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OPTIMIZATION OF BACTERIOCIN PRODUCTION BY LACTIC ACID BACTERIA FROM SPENT COFFEE GROUND HYDROLYSATE

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ABSTRACT

The instant coffee industry produces a considerable amount of spent coffee ground (SCG) as a residue that could be potentially utilized as a renewable lignocellulosic biomass feedstock for the production of bacteriocin. Bacteriocins have attracted much attention from academia and industry because of their possible applications as non-toxic additives in food preservation and prevention of food spoilage from food-borne pathogenic bacteria. The objective of this study was to evaluate the possibility of producing bacteriocin by *Lactobacillus plantarum* BCRC 10069 and *Lactobacillus paracasei* LCW23 using SCG as feedstock. The bacteriocin produced by *L. plantarum* and *L. paracasei* showed significant inhibitory activity against Gram-positive (*Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*). Response Surface Methodology (RSM) was used to optimize the factors involving in bacteriocin production such as pH, glucose concentration, and cysteine concentration. The optimum conditions for bacteriocin production from *L. plantarum* were found to be 15 mg/mL of SCG concentration, 0.059% (w/v) of cysteine content, and pH 7.1; while the optimum conditions for *L. paracasei* were 19.1 mg/mL of SCG concentration, 0.062% (w/v) of cysteine content, and pH 7.08. Under the optimal culture conditions, the specific activities of produced bacteriocins could respectively reach 1163 AU/mg and 1920 AU/mg after 24 h of inoculation.

Keywords: Bacteriocin, Antimicrobial activity, *Lactobacillus plantarum*, *Lactobacillus paracasei*, SCG.

1. INTRODUCTION

As a result of advances in technology and health care, the world's population and human life expectancy are both increasing. The world population is projected to reach 9.3 billion in 2050 (Grafton *et al*. 2015). Many experts have warned that food shortages will be a problem that must be faced in the future. Actually, around 800 million of the world population is in a state of food insecurity (Nelson *et al*. 2016). In recent years, some least developed countries also encounter many social problems as the consequence of rising food prices. However, the amount of food wasted each year by the countries with a more abundant food resource is quite staggering (Ghosh *et al*. 2016; Martínez Z. *et al*. 2014; Wunderlich and Martinez 2018). According to the report, approximately 4 billion tons of food can be produced globally each year, but nearly one-third of the food is wasted, including 45% of fruits and vegetables, 35% of seafood, 30% of cereal crops, 20% of dairy products and 20% of meat products. The problem of wasting food is usually caused by excessive purchases and improper preservation which in turn leads to food spoilage. These problems caused not only the problems of food security but also has some consequential health issues (Nelson *et al*. 2016). Therefore, many attempts were developed to effectively prolong the preservation period of food (Lipinski *et al*. 2013).

In the food market, chemical preservatives are commonly used as a food additive to prevent food spoilage. However, the long-term consumption of synthetic chemical preservatives will cause severe damage to human health (Lucera *et al*. 2012). Therefore, the development of effective natural preservatives has attracted many interests from academia and industry. The discovery of antibiotics is a great achievement for the improvement of food safety. Antibiotics is a substance, produced by microbial secretion in nature that can kill or inhibit the growth of bacteria (Kohanski *et al*. 2010). The antibiotics are also extensively applied in many fields including in medical treatment, livestock feed, and pest control. However, in recent years, many bacteria have slowly developed resistance to antibiotics because of the excessive use of antibiotics. There is a need to develop new approaches to prevent microbial contamination (Davidson *et al*. 2013).

