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ORIGINAL PAPER

Isolation, Characterisation and Fermentation Optimisation of Bacteriocin-Producing *Enterococcus faecium*

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Abstract

Purpose The aim of this work was to optimize a bacteriocin produced by *Enterococcus faecium* TJUQ1 and the potential application in vegetable wash solution.

Methods The agar well diffusion method was applied to screening strains with antibacterial activity. It was identificated based on physiobiochemical characteristics and 16S rDNA sequence analysis. Response surface method (RSM) was applied to optimize bacteriocin yield and discover the best production condition.

Results A total of 31 isolates of lactic acid bacteria (LAB) isolated from pickled Chinese celery were screened for bacteriocin production. Strain TJUQ1 showed an inhibition zone against *Listeria monocytogenes* CMCC 1595 as indicator by using agar well diffusion assay and identified as *E. faecium*. The culture parameters were further optimized using response surface methodology (RSM). By using Plackett–Burman (PB) design, beef extract, K_2HPO_4 and initial pH were found to be the most significant factors for bacteriocin activity. The effects of the three main factors on bacteriocin activity were further investigated using a central composite design (CCD) and the optimum composition was found to be beef extract 15.20 g/L, K_2HPO_4 1.93 g/L and initial pH 7.19. Optimum conditions were validated by experiment in which bacteriocin activity was increased 1.78-fold (816.87 ± 5.21 AU/mL) in 18 h fermentation. The bacteriocin-containing wash solutions showed activity against *L. monocytogenes* CMCC 1595 inoculated onto fresh-cut iceberg lettuce within the first 3 days of storage at 4 °C.

Conclusion A bacteriocin-producing strain TJUQ1 isolated from pickled Chinese celery was identified as *E. faecium*. The strain produced a high level of bacteriocin and the bacteriocin-containing wash solutions showed activity against *L. monocytogenes* CMCC 1595 inoculated onto fresh-cut iceberg lettuce.

Xiaoxiao Qiao and Renpeng Du contributed equally to this work and share the first authorship.

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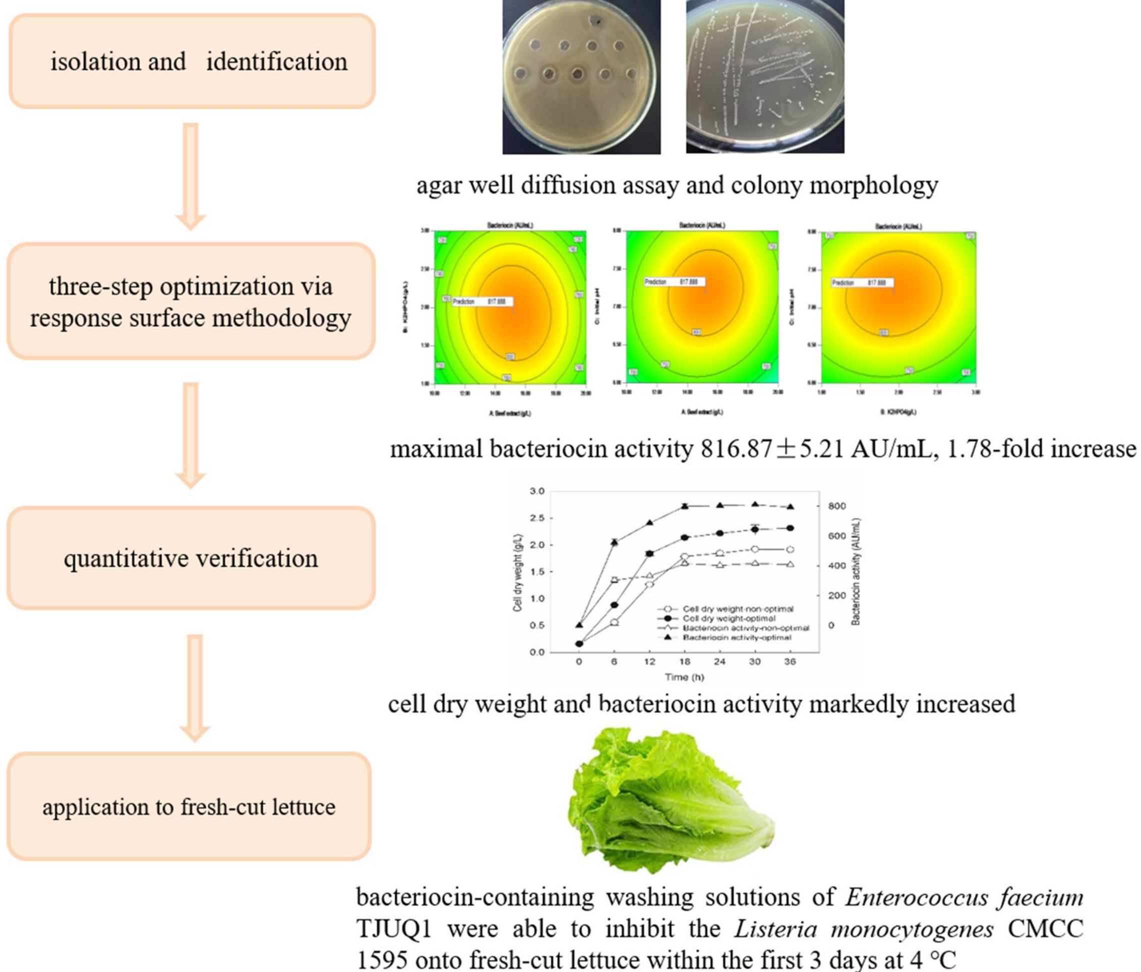
Graphical Abstract



