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In vitro Antioxidant and α-Glucosidase Inhibitory Activities of *Lactobacillus* spp. Isolated from Indonesian Kefir Grains

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Abstract

Background and Objective: In this study, nine *Lactobacillus kefiri* and one *Lactobacillus rhamnosus* isolates with kefir grain origin have been demonstrated to include potentials as probiotics. The aim of this study was to investigate ability of the isolates to produce compounds with α-glucosidase inhibitory and antioxidant activities and identify peptides with MW of ≤ 3 kDa in cell-free supernatants.

Material and Methods: All isolates were cultured in de Man, Rogosa and Sharpe broth media at 37 ºC for 24 h. Assessment of α-glucosidase inhibitory and antioxidant activities was carried out on cell-free supernatants. Assessment of optimum incubation time was carried out on two isolates with the highest α-glucosidase inhibitory and antioxidant activities. The two isolates were used to ferment reconstituted skim milk. Cell-free supernatant of the fermented skim milk was fractionated using filters of 10 and 3 kDa. Then, peptides in fractions of ≤ 3 kDa were identified.

Results and Conclusion: The highest α-glucosidase inhibitory activity was seen in *Lactobacillus rhamnosus* BD2 and *Lactobacillus kefiri* YK4 as 73.58 and 64.31%, respectively. The highest antioxidant activity was observed in *Lactobacillus kefiri* JK5 and *Lactobacillus kefiri* JK17 as 44.31 and 41.57%, respectively. When *Lactobacillus rhamnosus* BD2 and *Lactobacillus kefiri* YK4 were cultured in reconstituted skim milk, their α-glucosidase inhibitory activities respectively decreased to 25.72 and 36.16% while the antioxidant activities respectively increased to 74.53 and 80.92%. Fractionation of the cell-free supernatants from fermented reconstituted skim milk of *Lactobacillus kefiri* YK4 showed that the highest antioxidant activity was included in fractions greater than 10 kDa. Although fractions of 3 kDa or less exhibited quite high antioxidant activities. Identification of peptides in fractions of 3 kDa or less showed that the peptides were mostly derived form β-casein. Of these peptides, two peptides with sequences of FPPQSV and YQEPVLGPVRGPFPIIV have been reported to include antioxidant activities.

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1. Introduction

Diabetes mellitus is a serious health problem in most countries. Diabetes mellitus is a hyperglycemia disorder shown by a high glucose level in blood. This condition may be induced by insufficient secretion of insulin (type 1 diabetes mellitus) or insulin resistance (type 2 diabetes mellitus), which is reported more frequently [1]. Type 2 diabetes mellitus is associated with increases in blood glucose levels and can be triggered by oxidative stress conditions [2]. Blood glucose level can be controlled by α-glucosidase inhibitors [2]. The α-glucosidase is an enzyme in the small intestine epithelia that catalyzes oligosaccharide transformation to glucose before it is absorbed [3]. Inhibiting activity of this enzyme decreases glucose absorption and maintains blood glucose at normal levels [2]. Furthermore, oxidative stress is a condition when endogenous antioxidants produced by the human body are insufficient to neutralize free radicals [4]. Oxidative stress is harmful to people with type 2 diabetes mellitus because it can worsen performance of pancreas glands [2]. One of the solutions for the prevention of oxidative stress includes administration of

exogenous antioxidants [5]. Studies have reported that probiotic bacteria from *Lactobacillus* spp. can produce α-glucosidase inhibitor [6-10] and antioxidant compounds [11-13]. In this study, ten isolates of *Lactobacillus* spp. from Indonesian kefir grains have shown to be potential candidates as probiotics [14]. The objectives of this study were to assess ability of the isolates to produce α-glucosidase inhibitor and antioxidant activities and identify peptides in fractions of $\leq$ 3kDa from cell-free supernatants.

2. Materials and Methods

2.1 *Lactobacillus* strains

Lactobacillus (L.) kefiri (nine isolates) and *L. rhamnosus* (one isolate) were previously isolated from kefir grains in four regions of Indonesia, including Bogor, Bandung, Jakarta and Yogyakarta. The *L. rhamnosus* R23 previously reported as a potential probiotic with α-glucosidase inhibitory effects [15] was used for the assessment of α-glucosidase inhibitory activity as a reference culture.

