals ranging from 8.20 ± 0.52% to 8.53 ± 0.27%. The control used in the test was ascorbic acid with DPPH radical capturing ability of 97.71 ± 0.46% and significantly different from all tested *Lactobacillus* isolates. In this study, the ability of standard ascorbic acid to capture free radicals was comparable to 93.92 ± 0.28% (Farida *et al.*, 2017). *Lactobacillus rhamnosus* MY2 had the highest DPPH radical capturing ability, but it was not significantly different from other *Lactobacillus* isolates. The potential antioxidant activity of LAB has been reported in several studies (Shen *et al.*, 2011; Nyanzi *et al.*, 2015; Son *et al.*, 2017; Jang *et al.*, 2019). These studies show that CFE has a higher antioxidant activity than CFS. The results of this study are consistent with the findings of Nyanzi *et al*. (2015), who found that the methanol extract of several strains of *Lactobacillus acidophilus*, *L. rhamnosus* and *L. casei* from dairy products had antioxidant activity ranging from 36.92 ± 0.44% to 86.38 ± 0.54%, with an effective concentration against 50% DPPH ranging from 4.24 to 24.07 mg/mL.

The antioxidant activity of *Lactobacillus* isolates extracted with solvents was higher than that of unextracted LAB isolates and cell-free supernatants. This is probably because most of the components of antioxidant compounds are inside cells such as intracellular antioxidants and proteins (Lin and Yen, 1999). Several antioxidant enzymes, including SOD,

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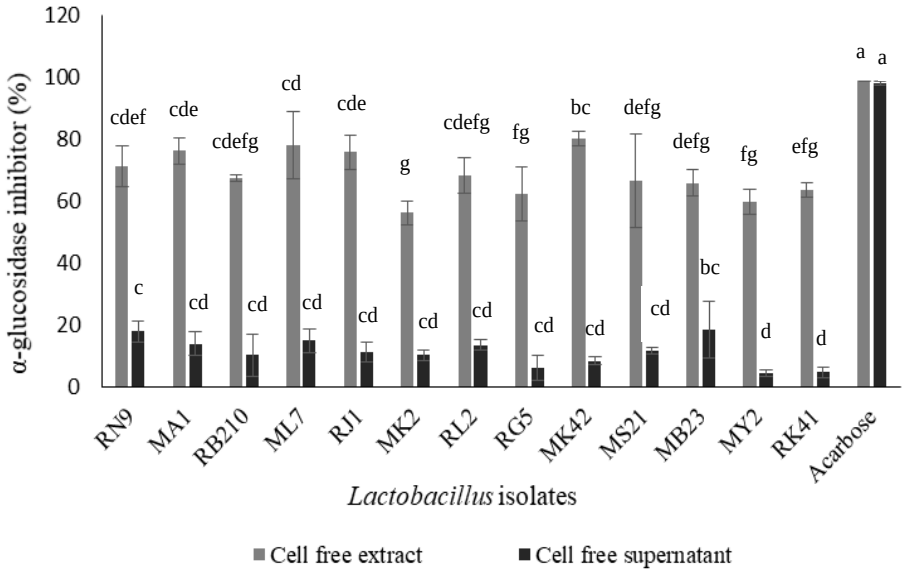

Figure 1: α-glucosidase inhibition (AGI) activity (%) of *Lactobacillus* isolates. Acarbose, an antidiabetic drug served as positive control.

