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Anja Meryandini
Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Dramaga 16680, Indonesia, ameryandini@apps.ipb.ac.id

Amor Tresna Karyawati
Department of Biology, Faculty of Science and Technique, Universitas Nusa Cendana, Kupang 85228, Indonesia

Lilis Nuraida
Department of Food Science and Technology, Faculty of Agricultural Technology, Institut Pertanian Bogor, Dramaga 16680, Indonesia

Yulin Lestari
Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Dramaga 16680, Indonesia

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Lactic Acid Bacteria from *Apis dorsata* Hive Possessed Probiotic and Angiotensin-Converting Enzyme Inhibitor Activity

Anja Meryandini[1*], Amor Tresna Karyawati[2], Lilis Nuraida[3], and Yulin Lestari[1]

1. Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor,
Dramaga 16680, Indonesia
2. Department of Biology, Faculty of Science and Technique, Universitas Nusa Cendana, Kupang 85228, Indonesia,
3. Department of Food Science and Technology, Faculty of Agricultural Technology, Institut Pertanian Bogor,
Dramaga 16680, Indonesia

[*]E-mail: ameryandini@apps.ipb.ac.id

Abstract

One source of bacteria that has not been widely explored is lactic acid bacteria originating from honeycomb (hive). Timor Island has a wealth of giant honey bees (*Apis dorsata*). Lactic acid bacteria from the *Apis dorsata* hive in Timor Island need to be investigated to obtain probiotic candidates with specific functional properties. Lactic acid bacteria were isolated from honeycomb by using de Mann, Rogosa, and Sharpe agar supplemented with 1% of $CaCO_3$. Bacterial isolates that formed clear zones and were Gram-positive and catalase-negative were determined as lactic acid bacteria. The probiotic candidates are isolates that are resistant to bile salts and low pH; susceptible to antibiotics; are able to aggregate, autoaggregate, and coaggregate; and have antibacterial activities. Isolate MC7 was selected as a probiotic candidate and had inhibitory activity against angiotensin-converting enzyme (ACE) with IC_{50} 263,098 ppm. Isolate MC7 showed weak inhibition against α-glucosidase activity. Molecular identification based on 16S rRNA gene showed that MC7 isolate was closely related to *Lactobacillus rhamnosus* with 100% similarity. Therefore, isolate MC7 was recommended as a probiotic candidate with a functional property as an inhibitor of ACE.

Keywords: angiotensin-converting enzyme, Apis dorsata, hive, lactic acid bacteria, probiotic

Introduction

Some lactic acid bacteria have an important role in maintaining human health and as probiotics. Probiotic bacteria can improve the human immune system, reduce cholesterol, ease lactose intolerance and allergies, and improve intestinal microbiota, as well as have anticancer and antidiarrhea properties [1-3]. Desirable probiotic properties that can be determined by *in vitro* tests are resistance to acids and bile salts, adhesion to the host's intestinal surface, ability to eliminate pathogens or reduce pathogenic adhesion, ability to produce substances that have an antagonistic effect on pathogens, and bile salt hydrolase activity [1].

Some probiotic bacteria have functional properties as inhibitors of angiotensin-converting enzyme (ACE). *Lactobacillus plantarum, L.* spp*., L. sakei* and *L. curvatus* are probiotics that produce extracellular peptide compounds that can inhibit ACE [4-6]. ACE is a dipeptidyl carboxy peptidase that catalyzes the reaction in which angiotensin I changes to angiotensin II, which

results in a hypertensive condition. ACE also plays a role in activating bradykinin, a vasodilator peptide. Inhibition of ACE can decrease blood pressure in the body; this principle can be used to decrease the high blood pressure of patients with hypertension.

Some probiotic bacteria have functional properties as inhibitor of α-glucosidase. *Lactobacillus rhamnosus, L. casei,* and *L. bulgaricus* have been known to produce exo-polysaccharides which can inhibit α-glucosidase enzyme [7], an enzyme found on the surface of the small intestine, and its function is to catalyze carbohydrate digestion. Inhibition of α-glucosidase activity can reduce blood glucose levels. This principle has been used in the production of antidiabetic compounds such as acarbose, which can be used by people with type-2 diabetes mellitus to reduce blood glucose levels.

