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Pediococcus pentosaceus as probiotic with cholesterol-lowering ability

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ABSTRACT

Aims: Hypercholesterolemia which is an elevated blood cholesterol level that considered as a major risk factor for cardiovascular disease, which is the leading cause of death in many countries. Therefore, lowering the cholesterol level is important to prevent the disease. Lactic acid bacteria (LAB) group are often used as probiotics for their health-promotion which include cholesterol-lowering effect. The purpose of this study was to evaluate the potency of *Pediococcus pentosaceus* as probiotic that could reduce cholesterol.
Methodology and results: All *Pediococcus pentosaceus* strains were able to survive in acid conditions and in the presence of 0.3% bile salts. These strains had antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella typhimurium* ATCC 14028. The LAB were also sensitive to chloram9phenicol and showed autoaggregation and coaggregation ability. *P. pentosaceus* E5, E7, and E8 were able to remove cholesterol with the highest activity showed by *P. pentosaceus* E7 (49.00 ± 2.83%). Dead cells and resting cells of *P. pentosaceus* E5, E7, and E8 (6-22%) also able to reduce the cholesterol but not as effectively as growing cells. Cholesterol lowering is often associated with bile salt hydrolase (BSH) enzyme activity, however none of the isolates were found BSH positive in this study.
Conclusion, significance and impact of study: The present study suggests that *P. pentosaceus* E7 has beneficial probiotic properties which can be exploited for probiotic product with cholesterol-lowering effect.

Keywords: Pediococcus pentosaceus, probiotics, cholesterol-lowering

INTRODUCTION

Cholesterol is an important structural component of animal cell membrane. However, elevated cholesterol levels is a major risk factor for coronary heart disease (Anila *et al*., 2016). Cardiovascular disease is the leading cause of death for million people worldwide. The risk of developing cardiovascular disease is reduced by 2-3% when cholesterol level is reduced by 1% (Alba *et al*., 2018). Although drugs such as statins, effectively decrease cholesterol level but its side effects are commonly reported (Miremadi *et al*., 2014). Myalgia is the most common side effect from statin use with rates 1-10% of patients (Ramkumar *et al*., 2016).

It has been proposed that consumption of probiotics products able to lower the serum cholesterol level. Probiotics are defined as viable microorganisms that exerts various beneficial effects to the host when ingested in an appropriate concentration. Therefore, the interest in using probiotics to reduce cholesterol level has increased from the last few decades (Anila *et al*., 2016). Probiotic bacteria mainly belonging to lactic acid bacteria (LAB) included *Lactobacillus* and *Pediococcus.* LAB are often used as probiotics for their health-promotion such as lowering-cholesterol, a series of *in vitro* tests must be applied as the selection criteria to identify potential probiotics.

The results from previous studies showed *Pediococcus pentosaceus* VJ56 from Idly batter was able

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to reduce 63% cholesterol (Vidhyasagar and Jeevaratnam, 2013); *Lactobacilllus plantarum* EM from kimchi was able to reduced 80.69% cholesterol (Choi and Chang., 2015); *P. pentosaceus* from fermented finger millet was able to reduce 34% cholesterol (Damodharan *et al*., 2015) and *P. pentosaceus* from breast milk was able to reduce the cholesterol for 15.76% (Nuraida *et al*., 2011). Several possible mechanisms for cholesterol removal by probiotics included cholesterol assimilation, adhesion of cholesterol to the cell membrane, conversion of cholesterol to coprostanol, and enzymatic deconjugation of bile salts (Tok and Aslim, 2010).

Hamida *et al*. (2015) had selected three strains of *P. pentosaceus* from spontaneous fermented corn as a probiotic candidate for chicken. However, this isolate has not been evaluated for its specific functional benefit and probiotic properties for human. Therefore, this study aimed to evaluate the potential of *P. pentosaceus* which isolated from spontaneous fermented corn as probiotics with cholesterol-lowering ability.