Keywords *Enterococcus faecium* · Response surface methodology · Bacteriocin · Optimization · Wash solution

Statement of Novelty

Bacteriocin is natural antibacterial peptides ribosomally biosynthesized by bacteria. In this study, a bacteriocin-producing strain TJUQ1 isolated from pickled Chinese celery was identified as *Enterococcus faecium*. To enhance the bacteriocin production, the culture parameters were optimized using RSM. Optimum conditions were validated by experiment in which bacteriocin activity was increased 1.78-fold (816.87 ± 5.21 AU/mL). The bacteriocin-containing wash solutions of TJUQ1 showed activity against *Listeria monocytogenes* CMCC 1595 inoculated onto fresh-cut iceberg lettuce within the first 3 days of storage at 4 °C. Therefore, *E. faecium* strains might be useful as natural biopreservatives against *L. monocytogenes* in food products.

Introduction

Emergence of antibiotic resistance and growing consumer trend towards foods containing biopreservatives gave rise to the search for natural antimicrobial substances for food preservation [1, 2]. In recent years, greater attention has been drawn towards application of bacteriocins as food biopreservative, they are innocuous due to their proteolytic degradation in gastrointestinal tract [3]. Bacteriocins are antimicrobial compounds produced by many different bacterial species. They are ribosomally-synthesized, proteinaceous compounds capable of controlling the growth of pathogenic and spoilage microorganisms, and those synthesized by lactic acid bacteria (LAB) have great potential as biopreservation agents in foods [4]. The genus *Enterococcus* belonging to the LAB presented as natural flora in the

human intestinal tract [5]. Nisin produced by *L. lactis* is the intensively studied and the only antibiotic approved in more than 50 countries to be used in food industry [6]. However, some bacteria may gain resistance to Nisin [7] or produce proteolytic substances that inhibit its efficacy [7, 8]. Therefore, microbiologists got interested to isolate and identify potential bacteriocin producing LAB that can be used to preserve food products and get commercial status.

The most important aspects in the bacteriocin study are production and purification. Most often, production of bacteriocin is very low and the complex growth media commonly used for bacteriocin production. It necessarily required optimization of production that can be dependent on environmental factors such as temperature, pH and media composition [9, 10]. Certain components, such as carbon sources, nitrogen sources, salts, metal ions and surfactants agents added to the Man, Rogosa and Sharpe (MRS) broth can affect bacteriocin production [1, 11]. These factors need to be evaluated to achieve optimized bacteriocin production for industrial applications. Response surface methodology (RSM) is an effective statistical technique that has been successfully applied in many areas, including the effects of distinct factors on bacteriocin production [12] and medium components and environmental conditions on the antimicrobial activity of bacteriocins produced by *Lb. plantarum* [13].

There is an increasing demand from consumers for natural and minimally processed foods, fresh-cut lettuce has been one of the most frequently requested commodities [14]. The increasing popularity of salad bars offering fresh-cut lettuce, has provided a new environment which support the growth of food-borne pathogens including *L. monocytogenes* [14, 15]. The objectives of this study were to isolate, identify and optimize bacteriocin-producing LAB from pickled Chinese celery, which probably against *L. monocytogenes* CMCC 1595 inoculated onto fresh-cut iceberg lettuce.