Many microorganisms can inhibit the growth of competitive microbial species by secreting bacteriocins into the surrounding environment. Bacteriocins are mainly composed of small molecular weight proteins (Hibbing *et al*. 2010). The antimicrobial activity of bacteriocins could be very useful in food preservation. Unlike antibiotics, there is no negative side effect found to be associated with the use of bacteriocin (Cleveland *et al*. 2001; Yang *et al*. 2014). Several lactic acid bacteria (LAB) are known to produce a variety of bacteriocins (Shi *et al*. 2016). LAB are "Generally Recognized as Safe (GRAS)" for human consumption and possess many health-promoting benefits such as enhancement of immune function and improvement of intestinal flora balance. Therefore, the application of bacteriocin produced by LAB had been suggested as a be very attractive approach for food preservation (Dobson *et al*. 2012; Zielińska and Kolożyn-Krajewska 2018).

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The utilization of lignocellulosic biomass as feedstock for bio-production is advocated as an approach to achieve sustainable production. The lignocellulosic biomass derived from the food processing wastes is a potential resource that can be obtained in large quantities. Presently, coffee is the second most traded commodity around the world and tons of spent coffee ground (SCG) were continuously generated by the instant coffee industry. SCG contains many ingredients such as lipids, Caffeine, and carbohydrates, which can be utilized as feedstocks for the productions of bioproducts. This study investigated the possibility of utilizing SCG hydrolysate as feedstock for LAB cultivation and bacteriocin production. Two LAB strains, *Lactobacillus plantarum* BCRC 10069 and *Lactobacillus paracasei* LCW23, were chosen as the bacteriocin producer based on the findings of other reports (Jiménez-Díaz *et al*. 1993; Lozo *et al*. 2004). Additionally, the Response Surface Methodology (RSM) was employed to optimize the experimental parameters for bacteriocin production.

2. MATERIALS AND METHODS

2.1 Chemicals and Enzymes

All the chemicals used in this study were analytical grade and purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The sulfuric acid (HPLC grade 50% solution) which was used to prepare the mobile phase for High-Performance Liquid Chromatography analysis was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Cellulase enzyme complex ACCELLERASE® 1500 was given by DuPont™ Genencor® Science (Rochester, NY, USA) as a gift sample and stored at 4°C until use.

2.2 Solvent Extraction of Spent Coffee Ground (SCG)

The SCG was obtained from a local store and dried at 50°C in a convection oven. The dry SCG was extracted by hexane to remove the lipids and hydrophobic substances using the Soxhlet extraction method. The solvent extracted biomass was then dried at 50°C and stored in Ziploc® bags at room temperature until further use.

2.3 Composition Analysis

To analyze lignin and structure carbohydrates, solvent extracted SCG was hydrolyzed by two-stage sulfuric acid hydrolysis modified from the NREL Laboratory Analytical Protocol (LAP) "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter *et al*. 2012). The acid hydrolysate was first neutralized using $CaCO_3$ followed by static precipitation at 4°C overnight. Then, the sample was filtered through a 0.45 μm syringe filter before HPLC analysis for determining the monosaccharide concentration. The HPLC used in this study was equipped with a Rezex ROA-organic acid column, a guard cartridge (Phenomenex Inc., Torrance, CA, USA), a RID, and a UV detector (λ = 210 nm). The protein content of SCG was estimated by converting nitrogen content obtained from the elemental analysis (Vario EL III, Elemental Analysensysteme GmbH) by multiplying a factor of 6.25.

2.4 Dilute Acid hyDrolysis and Enzymatic Saccharification of SCG

Dilute acid hydrolysis and enzymatic saccharification of SCG were performed using the optimal condition reported by Wang *et al*. (2016b). The concentration of monosaccharides was determined by using HPLC with the methods described in the previous section.

2.5 Bacterial Cultures and Media

Lactobacillus plantarum BCRC 10069 and *Lactobacillus paracasei* LCW23 were respectively obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan) and a local nutraceutical store. The LAB strains were grown at 30°C for 18 h in MRS broth (Difco, Detroit, MI, USA). The gram-negative bacteria *Escherichia coli* BL21 and the gram-negative bacteria *Bacillus cereus* A2 were used as the antimicrobial activity indicator strains, grown at 37°C for 24 h in Luria-Bertani (LB) broth. All the microbial strains were stored at −80°C in the presence of 20% (v/v) glycerol.