MATERIALS AND METHODS

Sources of microbes

Lactic acid bacteria (LAB) of *Pediococcus pentosaceus* E5, *P. pentosaceus* E7, *P. pentosaceus* E8 were isolated previously from spontaneous fermented corn and examined previously by Hamida *et al*. (2015). Pathogenic bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028) were obtained from Animal Biotechnology and Biomedical Laboratory, RCBIO, IPB University, Indonesia. While, *Lactobacillus rhamnosus* R23 which used as reference strain for cholesterol lowering was obtained from SEAFAST Center Laboratory, IPB University, Indonesia. All the LAB were grown in the Mann Rogosa Sharpe (MRS) broth media (Merck), while pathogenic bacteria were grown in the nutrient broth (NB) media.

Acid tolerance test

A low pH tolerance test was carried out based on Manini *et al*. (2016). MRSB was adjusted to pH 2 using 37% hydrochloric acid (HCl) and inoculated with 1% (10^7-10^8 CFU/mL) LAB culture for 2 h and incubated at 37 °C. The total amount of LAB was determined by the plate count method on MRS agar and incubated for 48 h at 37 °C. LAB survival rate was determined by the following equation:

$$\text{Survival (\%)} = \frac{\text{Log CFU of viable cells survived}}{\text{Log CFU of initial viable cells inoculated}} \times 100$$

Bile salt tolerance test

Bile salt tolerance was carried out based on Tokatli *et al*. (2015). MRS broth containing 0.3% (w/v) bile salt (HIMEDIA) was inoculated with 1% (10^7-10^8 CFU/mL) LAB culture and incubated for 6 h at 37 °C. Then, the total amount of LAB was determined by the plate count method on MRS agar after incubated for 48 h at 37 °C.

Antimicrobial activity assays

Antimicrobial activity was evaluated based on Shukla and Goyal (2014) and Wang *et al*. (2016). Briefly, the tested pathogenic bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 14028) were grown in NB medium and LAB was grown in MRS broth at 37 °C for 24 h. Then, the LAB culture was removed by centrifugation at 6000 × *g*, 4 °C for 10 min. The Petri dishes containing nutrient agar were prepared, previously inoculated with 200 µL the tested pathogenic bacteria. After the agar plates had been surface dried, sterilized paper disks were placed aseptically on the agar surface, then 20 µL CFS (cell-free supernatant) of LAB were applied to each disk. In another set of experiment, CFS was neutralized to pH 6.5 by 2 N sodium hydroxide (NaOH), then 20 µL of CFS were applied to each disk. The plates were incubated at 37 °C for 24 h and the zone of inhibition (mm) was measured and data interpretation referred to Zommiti *et al*. (2018).

Autoaggregation and coaggregation ability test

Autoaggregation and coaggregation ability tests were performed based on Seddik *et al*. (2017) and Ladha and Jeevaratnam (2018). LAB strains were grown for 24 h at 37 °C in MRS broth. After centrifugation (8000 × *g*, 10 min), the cells pellet were washed twice with 0.01 M, pH 7.2 phosphate buffer saline (PBS) and resuspended in PBS. Then, cell suspensions were mixed by vortexing and autoaggregation was determined after incubation at 37 °C for 2 h and 4 h, respectively. Subsequently, an aliquot of these suspensions was carefully removed from the upper layer of the suspension and its absorbance was read at 600 nm using spectrophotometer. The percentage of autoaggregation was calculated using the formula:

$$\text{Autoaggregation(\%)} = 1 - \left(\left(\frac{At}{A0}\right) \times 100\right)$$

where At represents absorbance value at time t = 2 h or 4 h and $A0$ represents absorbance value at t = 0 h.

Culture preparation for coaggregation test was carried out as previously described, 2 mL of LAB culture and 2 mL of pathogenic culture were mixed, then incubated at 37 °C for 2 h and 4 h. Subsequently, an aliquot of these suspensions was carefully removed, and its absorbance was read at 600 nm using spectrophotometer. The percentage of coaggregation was calculated using the formula:

$$\text{Coaggregation(\%)} = \left(\frac{Ax + Ay}{2}\right) - A(x+y) / \left(\frac{Ax + Ay}{2}\right) \times 100$$

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where *Ax* represents absorbance value of LAB culture; *Ay* represents absorbance value of pathogenic culture; *A* (*x* + *y*) represents absorbance value of the mixture suspension.