Common sources of lactic acid bacteria are milk and dairy products, animal digestive tracts, and plants. Another source of lactic acid bacteria is honeycomb (hive). *Lactobacillus* sp. Firm5, *L.* spp., and *L. kunkeei*

have been isolated from the *Apis mellifera* honeycomb in Tucson, an arid region in the USA [8]. *Lactobacillus kunkeei* YH-15, *L.* sp. Bma5 LMG P-24090, and *L.* sp. Taj Mustafa-1 UPMC 431 have been isolated from an *Apis dorsata* hive in Kedah, a tropical climate region in Malaysia [9]. Little information is known about the potential probiotic properties of lactic acid bacteria from honeycomb.

Timor Island, a tropical savanna climate region, has a considerable wealth of honey bee resources, especially giant honey bees (*Apis dorsata*). Previous research found that the *Apis dorsata* hive from Timor Island contained lactic acid bacteria such as *Lactobacillus kunkeei* strain YH-15 and *Lactococcus lactis* subsp. *tructae* strain L105 [10]. Exploration of lactic acid bacteria from *Apis dorsata* hive needs to be conducted to find a probiotic candidate that can produce inhibitor metabolites such as inhibitor of ACE or α-glucosidase.

Materials and Methods

Isolation of lactic acid bacteria from *Apis dorsata* hives in Kupang. *Apis dorsata* hives and flowers were collected from Kupang. The lactic acid bacteria were isolated from hives by using de Mann, Rogosa, and Sharpe (MRS) [7] agar supplemented with 1% CaCO$_3$, according to Prasirtsak et al. [11]. Isolates that formed a clear zone were characterized with Gram staining and catalase test. The isolated bacteria were stained by Gram staining method [12] and tested for the presence of catalase [13]. The isolates that formed a clear zone in MRS supplemented with CaCO$_3$ and were Gram-positive and catalase-negative were determined as lactic acid bacteria. The lactic acid bacteria were maintained in MRS broth supplemented with 40% glycerol and then stored in a freezer. The isolates were deposited in IPB Culture Collection (IPBCC) with collection number IPBCC b 20 1557 (MA15), IPBCC b 20 1558 (MC1), and IPBCC b 20 1559 (MC7).

Isolates' tolerance to bile salts and low pH. The isolates' tolerance to bile salts and low pH was evaluated by total plate count methods [7, 14]. The isolates were cultured for 18 hours in MRS broth at 37 °C. To determine tolerance to bile salts, 100 µL of the culture was inoculated in MRS broth supplemented by 0.3% (w/v) bile salts (Himedia, India), then incubated at 37 °C for 6 hours. Tolerance to low pH was assayed by inoculating 100 µL of bacterial suspension in MRS broth with pH 3 and then incubating it for 3 hours at 37 °C. The ability to tolerate the bile salts and low pH was determined based on the log number difference of colonies between MRS containing bile salts/low pH and MRS only.

Antibiotic susceptibility test. The susceptibility of lactic acid bacteria to antibiotics was tested by using the

Kirby-Bauer method. Amoxicillin and thiamphenicol were used in the susceptibility test. Inhibition zone criteria were determined based on the following criteria: sensitive (inhibition zone diameter >12 mm), intermediate (inhibition zone diameter between 5-12 mm), and resistance (inhibition zone diameter ≤4 mm) [15]. *Lactobacillus rhamnosus* R23, a probiotic with antidiarrhea property [16], was used as control.

Aggregation, autoaggregation, and coaggregation test. For the aggregation test, lactic acid bacteria isolates were cultured in MRS broth and incubated at 37 °C for 48 hours [17]. Positive aggregation can be detected from the precipitation of cell bacteria at the bottom of the tube and a clear supernatant. Lactic acid bacteria were tested by autoaggregation methods [18, 19]. Autoaggregation percentage can be calculated by the following formula:

$$\text{Autoaggregation (\%)} = 1 - (A_t/A_0) \times 100 \qquad (1)$$

where A_t represents an absorbance of 5 hours at 600 nm, and A_0 represents an absorbance of 0 hours at 600 nm. *Lactobacillus rhamnosus* R23 was used as control. The isolate's ability to coaggregate with pathogens was evaluated by conducting a coaggregation test [20] that involved three pathogenic bacteria (*Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, *S. typhymurium* ATCC 14028). The percentage of coaggregation can be calculated as follows:

$$\text{Coaggregation (\%)} = \{[(\text{Abs}_{pat}+\text{Abs}_{lab})/2] - \text{Abs}_{mix}\}/ [\text{Abs}_{pat}+\text{Abs}_{lab})/2] \times 100 \qquad (2)$$

where Abs_{pat} is the absorbance of pathogen, Abs_{lab} is the absorbance of lactic acid bacteria, and Abs_{mix} is the absorbance of a mixture pathogen and lactic acid bacteria. *L. rhamnosus* R23 was used as control.