2.6 Extraction of Bacteriocin

The bacteriocin secreted into the cell culture medium was first separated from cells by centrifugation (6000 × g) for 30 min at 4°C. The supernatant containing bacteriocin was precipitated by the addition of ammonium sulfate at cold condition. The precipitate was then suspended in 0.1 M potassium phosphate buffer (pH 7.0) and used for antibacterial activities by disc diffusion assay.

2.7 Determination of the Antibacterial Activity of Bacteriocin

Antibacterial activity of bacteriocin was evaluated against a gram-negative indicator strain *E. coli* BL21 and a gram-positive indicator strain *B. cereus* A2 using the disc diffusion assay (Savadogo *et al*. 2004; Tagg and McGiven 1971). Briefly, 50 μg of the sample was placed on each disc and all the tests were performed on Muller-Hinton agars which were inoculated with indicator bacteria (5×10^7 CFU/mL). The agar plates were then incubated at 37°C for 24 h and the zones of inhibition were measured and recorded.

2.8 Effect of Cysteine in MRS Broth on Bacteriocin Production

Bacteriocin production was assessed using MRS broth (Difco, Detroit, MI, USA) supplemented with 0.05% (w/v) of cysteine. These supplements were selected based on their influence on bacteriocin production. The supplement of cysteine was prepared and sterilized by filtration (0.22 m, Millipore Ind. Co. Ltd, USA) before adding into the sterilized MRS broth.

2.9 Effect of pH and Temperature on Bacteriocin Stability

The influence of pH on the bacteriocin stability was determined by adjusting the pH of the cell-free culture supernatant from 4.0 to 11.0 with sterile 1N HCl and 1N NaOH. The pH adjusted samples were left at room temperature for 30 min before examining by disc diffusion assay. The influence of temperature on bacteriocin stability was also evaluated by incubating cell-free culture supernatant at 25, 50, 70 and 100°C for 30 min. After incubation, the antimicrobial activity of the supernatant was determined as described before.

2.10 Optimization of MRS Supplementation Using Response Surface Methodology

Based on the experimental results and the findings reported in the other literature, the four variables (loading of SCG, cysteine concentration, pH and temperature) with the potential influence on bacteriocin production were investigated for their optimal conditions by RSM with a set of Box-Behnken design. The designated coding and actual value of factors, response variables, and the optimization criteria are listed in Table 1. Each variable

was evaluated at three coded levels (−1, 0, +1). A set of 17 experiments were carried out in duplicates (Table 2).

2.11 Data Analysis

The fundamentals of RSM was detailly explained in the literature published by Baş and Myers (Baş and Boyacı 2007; Myers *et al*. 2016). The factor levels used in this study were based on preliminary experimental results and converted to a coding scale of 1, 0, and −1 which represents the highest, the middle and the lowest level respectively. A quadric polynomial regression model was used to express Y as a function of the independent variables as Eg. (1):

$$E\{Y\} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

where $E\{Y\}$ is the expected value of the response variable, β_0 is the model intercept, X_i is the coded level for process factors, β_i is the parameter estimate for process factors, β_{ij} is the parameter estimate for the interaction between process factors, β_{ii} is the parameter estimate representing second-order effects of process factors. The satisfactory of the fitted models were examined and diagnosed by using Analysis of variance (ANOVA), Lack-of-Fit test and residual plots. Response surface plots were obtained using the fitted model obtained from the regression analysis. The test of statistical significance was based on a confidence level of 95.0%.

