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Medium Optimization Based on Statistical Methodologies for Pristinamycins Production by *Streptomyces pristinaespiralis*

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Abstract The optimization of nutrient levels for the production of pristinamycins by *Streptomyces pristinaespiralis* CGMCC 0957 in submerged fermentation was carried out using the statistical methodologies based on the Plackett–Burman design, the steepest ascent method, and the central composite design (CCD). First, the Plackett–Burman design was applied to evaluate the influence of related nutrients in the medium. Soluble starch and $MgSO_4 \cdot 7H_2O$ were then identified as the most significant nutrients with a confidence level of 99%. Subsequently, the concentrations of the two nutrients were further optimized using response surface methodology of CCD, together with the steepest ascent method. Accordingly, a second-order polynomial regression model was finally fitted to the experimental data. By solving the regression equation from the model and analyzing the response surface, the optimal levels for soluble starch and $MgSO_4 \cdot 7H_2O$ were determined as 20.95 and 5.67g/L, respectively. Under the optimized medium, the yield of pristinamycins in the shake flask and 5-L bioreactor could reach 1.30 and 1.01g/L, respectively, which is the highest yield reported in literature to date.

Keywords *Streptomyces pristinaespiralis* · Pristinamycins · Production medium · Optimization · Response surface methodology

Introduction

Antibiotics, dramatically lowering death and disability from infectious diseases, exert a great influence on the health care of our society. Unfortunately, the irrational use of

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antibiotics caused a rise of antimicrobial resistance, and now, it has become a very serious global public health problem [1]. Therefore, there is a growing demand for more specific and perspective antibiotics taking the multiresistant pathogens as a target.

Pristinamycins, a kind of such specific antibiotics produced by *Streptomyces pristinaespiralis*, were first discovered in France in 1962 [2, 3]. As a member of the streptogramin family of antibiotics, pristinamycins consist of two main components, pristinamycinIA and pristinamycinIIA, where the former is a group of B streptogramin (a depsipeptide) and the latter is a group of A streptogramin (a polyunsaturated macrolactone) [3]. These two components of pristinamycins synergicly interfere with the protein synthesis process of 70S bacterial ribosomes, which render the antibiotic potent activity against various pathogens, especially methicillin-, penicillin-, and vancomycin-resistant bacteria [4, 5]. Moreover, pristinamycins exhibit a prolonged postantibiotic effect [6, 7], so there has been an ever increasing interest of pristinamycins production in terms of both the strain improvement and the fermentation process. In the recent decades, cloning and analysis of genes involved in pristinamycins synthesis has become the focal point [8–10]. Whereas, in the studies on fermentation process, research teams have mainly focused on the metabolic characteristics of carbon and nitrogen sources and the induction effect of some lactones [11–14]. However, there are very few reports aimed at the regulation and optimization for pristinamycins fermentation. It was reported that under conventional batch conditions, the final yield of pristinamycins was just about 100mg/L with the buffered synthetic medium even by a spontaneous mutant [14].

It is well known that designing an appropriate fermentation medium is of critical importance in the production of secondary metabolites [15]. Prior knowledge and experience in developing a suitable basal medium may play an important role in further medium optimization. Previous studies of our team found that the mixed carbon sources and the mixed nitrogen sources helped increase pristinamycins production [16]. Nevertheless, systematical studies on medium optimization for pristinamycins production by *S. pristinaespiralis* have not been made so far.

The conventional method for optimization by the "one-variable-at-a-time" approach (OVAT) not only is laborious and time consuming but also can lead to the misinterpretation of results because the interaction between different factors is not taken into account. An alternative and more efficient procedure is the use of statistical methodologies. Statistical experimental methodologies are very useful tools for the selection of nutrients, as they can provide statistical models that help in understanding the interactions among the variables at varying levels and calculating the optimal level of each variable for a given target [17, 18]. For example, the Plackett–Burman design, the steepest ascent method, and response surface methodology (RSM) are often used as a whole strategy for various optimization processes in biotechnology fields [19–22]. Among these statistical methodologies, RSM is a powerful and most common technique for testing multiple variables and assessing the individual effect and interactions of these variables in a small number of experimental trials [23–25]. Although RSM has been increasingly used in fermentation, only few reports [26] focused on optimization of medium nutrients or fermentation process parameters for antibiotic production by this technique.

In this paper, RSM, the Plackett–Burman design, and the steepest ascent method, which are useful statistical methodologies, were adopted to optimize the medium for maximizing the production of this valuable antibiotic, viz. pristinamycins.