Hydrophobicity test. The hydrophobicity of lactic acid bacteria was tested by using microbial adhesion to solvent or MATS [17]. The hydrophobicity of the cell surface of lactic acid bacteria was calculated by the following equation:

$$H (\%) = (A - A_0)/A \times 100 \qquad (3)$$

where H is the hydrophobicity, A is the absorbance after 1 hour at 600 nm, and A_0 is the absorbance of 0 hours at 600 nm. *Lactobacillus rhamnosus* R23, a probiotic with antidiarrhea properties [13], was used as culture reference.

Antibacterial activity test. Lactic acid bacteria were grown in MRS broth for 48 hours, and then the supernatant was collected by centrifuging. The antibacterial activity of supernatant and neutralized supernatant was determined using agar well diffusion

method [21]. Three pathogen bacteria (*Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, *S. typhymurium* ATCC 14028) were obtained from the Laboratorium of Food Science (IPB University) and used in the anti-bacterial test. *Lactobacillus rhamnosus* R23, a probiotic with antidiarrhea properties [16], was used as control.

Identification of lactic acid bacteria. DNA extraction was performed from the bacterial cells by using Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). 16S rRNA genes were amplified by Polymerase Chain Reaction (PCR) by using primer pairs 63F and 1387R [22]. DNA products were sent to the *1ˢᵗ Base DNA Sequencing Division* (*Apical Scientific Sequencing*) for sequencing. The obtained DNA sequences were analyzed by performimg EzTaxon Biocloud [23]. The phylogenetic tree was analyzed by MEGA 7.0 [24] with neighbor joining method and 2000 bootstrap replications.

ACE inhibitor test. Lactic acid bacteria were cultured in MRS broth for 48 hours and then centrifuged. The supernatant was evaporated and then freeze dried. The freeze-dried supernatant was diluted in sterile water at different concentrations and used for ACE inhibitor measurement. Inhibitor activity against ACE was measured by using spectrophotometer method based on the reaction of enzyme and substrate [25]. Substrate hippuryl-histidyl-leucine was hydrolyzed by ACE to become hypuric acid. The release of hypuric acid was determined based on the absorbance value at 492 nm. The inhibition of ACE was calculated by the following formula:

$$\text{ACE inhibitory activity (\%)} = (B-A)/(B-C) \times 100 \qquad (4)$$

where A is the absorbance of the sample, B is the absorbance of the control, and C is the absorbance of the blank. Captopril was used as positive control. A high absorbance value of a sample indicates that more hypuric acid products are formed. This finding jndicates low sample inhibitory activity against ACE. IC_{50} was determined using a regression formula.

α-glucosidase inhibitor test. Sample preparation was performed similarly to the evaluation of the ACE inhibitor. Inhibitor α-glucosidase test was evaluated using spectrophotometer method [26]. Substrate p-Nitrophenyl-α-D-glucopyranoside was hydrolyzed by α-glucosidase to p-Nitrophenol. Acarbose (Bayer) was used as positive control. The inhibition of α-glucosidase was determined by using the following formula:

$$\text{Inhibition (\%)} = [(Ac - As)/Ac] \times 100 \qquad (5)$$

where Ac is the absorbance of the control and As is the absorbance of the sample. The absorbance value of the sample showed a number of p-Nitrophenol products as a result of substrate hydrolysis. High sample absorbance indicates more p-Nitrophenol products and lower inhibitory activity against α-glucosidase.

Results and Discussion

Isolation of lactic acid bacteria. A total of 135 isolates formed clear zones on the MRS agar supplemented by 1% CaCO₃, but only 81 isolates were Gram-positive and did not have catalase. Therefore, 81 isolates were determined as lactic acid bacteria isolates. Lactic acid bacteria inoculated on MRS agar supplemented with 1% CaCO₃ formed clear zones. Lactic acid that was produced by the lactic acid bacteria fermentation reacts with CaCO₃ to form calcium lactate. The clear zone around the colony of lactic acid bacteria indicates the presence of a water-soluble calcium-lactic compound.