Materials and Methods

Isolation of LAB from Pickled Chinese Celery

The sample used for strain isolation was collected from pickled Chinese celery (Kunming, Yunnan Province, China) (25.05°N, 102.73°E). The sample was stored at 4 °C in sterile containers and subjected to homogenise. The sample was diluted in a 1:10 ratio with 0.9% sterilized normal saline solution and then blended by Stomacher for 1 min. Approximately 200 μL of the diluted sample were pipetted into 5 mL of MRS broth incubated at 30 °C for 24 h. Aliquots (50 μL) of culture were maintained on MRS agar containing 0.3% (w/v) calcium carbonate [16]. The plates were incubated at 30 °C for 24 h. The strains were picked individually based on the clear zones on the plates and streaked on MRS agar medium to purify the isolates. The tested strain *L. monocytogenes* CMCC 1595 was cultured in Brain Heart Infusion (BHI) broth.

Screening of Bacteriocin-Producing LAB

The isolates was cultivated in 5 mL of MRS medium at 30 °C overnight and centrifuged for 5 min at 12,000×*g*. Cell Free Supernatants (CFS) was neutralized to pH 7.0 using 5 N NaOH to eliminate the inhibitory activity of organic acids. The antibacterial activity from H_2O_2 was excluded by the addition of 1 mg/mL catalase. CFS was heated (5 min) at 100 °C to inhibit enzyme activity [17].

The agar well diffusion method was applied to determinate an antibacterial activity produced from isolates. BHI soft agar preinoculated with test organisms cultivated to 10^6 colonies forming units (CFU) per milliliter, *L. monocytogenes* CMCC 1595 were used onto which wells with a 7 mm diameter were made. About 50 μL of each CFS was added into each well and incubated for 12 h at 30 °C [12]. The bacteriocin-producing ability of the isolates was verified by treating their CFS separately with proteinase K and adjusted to a final concentration of 1 mg/mL. The samples were incubated at 37 °C for 4 h. Inhibitory activity of the isolates was determined by agar well diffusion assay to calculate the activity of bacteriocin which was expressed as an Arbitrary Units per milliliter (AU/mL). Titers were defined as the reciprocal of the highest dilution that inhibited the growth of the indicator strain [18].

Identification of Selected Bacteriocin-Producing LAB

The identification of selected bacteriocin-producing strain was firstly based on physiobiochemical characteristics including morphological, cultural and physiological characteristics. Colony morphology was examined with stereoscope (Thktronix SM20, Shanghai, China) of colonies grown on MRS agar plate cultures after 48 h of growth. Individual form of strains were examined using system microscope (Olympus BX43, Beijing, China) on wet mounts of MRS broth at 30 °C for 24 h [19]. Gram-staining was examined after 24 h of incubation on MRS agar. Catalase activity and gas production from glucose were determined using the methods of Cang et al. [20]. Growth at different temperatures, pH, NaCl concentrations, urea concentrations were tested on MRS agar medium at 30 °C for 24 h. Production of esterase and urease and gelatine hydrolysis were tested as described by previous method [21]. The strain was characterized by sugar fermentation assays using API 50CH strips.

The strain was further confirmed by 16S rDNA sequence analysis. The species identity was carried out by PCR using universal primers 8F: 5′-AGAGTTTGATCATGG CTCAG-3′ and 1492R: 5′-ACGGTTACCTTGTTACGA CTT-3′. PCR conditions included a first denaturing step

for 3 min at 95 °C, followed by 30 cycles: denaturing for 30 s at 95 °C, annealing for 60 s at 55 °C and extension for 90 s at 72 °C and finally extension for 5 min at 72 °C [22]. The PCR products were sequenced by GENEWIZ (Tianjin, China). Sequence homologies were determined by blasting the obtained sequences against those in the databases of the National Center for Biotechnology Information (NCBI).

Optimization of Bacteriocin Production by Experimental Designs and Data Analysis

Plackett–Burman (PB) Design

Plackett–Burman design is a very useful method for identification of important variables in relatively low number tests as compared to traditional techniques [13]. Medium components and culture conditions were studied by single factor design before study. Here, the effect of 10 variables (Table. S1) on bacteriocin production was studied in 15 experimental trials (Table. S2). Each factor was examined in three levels: low (− 1), medium (0) and high (+ 1) levels based on PB design [23]. Bacteriocin activity was used as the response variable. PB experimental design was explained by Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + \beta_{10} X_{10} \quad (1)$$

where Y is the predicted response (bacteriocin activity AU/mL); X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} are the independent variables; β_1, β_2, β_3, β_4, β_5, β_6, β_7, β_8, β_9, and β_{10} are the linear coefficients. The variables at or above the 95% confidence level ($P < 0.05$) were deemed to have significant effect on response.

