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Optimization of Nutritional Factors for Nisin yield Improvement by *Lactococcus lactis* E15 using Corn Steep Liquor Powder as Nitrogen Source

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Abstract

In the present study, corn steep liquor powder (CSLP) was positively investigated as nitrogen source to replace the relatively costly peptone typically used for the production of nisin. Meanwhile the nutritional factors and nisin production in batch fermentation by *Lactococcus lactis* (*L. lactis*) E15were optimized using a series of statistical design of experiments and response surface methodology on the basis of the modified fermentation medium (CSLP-M). Results indicated that sucrose, Tween-80 and CSLP were significant factors for nisin production, and the first two had positive effects on bacteriocin production, while the last one was on the contrary. The optimum formula obtained for nisin production was composed of 1.97% (w/v) sucrose, 0.89% (w/v) CSLP, 1% (w/v) yeast extract, 0.5% (w/v) KH_2PO_4, 0.2% (w/v) NaCl, 0.03% (w/v) $MgSO_4 \cdot 7H_2O$, 0.5% (w/v) $CaCO_3$ and 0.28% (w/v) Tween-80 (X3) at pH 6.8. Under the optimized conditions, nisin titer reached a peak level of 3036 IU ml^{-1} at 14h in batch fermentation, which nearly increased by 52% compared with that in the initial medium. Besides, the controlled fed-batch fermentation in 1.0-L fermenter was carried out and maximum nisin titer of 5370 IU ml^{-1} after 16h was obtained, which might provide a potential strategy for increasing nisin yield in large-scale industrial production.

Keywords: *Lactococcus lactis*; Nisin production; Corn steep liquor powder; Fermentation; Optimization

Introduction

Lactococcus lactis, formerly also known as *Streptococcus lactis* [1], is a Gram-positive, facultatively anaerobe bacterium used extensively in the production of buttermilk and cheeses [2]. Nisin, a primary metabolite produced during the growth of several *L. lactis* strains, is a polycyclic peptide bacteriocin composed of 34 amino acid residues [3,4]. It has long been viewed as an excellent bio preservative widely used in food industry, primarily in dairy products, meat products, and canned and other processed foods, because of its superior antimicrobial activity, and lack of toxicity and side effects [5,6]. In addition, nisin emerges as a promising alternative in medical applications for bacterial infection in humans. It has been confirmed to be an anti-infective therapeutic agent against some inflammations of the skin or mucosa [7-10], especially in the oral cavity [11]. Moreover, a potent vaginal contraceptive has even been developed from nisin for future use in humans [12].

Now the industrial-scale production of nisin is achieved largely through fermentation by *L. lactis*, which is closely associated with the growth of producer cells. This bioprocess requires a large number of complex nutritional factors that has been well studied previously [13-17], including carbon source, nitrogen source, inorganic salts, etc., but the optimum fermentation conditions varied greatly due to differences in the nutrient compositions, experimental conditions and producer strains. Corn steep liquor powder (CSLP) containing a variety of crude proteins and trace elements is favorable for bacterial growth and its product accumulation [18,19], which could be used as an alternative culture medium to increase nisin production. In our preliminary experiments, the nisin yield of *L. lactis* E15 was estimated with the substitution of CSLP as nitrogen source, but further optimization is necessary.

Response surface methodology (RSM) is an efficient and economical strategy for screening optimal conditions for desirable responses [20-22]. It consists of a group of mathematical and statistical procedures that can be used to study the relationships between one or more responses and

multiple independent variables, and generates a mathematical model that can accurately predict desirable responses [23,24]. Currently RSM has been successfully applied to optimize the medium composition for nisin production for a natural nisin-producing *L. lactis* strain in batch and fed-batch fermentation systems [25,26].

To the best of our knowledge, there is no reports on the use of RSM to optimize the main nutritional factors containing CSLP as nitrogen source for nisin production. In this study, the effects of eight factors on nisin production of *L. lactis* E15 were studied in the modified fermentation medium (CSLP-M) and a sequence of experimental designs were employed to optimize the production of nisin by batch fermentation using CSLP as an alternative culture medium.