Table 1 The variables with coded and un-coded values for RSM design

Parameters	Design code	Value
SCG (mg/mL)	−1	10
	0	20
	1	30
Cysteine % (w/v)	−1	0
	0	0.05
	1	0.1
pH	−1	5
	0	7
	1	9

Table 2 Experimental table of Box-Behnken design

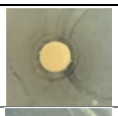
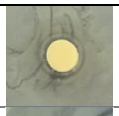
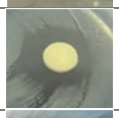
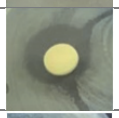
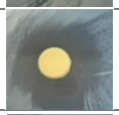
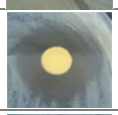
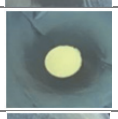
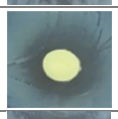
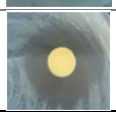
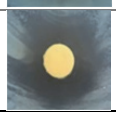
Experimental		SCG conc. (mg/mL)	Cysteine conc. (% w/v)	pH
Block1	Block 2			
1	18	30	0.05%	5.00
2	19	20	0.05%	7.00
3	20	20	0.05%	7.00
4	21	20	0.10%	5.00
5	22	20	0.10%	9.00
6	23	30	0.05%	9.00
7	24	30	0.00%	7.00
8	25	20	0.10%	7.00
9	26	20	0.05%	9.00
10	27	20	0.00%	9.00
11	28	20	0.05%	7.00
12	29	20	0.00%	5.00
13	30	20	0.05%	7.00
14	31	30	0.10%	7.00
15	32	20	0.05%	7.00
16	33	20	0.05%	5.00
17	34	20	0.01%	7.00

3. RESULTS AND DISCUSSION

3.1 Preliminary evaluation of antibacterial activity

The results (Table 3) showed that the bacteriocin produced by *L. plantarum* and *L. paracasei* in this study had considerable antibacterial activity against indicator gram-positive bacteria *B. cereus* A2 and gram-negative bacteria *E. coli* BL 21. The results also indicated that the bacteriocin produced in this study showed better antibacterial activity against gram-positive bacteria than gram-negative bacteria. This could be attributed to that the cell wall of gram-positive bacteria contains peptidoglycan. The bacteriocins produced by LAB usually can inhibit the synthesis of glycoprotein or form a complex with the synthesized glycoprotein and consequentially interfere with cell wall synthesis and leads to cell death (Perez *et al*. 2018; Wu *et al*. 2010).

Table 3 Assessment of the effect of cysteine supplement in the culture medium on the bacteriocin production

Culture supernatant	Inhibition zone (mm)			
	E. coli		*B. cereus*	
Control	ND		ND	
L. plantarum in MRS	3.7 ± 1.2		2.7 ± 0.6	
L. plantarum in MRS + 0.05% (w/v) cysteine	7.7 ± 0.3		8.2 ± 0.3	
L. paracasei in MRS	4.1 ± 1.0		4.2 ± 1.1	
L. paracasei in MRS + 0.05% (w/v) cysteine	8.0 ± 1.0		8.8 ± 0.3	

Control: MRS Medium containing 0.05% (w/v) cysteine without inoculation of LAB

The supplement of cysteine in the culture medium significantly increased the inhibitory activity of bacteriocins produced by two LAB strains. The previous literature also mentioned that the addition of cysteine in the culture medium can improve the synthesis of bacteriocin (Todorov 2009). Although LAB are facultative bacteria capable of growing in both anaerobic and aerobic conditions, they grow better in an anaerobic environment and the addition of cysteine in the growth medium can provide LAB an anaerobic environment and secrete bacteriocin (Malheiros *et al*. 2015). The highest inhibitory activities of bacteriocins produced by *L. plantarum* and *L. paracasei* were respectively about 8.0 mm and 8.8 mm when 0.5% (w/v) cysteine was supplied in the culture medium. The results were comparable with the data reported in another reference (Cheikhyoussef *et al*. 2009). Therefore, the supplement of cysteine in the culture medium was fixed as an essential condition in the following experiments.