2.2 The α- glucosidase inhibitory and antioxidant activities

2.2.1 Preparation of cell-free supernatants (CFS) [6]

Isolates were cultured in 10 ml of MRS (de Man, Rogosa and Sharpe) broth media (Oxoid, USA) at 37 ºC for 24 h. the MRS was more appropriate for lactic acid bacteria (LAB) because of its components of growth factors such as polysorbate, acetate, magnesium and manganese [16]. Supernatant was separated from the cells using centrifugation at 8000 ×g and filtration through 0.22-µm membrane filters (Minisart syringe filter, Sartorius, Germany). The pH was adjusted to 7.4 by adding 1 N NaOH.

2.2.2 Assessment of α-glucosidase inhibitory activity [10]

The mixture consisted of 25 µl of CFS, 150 µl of phosphate buffer solution (pH 7.4) (Oxoid, USA) and 75 µl of 20 mM p-nitrophenyl a-D-glucopyranoside (Sigma-Aldrich, USA). The mixture was incubated at 37 ºC for 10 min. Then, 50 µl of 0.2 U ml^{-1} α-glucosidase enzyme from *Saccharomyces cerevisiae* (Sigma-Aldrich, USA) were added to the mixture and incubated at 37 ºC for 10 min. After incubation, 1 ml of 0.1 M Na_2CO_3 (Merck, Germany) was added to the mixture. The absorbance was analyzed using microplate reader ($A_{405;}$ iMark, Biorad, USA). The α-glucosidase inhibitory activity of the sample was calculated as follows:

$$\alpha\text{-glucosidase inhibitory activity (\%)}=1 - \left[\frac{(C - D)}{(A - B)}\right] \times 100\%$$

Where, A was absorbance of the α-glucosidase enzyme, B was absorbance of the blank solution, C was absorbance of α-glucosidase enzyme with the sample and D was absorbance of the sample.

2.2.3 Assessment of antioxidant activity [6]

Approximately, 1 ml of CFS was mixed with 1 ml of 0.2 mM α-diphenyl-β-picrylhydrazy (DPPH) (Sigma-Aldrich, USA) solution and incubated for 30 min at dark followed by measurement of the absorbance using UV-vis spectrophotometry (A_{517}; Shimadzu, Japan) and distilled water as blank. The antioxidant activity was calculated as Eq. 1:

$$\text{Antioxidant activity (\%)} = 1 - \left[\frac{\text{Sample(A517)}}{\text{Blank(A517)}}\right] \times 100\% \quad (1)$$

2.3 Effects of incubation time

Two isolates with the highest α- glucosidase inhibitory and antioxidant activities were selected for the assessment of incubation time on the α-glucosidase inhibitory and antioxidant activities. Isolates were cultured in MRSB and incubated for 18, 24, 36 and 48 h. The CFS was assessed for α- glucosidase inhibitory and antioxidant activities [6]. The total LAB was enumerated at the end of incubation time. Methods for the preparation of cell-free supernatants were similar to the methods described previously. The LAB were enumerated on MRS agar incubated at 37 ºC for 48 h.

2.4 The α- glucosidase inhibitory and antioxidant activities of fermented skim milks

The two selected isolates were cultured in 12% w v^{-1} reconstituted skim milk (RSM) at 37 ºC for 24 h. Cell-free supernatants of the fermented skim milks (CFS-skim) were prepared using centrifugation at 8000 ×g. Hydrolyzed proteins of CFS-skim were assessed according to Church et al. [17] and protein contents of the RSM were analyzed according to Lowry et al. [18]. Moreover, α-glucosidase inhibitory and antioxidant activities were assessed using methods described previously.

2.5 Fractionation and identification of peptides sequences

2.5.1 Fractionation of reconstituted skim milks

A total of 15 ml of CFS-skim were transferred into a 10-kDa filter tube (Amicon Ultra-15, Merck, Germany) and centrifuged (4000 ×g, 30 min, 4 ºC). Solution was passed through 10-kDa filters, transferred into a 3-kDa filter tube (Amicon Ultra-4, Merck, Germany) and centrifuged similarly. All fractions were collected and the volume was adjusted to 15 ml by addition of water and then assessed for α-glucosidase inhibitory and antioxidants activities.