Materials and Methods

Bacterial strains and culture conditions

In the present study, *L. lactis* E15, a mutant nisin A producer, was obtained from a nisin-producing strain *L. lactis subsp*. *Lactis* ATCC 11454 by treatment with physical and chemical mutagens. *L. lactis* strain was propagated without aeration and pH control at 30 °C in M17 broth (OXOID, UK) supplemented with 0.5% (w/v) glucose (GM17 medium) in an orbital shaker at 100 rpm [27]. When necessary, *L. lactis* E15 with high yield of nisin A was selected on selective GM17 containing 500 IU ml^{-1}additional nisin and 0.004% sterilized bromocresol purple.

Micrococcus flavus NCIB 8166 was used as a nisin sensitive indicator bacterium in the nisin bioactivity assay and it was grown in Luria–Bertani (LB) broth at 37 °C with shaking at 200rpm. Prior to nisin bioactivity assay, the LB culture of the indicator bacteria was added into Nutrient Broth (NB) medium composed of 0.8% (w/v) tryptone, 0.5% (w/v) yeast, 0.5% (w/v) glucose, 0.5% (w/v) NaCl, 0.2% (w/v) Na_2HPO_4, 1% (v/v) Tween-20 and 1.2% (w/v) agar at pH 7.2 for plate preparation.

Fermentation Conditions

The initial fermentation medium was composed of 1.5% (w/v) sucrose, 1% (w/v) yeast extract, 1% (w/v) peptone, 0.5% (w/v) K_2HPO_4, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 0.2% (w/v) NaCl, 0.4% (w/v) $CaCO_3$ and 0.2% (w/v) Tween-80 at initial pH 6.8. While CSLP, just as a substitute for peptone, was added into the modified fermentation medium (CSLP-M).

Batch fermentation of *L. lactis* E15 strain was performed without pH control and aeration at 30 °C and a stirring rate of 100 rpm for 20 h in 250-ml flasks containing 50 ml of a series of different fermentation media. Fed-batch fermentation was carried out in a 1.0-L fermenter (INFORS, Switzerland) containing 800 ml of the final optimal fermentation medium simultaneously added 2 ml l^{-1} antifoamer at 30 °C and a stirring rate of 100 rpm for 20 h. Sucrose solution (50%, w/v) was fed at different time points by a manual control system into the broth to maintain the level of residual sucrose at 5-15g l^{-1} during the entire fermentation. Moreover, NaOH solution (22.5%, w/v) was added automatically into the fermenter to maintain a constant pH by a digital pH controller when the pH dropped to a value lower than the set-point of 6.8 due to the by-product of lactic acid. Prior to fermentation, seed culture was cultured twice at 30 °C for 8-10 h in GM17 broth and the fresh inoculum (5%, v/v) was inoculated into the above fermentation systems. Samples were collected aseptically every two hours from the fermentation systems for the subsequent analyses.

Experimental Designs

Previous investigations have shown that the major nutritional components affecting nisin production were sucrose, yeast extract, peptone, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $CaCO_3$ and Tween-80 [13-15,25,26]. CSLP is a cheap and easily available organic nitrogen source in the fermentation industry, which could be used as an alternative medium to increase nisin value. In the preliminary experiments, the CSLP-M medium containing CSLP instead of peptone as nitrogen source was positively examined in Figure 1, but still need further optimization.

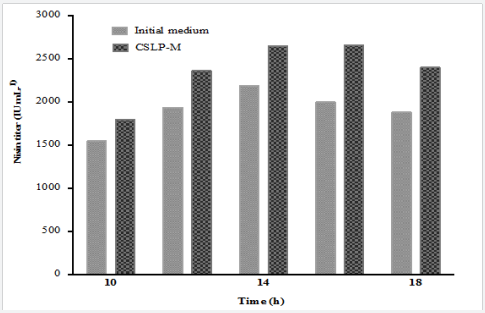

Figure 1: Influence of nitrogen sources on nisin production in the preliminary experiments. Initial medium contains 1% (w/v) peptone, and CSLP-M medium without peptone, but supplemented with 1% (w/v) corn steep liquor powder (CSLP).