Central Composite Design (CCD)

The three significant variables beef extract, K_2HPO_4 and initial pH from PB design were selected (Table. S3) and studied in CCD (Table. S4) to obtain their accurate optimal levels. The statistical software package Design Expert version 8.0 Stat-Ease Inc. (Minneapolis, USA) was used to analyze the experimental design. The relationship of the independent variables and the response were expressed according to Eq. (2):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i x_i + \sum_{i<j} \sum \beta_{ij} x_i x_j \quad (2)$$

where Y is the predicted response, β_0 is an intercept constant, β_i is the regression coefficients for linear effects, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficients of the variables, x_i, x_j are the coded independent variables and k is number of factors.

Experimental Validation of the Optimized Conditions

Strain TJUQ1 was cultured in MRS broth medium in Erlenmeyer flasks of 250 mL at 30 °C under static condition, which was inoculated at the ratio of 5.0% (v/v). Samples were withdrawn at different time intervals from 0 to 36 h. The cell dry weight and bacteriocin activity were estimated.

Preparation of Bacteriocin-Containing Wash Solutions and Application to Fresh-Cut Iceberg Lettuce

Whole iceberg lettuces were obtained on the day of their arrival from the grower, immediately transported to laboratory, and kept overnight at 4 °C. The sample was treated according to Allende et al. method [24]. The washing solutions were prepared by diluting 200 mL TJUQ1-CFS or Nisin in 1800 mL sterile tap water. The *L. monocytogenes* CMCC 1595 strain suspensions were then diluted in cold sterile water (4 °C) resulting in a final bacterial concentration 10^6 CFU/mL. The bacterial cocktail was then immediately applied to fresh-cut iceberg lettuce by immersing the lettuce in the bacterial solution for 30 min to allow adherence of the bacteria to the produce surface. The control sample was washed in sterile tap water while the each treatment was washed in bacteriocin-containing wash solutions for 2 min. Spin-dried samples were aseptically divided into 50 g bags of macro-perforated polypropylene film for storage over a 7-day period at 4 °C. For this, serial decimal dilutions from 1 g of the sample were made, and these were inoculated on BHI agar in duplicate. The plates were incubated at 30 °C for 24 h and the results expressed as Log CFU/g of fresh-cut iceberg lettuce.

Statistical Analysis

All tests were performed in triplicate according to a design matrix. JMP software (SAS Institute Inc., Version 9.0.2) was used for the statistical analysis, and Tukey's test was performed for determining the significant differences at 95% confidence interval ($p < 0.05$). SigmaPlot version 10.0 software was used for the statistical and chart analysis. Design Expert (version 8.0.0, Stat-Ease, Minneapolis, USA) was used for experimental design and data analysis.

Results and Discussion

Isolation and Screening of Bacteriocin-Producing LAB

A total of 31 isolates were screened for producing acid bacteria on MRS agar supplemented with $CaCO_3$. Among

31 isolates, only 4 isolates, named TJUQ1, TJUQ2, TJUQ3 and TJUQ4 exhibited bacteriocin activity against *L. monocytogenes* CMCC 1595. Bacteriocin activity was disappeared completely when CFS of the isolates were treated with proteinase K confirming the proteinaceous nature of the bacteriocin component. Similar results were obtained that the antimicrobial activity of supernatant of *Lb. plantarum* LD4 was not affected by catalase but was reduced in the presence of trypsin and protease [25].

Antimicrobial activity of CFS of TJUQ1, TJUQ2, TJUQ3 and TJUQ4 was maintained relatively stable by neutralization at pH 7 and treatment with catalase, indicating that the activity was not related to the production of either organic acids or hydrogen peroxide. Hwanhlem [17] reported that bacteriocin activity of CFS of CF1GI 15 was related to the production of organic acids but not related to hydrogen peroxide. A highest bacteriocin-activity strain designated as TJUQ1 was used in subsequent tests.

Identification of Selected Bacteriocin-Producing LAB

TJUQ1 was found to be Gram-positive, non-spore-forming cocci, catalase negative and formed circular, smooth and creamy colonies on MRS agar. Morphological observation of a 24 h culture of TJUQ1 grown on MRS agar revealed that it had the typical characteristics of genus *Enterococcus*. TJUQ1 was found to be positive for production of gas from glucose and negative for esterase, urease and gelatine hydrolysis. Strain TJUQ1 was observed to grow well between pH 6–9, with an optimum pH of 7–8. The range of temperature was determined to be 4–45 °C, with the optimum growth temperature at 30–37 °C. Strain TJUQ1 was observed to grow in presence of 0–6% NaCl (w/v) and 0–4% urea (w/v). The results of API 50CH indicated that TJUQ1 was typical *Enterococcus*. The strain was identified as *E. faecium* by morphological, physico-chemical tests as well as 16S rDNA sequence analysis, and named *E. faecium* TJUQ1.