Antibiotic susceptibility

The antibiotic susceptibility of *P. pentosaceus* E5, E7, and E8 were examined according to Lee *et al*. (2016) and Ilavenil *et al*. (2016). The strains were tested for their susceptibilities against streptomycin (25 µg), kanamycin (30 µg), chloramphenicol (30 µg) and ampicillin (25 µg). The Petri dishes containing MRS agar were prepared, previously inoculated with 200 µL (10^7-10^8 CFU/mL) LAB culture. After the agar plates had been surface dried, sterilized paper disks were placed aseptically on the agar surface, then 20 µL of antibiotics solution which had been filter sterilized with 0.22 µm membrane filter were applied to each disk. After 24 h of incubation at 37 °C, the diameters of inhibition zone (mm) were measured and data interpretation referred to Shukla and Goyal (2014).

Cholesterol removal by growing cells

The ability of LAB to reduce cholesterol was analyzed according to Shehata *et al*. (2016). In short, 1% (10^7-10^8 CFU/mL) of LAB cells grown overnight were inoculated into MRS broth supplemented with 0.3% (w/v) bile salt and 100 µg/mL of water-soluble cholesterol (PEG600, Sigma-Aldrich) which had been filter sterilized with 0.45 µm membrane filter and incubated for 24 h at 37 °C. Following incubation, the remaining cholesterol concentration of broth was determined. The cells were harvested by centrifugation (7000 × *g*, 15 min) and 1 mL of CFS was added to 1 mL of 33% (w/v) potassium hydroxide (KOH) and 2 mL of absolute ethanol. The mixture was shaken well for 1 min and then heated for 10 min at 60 °C water bath. After cooling, 3 mL of hexane and 2 mL of distillate water were added then mixed. The mixture was incubated at room temperature (28 °C) for 10 min for phase separation, after which 1 mL of a separated hexane layer was transferred into clean tube which was then evaporated using a nitrogen stream. Subsequently, 2 mL of *o*-phthalaldehyde (Sigma-Aldrich) was added, mixed, and stand at room temperature for 10 min. Following the addition of 2 mL of concentrated sulfuric acid and incubation for 10 min, the absorbance at 550 nm was read using a spectrophotometer. The cholesterol removal percentage was obtained by comparing the absorbance value with the control (cholesterol standard) as formula below (Ooi and Liong, 2010).

Cholesterol removal (%)

$$= \frac{\begin{pmatrix}\text{Control} - \text{Absorbance value of remaining} \\ \text{cholesterol in cultures}\end{pmatrix}}{\text{Control}} \times 100$$

Cholesterol removal by dead and resting cells

Cholesterol removal by dead and resting cells was determined according to Choi and Chang (2015). LAB was grown for 24 h in MRS broth, after centrifugation (7000 × *g*, 15 min) cell pellet were washed using sterile-distilled water. For cholesterol removal by resting cells, the cell pellet was suspended in 0.05 M PBS (pH 7.2) containing 0.3% (w/v) bile salt and 100 µg/mL of water-soluble cholesterol. For dead cell assay, the cell pellet was suspended in saline and heat-killed at 121 °C for 15 min. The dead cells were harvested, after which the pellet was suspended in MRS containing 0.3% (w/v) bile salt and 100 µg/mL of water-soluble cholesterol. All strains were incubated at 37 °C for 24 h, then remaining cholesterol concentration of broth was determined as previously described.

Qualitative determination of bile salts hydrolase (BSH) activity

BSH activity was determined based on Choi and Chang (2015). Active LAB culture (10 µL) was spotted on MRS agar containing 0.5% (w/v) taurodeoxycholic acid sodium salt (TDCA) (Sigma, Aldrich) and 0.037% (w/v) calcium chloride ($CaCl_2$). The agar plates were incubated at 37 °C for 48 h. The precipitation zone surrounding colonies indicated the presence bile salt hydrolase activity of bacteria.