2.5.2 Identification of peptides [19]

Identification of peptides was carried out using liquid chromatography-mass spectrometry (LC-MS) (Nano LC Ultimate 3000 Series Tandem Q System Exactive Plus Orbitrap HRMS, Thermo Scientific, USA). Sample was trapped in Thermo-Scientific 164649 Trap Column with a diameter of 30 µm and length of 5 mm and a capillary column (Pep Map RSLC C18; ES 800, Thermo Scientific,

USA) with dimensions of 75 µm × 15 cm, particle size of 3 µm and pore size of 100 µm. The eluents included H_2O/acetonitrile 98:2 formic acid (A) and H_2O/acetonitrile 2:98 0.1% formic acid (B). Flow rate was 300 ml min^{-1} with a gradient of 2-35% B for 30 min, 30-90% B for 15 min, 90% B for 15 min, 5% B for 30 min. The mass range was 200-2000 m z^{-1}. Results were analyzed using Proteome Discovered Software v.2.2 (Thermo Scientific, USA).

2.6 Statistical analysis

The viable counts of LAB were expressed as mean of colony forming units (CFU) ml^{-1} samples ±SD (standard deviation). One-way analysis of variance test followed by Duncan multiple range test were carried out at a significance level of 0.05 using SPSS Software v.24 (IBM Analytics, USA) (n = 3).

3. Results and Discussion

3.1 The α-glucosidase inhibitory and antioxidant activities

Results showed that LAB isolates included α-glucosidase inhibitory activities, ranging from 6.60% ±3.62 to 73.58% ±4.13 (Table 1). The highest activity was shown by *L. rhamnosus* BD2. The reference isolate of *L. rhamnosus* R23, which previously reported as a potential probiotic with α-glucosidase inhibitory activity [15] showed α-glucosidase inhibitory activity in the present study. The α-glucosidase inhibitory activities of the ten isolates were lower than those of acarbose (94.36% ±1.63), an α-glucosidase inhibitor drug for type 2 diabetes mellitus. No studies have been published on specific substances of α-glucosidase inhibitor compounds produced by *Lactobacillus* spp. However, *Lactobacillus* spp. is potential to include α-glucosidase inhibitor activity derived from its extracellular metabolites e.g. polysaccharide components [20] and peptides produced by its proteolytic activity [21]. Antioxidant activity of the LAB isolates varied. The highest activity belonged to *L. kefiri* JK5 (44.31% ±0.35) (Table 1). However, the percentage of LAB antioxidant activity was lower than that of 50 mg l^{-1} ascorbic acid (91.44% ±1.67). It has previously been reported that compounds including extracellular polysaccharide [22], glutathione [23], folate [11] and bioactive peptides [24] in CFS of LAB included antioxidant activity. These compounds might be responsible for antioxidant activity in the present study. Bioactive peptides with antioxidant activities produced by these isolates were characterized in the present study.

3.2 Effects of incubation time

The highest percentage of α-glucosidase inhibitory and antioxidant activities in MRSB were achieved after 48 h of incubation; however, no significances were seen from those of 24 and 36 h (Table 2). Number of *L. rhamnosus* BD2 and *L. kefiri* reached 9 log CFU ml^{-1} after 18 h of incubation. No significant increases were reported in colony counts after 18 h. However, bioactive compounds acting as α-glucosidase inhibitors and antioxidants such as lactic acid, antimicrobial compounds, extracellular polysaccharides and bioactive peptides were continuously produced during this period [16,25].

3.3 Protein and hydrolyzed protein contents of fermented reconstituted skim milks

Data showed that the protein contents of RSM was 1.19 mg ml^{-1} protein. After fermentation at 37 °C for 24 h, the hydrolyzed protein contents were almost similar between *L. rhamnosus* BD2 (4.67 mg ml^{-1}) and *L. kefiri* YK4 (4.83 mg ml^{-1}) (Table 3). These results indicated that both isolates secreted proteases during fermentation. Several LAB included proteolytic activities within 24 h and increased contents of peptides, which were mostly bioactive peptides [26]. The LAB included protease enzymes to hydrolysis proteins or polypeptides to oligopeptides or peptides [27]. High antioxidant activities were resulted from tripeptides from casein hydrolysis by LAB such as Pro-His-His [28].