Analytical Results of Optimizing Fermentation Conditions

Evaluation of Significant Variables Affecting Bacteriocin Activity by PB Design

Plackett–Burman design is a powerful technique for choosing important variables in bacteriocin activity [26]. Bacteriocin activity varied obviously from 521.70 ± 4.58 to 795.99 ± 3.75 AU/mL (Table. S2). This variation indicated the importance of medium optimization to get maximum bacteriocin activity. As was shown in regression analysis of PB design (Table 1), the model *P* value (0.0443) was significant and R^2 was close to 1 (0.9997) indicating 99.97% of the variability in the response could be explained by the model. The three significant variables beef extract (X_4, $P = 0.0274$), K_2HPO_4 (X_7, $P = 0.0283$) and initial pH (X_9, $P = 0.0177$) exhibited significant effect on bacteriocin activity. Hwanhlem et al. [17] found lactose and temperature were most important factors for the strain isolated from mangrove forests to produce bacteriocin with PB method. It might be that the source and characteristics of strains were different, which result in the difference of bacteriocin activity. According to Han et al. [26] reported, glucose and NaCl were first chosen from the 11 considered in the PB design for bacteriocin production. In this study, first-order regression was determined by multiple regression analysis and the correlation between the observed variables and bacteriocin production could be presented as Eq. (3):

$$\begin{aligned}\text{Bacteriocin activity (AU/mL)} = {} & 669.84 + 4.73 * X_1 \\ & + 0.42 * X_2 + 11.74 * X_3 + 35.23 * X_4 + 11.07 * X_5 \\ & + 17.74 * X_6 + 34.13 * X_7 - 32.62 * X_8 + 54.42 * X_9 \\ & + 6.67 * X_{10}\end{aligned} \tag{3}$$

where Y is the predicted response (bacteriocin activity, AU/mL), X_i is coded independent variable value. The high significance of the regression model was determined by the F-test with a low probability "*P*-value".

The results of PB design, experimental values (Table. S2, trial 1–12) and mean values (Table. S2, trial 13–15) were comparatively analyzed to see whether steepest ascent experiment was necessary or not. Under the condition of unequal variance, resulting from equality of variances ($P = 0.0055$), the result of T-test was significant ($P = 0.0019$). This meant that the data between experimental values and mean values exhibited extremely significant differences. The central point

Table 1 Results of the regression analysis of the PB design

Items	Coefficient	*F* value	*P* > |F|
Model		307.66	0.0443*
X_1	4.73	9.73	0.1975
X_2	0.42	0.077	0.8279
X_3	11.74	59.98	0.0818
X_4	35.23	539.86	0.0274*
X_5	11.07	53.27	0.0867
X_6	17.74	136.91	0.0543
X_7	34.13	506.72	0.0283*
X_8	− 32.62	462.83	0.0596
X_9	54.42	1287.83	0.0177*
X_{10}	6.67	19.35	0.1423

$R^2 = 0.9997$, Adjusted $R^2 = 0.9964$

*$P < 0.05$ indicated significant differences

**$P < 0.01$ indicated extremely significant differences

in PB design was close to maximal response area and no need to conduct steepest ascent experiment.

Interaction Between Variables and Optimization of Bacteriocin Activity by CCD

Central composite design was employed to investigate the interactions among the three selected factors [beef extract (X_4), K_2HPO_4 (X_7) and initial pH (X_9), Table. S3] and to determine their accurate optimal values and the results were listed in Table. S4. The model was examined by ANOVA (Table 2). The model was extremely significant ($P=0.0001$), lack of fit was not significant ($P=0.0689$) and R^2 was close to 1 (0.9273). These data collectively suggested that the model was a good fit. The quadratic terms of the three factors (beef extract, X_4^2; K_2HPO_4, X_7^2; initial pH, X_9^2) were significant ($P<0.05$), whereas interactions between beef extract and K_2HPO_4 (X_4X_7), beef extract and initial pH (X_4X_9), and K_2HPO_4 and initial pH, X_7X_9 were not significant ($P>0.05$) for bacteriocin activity (Table 2). A second-order polynomial mathematical model, combining the different interactions of low and high levels of different factors, is represented below: (Eq. 4).