Plackett–Burman design (PBD)

The Plackett-Burman design is a very efficient way for screening the main factors affecting response value among a large number of independent variables. In the present study, a series of experiments were designed to identify the most significant factors of eight variables in the initial formula for nisin production by *L. lactis* E15, and eight variables were coded according to the following equation:

$$X_i = (x_i - x_0) / \Delta x(1),$$

where Xi is the coded value of an independent variable, x_i is the real value of an independent variable, x_0 is the real value of an independent variable at the center point, and Δx is the step change value. The nisin titer ($IUml^{-1}$) was defined as response value (Y).

Steepest ascent design

This step was carried out to approach the optimum region of the response value to establish an effective fitted equation

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of the response surface. The direction and change range of the main factors toward predictive higher response values were determined according to the magnitude and sign of linear effect values of various factors [24,25].

Response surface methodology (RSM)

Response surface methodology (RSM) based on Box-Behnken experimental design (BBD) with three coded levels was adopted to determine the optimal conditions of the screened factors in PBD for improved nisin production. The coded levels of each factor and the design matrix are given in Table 5. The low, middle, and high levels of each variable were designated as -1, 0, and +1, respectively.

The whole optimization experiments were executed in 250-ml shake flasks with 50 ml of above designed media. All experiments were repeated for three times.

Analytical Methods

Residual sucrose concentration determination

After centrifugation and acid hydrolysis of the fermentation broth, the glucose concentration of the samples was measured by using an SBA Series of Biosensor Analyzer (Biology Institute of Shandong Academy of Science, China) and correlated with sucrose concentration.

Biomass

The biomass of the fermentation broth was estimated by the optical density at 600 nm (OD600) using a spectrophotometer (Tecan, Austria) after samples were diluted appropriately.

Lactic acid production

The production of lactic acid can directly lead to a decline in pH value offer mentation liquor, so the pH profiles were measured by using a pH meter (METTLER TOLEDO, Switzerland) to indirectly indicate the accumulation of lactic acid during the fermentation period.

Nisin bioactivity assay

Nisin titer was determined by a modified agar diffusion assay according to the method of Kong et al (2014). A standard nisin solution (10^3 IU ml^{-1}) was prepared by dissolving 0.01 g of nisin standard (10^6 IU g^{-1}, Sigma) in 10 ml of 0.02 MHCl. Fermentation samples were acidified to pH 2.0 by drop wise adding 2 M HCl solution to promote the stability and solubility of nisin. Subsequently, the acidified samples (1 ml) were boiled at 100 °C for 5 min followed by centrifugation at 12000 ×g for 10 min at 4 °C. The supernatants were then filtered through a 0.22 μm sterilized membrane filter (Millipore, USA) and stored at 4 °C until analysis. On the other hand, the testing plate was prepared by pouring 20 ml of molten NB medium (cooled to 40 °C-50 °C) pre-mixed with overnight culture of the indicator organism (approx.107CFU ml^{-1}) into sterile plate (Φ=90 mm), and allowed to solidify on a horizontal plane at room temperature. Afterwards, same holes were bored in each agar layer using a sterilized punch, and 100 μL of nisin standard solutions with gradient dilution or samples dilutions were added into the wells. After incubation at 37 °C for 24 h, a standard curve of nisin inhibition zones versus units of nisin standard (Sigma, USA) was drawn by measuring the diameters of inhibition zones caused by nisin standard solution. Accordingly, nisin titer of samples was calculated from this curve. Measurements of all samples were performed in triplicate using three different plates.

Statistical analysis

The Minitab 17.0 (Minitab Inc, USA) was employed for the experimental designs and subsequent regression analysis of the experimental data obtained [23]. The quality of the regression equations was judged statistically by the coefficient of determination R^2, and corresponding statistical significance was determined by a t-test. A value of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Plackett-Burman design

In this step, eight components were defined as different variables, and the concentration for each variable was appropriately enlarged as the ranges in Table 1. Subsequently, 12 experiments were designed to screen the significant factors of eight variables for nisin titer, and the results of the 2-level PBD was illustrated in Table 2, which indicated that there was a wide variation of nisin titer from 1910 to 2906 IUml-1with the different levels of the components in the media.