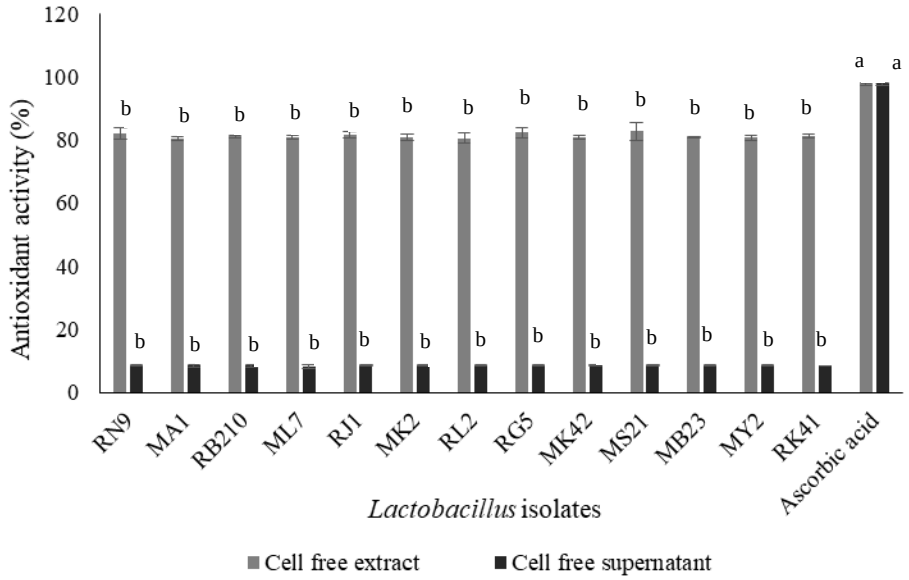

Figure 2: Antioxidant activity (%) of 13 *Lactobacillus* isolates. Ascorbic acid served as positive control.

NADH-oxidase and NADH peroxide, are important enzymatic defence systems against oxidative stress in LAB (Li *et al.*, 2012). The process of extracting the intracellular components of LAB results in the release of intracellular enzymes as CFE with antioxidant activity, such as *Lactobacillus plantarum* (Li *et al.*, 2012), several strains of *L. acidophilus*, *L. bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium longum* (Lin and Yen, 1999) and *Lactobacillus fermentum* (Kullisaar *et al.*, 2002). The antioxidant component other than intracellular enzymes is protein, which was successfully extracted from *Bifidobacterium animalis* 01 cells and has an antioxidant activity *in vitro* (Shen *et al.*, 2010). The antioxidant activity of several LAB strains was also associated with compounds produced on the cell surface such as extracellular polysaccharides produced by *Lactococcus lactis* subsp. *lactis* 12 (Pan and Mei, 2010) and *Bifidobacterium animalis* RH (Xu *et al.*, 2011), and also lipoteichoic acid on the surface of bifidobacteria (Yi *et al.*, 2009). This is supported by Li *et al.* (2012), who found that removing the compounds produced on the cell surface can reduce the DPPH radical capturing capacity. Intracellular enzymes and proteins, metabolite components that play a role in inhibiting DPPH radicals

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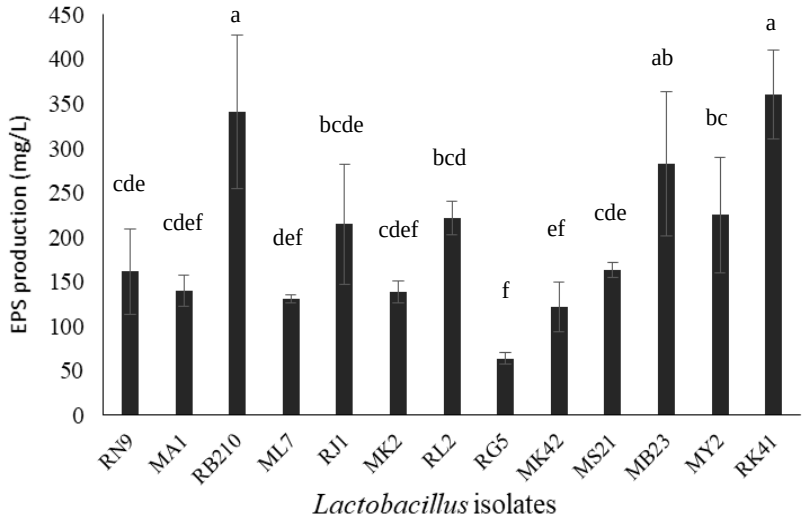

Figure 3: Production of exopolysaccharides (mg/L) of 13 *Lactobacillus* isolates.

from the ethyl acetate extract of *Lactobacillus plantarum* are L-3-(4-hydroxyphenyl) lactic acid (HPLA) and L-indole-3-lactic acid (ILA) (Suzuki *et al.*, 2013).