Table 1. The α-glucosidase inhibitory and antioxidant activities of *Lactobacillus* spp. isolated from Indonesian kefir grains grown in MRS broth for 24 h at 37 °C

No	*Lactobacillus* spp.	α-glucosidase inhibitory activity (%) cell free supernatant	Antioxidant activity (%)
1	*Lactobacillus kefiri BG8*	25.37 ±3.61 ^{g}	36.52 ±2.67 ^{f}
2	*Lactobacillus kefiri BG13*	48.82 ±5.91 ^{e}	15.65 ±0.66 ^{h}
3	*Lactobacillus rhamnosus BD2*	73.58 ±4.13 ^{b}	36.50 ±2.10 ^{f}
4	*Lactobacillus kefiri BD4*	21.71 ±5.08 ^{g}	17.96 ±2.40 ^{gh}
5	*Lactobacillus kefiri JK1*	6.60 ±3.62 ^{h}	32.58 ±1.80 ^{f}
6	*Lactobacillus kefiri JK5*	19.98 ±3.86 ^{g}	44.31 ±0.35 ^{e}
7	*Lactobacillus kefiri JK6*	57.02 ±2.42 ^{d}	20.39 ±0.87 ^{g}
8	*Lactobacillus kefiri JK17*	23.10 ±3.67 ^{g}	41.57 ±1.02 ^{e}
9	*Lactobacillus kefiri YK4*	64.31 ±3.69 ^{c}	34.15 ±2.77 ^{f}
10	*Lactobacillus kefiri YK7*	35.66 ±2.72 ^{f}	18.01 ±0.87 ^{gh}
11	*Lactobacillus rhamnosus R23**	70.02 ±5.70 ^{bc}	35.86 ±1.74 ^{f}
12	Acarbose 50 mg l^{-1}*	94.36 ±1.63 ^{a}	-
13	Ascorbic acid 50 mg l^{-1}*	-	91.44 ±1.67 ^{d}

*The reference samples. Means in the same column with different superscript letters are significantly different ($p < 0.05$) by Duncan's multiple range test

Table 2. The α-glucosidase inhibitory and antioxidant activities of the isolates from Indonesian kefir grains grown in MRS broth at 37 ºC for various incubation times

Isolate name	Incubation (h)	Colony count (CFU ml^{-1})	α-glucosidase inhibitory activity (%)	Antioxidant-DPPH activity (%)
			Cell-free supernatant	
Lactobacillus rhamnosus BD2	18	9.15 ±2.0 ×10^{8} ^{b}	18.47 ±4.98 ^{c}	16.32 ±5.06 ^{b}
	24	1.37 ±0.3 ×10^{9} ^{ab}	68.44 ±3.03 ^{a}	35.96 ±4.76 ^{a}
	36	1.40 ±0.2 ×10^{9} ^{ab}	68.96 ±2.68 ^{a}	40.32 ±4.39 ^{a}
	48	1.44 ±0.1 ×10^{9} ^{ab}	69.69 ±0.91 ^{a}	42.27 ±0.38 ^{a}
Lactobacillus kefiri YK4	18	1.46 ±0.2 ×10^{9} ^{ab}	15.56 ±0.76 ^{c}	10.85 ±1.39 ^{b}
	24	1.59 ±0.3 ×10^{9} ^{a}	60.11 ±3.40 ^{b}	36.97 ±1.51 ^{a}
	36	1.61 ±0.1 ×10^{9} ^{a}	60.77 ±0.61 ^{b}	37.30 ±3.75 ^{a}
	48	1.66 ±0.1 ×10^{9} ^{a}	60.72 ±4.82 ^{b}	38.22 ±4.84 ^{a}

Means in the same column with different superscript letters are significantly different ($p < 0.05$) by Duncan's multiple range test. DPPH = α-diphenyl-β-picrylhydrazy

3.4 The α-glucosidase inhibitory and antioxidant activities of fermented reconstituted skim milks

The RSM fermented by *L. rhamnosus* BD2 and *L. kefiri* YK4 showed higher antioxidant activities (74.53% ±2.06 and 80.92% ±3.03, respectively), compared to MRS broth; however, α-glucosidase inhibitory activity in fermented RSM was lower than that in MRS broth (Table 3). These results demonstrated that RSM was an appropriate medium for both isolates to produce metabolites with antioxidant activity. Antioxidant activity in milks fermented by LAB is thought due to bioactive peptides resulting from their proteolytic activity that breaks down milk proteins [24,29,30]. The MRSB was not an appropriate medium for the production of antioxidant peptides as the source of nitrogen was yeast extract, peptone and beef extract with partial hydrolysis. Hydrolyzing this nitrogen source might not produce peptides with antioxidant activities.

