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Effect of extracted pectin from fruit wastes on growth of *Pediococcus pentosaceus* RSU-Nh1 and *Lactobacillus plantarum* RSU-SO2

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Abstract Two strains of lactic acid bacteria identified as *Pediococcus pentosaceus* RSU-Nh1 and *Lactobacillus plantarum* RSU-SO2 were isolated from Thai fermented pork sausage (Nham) and fermented spring onion (Ton-Hom-Dong) respectively. The culture supernatant (CFS) of both strains exhibited antimicrobial activity against both Gram-positive and Gram-negative pathogenic bacteria. After being treated with alpha-chymotrypsin and trypsin, the antimicrobial activity of their CFS was eliminated, which suggests that the antimicrobial compound produced was antimicrobial peptide or bacteriocin. Pectin extracted from different fruit peels was supplemented in the bacterial growth medium to investigate the effect of pectin on the growth and antimicrobial production of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2. Pectin was extracted from pomelo, durian, passion fruit and salacca fruit peels with a pectin yield of 5.02 to 26.44%, degree of esterification of 80.84 to 86.68%, and galacturonic acid content of 52.36 to 64.23%. Supplementation with pomelo and passion fruit pectin exhibited the highest improvement of bacterial growth and antimicrobial production of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2. These findings suggested the potential application of these pectin extracts as prebiotics to promote the growth and antimicrobial production of lactic acid bacteria.

Keywords: Pectin, Fruit waste, Lactic acid bacteria, Bacteriocin

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Introduction

Pectin is a naturally-occurring biopolymer that has been used in the pharmaceutical and food industries as a stabilizer, emulsifier, gelling agent, thickening agent and prebiotic (Kaur *et al.,* 2018). It is a complex polysaccharide that plays a significant role as a structural element in plant cells. Pectin consists mainly of galacturonic acid chains that are linked by α-(1-4) glycosidic bonds, which are partially esterified as methyl esters (Donald, 2001). Pectin occurs in the primary cell wall and intracellular membrane of plant cells. It is largely present in the peel of fruits (Flutto, 2003). Commercial pectin can be obtained from citrus peel and apple pomace (Girma and Worku, 2016). A number of studies have reported pectin from different types of fruit peels, such as apple (Virk and Sogi, 2004), banana (Happi Emaga *et al.,* 2008), passion fruit (Seixas *et al.,* 2014), durian (Foo and Hameed, 2011) and citrus fruits (Wang *et al.,* 2008). Other types of fruit peel waste could be potential sources for pectin as well.

Pectin is considered a dietary fiber which is divided into soluble and insoluble fibers according to solubility (Yanniotis *et al.,* 2007; Palin and Geitmann, 2012). As such, soluble fibers are well known to be very useful for the digestive system (Slavin, 2013). In the human gastrointestinal (GI) tract, pectin exhibits excellent swelling and erosion properties (Kaur *et al.,* 2018). Moreover, it is capable of holding water and forming gel, which eventually leads to the binding of bile acids and ions. The gel-forming capability of pectin is determined as a possible mechanism for its beneficial health effects, such as the enhancement of glucose, lipid and cholesterol metabolism (Mudgil, 2017; Gunness and Gidley, 2010; Pluschke *et al.,* 2018).

Because of its non-digestible fiber polysaccharide property, pectin can stimulate the growth and activity of beneficial bacteria (Chatterjee *et al.,* 2016). It can pass undigested through the upper part of the GI tract and induce the activity of beneficial microbes that colonize in the large intestine by acting as growth substrate for them (Hutkins *et al.,* 2016). Commonly, the GI tract contains a number of microflora including both harmful and advantageous bacteria. Pathogenic bacteria in the human GI tract can lead to health problems in the host, such as liver damage, infections and diarrhea (Roberfroid, 2003; Sen *et al.,* 2014). These bacteria may produce some substances that enhance the risk of selective growth and reduce the growth rate of useful bacteria in the colon (Chatterjee *et al.,* 2016).