3.2 The Influences of pH and Temperature on the Stability of Bacteriocin

To assess the pH effect on the stability of bacteriocins produced by the two LAB strains in this study, the inhibitory activities of bacteriocins against *E. coli* BL21 were examined at different pH conditions by antibacterial activity measurement (Fig. 1A). The bacteriocins produced in this study were stable in the pH range of 4-10 but the inhibitory activities were completely lost at pH 11. The pH stability of bacteriocins produced in this study was also comparable with other bacteriocin reported by others (Gong *et al*. 2010; Mostafa *et al*. 2015). Since the growth of microorganism typically occurs at the pH range of between 4-10, the pH stability of bacteriocins produced in this study makes them useful for food processing and preservation.

The results of the temperature effect on the inhibitory activities of bacteriocins against *E. coli* is shown in Fig. 1B. The bacteriocins produced by *L. plantarum* and *L. paracasei* in this study showed good thermal stability between the range from 25-100℃. The thermal stability of the bacteriocins was also comparable with other reports (Repally *et al*. 2018; Zacharof and Lovitt 2012). The thermal stability of bacteriocin is very important because food processing often involves a lot of heating steps, the wide-range thermal stability of bacteriocin is more favorable in the food industry. Overall, the extensive pH stability and thermal stability of the bacteriocin produced by *L. plantarum* BCRC 10069 and *L. paracasei* LCW23 make them potential additives to be used as food preservatives.

3.3 SCG Hydrolysate as Carbon Source for LAB Cultivation and Bacteriocin Production

Table 4 shows the yields of monosaccharides from the acid hydrolysis and enzymatic saccharification of SCG, around 60 g of monosaccharides could be yielded from a gram of SCG. Based on the data reported in other references (Mussatto *et al*. 2011; Wang *et al*. 2016a), mannose is the most abundant monosaccharide presented in the SCG, which is accounted for more than a third of the total carbohydrates. The content of carbohydrates in SCG is comparable with other common lignocellulosic biomass, but the content of lignin is relatively low. In addition, the monosaccharides in SCG are majorly composed of hexoses, including glucose, mannose, and galactose, which can be easily assimilated by LAB for their growth metabolism and metabolite production (Kosugi *et al*. 2010; Moon *et al*. 2012). Base on the characteristics of SCG, the SCG hydrolysate could be a promising carbon source for microbial fermentation.

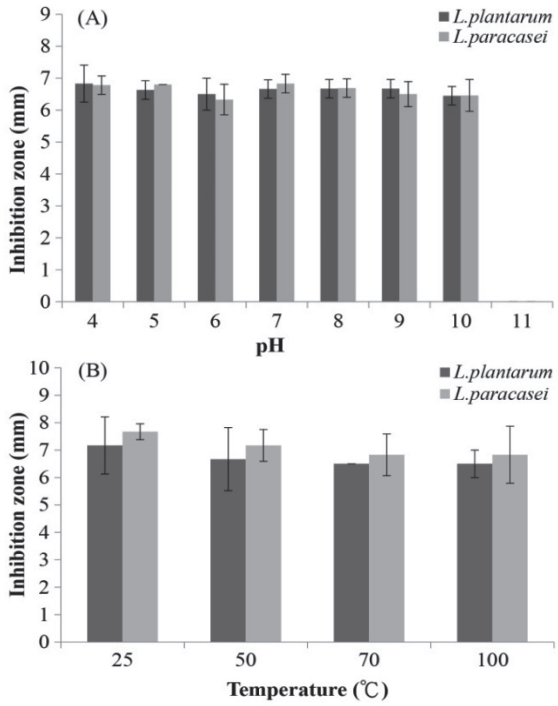


Fig. 1 Influence of (A) pH and (B) temperature on inhibitory activities of bacteriocins against *E. coli* BL21

Table 5 showed that the antibacterial activities of bacteriocins produced by *L. plantarum* BCRC 10069 and *L. paracasei* LCW23 cultured in the medium containing SCG hydrolysate as the carbon source. SCG is a type of waste generated in large quantity by the instant coffee industry, the utilization of SCG as feedstock could potentially reduce the cost of raw materials and achieve circular economics by biological refining (Maina *et al*. 2017). Based on the results obtained in this study, using SCG hydrolysate as an alternative carbon source did not affect LAB growth and the production of bacteriocin. The bacteriocins produced in the medium containing SCG hydrolysate possessed inhibitory activities against *E. coli* BL21 equivalent to the bacteriocins produced in the MRS medium.