Isolates' tolerance to bile salts and low pH. All isolates can grow on MRS agar supplemented with 0.3 % bile salts (Himedia). Ten isolates were selected as tolerant isolates to bile salts and low pH. Three of 10 isolates of lactic acid bacteria were selected as the best isolates on the basis of their tolerance (Figure 1). The three isolates were MA15, MC1, and MC7 and they can grow in MRS supplemented with bile salts that had a similar condition as the human intestine and in media with low pH such as the human gastric juice. This condition is important in enabling the three isolates to pass through the digestive tract. Bile salts are harmful to bacteria cells because they can damage the structure of the cell membrane [7]. The average concentration of bile salts in the human intestine is 0.3% (w/v) [27]. MA15, MC1 and MC7 can grow in MRS supplemented with 0.3% (w/v) bile salts and are thus tolerant to bile salts in the human intestine. The human stomach has low pH (2.5−3.5), and food travels through the stomach for 2−4 hours [28]. The 29 *Lactobacillus* strains of dairy origin were able to survive at pH 3.0 [29]. *Lactobacillus reuteri*, *L. rhamnosus* G5435, *L. acidophilus* 388, and *L. delbrueckii* subsp. *bulgaricus* 11842 can survive during simulated gastric transit at pH 3 for 3 hours [30]. The three isolates from *Apis dorsata* hives can survive in MRS with pH 3.0, thereby suggesting that the isolates can pass through and enter the human intestine.

Antibiotic susceptibility test. The three isolates of lactic acid bacteria and *Lactobacillus rhamnosus* R23 (as control) are susceptible to amoxicillin and thiamphenicol. The inhibiton zone diameter of antibiotics toward the three lactic acid bacteria and positive control ranges from 21 mm to 28 mm (Table 1), thus indicating that the bacteria are sensitive to antibiotics. Moreover, the bacteria are more sensitive to amoxicillin than to thiamphenicol. The control bacterium is more sensitive than the three lactic acid bacteria. The three lactic acid bacteria isolates are sensitive to amoxicillin and thiamphenicol. The sensitivity of bacteria to antibiotics is important in selecting probiotics because these properties prevent the bacteria from transferring resistant genes to pathogenic bacteria. Resistant bacteria can transfer resistant properties to other bacteria through

horizontal gene transfer [31]. The three mechanisms of horizontal gene transfer are transformation, conjugation, and transduction. Transformation is a gene transfer process in which cells take up free DNA directly from their environment, and then the DNA is derived from a donor bacterium and taken up by a recipient bacterium [32]. Plasmid or other self-transmissible DNA elements transfer themselves and sometimes other DNA into another bacterial cell in a process called conjugation [32]. Bacteriophages sometimes transfer bacterial DNA from one cell to another in a process called transduction, in which a phage carries DNA from one bacterium to another [32].

Table 1. Results of Antibiotic Susceptibility Test

Isolate	Amoxicillin (25 µg/mL)		Thiamphenicol (30 µg/mL)	
	Inhibition zone (mm)	Criteria[a]	Inhibition zone (mm)	Criteria[a]
MA15	25	sensitive	24	sensitive
MC1	22	sensitive	21	sensitive
MC7	27	sensitive	25	sensitive
LrR23	28	sensitive	25	sensitive

[a]Based on the criteria of Mukherjee (1988), i.e., sensitive (diameter of inhibition zone >12 mm), intermediate (diameter of inhibition zone between 5−12 mm), and resistance (diameter of inhibition zone ≤4 mm).

Table 2. Result of Aggregation, Autoaggregation and Coaggregation Test

Isolate	Aggregation[a]	Autoaggregation (%)	Coaggregation (%) with pathogen		
			E. coli	*S. aureus*	*S. typhymurium*
MA15	+	91.2	90.47	92.30	92.00
MC1	+	88.4	-13.33[b]	88.57	76.47
MC7	+	91.9	-56.09[b]	95.65	86.66
LrR23	+	95.6	-26.67[b]	80.00	89.47

[a]Positive aggregation is found if sediment-like sands formed on the bottom of the tube and the upper layer of the supernatant is clear. [b]Negative values indicate no coaggregation activity.