3.5 Antioxidant activities of various fractions of reconstituted skim milks

Results showed that CFS-skim fraction of > 10 kDa included the highest antioxidant activity (85.06% ±0.38) with no significances, compared to unfractionated CFS-skim (80.92% ±3.03). Furthermore, CFS-skim fractions with molecule sizes of ≤ 10 to > 3 and ≤ 3 kDa showed lower antioxidant activities (Table 4). Sabeena-Farvin et al. [26] investigated peptide contents of yoghurt and showed that fractions of > 10 and 10^{-3} kDa contained larger quantities of threonine and serine amino acid residues from α-lactalbumin and β-casein than valine, proline and histidine. Fractions of < 3 kDa contained amino acid residues of tyrosine, glutamine, tryptophan, histidine and leucine from casein. The researchers [26] reported that the antioxidant peptide was identified as GPVRGPFPII in fractions of > 10 kDa, AVPYPQR in fractions of 3–10 kDa and IPIQYVL in fractions of < 3 kDa.

Table 3. Protein and peptide concentrations and α-glucosidase inhibitory and antioxidant activities in RSM fermented by *Lactobacillus rhamnosus* BD2 and *Lactobacillus kefiri* YK4

Isolate name	Protein in skim milk (mg ml^{-1})	Peptide concentration after 24 h of fermentation at 37 ºC (mg ml^{-1})	α-glucosidase inhibitory activity (%)	Antioxidant-DPPH activity (%)
Lactobacillus rhamnosus BD2	1.19	4.67 ^{a}	25.72 ± 3.05 ^{a}	74.53 ± 2.06 ^{a}
Lactobacillus kefiri YK4		4.83 ^{a}	36.16 ± 2.81 ^{a}	80.92 ± 3.03 ^{a}

Means in the same column with different superscript letters are significantly different ($p < 0.05$) by Duncan's multiple range test. DPPH = α-diphenyl-β-picrylhydrazy

Table 4. Antioxidant activities of various fractions of the cell-free supernatants from fermented skim milks by *Lactobacillus kefiri* YK4

Isolate name	Molecule weight	Antioxidant activity (%)
Lactobacillus kefiri YK4	Unfractionated cell-free supernatant	80.92 ±3.03 ^{a}
	> 10 kDa	85.06 ±0.38 ^{a}
	≤10 - >3 kDa	61.51 ±1.96 ^{b}
	≤ 3 kDa	54.48 ±0.86 ^{c}

Means in the same column with different superscript letters are significantly different ($p < 0.05$) by Duncan's multiple range test

3.6 Sequences of peptides with antioxidant activities

Identification of peptides was carried out for fractions of ≤ 3 kDa from RSM fermented by *L. kefiri* YK4. Selected fractions ≤ 3 kDa was based on the availability of LC-MS instrument, which was only appropriate for the fractions of ≤ 3 kDa. Furthermore, results demonstrated that the peptides were derived from β-casein, κ-casein, α_{S1}-casein, α_{S2}-casein, α-lactalbumin and β-lactalbumin; however, their coverage percentages varied. The greater the coverage percentages, the more reliable the protein identification results. The minimum recommended coverage percentage for protein identification using LC-MS method was 70% [31]; therefore, only those with percentage coverage of above 70% were presented in Table 5 with dominant peptides were derived from β-casein. Several studies have reported antioxidant peptides from fermented milks [32-34]. Antioxidant peptides from this study were then investigated for the fractions of ≤ 3 kDa and limited to the major protein with coverage percentages more than 70%. The antioxidant peptides of FPPQSV and YQEPVLGPVRGPFPIIV were found from β-casein (P02666) (Table 6). In addition to the highlighted peptides, a peptide with sequence of IQY was known to include antioxidant activities [32]. The IQY sequence was partly in peptides in the present study, KYIPIQYV and KYIPIQY derived from κ-casein (A0A140T8A9). Other peptides such as GPVRGPFPII, IPIQYVL and dan DKIHPF [26] have been identified as antioxidants. The peptide sequence was identified in the present study were still bound into valine and leucine at the end of the chain (Table 6). Antioxidant activity of antioxidant peptides was affected by its amino acid sequence, composition and concentration [35]. Tyrosine, tryptophan, methionine, lysine, cysteine and histidine were amino acids that provided antioxidant activities [26]. Therefore, peptides identified from fractions of ≤ 3 kDa included those amino acids; therefore, they were still potential to include antioxidant activities. However, further verifications are needed by constructing the sequences and analyzing their activities.