3.4 Optimization of bacteriocin production by RSM

Table 6 is the results of the Analysis of variance (ANOVA) for the full quadratic models generated by the designed experiments.

Table 4 The yields of monosaccharide in SCG

Sugar	Yield (g/gram of SCG)
Glucose	12.13 ± 1.23
Xylose + Mannose + Galactose	40.15 ± 2.31
Arabinose + Rhamnose	3.67 ± 1.79
Total	55.95 ± 2.47

Table 5 Assessment of the inhibitory activity of bacteriocins produced by LAB in the medium containing SCG hydrolysate against indictor organisms

Culture supernatant	Inhibition zone (mm)	
	E. coli	*S. cereus*
Control	ND	ND
L. plantarum	7.0 ± 0.3	7.5 ± 1.3
L. paracasei	8.1 ± 0.4	8.5 ± 0.5

Control group: SCG hydrolysate medium containing 0.05% (w/v) cysteine without inoculation of LAB.

Table 6 The analysis of variance (ANOVA) tables for the full quadratic model

(A) *L. plantarum* BCRC 10069

Source	SS	DF	MS	F-value	p-value
Block	7.35E-03	1		7.353E-03	
Model	124.07	7	17.72	6240.33	＜0.0001
A	0.074	1	0.074	26.14	＜0.0001
B	17.1	1	17.10	6019.90	＜0.0001
C	0.76	1	0.76	268.02	＜0.0001
AB	1.25E-03	1	1.250E-03	0.4553045	0.5066
AC	6.61E-03	1	6.613E-03	2.4085607	0.1343
BC	0.034	1	0.034	11.90	0.0020
A	12.58	1	12.58	4428.29	＜0.0001
B	58.84	1	58.84	20716.79	＜0.0001
C	24.83	1	24.83	8741.96	＜0.0001
Residual	0.071	25	2.840E-03		
Lack of Fit	0.050	17	2.967E-03	1.15	0.4378
Pure Error	0.021	8	2.570E-03		
Cor Total	124.15	33			

(B) *L. paracasei* LCW23

Source	SS	DF	MS	F-value	p-value
Block	0.016	1	0.016		
Model	126.93	8	15.87	5779.31	＜0.0001
A	0.038	1	0.038	13.85	0.0011
B	17.06	1	17.06	6213.20	＜0.0001
C	0.53	1	0.53	191.47	＜0.0001
AB	0.029	1	0.029	10.49	0.0035
AC	2.00E-04	1	2.00E-04	0.07	0.7937
BC	0.14	1	0.14	49.25	＜0.0001
A^2	12.15	1	12.15	4424.53	＜0.0001
B^2	59.63	1	59.63	21720.57	＜0.0001
C^2	27.24	1	27.24	9922.07	＜0.0001
Residual	0.066	24	2.745E-03		
Lack of Fit	0.054	16	3.345E-03	2.17	0.1348
Pure Error	0.012	8	1.545E-03		
Cor Total	127.01	33			

A：Total carbohydrate concentration of the SCG Hydrolysate (mg/mL)
B：L-Cysteine content (w/v)
C：pH value
SS：Sum of Squares
DF：Degree of Freedom