Table 1: The coded values and levels of defined variables for Plackett-Burman design.

Factors	Coded Variables	Levels (g l-1)	
		Low Level (-1)	High Level (+1)
Sucrose	X1	15	20
Yeast extract	X2	10	15
corn steep powder	X3	10	15
KH_2PO_4	X4	5	7.5
$MgSO_4$	X5	0.2	0.3
NaCl	X6	2	3
$CaCO_3$	X7	4	5
Tween-80	X8	2	3

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Table 2: The design matrix and experimental results of Plackett-Burman design with nisin titer as response value (Y).

Run	X1	X2	X3	X4	X5	X6	X7	X8	Y (IU ml-1)
1	1	-1	1	-1	-1	-1	1	1	2586
2	1	1	-1	1	-1	-1	-1	1	2906
3	-1	1	1	-1	1	-1	-1	-1	2117
4	1	-1	1	1	-1	1	-1	-1	2212
5	1	1	-1	1	1	-1	1	-1	2782
6	1	1	1	-1	1	1	-1	1	2530
7	-1	1	1	1	-1	1	1	-1	1910
8	-1	-1	1	1	1	-1	1	1	2398
9	-1	-1	-1	1	1	1	-1	1	2456
10	1	-1	-1	-1	1	1	1	-1	2803
11	-1	1	-1	-1	-1	1	1	1	2564
12	-1	-1	-1	-1	-1	-1	-1	-1	2536

The regression analysis of the PBD of eight factors shown in Table 3 exhibited thatX1 (sucrose, P = 0.007), X3 (CSLP, P = 0.004) and X8 (Tween-80, P = 0.03) had the greatest impacts on nisin production at the probability level of 95%. The concentration of sucrose strongly affected nisin production, and the high level of sucrose (2.25%, w/v) allowed the strain to produce a greater nisin titer than the low level of sucrose (1.5%, w/v). It has been considered that sucrose with carbon source have an important influence on nisin biosynthesis due to the genetic linkage between sucrose metabolism and nisin production on the chromosome, and the regulation of carbon metabolism appeared to be a major control mechanism for nisin biosynthesis [13]. Similarly, the high level of X8 (Tween-80) (0.3%, w/v) was more beneficial tonisin synthesis. Huot [28] proved that Tween-80 supply could suppress bacteriocin cell adhesion, which was of vital importance for nisin production.

Table 3: The regression analysis of Plackett-Burman design.

Factors	Effect Value	Coefficient	Standard Error	T-Value	P-Value
Model		2482.5	22.94	108.21	0.000***
X1	304.7	152.3	22.94	6.64	0.007***
X2	-32	-16	22.94	-0.7	0.536
X3	-380.7	-190.3	22.94	-8.3	0.004***
X4	-80.3	-40.2	22.94	-1.75	0.178
X5	63.7	31.8	22.94	1.39	0.259
X6	-140	-70	22.94	-3.05	0.055*
X7	49.3	24.7	22.94	1.08	0.361
X8	178.3	89.2	22.94	3.89	0.03**

Asterisk (*) indicates the significance level; R2 = 97.96%, R2 (adj) = 92.52%

In addition, CSLP as a nutritious organic nitrogen source is favorable for bacterial growth and its product accumulation, but nisin production decreased with the enhanced concentration of CSLP in this study, which perhaps due to excessive CSLP would inhibit nisin biosynthesis. Furthermore, the t-value indicated that X5 ($MgSO_4 \bullet 7H_2O$) and X7($CaCO_3$) had positive effects on nisin production and set at their high levels, whereas X2 (yeast extract), X4 (K_2HPO_4) and X6 (NaCl) had negative effects and set at their low levels. The regression coefficients were calculated and a fitted equation was obtained as follow:

Y=2482.5+152.3X1-16X2-190.3X3-40.2X4+31.8X5 - 70X6+24.7X7+89.2X8(1), where Y is nisin titer as response value. The coefficient of determination $R^2$of the model was calculated to be 0.9796, which means that the model could explain 97.96% of the variation in the results.