$$\begin{aligned}\text{Bacteriocin activity (AU/mL)} = {} & 815.58 + 2.76 * X_4 \\ & - 7.90 * X_7 + 21.21 * X_9 - 6.34 * X_4 * X_7 \\ & + 15.46 * X_4 * X_9 + 10.01 * X_7 * X_9 - 73.63 * X_4^2 \\ & - 46.38 * X_7^2 - 56.51 X_9^2 \qquad (4)\end{aligned}$$

The interaction of factors can be visualized by the shape of contour plot, and elliptical plots suggest significant stronger interaction between variables than circle ones. According to the response counter plot (Fig. 1), it could be clearly seen that the bacteriocin activity almost constantly increased in the designed range of beef extract from 14.0 to 16.0 g/L, K_2HPO_4 from 1.5 to 2.0 g/L and initial pH around 7.0. The predicted maximal bacteriocin activity reached 817.89 AU/mL. This maximum could be obtained when the accurate values of beef extract (X_4), K_2HPO_4 (X_7) and initial pH (X_9) were 15.20 g/L, 1.93 g/L and 7.19, respectively. CCRD was employed to optimize the independent variables, quantity of prebiotic ingredient and temperature to maximize the cell viability and antilisterial activity [11, 15].

Table 2 Results of the regression analysis of the CCD

Items	Coefficient	*F* value	*P* > |F|
Model		14.18	0.0001**
X_4	2.76	0.094	0.7656
X_7	−7.90	0.77	0.4003
X_9	21.21	5.56	0.0401*
X_4*X_7	−6.34	0.29	0.6016
X_4*X_9	15.46	1.73	0.2177
X_7*X_9	10.01	0.73	0.4142
X_4^2	−73.63	70.68	<0.0001**
X_7^2	−46.38	28.05	0.0003**
X_9^2	−56.51	41.63	<0.0001**
Lack of fit		4.26	0.0689

* $P<0.05$ indicated significant differences

** $P<0.01$ indicated extremely significant differences

$R^2=0.9273$, Adjusted $R^2=0.8620$

Experimental Validation of the Optimized Culture Variables

Six repeated experiments were conducted under the above mentioned conditions to verify the predicted maximal response value. The results of the six replicants were 815.21, 816.32, 817.91, 817.65, 815.45 and 818.65 AU/mL. T-test showed that there were no significant differences between the predicted maximum (817.89 AU/mL) and the six experimental values ($P=0.9325$). This study therefore suggests that the final optimal culture conditions would be 30 g/L sucrose, 15 g/L tryptone, 6 g/L yeast extract, 15.20 g/L beef extract, 1.93 g/L K_2HPO_4, 4 g/L anhydrous sodium acetate, 1.5 g/L ammonium citrate, 0.2 g/L K_2HPO_4, 2 mmol/L $ZnCl_2$, 1.0 mL/L Tween80, initial pH 7.19, loading volumes 120 mL/250 mL, inoculation quantity of 5% (v/v) and at 30 °C in 18 h fermentation under static condition. Time course of cell dry weight and bacteriocin activity of TJUQ1 under non-optimized and optimized condition were observed (Fig. 2). When TJUQ1 was grown in the optimized medium, the cell dry weight of the culture medium rapidly increased from 0.16 ± 0.01 to 2.25 ± 0.11 g/L. Maximum bacteriocin activity (816.87 ± 5.21 AU/mL) was about 1.782-fold compared with that under non-optimized condition (460 ± 3.24 AU/mL) in 18 h fermentation. It has been reported that the optimal conditions for increasing bacteriocin production may vary with different strains. Hwanhlem et al. [17] optimised bacteriocin from *E. faecalis* KT2W2G by RSM, which the optimum composition was lactose 14.85 g/L and temperature 25.59 °C, the antifungal specific activity was 640 AU/mL lower than our results. Radha et al. [12] assumed that starch (3%), casein (3%), $FeSO_4$ (0.3%) and Tween 20 (0.24%) w/v supplemented to MRS media were optimum for *Lactobacillus delbrueckii* subsp *bulgaricus*. Bacteriocin concentration before and after optimization was found to be 178.8 AU/mL and 310 AU/mL, respectively. We optimised bacteriocin from *E. faecium* TJUQ1 using RSM for the first time and gained a higher bacteriocin activity. We assumed that the strains, growth conditions, the structure and characteristics of bacteriocin and