Diabetes and its complications are linked to increased oxidative stress caused by free radical formation. Hyperglycemia results in oxidative stress, which increases glycosylation and oxidation of proteins involved in the pathogenesis of diabetes complications (Monnier *et al.*, 2006). Diabetes complications are characterized by increased production of reactive oxygen species (ROS) as a result of changes in various metabolic pathways (Yadav *et al.*, 2007). Excess free radicals can harm cell function, including pancreatic β cells (Kaneto *et al.*, 2005), endothelial cells (Soro-Paavonen and Forbes, 2006), muscle and nerve cells (Vincent *et al.*, 2005). Probiotics are known to have health benefits because they have antioxidant activity and help to reduce oxidative damage. Lactic acid bacteria can fight ROS (reactive oxygen species), including peroxide radicals (Stecchini *et al.*, 2001), superoxide anions and hydroxyl radicals (Kullisaar *et al.*, 2002; Shen *et al.*, 2011). Several studies have shown that different strains of probiotic bacteria can have the antioxidant capacity through various mechanisms. Antioxidant modulation by probiotics occurs through the following mechanisms: (1) ability of probiotics to chelate metal ions, (2) antioxidant activity of probiotics themselves, (3) producing antioxidant metabolites, (4) regulating host antioxidant activity, (5) regulating increased levels of host antioxidant metabolites, (6) regulating signaling pathways, (7) decreasing the activity of ROS-producing enzymes and (8) regulating the gut microbiota (Wang *et al.*, 2017).

Production of crude exopolysaccharides (EPS)

Exopolysaccharides (EPS) are long-chain polysaccharides with a high molecular weight produced by microbes and secreted out of cells to perform a variety of functions such as cell adhesion, cell protection from external environmental stress and nutrition during deficiency (Kumar *et al.*, 2007). The ability of *Lactobacillus* strains to produce crude EPS can be seen in Figure 3.

In Figure 3, it is known that all *Lactobacillus* isolates were able to produce EPS. According to the statistical analysis, the type of *Lactobacillus* isolates had a significant difference (P<0.01) in EPS weight. The resulting EPS weight ranged from 63.87 ± 6.82 mg/L to 360.13 ± 50.01 mg/L. *Lactobacillus paracasei* RK41 isolates produced the most EPS (360.13 ± 50.01 mg/L), which was not significantly different (P>0.05) from *Lactobacillus plantarum*1 RB210 and *Lactobacillus pentosus* MB23 isolates, which produced EPS levels of 340.63 ± 86.16 mg/L and 282.50 ± 80.58 mg/L, respectively. The EPS weight produced in this study had various values. This may be due to differences in the *Lactobacillus* isolates used. The potential of LAB in producing EPS has been reported in several studies. The results of this study are in line with the research conducted by Ruas-Madiedo *et al.* (2002), which stated that *Streptococcus thermophilus* in milk fermentation was able to produce EPS ranging from 50-350 mg/L, while *Lactobacillus delbrueckii* ssp. *Bulgaricus* was able to produce EPS of 60-150 mg/L. The yield of crude and pure EPS from *Lactobacillus rhamnosus* RW-9595M was 2.0 ± 0.1 g/L and 17.4 ± 0.3 g/L, respectively (Doleyres *et al.*, 2005). *Bifidobacterium longum* JBL05 grown in media containing skim milk and lactose produced EPS of 1.3 g/L (Kohno *et al.*, 2009). Yoghurt fermented with the addition of *Streptococcus thermofilus* produced crude EPS of 37.43 mg/100 g yoghurt (Ramchandran and Shah, 2009b). *Lactobacillus plantarum* RJF_4 grown on medium containing lactose and precipitated with acetone was able to produce EPS of 1.5 g/L (Dilna *et al.*, 2015). Many factors influence the different amount of EPS produced by each LAB, including the type of LAB, growth conditions,

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Table 3: Production of acetic acid (mmol/L) of *Lactobacillus* isolates on various carbon sources.

Lactobacillus isolates	Acetic acid (mmol/L)			Means
	FOS[1]	GOS[2]	Inulin	
*L. plantarum*1 RN9	117.62 ± 30.49	187.58 ± 17.54	91.51 ± 1.84	132.24 ± 49.67[a]
*L. plantarum*1 MA1	125.27 ± 54.07	171.33 ± 9.74	94.72 ± 1.08	130.44 ± 38.57[a]
*L. plantarum*1 RB210	145.82 ± 59.43	143.36 ± 17.89	144.43 ± 27.18	144.54 ± 1.23[a]
*L. plantarum*1 ML7	96.17 ± 15.66	124.88 ± 28.21	94.44 ± 19.06	105.16 ± 17.10[a]
*L. plantarum*1 RJ1	142.88 ± 33.94	183.90 ± 130.10	108.79 ± 1.80	145.19 ± 37.61[a]
*L. plantarum*1 MK2	107.74 ± 18.37	178.23 ± 49.60	105.64 ± 7.48	130.54 ± 41.31[a]
L. paracasei RL2	118.54 ± 6.58	195.57 ± 29.05	155.13 ± 29.62	156.41 ± 38.53[a]
L. pentosus RG5	124.09 ± 18.39	179.30 ± 50.41	115.59 ± 5.28	139.66 ± 34.59[a]
L. pentosus MK42	120.51 ± 21.57	128.06 ± 43.55	97.64 ± 2.80	115.40 ± 15.84[a]
L. pentosus MS21	197.22 ± 28.79	144.44 ± 4.91	117.26 ± 12.96	152.97 ± 40.66[a]
L. pentosus MB23	110.66 ± 26.39	169.25 ± 9.52	126.01 ± 10.01	135.31 ± 30.38[a]
L. rhamnosus MY2	120.00 ± 14.41	166.05 ± 24.11	86.34 ± 31.94	124.13 ± 40.02[a]
L. paracasei RK41	120.07 ± 36.46	173.88 ± 10.13	129.54 ± 6.36	141.16 ± 28.73[a]
Means	126.66 ± 24.89[bc]	165.06 ± 22.70[a]	112.85 ± 21.19[c]	