Although there are several reports on pectin extraction and characterization from fruits, there remains a lack of information on prebiotics studies. Pectin from fruit peels have been used as prebiotics in food (Ho *et al.,*

2017). It is also applied for enhancing the growth rate of useful bacteria (Chatterjee *et al.,* 2016). Furthermore, prebiotics can improve metabolic activity and induce the synergistic effects of beneficial bacteria like *Bifidobacteria* and *Lactobacilli*, which are well known as probiotics (Gibson *et al.,* 2017; Slavin, 2013; Pandey *et al.,* 2015; Sen *et al.,* 2014). Particularly, the group of lactic acid bacteria (LAB) is mainly present in the intestinal tract of healthy animals and humans, such as *Lactobacillus*, *Lactococcus* and *Streptococcus* sp. (Vaughan *et al.,* 2005). These bacteria are gram-positive and can promote the health of a host by enhancing absorption and the digestion system of essential nutrients, inducing the immune system, and inhibiting the growth of pathogenic bacteria, such as in genera of *Aeromonas*, *Escherichia* and *Staphylococcus* (Vaughan *et al.,* 2005; Chatterjee *et al.,* 2016).

The aim of this study was to determine the effect of pectin extracted from different fruit peels on the growth and antimicrobial production of LAB. In this study, two lactic acid bacteria: *Pediococcus pentosaceus* RSU-Nh1 and *Lactobacillus plantarum* RSU-SO2 were used as representatives of bacteriocinogenic lactic acid bacteria.

Materials and Methods

Plant materials and sample preparation

Fruit peels of pomelo (*Citrus grandis* (L.) *Osbeck*), durian (*Durio zibethinus* Murray), passion fruit (*Passiflora laurifolia* Linn.) and salacca fruit (*Salacca zalacca* (Gaertn.) Voss) were collected from the local Si Mum Mueang Market (Rangsit) in Pathum Thani, Thailand. The green skin of pomelo and durian peels was removed before further processing. All peels were dried at 65°C in a hot-air oven (Binder, Model FD 115, Germany) overnight. The dried peels were mashed and boiled in 95% ethanol at a peel-to-ethanol ratio of 1:1 (w/v). Peel boiling was performed at 80°C for 10 min. Ethanol was then removed by squeezing and the treated peels were washed three times with distilled water. All treated samples were dried at 65°C in a hot-air oven until the weight was constant. Finally, the samples were milled to a sieve size of 40 mesh and kept at -20°C for pectin extraction.

Pectin extraction

The peel powder of each sample was extracted using a modified method from Larptansuphaphol *et al.* (2013). Firstly, 100 g of sample was added into 0.05 M HCl at a ratio of 1:12 (w/v) and then heated at 95°C for 1 h. Secondly,

it was filtered through a perlon filter cloth. The permeate was collected and the peel cake was kept to repeat the extraction steps. Then, 95% ethanol was added into the collected permeate at a ratio of 1:1 (v/v) and incubated at room temperature for 15 h for pectin precipitation. Pectin was filtered through the two-layer perlon filter cloth and washed three times with 95% ethanol. Subsequently, it was washed three times with 50% acetone and dried in an oven at 60°C to yield a powder form of pectin, after which it was kept at -20°C for further experiments. Each pectin extraction was carried out in triplicate.

Determination of degree of esterification

The degree of esterification (DE) of pectin was determined using the titrimetric method according to Food Chemical Codex (Fcc, 2004; USP, 2003). Dried pectin (0.5 g) was moistened with 2 ml of 95% ethanol and dissolved in 100 ml of carbon dioxide-free water. Two drops of phenolphthalein were then added into the suspension as an indicator. The free carboxyl groups were determined by titration with 0.1 M NaOH, while the volume of 0.1 M NaOH used was recorded as N_0. Then, 10 ml of 0.1 M NaOH was added and stirred at room temperature for 15 min, followed by the addition of 10 ml of 0.5 M HCl and shaken until the pink color disappeared. The excess HCl was titrated with 0.1 M NaOH. The number of esterified carboxyl groups was calculated from the volume of NaOH solution used for the titration and was recorded as N_1. DE was calculated according to the following equation:

$$\%DE = [N1/(N_0+N_1)]\times100$$

Determination of galacturonic acid contents

Galacturonic acid content assay was carried out according to the method described by Sukboonyasatit *et al.* (2018). Briefly, 0.1 g of pectin powder was dissolved in 100 ml of 0.05 M NaOH for 30 min. Ten ml of the solution was then diluted to 100 ml in distilled water. Subsequently, 2 ml of the diluted sample was mixed with 1 ml of 0.1% carbazole and 12 ml of sulphuric acid. The mixture was mixed thoroughly and incubated at room temperature for 25 min. Absorbance values were recorded at 525 nm using a spectrophotometer (Thermo Fisher Scientific-GENESYS 20, USA) and compared to a standard curve created using known concentrations of galacturonic acid. Galacturonic acid content was expressed as percentage of galacturonic acid in pectin powder.

Bacterial strains and growth conditions

Two strains of lactic acid bacteria: *Pediococcus pentosaceus* RSU-Nh1and *Lactobacillus plantarum* RSU-SO2 were used in this study. *Ped. pentosaceus* RSU-Nh1was isolated from Thai fermented pork sausage (Nham) while *Lb. plantarum* RSU-SO2 was isolated from fermented spring onion (Ton-Hom-Dong). Both strains were kept in deMan Rogosa Sharpe (MRS) broth (Merck, Darmstadt, Germany) supplemented with 40% glycerol at -20 ℃. Before use, the strains were propagated in MRS broth at 30 ℃ for 16 h without agitation. The indicator strains were propagated according to the growth conditions shown in Table 1.

Table 1. List of indicator strains and their growth conditions

Indicator species[1/]	**Medium**[2/]	**Temperature (℃)**[3/]
Gram-positive bacteria		
Bacillus coagulans TISTR 1447	NB	37
Bacillus subtilis subsp. subtilis JCM 1465^T	NB	37
Listeria innocua ATCC 33090^T	NB	37
Staphylococcus aureus TISIR 118	NB	37
Gram-negative bacteria		
Escherichia coli ATCC 780	NB	37
Salmonella enterica serovar Enteritidis DMST 17368	NB	37
Vibrio vulnificus 1809	TSB+1.5% NaCl	30*
Vibrio algennolyticus Va	TSB+1.5% NaCl	30*
Vibrio harveyi Vh1	TSB+1.5% NaCl	30*
Vibrio harveyi AQVH01	TSB+1.5% NaCl	30*
Vibrio harveyi VH SURAT	TSB+1.5% NaCl	30*
Vibrio harveyi VH 1526	TSB+1.5% NaCl	30*
Vibrio harveyi VH 639	TSB+1.5% NaCl	30*
Vibrio harveyi VH 1807	TSB+1.5% NaCl	30*
Vibrio parahaemolyticus DMST 26792	TSB+1.5% NaCl	30*
Vibrio parahaemolyticus Vp1	TSB+1.5% NaCl	30*
Vibrio parahaemolyticus Vp AHPN(+) 1681	TSB+1.5% NaCl[a]	30

1/: JCM = Japanese Culture of Microorganism, Wako, Japan; ATCC = American Type Culture Collection, Rockville, MD; DMST = Department of Medical Sciences, Thailand; TISTR = Thailand Institute of Scientific and Technological Research

2/: NB = Nutrient broth (Difco, France), TSB = Tryptic soy broth (Difco, France)

3/: The asterisk symbol "*" indicates the need for agitation at 150 rpm during cultivation.