The statistical results indicated that all three factors had significant effects ($p < 0.0001$) on the bacteriocin production by *L. plantarum* BCRC 10069 while only pH and cysteine concentration had significant effects on the bacteriocin production by *L. paracasei* LCW23. The Fig. 2A is the response surface plot that shows the effects of pH value and cysteine concentration on the bacteriocin production by *L. plantarum* in the medium containing SCG hydrolysate. The results showed that the antibacterial activity was low without the addition of cysteine and increased gradually after the addition of cysteine to around 0.05% w/v. The effect of cysteine on bacteriocin production was studied and pointed out in a reference reported by Malheiros *et al*. (2015). Cysteine was commonly used as an amino acid supplement and a reducing agent in the microbial growth medium (Fukushima *et al*. 2002). Cysteine is capable of providing a good anaerobic environment for the growth of the microorganism. Therefore, the addition of cysteine can provide a better environment for lactic acid bacteria to grow and produce bacteriocin. The results also indicated that pH in the neutral range was much favor for bacteriocin production, which was in agreement with the results found in other reports (Barman *et al*., 2018; Kumar *et al*. 2012). The optimal condition derived from the model for bacteriocin production by *L. plantarum* was 15.0 mg/mL SCG hydrolysate, 0.059% (w/v) cysteine content, and pH 7.1. Under the optimal conditions, the bacteriocin produced by *L. plantarum* had the inhibitory activity against *E. coli* BL21 was around 8.1 mm based on the disc diffusion assay. For the optimization of bacteriocin production by *L. paracasei,* a similar trend was found based on the statistical result and response surface plot (Fig. 2B). The optimum condition for bacteriocin production by *L. paracasei* LCW23 was 19.1 mg/mL SCG hydrolysate, 0.062% (w/v) cysteine content, and pH 7.1. The inhibitory activity was around 9.9 mm based on the disc diffusion assay.

3.5 Scale-up of bacteriocin production under optimum conditions

Cultivation of LAB in a 1 L bioreactor with 700 ml working volume was employed to validate the optimal conditions for bacteriocin production derived from the experimental design with RSM. Figure 3 shows the cell growth of LAB strains, the consumption of monosaccharides, and the antibacterial activity of protein purified from the culture medium. The results showed that both LAB strains could reach maximum cell density in 24 hours while the sugar concentration decreased correspondingly. The production of bacteriocins in this study displayed primary metabolite kinetics with a peak antibacterial activity at 24 hours after inoculation and then slightly decreased from 24 to 36 hours. The decrease of antibacterial activity might be attributed to the protease degradation of bacteriocin (Ivanova *et al*. 2002). The results of the disc diffusion assay showed that the peak antibacterial activities against indicator strain *E. coli* BL21 were around 8 mm and 9.33 mm respectively for the bacteriocin produced by *L. plantarum* and by *L. paracasei*. The obtained peak antibacterial activities were close to the predicted value derived from RSM models. The activities of bacteriocins partially purified from the culture medium were also determined and reported in Table 7. The activities of bacteriocins produced by *L. plantarum* BCRC 10069 and *L. paracasei* LCW23 were 1163 AU and 1920 AU per mg protein, respectively. The results were comparable to some bacteriocins reported in other studies (Ge *et al*. 2016; Mahdi *et al*. 2017). The MIC value of bacteriocin produced by *L. plantarum* and *L. paracasei* were determined against indicator strain *E. coli* BL21 and the results were given in Fig. 4. The results showed that the bacteriocins produced in this study can deter the growth of indicator strain when the applied dosage was at 50 AU/mL. When the applied dosage was higher than 100 AU/ml, the growth of the indicator bacteria was inhibited.

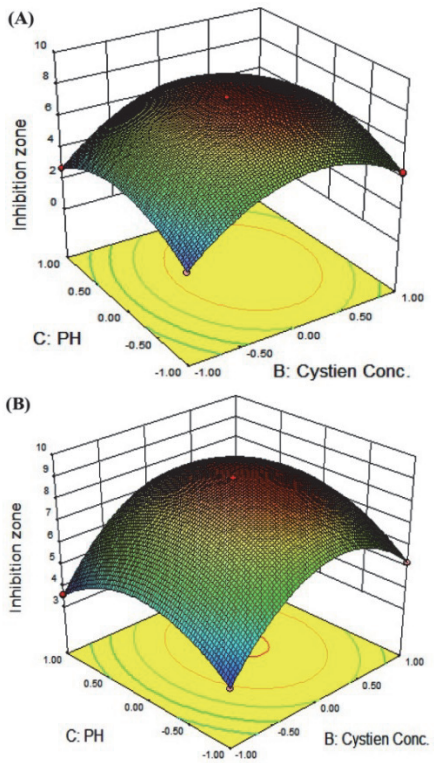


Fig. 2 Response surface plots of the effects of pH and cystein concentration on the bacteriocin production (A) L. plantarum BCRC 10069 and (B) L. paracasei LCW23