Table 5. Profiles of ≤ 3 kDa fractions from RSM fermented by *Lactobacillus kefiri* YK4 for 24 h at 37 °C

Mother protein	Coverage (%)	Total peptides identified	Amino acid residues	Dominant peptide
β-kasein (J9UHS4*)	94	122	77	DELQDKIHPFAQTQ
κ-kasein (E7E1P8*)	87	49	141	KTEIPTINT
β-kasein (P02666*)	85	272	224	DELQDKIHPFAQTQ
κ-kasein (A0A140T8A9*)	74	64	190	KTEIPTINT
α_{S2}-kasein (P02663*)	53	40	222	AVRSPAQILQ
α_{S1}-kasein (P02662*)	50	112	214	AVRSPAQILQ
Glycosylation (P80195*)	49	28	153	KPWIQPK
α_{S1}-kasein (B5B3R8*)	44	81	214	AAGGPGAPADPGRPT
α-laktalbumin (B6V3I5*)	24	6	142	AQPTDASAQFIRN
β-laktoglobulin (B5B0B4*)	19	7	178	PAPPPPPPP
Osteopontin (P31096*)	11	7	278	DEALEKF
Butyrophilin (P18892*)	7	3	526	TWLKPDPS
β-2 mikroglobulin (P01888*)	7	1	118	FHTSGYDTQ
Imunoglobulin (Q3SYR8*)	6	1	157	PPPPPPPPP
Myozenin (F1N0W6*)	6	1	245	KEGNGIGVN

* Protein access code at https://www.uniprot.org/. A: Alanine, D: Aspartic Acid, E: Glutamic Acid, F: Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y: Tyrosine

Table 6. Identification of peptides with potentials of antioxidants (≤ 3 kDa) from *Lactobacillus kefiri* YK4

Peptides antioxidant from references	Peptides antioxidant from sample	m/z (Da)	Retention time (min)	Mother protein
FPPQSV[1]	FPPQSV	494.27	22.35	β-kasein (P02666*)
	VMFPPQSVL	509.27	22.41	
YQEPVLGPVR[1]	YQEPVLGPVRGPFP	778.41	19.83	
	YQEPVLGPVRGPFPI	834.95	21.85	
YQEPVLGPVRGPFPIIV[2]	YQEPVLGPVRGPFPIIV	941.03	23.68	
VYPFPGPIPN [3]	SLVYPFPGPIPN	650.84	23.30	
	SLVYPFPGPIPNS	694.36	23.08	
	QSLVYPFPGPIPN	714.87	23.02	
	TQSLVYPFPGPIPN	765.39	22.94	
	LVYPFPGPIPNSLPQN	876.96	23.40	
IQY[1]	KYIPIQYV	512.29	17.41	κ-kasein (A0A140T8A9*)
	KYIPIQY	462.76	16.01	

* Protein access code at https://www.uniprot.org/. [1]Conway et al. [32]; [2]Sandré et al. [33]; [3]Eisele et al. [34]. A: Alanine, D: Aspartic Acid, E: Glutamic Acid, F: Phenylalanine, G: Glysin, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonin, V: Valine, W: Triptophan, Y: Tyrosine.

4. Conclusion

In general, ten *Lactobacillus* spp. isolated from Indonesian kefir grains were able to produce compounds with α-glucosidase inhibitory and antioxidant activities with various degrees. Of these isolates, *L. rhamnosus* BD2 and *L. kefiri* YK4 were the best isolates to produce α-glucosidase inhibitory and antioxidant activities. The RSM was the best media to produce compounds with antioxidant activities. Antioxidant activity in skim milk is attributed to peptides resulted from the proteolytic activity of cultures that affects mostly β-casein. Identification of peptides in fractions of ≤ 3 kDa showed that peptides included similar sequences with already known antioxidant peptides. Others peptides included sequences with well-known antioxidant activities. In general, the present study suggests that *L. rhamnosus* BD2 and *L. kefiri* YK4 can be used as starter cultures to produce fermented milks with high antioxidant activities.