RESULTS AND DISCUSSION

Acid tolerance test

It is well known that probiotic bacteria should be capable of surviving passage through the gastrointestinal tract based on acid tolerance to human gastric juice in the small intestine (Choi and Chang, 2015). In this study, *P. pentosaceus* E5, *P. pentosaceus* E7, *P. pentosaceus* E8 were shown to have more than 85% of survival rate in MRS broth with pH 2 for 2 h (Table 1). The results are in accordance with *P. pentosaceus* LJR1, *P. pentosaceus* LJR5, and *P. pentosaceus* LJR9 which have a survival rate of more than 75% (Ladha and Jeevaratnam, 2018), but in contrast to Osmanagaoglu *et al*. (2010), who reported *P. pentosaceus* OZF was not resistant to acidic condition. The decrease of the cells number can be associated with the impact of the production of H^+ ions by acid in the cell wall and metabolism of the isolates, however, acid tolerance was strain dependent (Ayyash *et al*., 2018).

Table 1: Survival rate (%) of LAB in MRS broth pH 2 after incubated for 2 h at 37 °C.

LAB	Survival rate (%)*
P. pentosaceus E5	85.21 ± 0.82
P. pentosaceus E7	88.68 ± 1.58
P. pentosaceus E8	90.41 ± 1.18

*Means of triplicate ± standard deviation is shown.

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Bile salt tolerance test

Tolerance to bile salt was a prerequisite for microbial colonization and metabolic activity in small intestine of the host (Shehata *et al*., 2016). *P. pentosaceus* E5, E7 and E8 strains exhibited good bile salt tolerance with increased in cells number for about 1 log CFU/mL from initial cell number of 7 log CFU/mL to 8 log CFU/mL (Table 2). Ladha and Jeevaratnam (2018) and Damayanti *et al.* (2014) reported that *P. pentosaceus* LJR5 and *P. acidilactici* R01, R02 had more than 100% survival after 2 h incubated in MRS broth with bile salt, in contrast to *P. ethanolidurans* (0%) (Tokatli *et al*., 2015). Bile salt can act as antimicrobial agent via disintegration of bacterial membranes. Resistance to bile salt can involves several mechanisms including bile salt efflux, hydrolysis of bile salt, and changes in membrane and cell wall components (Ruiz *et al*., 2013).

Table 2: Increased in cell number (with initial cell number 7 log CFU/mL) of LAB in MRS broth with 0.3% bile salt after incubated for 6 h at 37 °C.

LAB	Increased in cell number (Log CFU/mL)*
P. pentosaceus E5	1.05 ± 0.03
P. pentosaceus E7	1.31 ± 0.03
P. pentosaceus E8	1.04 ± 0.05

*Means of triplicate ± standard deviation is shown.

Antimicrobial activity assays

Table 3 showed that CFS without neutralization was able to inhibit all pathogenic bacteria with inhibition zone ranging from 2.06 ± 0.44 to 5.00 ± 0.35 mm. Damodharan *et al.* (2015) reported that *P. pentosaceus* KID7 was able to inhibit Gram positive and Gram negative pathogens with the highest activity against *S. aureus* while Noohi *et al.* (2016) found that *P. pentosaceus* P3 and P6 had the highest inhibitory activity against *S. typhimurium*. Neutralized CFS of *P. pentosaceus* E7 was unable to inhibit *E. coli* and *S. aureus* (Table 3), and this result was contradicting with *L. bulgaricus* (Georgieva *et al*., 2015). Overall, CFS without neutralization and neutralized CFS had different antimicrobial activity. CFS was neutralized in order to eliminate putative effect of produced organic acid. The observed inhibition for some strains after elimination of the putative effects of lactic acid raised the question for possible production of other inhibitor substances, such as hydrogen peroxide, bacteriocin and bacteriocin-like substances (Georgieva *et al*., 2015). Hamida *et al.* (2015) showed that neutralized CFS of *P. pentosaceus* E5, E7, and E8 lose their inhibitory activity againts *Enterococcus casseliflavus* after added with proteinase-K, it seems that the antimicrobial compounds are protein such as bacteriocin.