Antibacterial activity assay

The antimicrobial activity of cell free supernatant of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was evaluated by the spot-on-lawn method, as described by Ennahar *et al.* (2001), with some modifications. Briefly, the bacteria were grown in 5 ml of MRS broth and incubated at 30 ℃ for 16 h. The culture supernatant was collected by centrifugation at 12,000 rpm for 15 min and then filtered through 0.2 µm pore size membrane filter (Pall, USA) to produce a sterilized cell free supernatant (CFS). Subsequently, the CFS was two-fold serially diluted in sterilized distilled water. Ten µl of each diluted sample was spotted on the surface of agar plates, which were overlaid with 6 ml of soft agar (1% agar) with 0.6% of the fresh indicator strains. The titer of inhibition zones on the bacterial lawn were examined after overnight incubation and expressed as activity units per milliliter (AU/ml).

Effect of enzymes on antimicrobial activity

The effect of enzymes on the antimicrobial activity of CFS was evaluated according to the method of Gao *et al.* (2010) and Rumjuankiat *et al.* (2010). The CFS was treated with three proteolytic enzymes, including alpha-chymotrypsin, trypsin (Sigma-Aldrich, St. Louis, MO, USA) and pepsin (Fisher Scientific Inc., Pittsburgh, PA, USA) at a final concentration of 1 mg/ml. The pH of samples was adjusted to 8.5 before being treated with alpha-chymotrypsin and trypsin, and to 3.0 before being treated with pepsin. The mixture of CFS and enzyme was incubated at 37 ℃ for 3 h. The enzymatic reaction was terminated by heating at 100 ℃ for 5 min. The residual antimicrobial activity of CFS was evaluated by the spot-on-lawn method using *Bacillus coagulans* TISTR 1447 as an indicator strain. The antimicrobial activity of untreated CFS was defined as 100% residual activity.

Effect of pectin on the growth of lactic acid bacteria and their antimicrobial production

The effect of pectin on the growth and antimicrobial production of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was evaluated according to the modified method of Chatterjee *et al.* (2016). One percent inoculum of each tested bacterium was cultured in MRS broth containing 0.4% pectin extracted from different fruit peels and commercial pectin. The culture was incubated at 30 ℃ for 48 and 60 h. Viable count of tested bacterium was determined using drop plate methods as described by Herigstad *et al.* (2001).

The antimicrobial activity of CFS obtained after 48 and 60 h of incubation was evaluated by the spot-on-lawn method described above using *B. coagulans* TISTR 1447 and *Escherichia coli* ATCC 780 as indicator strains.

Statistical analysis

Data was presented as mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was performed to determine the differences between groups. Duncan's multiple range test was performed to identify the differences between groups when significance levels at $p < 0.05$ were detected. All statistical analysis were performed using SPSS for Windows version 16.0.

Results

Pectin yield and chemical properties

In this study, the peels of pomelo, durian, passion fruit and salacca fruit were used for the extraction of pectin since they seem to be rich sources of pectin and are commonly found as waste products in local markets. As shown in Table 2, the yield of pectin extracted from different fruit peels was significantly different ($p < 0.05$). Among the 4 fruit peels used in this study, pomelo provided the highest yield of pectin, which was 26.44 ± 1.50%, while the yields of pectin from durian peel, passion fruit peel and salacca fruit peel were 20.24 ± 3.06%, 15.22 ± 1.49% and 5.02 ± 0.45%, respectively.

The degree of esterification (DE) and galacturonic acid content for pectin extracted from different fruit peels are shown in Table 2. The DE of pectin extracted in this study ranged from 80.84 ± 5.82% to 86.68 ± 1.51%. Durian peel pectin had the highest DE, while pomelo pectin had the lowest DE. Galacturonic acid content of all extracted pectin was not significantly different ($p > 0.05$), which ranged from 52.36 ± 5.25 % to 64.23 ± 6.12 %.