Table 3. Hydrophobicity Properties of Lactic Acid Bacteria

Isolate	Hydrophobicity (%)	[a]Criteria
MA15	8.17	Not hydrophobic
MC1	3.66	Not hydrophobic
MC7	8.93	Not hydrophobic
LrR23	11.5	Not hydrophobic

Note: [a]Hydrophobicity criteria according to Santos *et al.* (1990): hydrophobicity >50% (strongly hydrophobic), hydrophobycity of 20−50% (moderately hydrophobic), hydrophobicity <20% (not hydrophobic).

Table 4. Results of Antibacterial Test of Lactic Acid Bacterial Supernatant

Isolate	Diameter of inhibition zone (mm)					
	S. aureus		*S. typhymurium*		*E.coli*	
	a	b	a	b	a	b
MA15	15	13	9	10	9	11
MC1	7	8	9	10	8	11
MC7	17	15	10	11	10	11
LrR23	26	25	13	13	22	13

Notes: a = supernatant; b = neutralized supernatant.

Table 5. Identification of Lactic Acid Bacteria Based on 16S rDNA Sequence Data

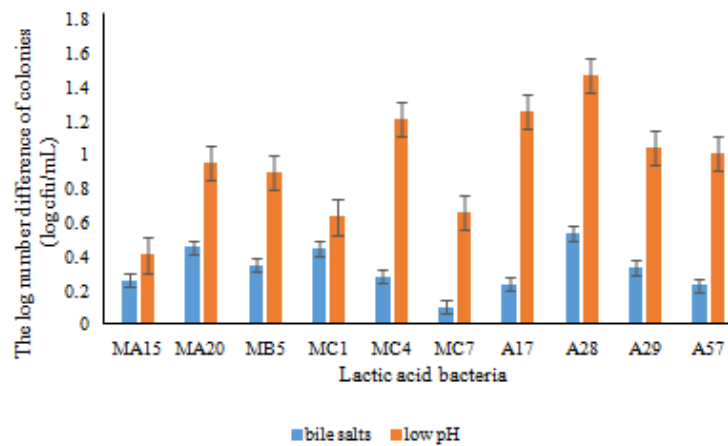| Isolate	Species	Strain	Similarity (%)	Accession number
MA15	*Enterococcus durans*	NBRC 100479	99.91	BCQB 01000108
MC1	*Enterococcus durans*	NBRC 100479	99.91	BCQB 01000108
MC7	*Lactobacillus rhamnosus*	JCM 1136	100.00	BALT 01000058

Figure 1. Selection of Lactic Acid Bacteria Based on the Test Result of Resistance to Bile Salts and Low pH. Three Isolates (MA15, MC1, MC7) were Selected as Bacteria that are Resistant to Bile Salts and Low pH.
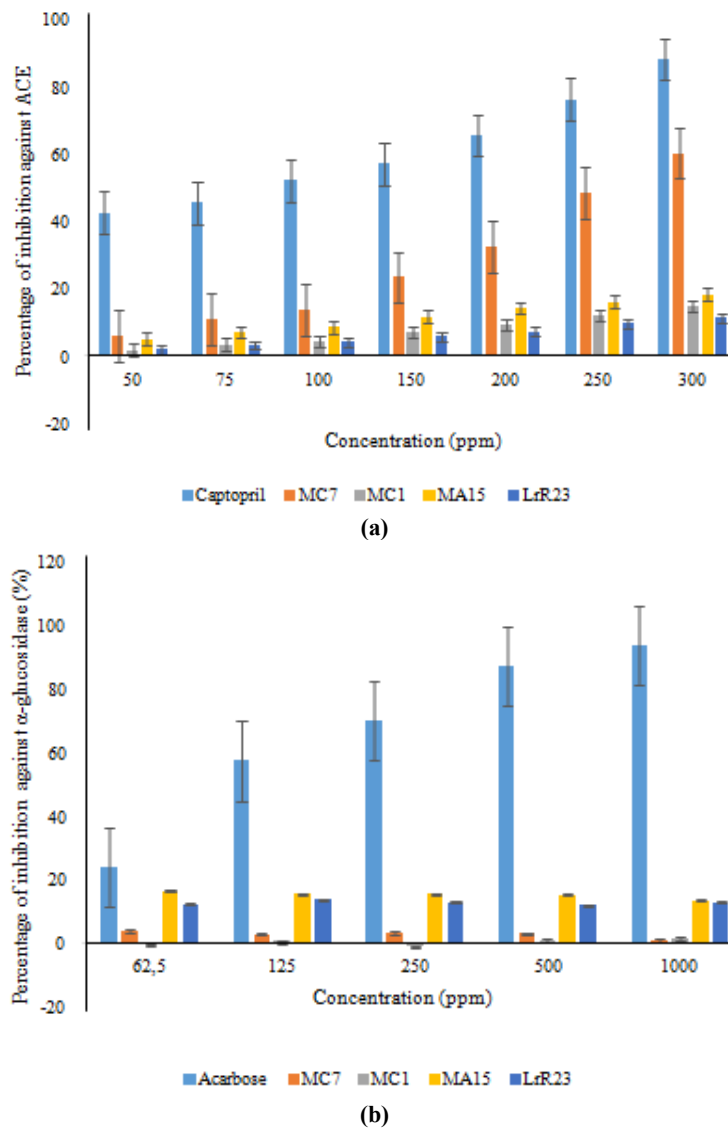