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6. Conflict of Interest

The authors declare no conflict of interest.

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فعالیت ضداکسایشی و مهاری آلفا-گلوکوزیداز گونه‌های *لاکتوباسیلوس* جدا شده از دانه های کفیر اندونزیایی در شرایط برون‌تن

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واژگان کلیدی

- گونه‌های *لاکتوباسیلوس*
- دانه کفیر
- پپتیدها
- مهارکننده‌های آلفا-گلوکوزیداز
- ضداکسایندها

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چکیده

سابقه و هدف: در این مطالعه، نشان داده شده است که نه گونه *لاکتوباسیلوس کفیری* و یک گونه *لاکتوباسیلوس رامنوسس* جدا شده از دانه کفیر به عنوان زیست‌یار[1] به‌شمار می‌آیند. هدف مطالعه حاضر بررسی توانایی گونه‌های جدا شده در تولید ترکیباتی با فعالیت‌های مهار آلفا-گلوکوزیداز و ضداکسایشی[2] و نیز شناسایی پپتیدهای دارای وزن مولکولی کمتر یا مساوی ۳ کیلودالتون در روماندهای[3] فاقد سلول بود.

مواد و روش ها: تمام گونه‌های جدا شده در محیط‌های کشت دمان، روگوسا و شارپ براث در درجه حرارت ۳۷ درجه سلسیوس به مدت ۲۴ ساعت کشت داده شدند. ارزیابی فعالیت‌های مهاری آلفا-گلوکوزیداز و ضداکسایشی بر روی روماند فاقد سلول انجام شد. دو گونه جداشده برای تخمیر شیر بدون چربی بازساخته[4] مورد استفاده قرار گرفتند. روماند فاقد سلول شیر بدون چربی تخمیر شده با فیلترهای ۱۰ و ۳ کیلودالتون جزءبه‌جزء[5] شد. سپس، پپتیدهای موجود در جز با وزن مولکولی کمتر یا مساوی ۳ کیلودالتون شناسایی شدند.

یافته‌ها و نتیجه‌گیری: بیشترین فعالیت مهار آلفا-گلوکوزیداز در *لاکتوباسیلوس رامنوسس* BD2 و *لاکتوباسیلوس کفیری* YK4 به ترتیب ۷۶/۵۸ و ۶۴/۳۱ درصد مشاهده شد. بیشترین فعالیت ضداکسایشی در *لاکتوباسیلوس کفیری* JK5 و *لاکتوباسیلوس کفیری* JK17 به ترتیب به میزان ۴۴/۳۱ و ۴۱/۵۷ درصد ملاحظه شد. هنگامی که *لاکتوباسیلوس رامنوسس* BD2 و *لاکتوباسیلوس کفیری* YK4 در شیر بدون چربی بازساخته کشت داده شدند، فعالیت‌های مهار آلفا-گلوکوزیدازی آنها به ترتیب ۲۵/۷۲ و ۳۶/۱۶ درصد کاهش یافت، در حالی که فعالیت ضداکسایشی به ترتیب به میزان ۷۴/۵۳ و ۸۰/۹۲ درصد افزایش یافت. جزءبه‌جزء کردن روماند فاقد سلول شیر بدون چربی بازساخته تخمیرشده با *لاکتوباسیلوس کفیری* YK4 نشان داد که بیشترین فعالیت ضداکسایشی مربوط به اجزای با وزن مولکولی بیشتر از ۱۰ کیلودالتون می‌باشد. اگرچه، اجزای با وزن مولکولی ۳ کیلودالتون و کمتر فعالیت ضداکسایشی کاملا بالایی نشان دادند. شناسایی پپتیدها در اجزای با وزن مولکولی ۳ کیلودالتون و کمتر نشان داد که اغلب مشتق بتا-کازیین بودند. در میان این پپتیدها، دو پپتید با توالی FPPQSV و YQEPVLGPVRGPFPIIV با فعالیت ضداکسایشی گزارش شدند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] Probiotic
[2] Antioxidant
[3] Supernatants
[4] Reconstituted
[5] Fractionated