Table 2. Chemical properties and yield (%) of pectin from different fruit peels

Pectin sources	Degree of esterification (DE; %)	Galacturonic acid (%)	Yield (%)
Pomelo peel	80.84 ± 5.82$^{ab1/}$	52.36 ± 5.25	26.44 ± 1.50^{a}
Durian peel	86.68 ± 1.51^{b}	56.31 ± 2.35	20.24 ± 3.06^{b}
Passion fruit peel	83.91 ± 2.65^{ab}	54.05 ± 11.16	15.22 ± 1.49^{c}
Salacca peel	81.93 ± 0.88^{ab}	64.23 ± 6.12	5.02 ± 0.45^{d}

1/: Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Antimicrobial activity of lactic acid bacteria

The antimicrobial activity produced by *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was evaluated by the spot-on-lawn method. The cell free culture supernatant of both strains was tested against Gram-positive and Gram-negative bacteria. Among 17 tested bacterial indicators, *Bacillus coagulans* TISTR 1447 was the most sensitive bacteria to the CFS of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 (Table 3).

Table 3. Antimicrobial spectra of CFS of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2

Indicator bacteria	Antimicrobial activity (AU/ml)[1/]	
	RSU-Nh 1	RSU-SO2
Gram-positive bacteria		
Bacillus coagulans TISTR 1447	800	800
Bacillus subtilis subsp. subtilis JCM 1465^T	NT	200
Listeria innocua ATCC 33090^T	NT	NA
Staphylococcus aureus TISIR 118	200	NT
Gram-negative bacteria		
Escherichia coli ATCC 780	100	100
Salmonella enterica serovar Enteritidis DMST 17368	NT	100
Vibrio Vulnificus 1809	100	NT
Vibrio algennolyticus Va	100	NT
Vibrio harveyi Vh1	NA	NA
Vibrio harveyi AQVH01	NA	NA
Vibrio harveyi VH SURAT	100	NT
Vibrio harveyi VH 1526	200	200
Vibrio harveyi VH 639	100	NT
Vibrio harveyi VH 1807	200	200
Vibrio parahaemolyticus DMST 26792	100	NT
Vibrio parahaemolyticus Vp1	100	NT
Vibrio parahaemolyticus Vp AHPN(+) 1681	100	NT

1/: NT = Not tested yet, NA = No antimicrobial activity detected

Effect of enzymes on antimicrobial activity

In order to investigate whether the antimicrobial compound produced by *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was an antimicrobial peptide, CFS of both bacteria was treated with proteases. The decrease in antimicrobial activity after being treated with protease suggested the

proteinaceous nature of antimicrobial molecules. As shown in Table 4, the antimicrobial activity of both CFS *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was completely eliminated after treatment with alpha-chymotrypsin and trypsin. Meanwhile, pepsin did not affect the antimicrobial activity of the CFS.

Table 4. Effect of enzymes on antimicrobial activity of CFS

Treatments	**Diameter of inhibition zone (mm)**[1/]
Control	
Buffer pH 3.0	10
Buffer pH 8.5	-
Ped. pentosaceus RSU-Nh 1	10
Lb. plantarum RSU-SO2	10
***Ped. pentosaceus* RSU-Nh1**	
Alpha-chymotrypsin	-
Pepsin	10
Trypsin	-
***Lb. plantarum* RSU-SO2**	
Alpha-chymotrypsin	-
Pepsin	10
Trypsin	-

1/: "-" indicates no inhibition zone.

Effect of pectin on growth of lactic acid bacteria

Table 5 shows the effect of pectin supplementation on bacterial growth.