(a)

(b)

Figure 2. (a) Inhibition of Lactic Acid Bacteria Supernatant and Captopril against ACE; (b) Inhibition of Lactic Acid Bacteria Supernatant and Acarbose against α-glucosidase

Aggregation, autoaggregation and coaggregation test. The percentage of autoaggregation was between 88.4% and 95.6% (Table 2). The percentage of coaggregation between the three lactic acid bacteria isolates and two pathogen bacteria was high (Table 2). The lactic acid bacteria formed an aggregate with *Staphylococcus aureus* and *Salmonella thyphymurium.* The autoaggregation percentage correlates to the ability of the lactic acid bacteria to form aggregate among the cells of lactic acid bacteria. The ability of lactic acid bacteria to form aggregation and autoaggregation is necessary for the adherence of lactic acid bacteria to the surface of intestine walls [19]. Coaggregation between lactic acid bacteria and pathogen can inhibit pathogens from adhering to the surface of intestine walls. Therefore, the host could not be infected by pathogens because the pathogens were eliminated by the lactic acid bacteria. The high coaggregation ability of the isolates against pathogen can prevent pathogens from attaching to the host epithelial tissue [19]. These abilities are important in supporting the adherence of lactic acid bacteria on the surface of the intestinal wall.

Hydrophobicity test. The selected isolates (MA15, MC1, MC7) and *L. rhamnosus* R23 are not hydrophobic. These results are consistent with the results from previous research, which stated that *L. rhamnosus* R23 as a probiotic candidate is not hydrophobic or hydrophilic [18]. Although hydrophilic, *L. rhamnosus* R23 can adhere to the surface of rat intestinal epithelium [18]. The adhesive ability of *Lactobacilli* is not dependent on hydrophobicity and no correlation exists between the hydrophobicity of the cell surface and adhesive ability [18]. Therefore, the hydrophylic properties of bacteria do not affect the ability of bacteria to adhere to the surface of the human intestine. This fact is supported by probiotics such as *L. acidophylus* LA1, *L. casei* Shirota, and *Lactobacillus rhamnosus* GG which had been proved to deliver beneficial health effects despite their low adhesive properties (3.2−12.6%) [17].

Antibacterial activity test. The supernatant of MC1, MC7, and MA15 exhibited antibacterial activity against pathogen bacteria (Table 4). The antibacterial activity of the control (*L. rhamnosus* R23) was higher than that of others. *L. rhamnosus* R23 possessed broad-spectrum antibacterial activity that can inhibit Gram-positive and Gram-negative bacteria. MA15, MC1, and MC7 also showed a broad-spectrum antibacterial activity, but it was lower than that of the control. The supernatant of lactic acid bacteria (MA15, MC1, MC7) showed antibacterial activity against pathogen bacteria. Therefore, the three isolates are expected to be able to eliminate pathogens from the human digestive tract. The lactic acid bacteria can decrease pH in MRS broth because the bacteria produce lactic acid, which can inhibit the three pathogen bacteria (*Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, *S. typhymurium*

ATCC 14028). The neutralized supernatant showed antibacterial activity against pathogenic bacteria, thereby indicating that the supernatant contained antibacterial compounds other than acid. This result was in agreement with that of a previous study [16], *L. rhamnosus* R23 has antibacterial activity against *E. coli* K1.1 (enteropathogenic bacteria that cause diarhea). Daily administration of *L. rhamnosus* R23 protected the mice from diarrhea even though they were infected by *E. coli* K1.1 [16].