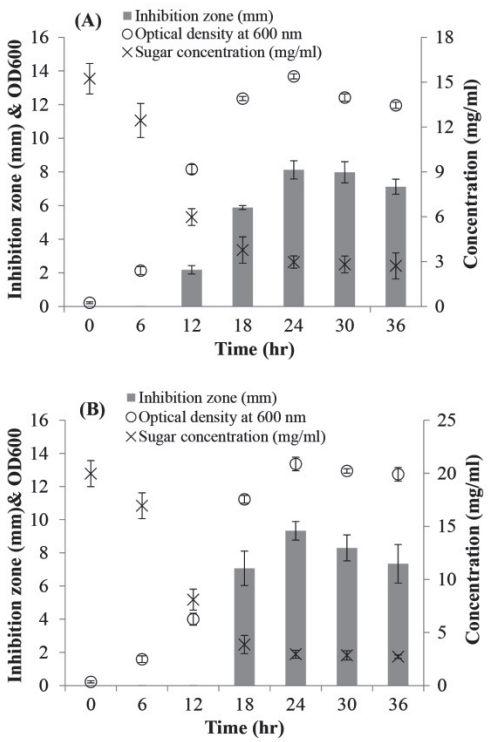


Fig. 3 The growth of LAB and the production of bacteriocin under optimum culture condition: (A) *L. plantarum* BCRC 10069 and (B) L. *paracasei* LCW 23

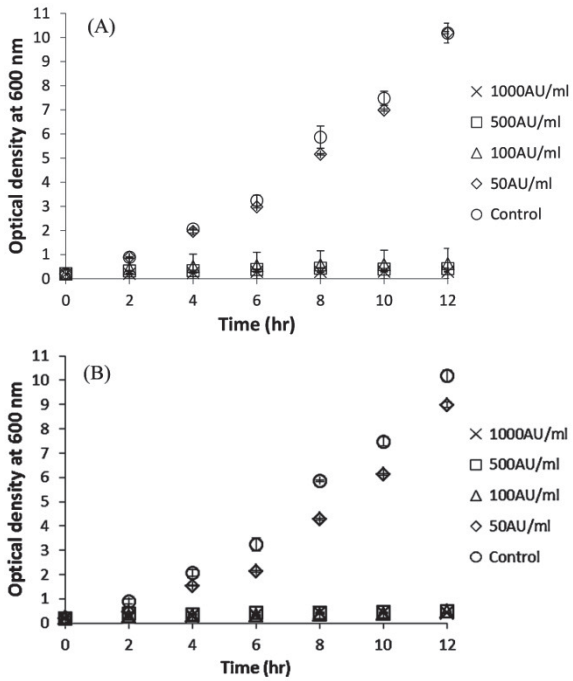


Fig. 4 The minimum inhibitory concentration of bacteriocin produced by (A) *L. plantarum* BCRC 10069 and (B) *L. paracasei* LCW23 against *E. coli* BL21

4. CONCLUSION

In this study, the bacteriocins were produced by *L. plantarum* BCRC 10069 and *L. paracasei* LCW23 which were grown

in medium containing SCG hydrolysate as carbon source. The results confirmed that the bacteriocin produced by *L. plantarum* BCRC 10069 and *L. paracasei* LCW23 exhibited good inhibitory activities on gram-positive and gram-negative indicator bacteria. The utilization of SCG hydrolysate as a carbon source in the medium could support the growth of LAB and the production of bacteriocins. Overall, this study confirmed that the SCG could be utilized as a renewable biomass feedstock for bacteriocin production.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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