Table 5. Effect of pectin on the growth of lactic acid bacteria

LAB species	Pectins	Viable count (CFU/ml) [1/]		
		0 h	48 h	60 h
Ped. pentosaceus RSU-Nh1	Control	5.19 ± 0.08^{Aa}	6.80 ± 0.14^{Ab}	5.49 ± 0.07^{Aa}
	passion fruit	5.31 ± 0.09^{Aa}	9.62 ± 0.21^{Dc}	6.23 ± 0.09^{Bb}
	Durian	5.31 ± 0.09^{Aa}	8.92 ± 0.04^{Cc}	6.43 ± 0.05^{Cb}
	Salacca	5.31 ± 0.09^{Aa}	8.68 ± 0.12^{Cc}	6.23 ± 0.00^{Bb}
	Pomelo	5.31 ± 0.09^{Aa}	9.94 ± 0.14^{Dc}	6.73 ± 0.05^{Db}
	Commercial	5.31 ± 0.09^{Aa}	7.68 ± 0.12^{Bc}	6.55 ± 0.03^{Cb}
Lb. plantarum RSU-SO2	Control	5.58 ± 0.16^{Aa}	7.92 ± 0.11^{Ac}	6.88 ± 0.16^{Ab}
	passion fruit	5.58 ± 0.16^{Aa}	10.47 ± 0.10^{Cc}	8.84 ± 0.09^{Cb}
	Durian	5.58 ± 0.16^{Aa}	10.03 ± 0.05^{Bc}	6.92 ± 0.11^{Ab}
	Salacca	5.58 ± 0.16^{Aa}	10.48 ± 0.01^{Cc}	6.92 ± 0.21^{Ab}
	Pomelo	5.58 ± 0.16^{Aa}	9.76 ± 0.10^{Bc}	6.95 ± 0.00^{Ab}
	Commercial	5.58 ± 0.16^{Aa}	9.85 ± 0.36^{Bc}	7.36 ± 0.04^{Bb}

1/: Values are expressed as the mean ± standard deviation. Uppercase letters show significant differences ($p < 0.05$) in the same column. Lowercase letters show significant differences ($p < 0.05$) in the same row.

For both strains of lactic acid bacteria, the maximum viable count was detected at 48 h of incubation. Pectin supplementation significantly improved bacterial growth in the MRS broth ($p < 0.05$). In addition, the source of pectin significantly affected the growth of tested bacteria, especially after 48 h of incubation ($p < 0.05$). Pomelo and passion fruit pectins were the most effective for enhancing the growth of *Ped. pentosaceus* RSU-Nh1, while salacca and passion fruit pectins were the most effective for *Lb. plantarum* RSU-SO2. The viable count of both strains in all treatments significantly decreased after 60 h of incubation ($p < 0.05$). However, the effect of pectin supplementation on bacterial growth was still detected at this time point.

Effect of pectin on the production of antimicrobial activity

The effect of pectin on the production of antimicrobial activity was evaluated. CFS of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was collected after 48 and 60 h of incubation in MRS, with and without pectin supplementation. *B. coagulans* TISTR 1447 and *E. coli* ATCC 780 were used as representatives of Gram-positive and Gram-negative bacterial indicators, respectively, which were used for the evaluation of the antimicrobial activity produced in the CFS. As shown in Table 6, passion fruit pectin significantly improved the antimicrobial activity produced by *Ped. pentosaceus* RSU-Nh1 against *B. coagulans* TISTR 1447. In the case of *Lb. plantarum* RSU-SO2, passion fruit, salacca and pomelo pectins did not. Considering the antimicrobial activity against *E. coli* ATCC 780, pomelo pectin was the most effective for enhancing antimicrobial production by *Ped. pentosaceus* RSU-Nh1. Passion fruit and salacca pectins were the most effective for *Lb. plantarum* RSU-SO2. In addition, the results revealed that the antimicrobial activity produced by *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 was maximized at 48 h of incubation. After 60 h, the antimicrobial activity of CFS of *Lb. plantarum* RSU-SO2 decreased slightly in some treatments.