Note: [1] = Fructooligosaccharide; [2] = Galactooligosaccharide.
Value were means ± standard deviation of two replicates (n=2). Value in the same column or line (a-c) with different superscript were significantly different (P<0.05).

nutrient sources/carbon sources, method of determining EPS and type of EPS produced (Ramchandran and Shah, 2009a).

EPS produced by microorganisms have played an essential role in biological and physiological functions such as attachment/adhesion to the interior of the intestine (Abbad Andaloussi *et al.*, 1995), promotion of colonization (Roberts *et al.*, 1995), has an antimutagenesis effect (Sreekumar and Hosono, 1998), lower cholesterol levels, immunomodulation and antitumor activity (Hugenholtz and Smid, 2002; Welman and Maddox, 2003), having antidiabetic effect in this case AGI activity (Ramchandran and Shah, 2009a) and having α-amylase inhibitory activity (Dilna *et al.*, 2015). In this study, high EPS production by *Lactobacillus plantarum*1 RB210 was shown to have high AGI activity. This is possible because EPS has a similar shape to the α-glucosidase substrate, allowing it to survive competitively against the enzyme's action, even though the mechanism is not fully understood. The ability of LAB to inhibit α-glucosidase contributes to blood glucose reduction and may have antidiabetic property (Chen *et al*., 2014). One of the drawbacks of EPS produced by LAB is the relatively small amount of production. However, although the amount of EPS produced by LAB is relatively low, it can still contribute to health improvement (Doleyres *et al*., 2005).

Production of short chain fatty acid (SCFA)

Intestinal microflora plays a role in the development of diabetes depending on the composition and function of microflora in the digestive tract. One of the mechanisms of probiotics as antidiabetic is the ability of LAB to produce SCFA as a product of fermentation in the large intestine. During the growth, LAB isolates consumed carbon source to produce energy and organic acids as a by-product. Here, the carbon sources used are FOS, GOS and inulin to substitute glucose. The SCFA measured included acetic acid, propionic acid and butyric acid. Based on the measurement results, it was known that all LAB isolates were able to produce acetic acid, but not all isolates were able to produce propionic acid and butyric acid (Table 3, 4 and 5).

Acetic acid

Based on statistical analysis (Table 3), the type of substrate had a significant effect (P<0.01) on the acetic acid produced during growth, while the interaction and type of isolate had no significant effect (P>0.05) on the acetic acid produced during growth. The levels of acetic acid produced ranged from 105.16 ± 17.10 mmol/L to 156.41 ± 38.53 mmol/L. *Lactobacillus paracasei* RL2 produced the highest acetic acid, but it was not significantly different from the other isolates. Based on the carbon source used, the yield of acetic acid ranged from 126.66 ± 24.89 mmol/L to 165.06 ± 22.70 mmol/L. The use of GOS as a carbon source produced the highest acetic acid and was significantly different (P<0.05) with acetic acid produced from FOS and inulin carbon sources.

Propionic acid

Based on Table 4, not all LAB isolates were able to produce propionic acid. Propionic acid produced from the use of prebiotic FOS as a carbon source ranged from 0.07 mmol/L to 0.27 mmol/L. The highest propionic acid was produced by *L. plantarum*1 RB210 and there were 5 isolates that were unable to produce propionic acid, i.e., *L. plantarum*1 MA1, *L. plantarum*1 ML7, *L. pentosus* MK42, *L. plantarum*1 MK2 and *L. paracasei* RK41. The use of GOS as a carbon source produced the highest propionic acid by *L.* plantarum1 MA1 at 0.13 mmol/L

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Table 4: Production of propionic acid (mmol/L) of *Lactobacillus* isolates on various carbon sources.