Moreover, the maximal effect was also presented in the upper portion and then decreased progressively to the minimal effect in the Pareto chart (Figure 2), which also showed that the most important factors determining nisin titer were X1 (sucrose), X3 (CSLP) and X8 (Tween-80) at the probability level of 95% (P<0.05). Yet, although sucrose, CSLP and Tween-80 were recognized as three significant factors based on the levels of CSLP-M medium, the optimal level of each significant factor was still unknown at this stage. Thus, they need to be determined by the following optimization experiments.

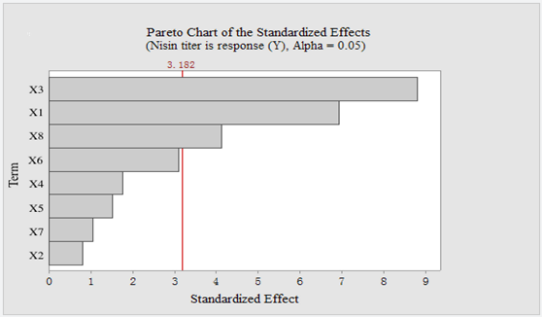

Figure 2: Pareto chart for the standardised main effects on nisin titer in the Box-Behnken design.

Steepest ascent design

Table 4: The design matrix and experimental results of steepest ascent experiment with nisin titer as response value (Y).

Run	X1 (gl-1)	X3 (gl-1)	X8 (gl-1)	y (IU ml-1)
1	15	15	2	2547
2	16.5	13	2.2	2634
3	18	11	2.4	2750
4	19.5	9	2.6	2891
5	21	7	2.8	2786
6	22.5	5	3	2709

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In this section, the starting points of the path chosen for experimental design were 1.5% X1 (sucrose, w/v), 0.2% X8 (Tween-80, w/v) and 1.5% X3 (CSLP, w/v) according to the results of PBD, and five equally spaced points along the path were then selected by increasing the concentrations of sucrose and Tween-80 and decreasing the concentration of CSLP. The directions of changing the three variables and corresponding nisin titer obtained in these experiments are summarized in Table 4. It is clearly observed that the peak of nisin titer was reached at Run 4, which indicated that the response value approached the neighborhood of the optimum medium, and this medium was then chosen for the next optimization.

Response surface methodology

The central point's chosen from the above steepest ascent experiment for experimental design were1.95% sucrose (w/v), 0.26% Tween-80 (w/v) and 0.9% CSLP (w/v). The BBD and the corresponding experimental responses were listed in Table 5 and Table 6, respectively. Regression analysis was showed in Table 7 and a second-order polynomial equation fitting the response function was obtained:

Table 5: The coded values and levels of defined variables for Box-Behnken design.

Factors		Levels (g l^{-1})	
	Low Level (-1)	Central Point (0)	High Level (+1)
X1	18	19.5	21
X3	7	9	11
X8	2.2	2.6	3

Table 6: The design matrix and experimental results of Box-Behnken design with nisin titer as response value (Y).

Run	X1(gl^{-1})	X3(gl^{-1})	X8(gl^{-1})	Y (IU ml^{-1})
1	-1	-1	0	2559
2	1	-1	0	2687
3	-1	1	0	2622
4	1	1	0	2623
5	-1	0	-1	2526
6	1	0	-1	2504
7	-1	0	1	2636
8	1	0	1	2743
9	0	-1	-1	2466
10	0	1	-1	2549
11	0	-1	1	2811
12	0	1	1	2782
13	0	0	0	2889
14	0	0	0	2914
15	0	0	0	2855

Table 7: The regression analysis of Box-Behnken design.