Table 3: Antimicrobial activity of LAB against pathogen on MRS agar after incubated for 24 h at 37 °C.

LAB	Without neutralization CFS (pH ± 4)			Neutralized CFS (pH 6.5)		
	S. aureus	*S.typhimurium*	*E. coli*	*S. aureus*	*S.typhimurium*	*E. coli*
P. pentosaceus E5	++	+	++	+	+	+
P. pentosaceus E7	++	++	++	-	+	-
P. pentosaceus E8	+++	++	++	+	+	-

Interpretation of inhibition zone diameter based on Zommiti *et al.* (2018): (-): no inhibition zone; (+): <3 mm; (++): 3-6 mm; (+++): > 6mm.

Autoaggregation and coaggregation ability

The highest autoaggregation ability (54.85 ± 1.03 %) was found in *P. pentosaceus* E7 after 4 h and *P. pentosaceus* E8 (26.97 ± 0.15 %) after 2 h of incubation in PBS (Table 4). The autoaggregation ability of *P. pentosaceus* E7 was better than *P. pentosaceus* A24 (40.4%) reported by Lee *et al.* (2014), but lower than *P. pentosaceus* LJR1 (81%) (Ladha and Jeevaratnam, 2018) after 4 h of incubation. According to Wang *et al.* (2010), strong autoaggregation ability must be higher than 40% while weak autoaggregation is defined for 10% or less. In contrast, Rahman *et al.* (2008) claimed that strong autoaggregation ability is about 70%. Thus, it is difficult to categorize the standard autoaggregation value for *Pediococcus* sp. (Zommiti *et al*., 2018).

On the other hand, the strongest coaggregation ability at 2 h of incubation was found in *P. pentosaceus* E7 with *S. typhimurium*, which was 29.73% (Figure 1). The coaggregation ability of *P. pentosaceus* E7 was better than *P. pentosaceus* OZF with *S. typhimurium* (6.26%) after incubation of 5 h (Osmanagaoglu *et al*., 2010). *P. pentosaceus* E5 with *E. coli* showed the strongest

Table 4: Autoaggregation ability of LAB in PBS after incubated for 2 and 4 h at 37 °C.

LAB	Autoaggregation (%)*	
	2 h	4 h
P. pentosaceus E5	15.46 ± 3.31	26.58 ± 2.48
P. pentosaceus E7	13.03 ± 3.98	54.85 ± 1.03
P. pentosaceus E8	26.97 ± 0.15	40.88 ± 2.04

*Means of triplicate ± standard deviation is shown.

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coaggregation ability (71.01%) after incubated for 4 h as similar as reported by Osmanagaoglu *et al.* (2010). The coaggregation ability is important in inhibiting the growth of pathogens (Ladha and Jeevaratnam, 2018). It has been reported protein, glycoprotein, teichoic acid, and lipoteichoic acid of bacteria cell wall play important role in co- and auto-aggregation of pathogens and LAB (Tuo *et al.*, 2013).