Discussion

Commercial pectin is mostly extracted from citrus peel or apple pomace, which are by-products from juice manufacturing. Citrus peels contain the highest amount of pectin at approximately 20-30% (Wang *et al.*, 2014; Nesic and Seslija, 2017; Srivastava and Malviya, 2011). In Thailand, pectin is also extracted from the peels of fruit such as Hom Thong banana, papaya, lime, mango and pomegranate fruit with a yield of 14.04, 2.23, 16.36, 9.0 and 14.0%, respectively (Sengkhamparn *et al.*, 2019; Amnuaysin *et al.*, 2018; Boonrod *et*

al., 2006; Larptansuphaphol *et al.*, 2013). Our results revealed that, among the four fruit peels used, pomelo peels showed the maximum yield of pectin (26.44±1.50%), which was higher than those in previous reports. Pectin recovered from pomelo peel has been reported at 16.74% (Roy *et al.*, 2018) and 23.83% (Quoc *et al.*, 2015). The difference in pectin yield may be due to the different extraction procedures, conditions and solvents used (Yeoh *et al*., 2008; Monsoor, 2005). Hydrochloric acid has been suggested by several authors as a suitable solvent to increase the recovery of pectin during extraction (Kalapathy and Proctor, 2001; Roy *et al.*, 2018). Salacca peel showed the thinnest peel compared with the others used in this study. As expected, it yielded the lowest pectin with 5.02 ± 0.45%. However, this is the first report of pectin extraction from salacca peel. To the best of our knowledge, only one study has previously reported on the recovery of total dietary fibers, not neat pectin, which is approximately 6.33-6.79% (Lestari *et al.*, 2003).

Table 6. Effect of pectin on the production of antimicrobial activity

LAB species	Pectin	Antimicrobial activity of CFS (AU/ml)			
		48 h		60 h	
		B. coagulans TISTR 1447	*E. coli* 780	*B. coagulans* TISTR 1447	*E. coli* 780
Ped. pentosaceus RSU-Nh1	Control	800	100	800	100
	passion fruit	1,600	100	1,600	100
	Durian	800	100	800	100
	Salacca	800	200	800	200
	Pomelo	800	400	800	400
	Commercial	800	100	800	100
Lb. plantarum RSU-SO2	Control	800	100	400	100
	passion fruit	1,600	400	800	400
	Durian	800	100	400	100
	Salacca	1,600	200	1,600	200
	Pomelo	1,600	400	400	400
	Commercial	800	100	800	100

DE and galacturonic acid content of pectin are the parameters which could describe the functional property and quality of pectin. Our study showed that the DE of pomelo, durian, passion fruit and salacca pectins were 80.84 ± 5.82, 86.68 ± 1.51, 83.91 ± 2.65 and 81.93 ± 0.88%, respectively. Based on DE, pectin can be divided into two groups, low-methoxyl (LM) and high-methoxyl (HM) pectins. Pectin with DE of less than 50% is designated as LM, while those with DE higher than 50% are considered to be HM pectin (Sayah *et al*.,

2016; Mesbahi *et al*., 2005). Their gel-forming ability is different. LM pectins can form gel in the presence of divalent metal cations, usually calcium (Srivastava and Malviya, 2011). Meanwhile, HM pectins have the ability to form gels with acid at low pH (pH < 4.0) and stabilize by hydrophobic interactions (Verrijssen *et al*., 2014). Moreover, DE value above 72% causes more rapid-set pectin, while pectin with a DE of 58-65% is slow-set pectin (Maran *et al*., 2013). In this study, all pectins extracted from different fruit peels were classified as HM pectin with rapid-set type.

Galacturonic acid content of pomelo, durian, passion fruit and salacca pectins were 52.36 ± 5.25, 56.31 ± 2.35, 54.05 ± 11.16 and 64.23 ± 6.12 %, respectively. A higher percentage of galacturonic acid means the higher purity of pectin extracted (Sotanaphun *et al.*, 2012). In our study, salacca pectin showed the highest content of galacturonic acid, suggesting that salacca pectin has the highest purity when compared with other pectins. It might be explained by the fact that salacca peel is composed of small amounts of other organic matter. Thus, there is no high interference in pectin extraction. Roy *et al.* (2018) suggested that galacturonic acid content of higher than 65% might be a preferable quality for pectin. However, the galacturonic acid values were dependent on the temperature and extraction condition (Wang *et al*., 2014).