Term	Coefficient	Standard Error	t-value	P-value
model	2886	24.4	118.286	0
X1	26.75	14.94	1.79	0.133
X3	6.63	14.94	0.443	0.676
X8	115.88	14.94	7.756	0.001***
X1*X1	-156.5	21.99	-7.116	0.001***
X3*X3	-106.75	21.99	-4.854	0.005***
X8*X8	-127.25	21.99	-5.786	0.002***
X1*X3	-31.75	21.13	-1.503	0.193
X1*X8	32.25	21.13	1.526	0.187
X3*X8	-28	21.13	-1.325	0.242

Asterisk (*) indicates the significance level

R2 = 97.04%, R2(adj) = 91.70%

$$Y=2886 +26.75X1+6.63X3+115.88X8-156.5X1^2-106.75X3^2-127.25X8^2-31.75X1X3+32.25X1X8-28X3X8 \quad (2)$$

In this model, the t-test and P-values were used to identify the effect of each factor on nisin titer. The terms, X8, $X1^2$, $X3^2$and $X8^2$, had significant effects on nisin titer ($P < 0.05$), while the other terms showed a less obvious influence on nisin titer ($P > 0.05$). The fitness of the regression equation was examined by the coefficient of determination R^2, which was calculated to be 0.9704, indicating that 97.04% of the variability in the responses could be explained by the model. Also the adjusted determination coefficient (Adj R^2 = 91.70%) was very high to support a very reliable correlation of the model. Hence, it was reasonable to use the regression model to analyze the variables for the experimental responses.

The effects of sucrose, CSLP and Tween-80 on nisin production were also investigated by the 3-dimensional response surface. In Figure 3, the curves with obvious convexity of the sucrose, CSLP and Tween-80 against nisin titer could explain the results of the statistical analyses. It could be calculated from the regression equation (3) and the curves that nisin production reached its maximum at a combination of the coded levels 0.140 (X1), -0.053 (X3), and 0.479 (X8). Accordingly, the fitted model predicted a maximum response value of nisin titer (2916 $IUml^{-1}$) at the optimal concentrations of 1.97% (w/v) sucrose, 0.89% (w/v) CSLP, 1% (w/v) yeast extract, 0.5% (w/v) KH_2PO_4, 0.2% (w/v) NaCl, 0.03% (w/v) $MgSO4•7H_2O$, 0.5% (w/v) $CaCO_3$ and 0.28% (w/v) Tween-80.

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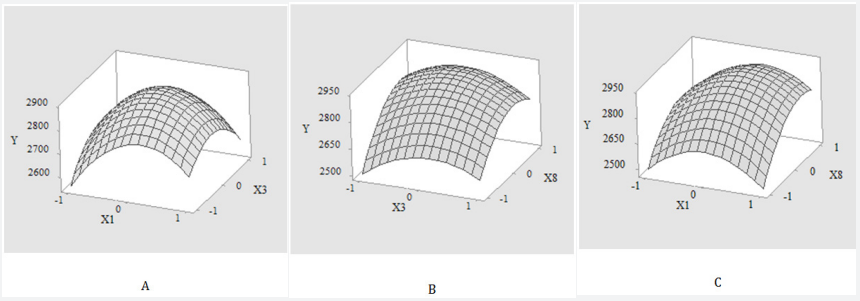

Figure 3: Response surface plots of the interactions of X1 (sucrose) and X3 (CSLP) (A), X1 (sucrose) and X8(Tween-80); (B), X3 (CSLP) and X8(Tween-80); (C) on nisin production (Y). The coded values of the independent variables were obtained according to the equation (1): X1 = (x1 – 19.5) / 1.5; X3 = (x3 – 9) / 2; X8 = (x8 – 2.6) / 0.4.

Validation of the optimal medium

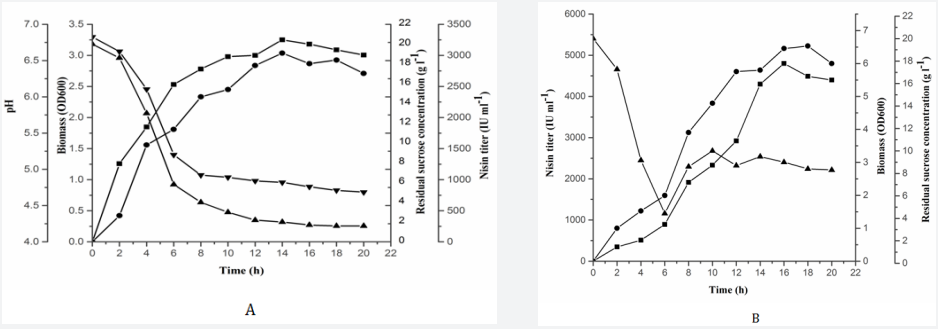

Figure 4: Kinetics analysis of nisin production by *L. lactis* E15 in batch fermentation system using the optimal medium (A) and in fed-batch fermentation system using the optimal medium and constant pH (6.5); (B). The symbols were used: pH (*solid inverted triangle*), Biomass (OD600) (*solid square*), Residual sucrose concentration (*solid triangle*), and nisin titer (*solid circle*).