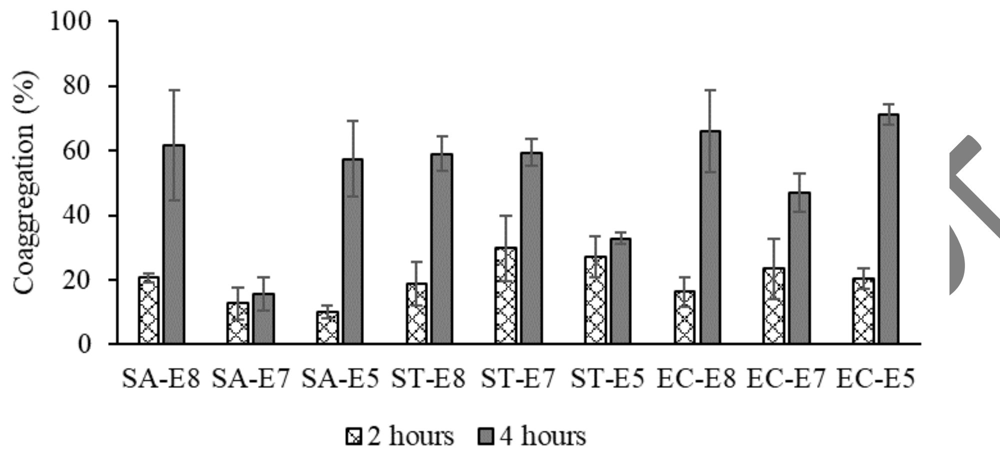

Figure 1: Coaggregation ability of LAB with pathogens in PBS after incubated for 2 and 4 h at 37 °C. SA: *S. aureus;* ST: *S. typhimurium;* EC: *E. coli;* E5: *P. pentosaceus* E5; E7: *P. pentosaceus* E7; E8: *P. pentosaceus* E8.

Antibiotics susceptibility test

All *P. pentosaceus* were sensitive to chloramphenicol, but resistant to ampicillin, streptomycin, and kanamycin (Table 5). The result of this study was in accordance with *P. pentosaceus* VJ35 (Vidhyasagar and Jeevaratnam, 2013) and *P. pentosaceus* KID7 (Damodharan *et al.*, 2015) with slight variations. *Pediococcus* sp. intrinsically is resistant to various groups of antibiotics, including β-lactams, cephalosporins, aminoglycosides, glycopeptides, streptomycin, kanamycin, tetracycline, and sulfatrimethoprim (Zommiti *et al.*, 2018). When resistance is intrinsic which acquired as the results of chromosomal mutation, probiotic bacteria do not constitute a safety concern because the antibiotic resistance is only passed onto the next generation via the organism's genetic material (Pereira *et al.*, 2018). Antibiotic resistance of probiotics is considered a safety issue when the risk of gene transfers is present (Gueimonde *et al.*, 2013). The transmissible resistance genes are attributed to plasmid containing resistance genes, which may be transferred to intestinal pathogens, and may give rise to negative consequences when humans receive antibiotic therapy. In contrast to intrinsic resistance, it might be considered advantageous, because probiotics with the property of intrinsic resistance could maintain the natural poise of intestinal microbiota (Bacha *et al.*, 2010). Therefore, LAB with intrinsic resistance to antibiotics is generally can be used as a probiotic microorganism (EFSA, 2012).

Table 5: Susceptibility of LAB to antibiotics on MRS agar after incubated for 24 h at 37 °C.

LAB	Interpretation of LAB to antibiotics*			
	Chloramphenicol	Ampicilin	Streptomycin	Kanamycin
P. pentosaceus E5	S	R	R	R
P. pentosaceus E7	S	R	R	R
P. pentosaceus E8	S	R	R	R

*Interpretation of inhibition zone diameter was based on Shukla and Goyal (2014): Resistant (R): 0-2 mm; Moderate (M): 3-6 mm; Sensitive(S): 7-13 mm.

Cholesterol removal ability by growing, resting, and dead cells

All the tested strains exhibited higher ability to reduce cholesterol than *L. rhamnosus* R23, the reference strains (Figure 2). *P. pentosaceus* E7 had the highest cholesterol removal ability in growing cells (49%), dead cells (15%), and resting cells (22.2%). It shows that the cholesterol removal ability of *P. pentosaceus* E7 growing cells was lower than *P. pentosaceus* VJ56 (63%) (Vidhyasagar and Jeevaratnam, 2013) and *P. pentosaceus* LAB6 (58%) (Syakila *et al.*, 2018) but better than *P. pentosaceus*

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KACC 12311 (28%) (Damodharan *et al.*, 2015). It has been reported that cholesterol removal by probiotics were strain dependent (Syakila *et al.*, 2018). Growing cells were able to reduce cholesterol from media via incorporation and conversion of cholesterol to coprostanol (Lye *et al.*, 2010).