Identification of lactic acid bacteria. The lactic acid bacteria, MA15, and MC1 isolates were closely related to *Enterococcus durans* with 99.91% similarity as indicated by molecular identification based on 16S rDNA sequence data (Table 5), whereas the MC7 isolate was closely related to *Lactobacillus rhamnosus* with 100% similarity. The three isolates of lactic acid bacteria were registered in GenBank (accession number MH656781 [MA15], MH656782 [MC1], and MH656783 [MC7]). MA15 and MC1 isolates are closely related to *Enterococcus durans* NBRC 100479. Both MA15 and MC1 share the same genus with the pathogens *Enterococcus faecalis* and *Enterococcus faecium,* which cause enterococcal endocarditis in humans [34]. *E. durans* is non-faecalis enterococci found in the intestines of animals [34]. These bacteria rarely endocarditis in humans. *Enterococcus durans* EP1 has been recommended as a probiotic with antiinflammation properties [35]. *E. durans* EP1 is not virulence, sensitive to vancomycin, and non-α-hemolytic [35].

ACE inhibitor test. A high concentration of the MC7 supernatant corresponds to a high percentage of ACE inhibition (Fig. 2), thereby indicating that the MC7 supernatant contains metabolites that can inhibit ACE activity. The IC_{50} of the MC7 supernatant and of captopril was 263.098 ppm and 98.501 ppm, respectively. Isolate MC7 is recommended as a probiotic given its functional property as an ACE inhibitor. The supernatant had inhibitor activity to ACE with IC_{50} of 263.099 ppm, while captopril had IC_{50} of 98,501 ppm. This IC_{50} of MC7 supernatant was higher than IC_{50} of captopril (98.501 ppm), thereby indicating that the inhibitor activity of isolate MC7 against ACE was lower than that of captopril. Captopril contains pure compounds, while the MC7 supernatant contains mixture compounds. The MC7 supernatant can inhibit ACE although the supernatant was not concentrated yet. Therefore, the MC7 supernatant needs to be extracted to increase its inhibitor activity against ACE. For example, after extraction and fractionation of fraction 27 of *L. sakei* CRL 1862 and fraction 30 of *L. curvatus* CRL 705, two lactic acid bacteria were isolated from traditional sausage; they have the active peptide FISNHAY and exhibit inhibitor activity of 54±2.3% and 50±3.1%, respectively, agaist ACE [6].

α-glucosidase inhibitor test. The MC1, MC7, and MA15 isolates and the control had weak α-glucosidase inhibitor activity. Acarbose had the strongest α-glucosidase inhibitor activity, while the supernatant MC7 isolate had weak α-glucosidase inhibitor activity (Fig.2). The inhibitor activity of supernatant MC7 ranges from 0.58% to 3.55%. In this research, the supernatant of isolate MC7 was a weak inhibitor against α-glucosidase activity. This condition is probably caused by the fact that the supernatant of isolates was not concentrated yet. The supernatant can be extracted with organic solvent to separate the compounds that have inhibitory activity against α-glucosidase. *Lactobacillus rhamnosus* Z7 (isolated from human feces) has been recommended as a probiotic with a functional property as inhibitor of α-glucosidase. The supernatant of the bacteria can inhibit α-glucosidase with a percentage of 29.21 ± 0.99 % [7]. The inhibitor activity of *L. rhamnosus* Z7 supernatant against α-glucosidase was almost equal to that of inhibitor of commercial probiotic, i.e. *L. rhamnosus* GG ATCC 53103 (Valio Ltd., Helsinki, Finland), which has inhibitor activity against α-glucosidase of $29.57 \pm 1.38\%$ [7].

Conclusion

Isolate MC7 is closely related to *Lactobacillus rhamnosus*, and isolates MC1 and MA15 are closely related to *Enterococcus durans*. The isolates MC1 and MA15 can be recommended as probiotics, but their hemolytic activity needs further investigation to determine if they are not pathogenic. The isolates MC1 and MA15 are weak inhibitors against ACE and α-glucosidase. Isolate MC7 exhibited inhibitor activity against ACE with IC_{50} 263.099 ppm, but it weakly inhibited α-glucosidase. Isolate MC7 is recommended as a probiotic with a functional property as inhibitor of ACE. This research has some limitations and can be expanded in different aspects, such as the extraction of bioactive compounds and the determination of bioactive compounds and bile salt hydrolase activity.

Acknowledgments

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