In order to confirm the validity of the model equation for predicting maximum nisin production, the validation experiment was performed in triplicate under the optimal condition for nisin production by batch fermentation. The observed experimental data was illustrated in Figure 4A. The highest average nisin yield of 3037IU $ml^{-1}$was obtained at 14 h, which was in good agreement with the model predicted maximum value of 2916 IU ml^{-1} within the range of the permitted errors ($P > 0.05$) and almost increased by 52% compared with that in the initial medium (1992IU ml^{-1}, Figure 1). This result confirmed that the optimized medium favored the production of nisin.

Simultaneously, the kinetics analysis showed that the consumption of sucrose, production of nisin and lactic acid in the lag phase (0-2h) were very slow, while in exponential growth phase, the nisin titer and OD600 increased quickly and reached a maximum value of 3036.59IU ml^{-1} and 3.0 at 14h, respectively. And the sucrose was rapidly consumed and decreased to a low level of 2g l^{-1} after 14h. It has been reported that nisin production shows primary metabolite kinetics and the maximum nisin production is related to biomass formation of the producers [13,16,29]. However, the batch fermentation produced a lower biomass after 14 h, which might be attributed to a low sucrose level and acid inhibition (low pH stress) accumulated by lactic acid. The effect of pH on the growth and nisin production of lactic acid bacteria was well investigated [16,30], so pH regulation of nisin biosynthesis must be taken into consideration. And the peak value of nisin titer dropped significantly after 14h, this might be due to the proteolytic inactivation, protein aggregation and the adsorption of nisin molecules to the cell surface of the producer cells [31,32].

Fed-batch fermentations

Additionally, different results of the fed-batch fermentation in 1.0-L fermenter by the above strategy described in Materials and Methods were shown in Figure 4B. The biomass curve exhibited a greater biomass and a longer exponential growth phase compared with that in the batch fermentation, and the cell growth (OD600) reached its maximum of approximately 6.0 at 16 h. The production of nisin raised with the rapid growth of the producer cells during the exponential growth phase by manual sucrose control, and the maximum nisin titer of 5370IU ml^{-1} was obtained after 16 h. But, the cell growth and sucrose consumption appeared to be terminated owing to the end-product inhibition. Thus, compared with the batch culture, a greater biomass and a longer exponential growth phase caused by the fed-batch fermentation strategy combined sucrose control and constant pH could favor the cell growth and accumulation of nisin under the optimal medium condition [33-35].

Conclusion

In this study, the modified medium containing CSLP instead of peptone as nitrogen source was positively investigated

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for further optimization through the statistically designed optimization. We obtained the final optimal medium for nisin production composed of 1.97% (w/v) sucrose, 0.89% (w/v) CSLP, 1% (w/v) yeast extract, 0.5% (w/v) KH_2PO_4, 0.2% (w/v) NaCl, 0.03% (w/v) $MgSO_4$•7H2O, 0.5% (w/v) $CaCO_3$ and 0.28% (w/v) Tween-80 (X3). The yield of nisin produced by *L. lactis* E15 in batch fermentation system at 14 h was increased from an average of 1992IU ml^{-1} to 3037IU ml^{-1}, and the optimized condition was used for constant pH fermentation in fed-batch fermentation system, where a maximum activity of 5370IU ml^{-1} was achieved at pH 6.5 after 16 h. Compared with the initial medium, the substitution of CSLP as nitrogen source successfully produces an optimized medium for nisin production by *L. lactis* E15, which might reduce the fermentation cost. Therefore, this study provides a potential strategy for increasing nisin yield in large-scale industrial production.

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