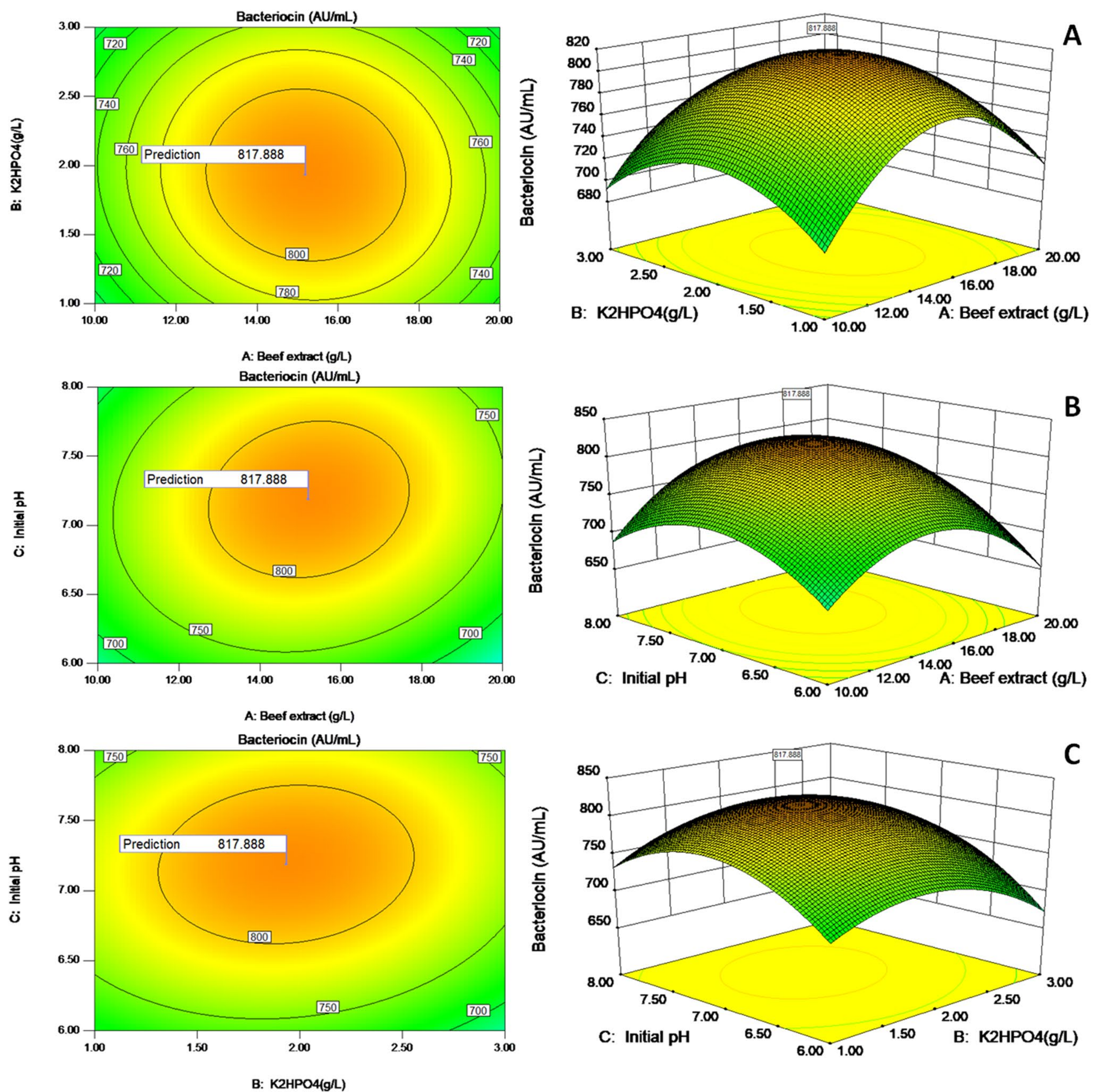


Fig. 1 Response surface and corresponding contour plots showing the effects of **a** beef extract and K_2HPO_4, **b** beef extract and initial pH, **c** K_2HPO_4 and initial pH on bacteriocin activity (AU/mL)

indicators can account for the differences of antibacterial effect. Meanwhile, the highest bacteriocin activity was reached at 18 h, shorter than the fermentation period of many other microbial strains, such as *Bacillus* sp. BH072 60 h [27], *Lactobacillus viridescence* NICM 2167 48 h [12], which was a desirable advantage during industrial application.