Materials and Methods

Microorganism and Culture Method

S. pristinaespiralis CGMCC 0957 was preserved as a frozen spore suspension in 20% glycerol at −70 °C. One milliliter of spore suspension was inoculated on agar medium for sporulation. The spore culture, as well as the seed culture, was prepared as in previous literature [27]. Then, 6% (v/v) of the seed culture was inoculated into a 250-mL flask containing 25mL production medium based on the experimental designs. The fermentation was carried out at 28 °C on a rotary shaker at 220rpm for 65–70h. All experiments were carried out in triplicate, and the average yield of pristinamycins was given in each table. Scale-up studies were conducted at a 5-L bioreactor (KBT, Korea) with 3.5L working volume. The operating conditions were maintained at 28 °C for 72h with an aeration rate of 1VVM and stirrer speed of 300rpm at beginning. The dissolved oxygen level was maintained above the minimum of 30% air saturation during the fermentation process by adjusting the stirrer speed to 300–600rpm accordingly.

Analytical Procedures

To determine the yield of pristinamycins, the broth in the shake flask was extracted with acetone and analyzed by high-performance liquid chromatography [28]. The broth in the bioreactor was periodically collected and centrifuged at 2,500 × *g* for 15min to obtain the mycelia and the supernatant. The mycelia were then washed twice in deionized water and dried to constant weight. The supernatant was simultaneously analyzed for the reducing sugar and amino nitrogen concentrations by the dinitrosalicylic acid method and the formaldehyde titration method, respectively. The pH value was monitored by the digital pH electrode (Hamilton) on line.

Experimental Design and Data Analysis

Plackett–Burman Design

The Plackett–Burman design [29], useful for picking the one or two most important factors by screening up to $N - 1$ factors with N experiments, is efficient for medium optimization [30, 31]. The Design Expert software (Version 7.0.0, Stat-Ease, Minneapolis, USA) was used for the experimental design and the analysis of variance (ANOVA) for the data. The statistical confidence level above 95% was accepted in this experiment. The variables with less significant effect (confidence level less than 95%) were not included in the optimization experiments thereafter but instead were used in all trials at their low levels in consideration of lowering cost.

The Steepest Ascent Method

The method of steepest ascent is a procedure for moving sequentially along the path of steepest ascent to the region of the optimum, that is, in the direction of the maximum

increase in the response [32]. Based on the results obtained from the Plackett–Burman design, the fitted first-order model is given as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i \tag{1}$$

where Y is the predicted response, β_0 is the constant coefficient, β_i is the linear coefficient, and X_i is the coded independent variable. The direction of steepest ascent was the direction in which Y increased most rapidly. The zero level of selected variables in the Plackett–Burman design was identified as the base point of the steepest ascent path. The steps along the path were determined by the estimated coefficient ratio from Eq. 1, together with the practical experience. Once the path of steepest ascent no longer led to an increase, this procedure should be discontinued in favor of a more elaborate experiment. Thus, the steepest ascent method allowed coming closer to the optimal level and locating a new experimental region.

Central Composite Design

The central composite design (CCD), a response surface design, contains an imbedded factorial or fractional factorial matrix with center points and star points to allow estimation of the curvature [33]. For the two factors, this design is made up of a full 2^2 factorial design with its four cube points, augmented with five replications of the center points (all factors at zero level) and the four star points, that is, points with one factor having an axial distance to the center of $\pm\alpha$, whereas the other factor at zero level. If the factorial is a full factorial, then $\alpha = \left[2^k\right]^{1/4}$ (k is the number of the factors). In this experiment, there were two factors ($k = 2$), so the axial distance α was chosen to be 1.414 to make this design orthogonal. The Design Expert software (Version 7.0.0, Stat-Ease) was also used here for experimental design and regression analysis. The observed data are fitted into the second-order polynomial equation by multiple regression procedure:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \tag{2}$$

where Y is the predicted response, β_0 is the intercept coefficient, β_i is the linear coefficient, β_{ii} is the squared coefficient, β_{ij} is the interaction coefficient, and X_i and X_j are the coded independent variables for statistical calculations according to the following equation:

$$X = (U - U_0)/\Delta U \tag{3}$$

where X is the coded value of the independent variable, U is the real value of the independent variable, U_0 is the real value of the independent variable on the center point, and ΔU is the step change value. The optimal levels were obtained by solving the regression in Eq. 2 and also by analyzing the response surface plot.