LAB has been commercially used as a probiotic for human and farm animals for decades. They play an important role as a pathogen eliminator since they can produce various antimicrobial substances, especially bacteriocins which are generally recognized as safe (GRAS) (Pringsulaka *et al.*, 2011; Cocolin and Ercolini, 2008). LAB species are generally found in fermented food products. In Thailand, LAB species have usually been isolated from Nham and pickles. The isolated species included *Lb. plantarum, Lb. pentosus, Lb. sakei, Ped. acidilactici, Weissella* and *Ped. Pentosaceus* (Thongsanit *et al*., 2009; Kingcha *et al*., 2012; Jampaphaeng *et al*., 2017; Pringsulaka *et al*., 2011). Recently, two LAB strains identified as *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 were isolated from Thai fermented pork sausage (Nham) and fermented spring onion (Ton-Hom-Dong), respectively. The CFS of both species showed broad antimicrobial spectra against both Gram-positive and Gram-negative bacteria. The antimicrobials presented in CFS were sensitive to proteases, including alpha-chymotrypsin and trypsin, suggesting that the antimicrobial activity is due to the action of antimicrobial peptide or bacteriocin (Rumjuankiat *et al.*, 2015; Wu *et al*., 2004). Almost all of the tested bacterial indicators are sensitive to the CFS of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2. The bacterial indicators are pathogenic bacteria generally found in food, humans and animals. *B. coagulans* has been reported to cause the spoilage of canned food (Andr é *et al*., 2017). *Escherichia coli* is a cause of

human diseases such as diarrhea, urinary tract infections (UTI), meningitis and septicemia (Bédanger *et al*., 2011; Kaper *et al*., 2004). *Salmonella* is usually found to contaminate raw chicken eggs and cause the human disease called salmonellosis (Cao *et al*., 2014). Some *Vibrio* species cause serious disease in humans and animals and usually contaminates seafood (Froelich and Noble, 2016; Oliver *et al*., 2013). Our findings suggest the potential use of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 as antagonistic strains against pathogenic bacteria in food and protiobic applications.

Pectin has been used as a prebiotic due to its ability to selectively promote the growth of beneficial strains of LAB. Only strains with the 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase enzyme can utilize pectin through the catalysis of galacturonate, a major monomeric constituent in pectic polysaccharides metabolism (Slováková *et al.,* 2002). Our study found that pectin extracted from pomelo, durian, passion fruit and salacca peels significantly improved the growth of *Ped. pentosaceus* RSU-Nh1 and *Lb. plantarum* RSU-SO2 and enhanced their antimicrobial production. Passion fruit and pomelo peels were found to be the most effective. Our findings were similar to those reported by Chatterjee *et al*. (2016), who reported that the addition of 0.4% pectin from different kinds of fruit waste (*Musa* sp., *Citrus limetta*, rind of *Citrullus lanatus*, putrefied fruits of *Solanum lycopersicum* and *Psidium guajava*) enhanced the growth of LAB, including *Lactobacillus casei, L. acidophilus* and *Bifidobacterium bifidum*. The ability of some strains of LAB to hydrolyse pectin has been reported (Vidhyasagar *et al*., 2013). The evidence supports our findings that LAB can utilize pectin as a nutrient source for bacterial growth. Since bacteriocin production is correlated with the growth of bacteriocin-producing bacteria, the improvement of bacterial growth can, therefore, improve the production of bacteriocin.

Conclusion

In conclusion, the highest yield of extracted pectin was obtained from pomelo fruit waste peel (26.44%) with the degree of esterification value of 80.84% and galacturonic acid contents of 52.36%. Pectin from pomelo and passion fruit waste has the potential to be used as a prebiotic to enhance the growth of LAB as well as increase bacteriocin production. Further study on large-scale production is required for further application as a prebiotic in the food industry.

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