Effect of Bacteriocin-Washing Treatments on *L. monocytogenes* CMCC 1595 Populations Inoculated Onto Fresh-Cut Iceberg Lettuce

Cell proliferation kinetics of *L. monocytogenes* CMCC 1595 was shown in Fig. 3. The estimated population of *L. monocytogenes* CMCC 1595 obtained in fresh-cut lettuce after inoculation was 5.2 ± 0.4 log CFU/g (data not shown). Washing with tap water (control) reduced *L. monocytogenes*

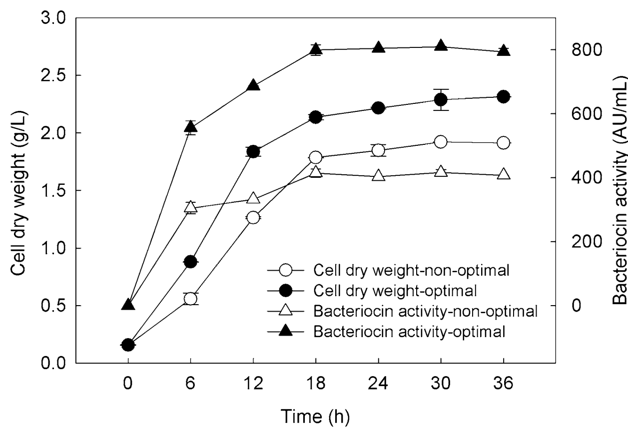


Fig. 2 Time courses for cellular growth and bacteriocin activity by *E. faecium* TJUQ1 were experimented, including cell dry weight-non-optimal condition (open circle), cell dry weight-optimal condition (filled circle), bacteriocin activity-non-optimal condition (open triangle), bacteriocin activity-optimal condition (filled triangle)

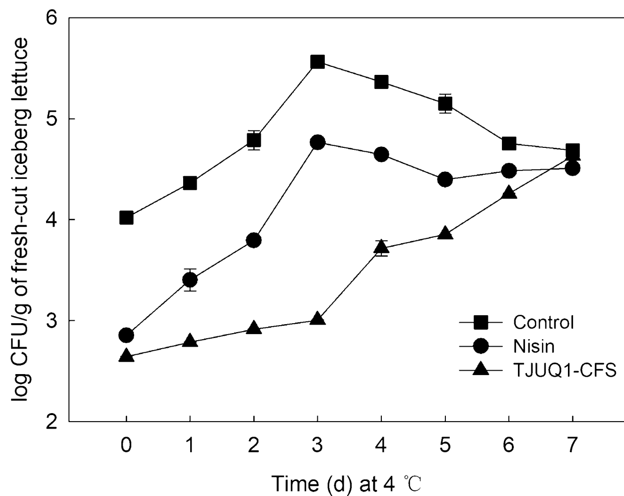


Fig. 3 Survival of *L. monocytogenes* CMCC 1595 populations on inoculated fresh-cut iceberg lettuce treated with washing solutions containing Nisin (filled circle) and TJUQ1-CFS (bacteriocin) (filled triangle) and stored at 4 °C for 7 days in macroperforated plastic bags. Sterile tap water was used as control washing solution (filled square)

CMCC 1595 by 1.1 ± 0.2 log units, while bacteriocin solutions decreased the pathogen populations to 2.5–3.0 log CFU/g which more efficient than pediocin PA-1 [24]. Within the first 3 days of storage, bacteriocin solution prevented *L. monocytogenes* CMCC 1595 proliferation. However, its effectiveness decreased throughout storage. No differences were observed between control and any of the bacteriocin solution washes after 7 days of storage at 4 °C. The result indicated that the bacteriocin-containing washing solutions were able to reduce the pathogen counts in fresh-cut iceberg lettuce within the first 3 days of storage at 4 °C. Similar results were found that the live-*Enterococcus*-doped film showed a more remarkable activity than nisin- and enterocin-doped films over long times both at 4 °C and 22 °C [28]. Therefore, it has preservative properties and can be used as a bio-preservative which can take the place of chemical preservatives which have side effect on health of the consumer.

Conclusion

In this study, a bacteriocin-producing strain TJUQ1 isolated from pickled Chinese celery was identified as *E. faecium*. To enhance the bacteriocin production, the culture parameters were optimized using RSM. By using PB design, beef extract, K_2HPO_4 and initial pH were found to be the most significant factors for bacteriocin activity. The three main factors were further investigated using CCD. Optimum conditions were validated by experiment in which bacteriocin activity was increased 1.78-fold (816.87 ± 5.21 AU/mL). The bacteriocin-containing wash solutions of TJUQ1 showed activity against *L. monocytogenes* CMCC 1595 inoculated onto fresh-cut iceberg lettuce within the first 3 days of storage at 4 °C.

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