Lactobacillus isolates	Propionic acid level (mmol/L)		
	FOS[1]	GOS[2]	Inulin
*L. plantarum*1 RN9	0.09 ± 0.12	0.00 ± 0.00	0.08 ± 0.11
*L. plantarum*1 MA1	0.00 ± 0.00	0.13 ± 0.01	0.20 ± 0.28
*L. plantarum*1 RB210	0.27 ± 0.01	0.00 ± 0.00	0.40 ± 0.31
*L. plantarum*1 ML7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
*L. plantarum*1 RJ1	0.07 ± 0.09	0.07 ± 0.10	0.25 ± 0.35
*L. plantarum*1 MK2	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.37
L. paracasei RL2	0.23 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
L. pentosus RG5	0.22 ± 0.08	0.08 ± 0.11	0.19 ± 0.06
L. pentosus MK42	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.10
L. pentosus MS21	0.09 ± 0.13	0.00 ± 0.00	0.07 ± 0.09
L. pentosus MB23	0.18 ± 0.04	0.00 ± 0.00	0.09 ± 0.12
L. rhamnosus MY2	0.08 ± 0.11	0.00 ± 0.00	0.09 ± 0.13
L. paracasei RK41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Note: [1] = Fructooligossacharide; [2] = Galactooligossacharide.

Table 5: Butyric acid production (mmol/L) of *Lactobacillus* isolates on various carbon sources.

Lactobacillus isolates	Butyric acid level (mmol/L)		
	FOS[1]	GOS[2]	Inulin
*L. plantarum*1 RN9	0.03 ± 0.02	0.13 ± 0.71	0.13 ± 0.00
*L. plantarum*1 MA1	0.09 ± 0.13	0.05 ± 0.78	0.12 ± 0.17
*L. plantarum*1 RB210	0.07 ± 0.04	0.00 ± 0.00	0.18 ± 0.11
*L. plantarum*1 ML7	0.07 ± 0.09	0.12 ± 0.07	0.06 ± 0.08
*L. plantarum*1 RJ1	0.14 ± 0.00	0.07 ± 0.10	0.17 ± 0.07
*L. plantarum*1 MK2	0.00 ± 0.00	0.12 ± 0.02	0.22 ± 0.08
L. paracasei RL2	0.07 ± 0.01	0.09 ± 0.01	0.15 ± 0.01
L. pentosus RG5	0.09 ± 0.01	0.06 ± 0.08	0.12 ± 0.03
L. pentosus MK42	0.08 ± 0.01	0.05 ± 0.07	0.12 ± 0.01
L. pentosus MS21	0.12 ± 0.00	0.08 ± 0.02	0.14 ± 0.01
L. pentosus MB23	0.09 ± 0.02	0.09 ± 0.01	0.12 ± 0.01
L. rhamnosus MY2	0.14 ± 0.08	0.05 ± 0.06	0.16 ± 0.01
L. paracasei RK41	0.05 ± 0.06	0.00 ± 0.00	0.06 ± 0.08

Note: [1] = Fructooligossacharide; [2] = Galactooligossacharide.

and there were 10 isolates that did not produce propionic acid. The use of inulin as a carbon source produced propionic acid ranging from 0.07 mmol/L to 0.40 mmol/L and there were only 3 isolates that did not produce propionic acid, i.e., *Lactobacillus paracasei* RL2, *Lactobacillus plantarum*1 ML7 and *Lactobacillus paracasei* RK41.

Butyric acid

Butyric acid production of *Lactobacillus* isolates on various carbon sources can be seen in Table 5. Butyric acid produced from FOS carbon sources ranged from 0.03 mmol/L to 0.14 mmol/L. *Lactobacillus plantarum*1 RJ1 and *L. rhamnosus* MY2 produced the highest butyric acid at 0.14 mmol/L, while *L. plantarum*1 MK2 was not detected. In Table 5, it can be seen that the use of GOS as a carbon source produced butyric acid ranging from 0.05 mmol/L to 0.13 mmol/L. *L. plantarum*1 RN9 produced the highest butyric acid at 0.13 mmol/L, while butyric acid was not detected in *L. plantarum*1 RB210 and *L. paracasei* RK41. The use of prebiotic inulin produced butyric acid ranging from 0.06 mmol/L to 0.22 mmol/L. *Lactobacillus plantarum*1 MK2 produced the highest butyric acid of 0.22 mmol/L and all isolates were able to metabolize inulin to produce butyric acid.