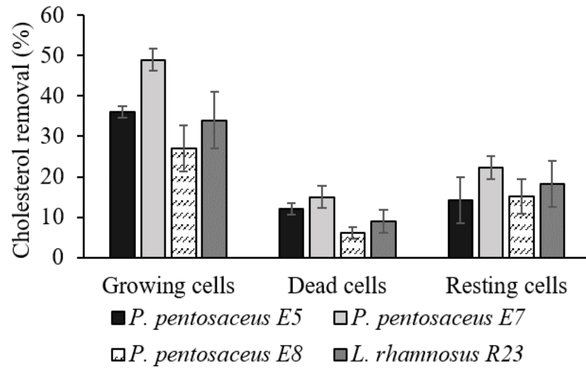

Figure 2: Cholesterol removal (%) by LAB in MRS broth (for growing and dead cells) and PBS (for resting cells) containing 100 µg/mL cholesterol and 0.3% bile salt after incubated for 24 h at 37 °C.

Cholesterol removal by dead cells and resting cells has been reported previously (Vidhyasagar and Jeevaratnam, 2013; Iranmanesh *et al.*, 2014). The ability of dead cells and resting cells to reduce cholesterol was due to binding of cholesterol to the cell membrane (Anila *et al.*, 2016). Iranmanesh *et al.* (2014) reported that the decrease in cholesterol by dead cells increased with increasing number of cells. Cholesterol binding to LAB varies among strains and species and hypothesized that these differences in binding abilities can be attributed to chemical and structural properties of cell wall peptidoglycans containing amino acids capable of binding to cholesterol (Choi and Chang, 2015). Syakila *et al.* (2018) showed that cholesterol strongly adhered to cell membranes of *P. pentosaceus* LAB12 as observed by fluorescently tagged cholesterol under a confocal microscope.

Qualitative determination of BSH activity

In this study, none of the strains were found to be BSH positive and the results was similar to 12 isolates from cheese (Sedlackova *et al.*, 2015) and 5 isolates from food fermentation (Anila *et al.*, 2016). LAB from the gastrointestinal tract are more likely to be BSH positive, as compared to those without exposures to bile salts (Sedlackova *et al.*, 2015). Miremadi *et al.* (2014) found that 14 isolates of *Lactobacilli* and *Bifidobacteria* from human possessed BSH activity, while *Pediococci* from fermented vegetables were only 10% to be BSH positive (Abriouel *et al.*, 2012). Other findings suggest that BSH activity is influenced by specific substrates, bile salt formed from glycine is more easily hydrolyzed than bile salt from taurine (Hu *et al.*, 2018). A total of 243 isolates tested by Ru *et al.* (2018) showed higher BSH activity on the GDCA (glycodeoxycholic acid) substrate (68%) compared to TDCA (taurodeoxycholic acid) (23%). Tokatli *et al.* (2015) stated that the cholesterol-lowering effect of the strains tested is not always related to the ability of deconjugated bile salt such as *P. pentosaceus* VJ31 and VJ35, those which are BSH negative, but able to reduce cholesterol by more than 67% (Vidhyasagar and Jeevaratnam, 2013). Other mechanisms for cholesterol lowering was conversion of cholesterol to coprostanol (Lye *et al.*, 2010).

CONCLUSION

In this study, three strains of *P. pentosaceus* have a good probiotic properties (acid tolerance, bile salt tolerance, antimicrobial activity, autoaggregation and coaggregation ability, and antibiotics susceptibility) and cholesterol-lowering ability. Although they are BSH negative, *P. pentosaceus* E7 showed the highest cholesterol-lowering ability. This strains could be potentially used in the development of probiotic product with their functional properties in cholesterol-lowering effect. Based on the finding from this study, further *in vitro* studies are needed to determine the mechanism involved in the reduction of cholesterol and *in vivo* study is necessary to prove the hypercholesterolemic effect.

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