Production of SCFA is the result of carbohydrate metabolism by LAB, especially carbohydrates that cannot be broken down by digestive enzymes. 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 carbon source for its growth. Acetic acid, propionic acid and butyric acid are the main SCFAs produced by the metabolism of nondigestible carbohydrates. SCFA has the ability to control the body's metabolism, including regulating insulin sensitivity, hormone secretion in the digestive tract and other metabolic processes (Estiasih *et al.*, 2012). According to Wong *et al.* (2006), the equally important roles of SCFA include the role as nutrients for the colonic epithelium, as

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colonic modulators and intracellular pH, cell volume and other functions related to ion transport, and as regulators of proliferation, differentiation and gene expression. Acetic acid is the main SCFA produced. Acetate will enter the peripheral circulation and metabolized by peripheral tissues which can increase cholesterol synthesis. Acetic acid can also be used as an energy source for large non-hepatic tissues (Puddu *et al.*, 2014). Butyric acid serves as the main energy source for colonocytes, nourishing the colonic mucosa and preventing colon cancer by promoting cell division (Puddu *et al.*, 2014), cell cycle arrest and changes in colonocyte apoptosis, inhibiting histone enzyme deacetylase, and reducing the transformation of primary to secondary bile acids as a result of colonic acidification. Propionic acid is mostly taken up by the liver and acts as a gluconeogenerator which has been proven to inhibit cholesterol synthesis. Propionic acid can inhibit gluconeogenesis activity and increase glycolysis in rats (Henningsson *et al*., 2002; Puddu *et al.*, 2014). Therefore, substrates that can reduce the acetate-propionate ratio can reduce serum lipids and cardiovascular disease risk (Wong *et al.*, 2006).

Among the three types of SCFA produced, butyric and propionic acid plays an important role in blood glucose regulation and lipid metabolism (Puddu *et al.*, 2014; Yovananda and Estiasih, 2016). The SCFA produced can modulate digestive hormones/incretin hormones such as glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which play a role in glucose and energy balance (Cani *et al.*, 2014). GLP-1 hormone is secreted by intestinal L cells (Puddu *et al.*, 2014). GLP-1 can reduce blood glucose levels during hyperglycemia by stimulating insulin secretion, increasing insulin sensitivity, reducing glucose dependence and maintaining pancreatic β cell function (Puddu *et al.*, 2014; Tilg and Moschen, 2014). This hormone can stimulate satiety and delay gastric emptying through a central mechanism, thereby reducing postprandial glucose levels (Wang *et al.*, 2015).

CONCLUSION

In conclusion, our results show definitively that *Lactobacillus* isolates from kombucha tea, dadih and bamboo of dadih container have considerable α-glucosidase inhibitor, antioxidant activity and the ability to produce of EPS and SCFA. The tested *Lactobacillus* isolates were able to inhibit α-glucosidase with varying abilities between 56.36 ± 3.76% until 80.32 ± 2.20% for CFE and 4.43 ± 1.13% until 18.47 ± 9.09% for CFS. Antioxidant activity varies between 80.33 ± 0.55% until 82.75 ± 2.91% for CFE and 8.20 ± 0.52% until 8.53 ± 0.27% for CFS. Production of EPS varies between 63.87 ± 6.82 mg/L to 360.13 ± 50.01 mg/L. All *Lactobacillus* isolates with FOS, GOS and inulin as carbon source were able to produce acetic acid but not all *Lactobacillus* isolates were able to produce propionic and butyric acid. Based on the four tests, there were 7 isolates of *Lactobacillus* with different advantages that had the potential to be further developed, i.e., *Lactobacillus pentosus* MK42, *Lactobacillus pentosus* MB23, *Lactobacillus rhamnosus* MY2, *Lactobacillus paracasei* RK41, *Lactobacillus plantarum*1 RB210, *Lactobacillus paracasei* RL2 and *Lactobacillus plantarum*1 MK2. This is a promising preliminary study because the use of indigenous *Lactobacillus* isolate has the potential as an antidiabetic agent, although the causative compound is still unknown. Therefore, more in-depth studies involving experimental animals and diabetic patients with *Lactobacillus* isolates, which are antidiabetic candidates in this study, are required.

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