Results and Discussion

Screening Design of Important Medium Nutrients

Based on our previous results of the OVAT approach [16], a total of ten medium nutrients were selected to test at two levels, a high (+) and a low (−) level, as shown in Table 1. In the

Table 1 Medium nutrients for screening by using the Plackett–Burman design.

Nutrient code	Nutrient	Levels	
		Low (−)	High (+)
A	Soluble starch	40[a]	60
B	Glucose	10	15
D	Soybean flour	25.0	37.5
E	Peptone	5.0	7.5
G	Zein flour	10	15
H	Yeast extract	3.0	4.5
K	$(NH_4)_2SO_4$	1.50	2.25
L	$MgSO_4{\cdot}7H_2O$	3.50	5.25
N	KH_2PO_4	0.2	0.3
O	$NaNO_3$	0.750	1.125

[a] The data were the concentration (g/L) of different nutrients in the medium.

table, 15 variables, ten for the nutrient variables and five for the dummy variables D_1–D_5, were introduced to fill out the 15 × 16 Plackett–Burman design matrix (Table 2). Dummy variables were used as the measure of variability for the Plackett–Burman design. No changes were made to these dummy variables, and they did not influence the outcome of the experiment.

The ANOVA for the experimental designs showed that soluble starch and $MgSO_4{\cdot}7H_2O$ in the tested range had a confidence level above 99%, and other nutrients had a confidence level less than 95% (Table 3). Therefore, soluble starch and $MgSO_4{\cdot}7H_2O$ were both considered to be the significant nutrients for pristinamycins production, and other nutrients may play a minor role in pristinamycins production.

Table 2 Experimental results using the Plackett–Burman design of 16 trials with yield of pristinamycins as response.

Trial	A	B	D_1[a]	D	E	D_2	G	H	D_3	K	L	D_4	N	O	D_5	Yield (g/L)
1	+1	−1	−1	+1	+1	−1	−1	+1	+1	−1	−1	+1	+1	−1	−1	0.36
2	−1	+1	−1	+1	+1	−1	+1	−1	+1	−1	+1	−1	−1	+1	−1	0.77
3	+1	−1	+1	−1	−1	+1	−1	+1	+1	−1	+1	−1	−1	+1	−1	0.41
4	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	0.44
5	−1	−1	+1	−1	+1	−1	+1	+1	−1	+1	−1	+1	−1	+1	−1	0.65
6	−1	+1	+1	+1	−1	−1	−1	+1	−1	−1	−1	−1	+1	+1	+1	0.52
7	−1	+1	+1	−1	−1	+1	+1	−1	+1	−1	−1	+1	+1	−1	−1	0.59
8	+1	+1	−1	+1	−1	+1	−1	−1	−1	+1	−1	+1	−1	+1	−1	0.30
9	−1	−1	+1	+1	+1	+1	−1	−1	+1	+1	−1	−1	−1	−1	+1	0.68
10	+1	+1	−1	−1	−1	−1	+1	+1	+1	+1	−1	−1	−1	−1	+1	0.36
11	+1	+1	+1	−1	+1	−1	−1	−1	−1	+1	+1	−1	+1	−1	−1	0.40
12	−1	−1	−1	+1	−1	+1	+1	+1	−1	+1	+1	−1	+1	−1	−1	1.10
13	+1	−1	+1	+1	−1	−1	+1	−1	−1	−1	+1	+1	−1	−1	+1	0.54
14	−1	+1	−1	−1	+1	+1	−1	+1	−1	−1	+1	+1	−1	−1	+1	0.84
15	+1	−1	−1	−1	+1	+1	+1	−1	−1	−1	−1	−1	+1	+1	+1	0.32
16	−1	−1	−1	−1	−1	−1	−1	−1	+1	+1	+1	+1	+1	+1	+1	0.99

[a] D_1–D_5 were dummy variables; other symbols were the same as those in Table 1.

Table 3 Identifying the significant nutrients for pristinamycins production using the Plackett–Burman design.

Source	Sum of squares	Degree of freedom	Mean square	*F* value	Prob>*F*
Model	0.840	10	0.084	9.28	0.0120*
Soluble starch (A)	0.570	1	0.570	62.83	0.0005**
Glucose (B)	0.043	1	0.043	4.77	0.0807
Soybean flour (D)	1.122E−03	1	1.122E−03	0.12	0.7387
Peptone (E)	7.482E−03	1	7.482E−03	0.83	0.4043
Zein flour (G)	4.290E−03	1	4.290E−03	0.48	0.5212
Yeast extract (H)	5.290E−04	1	5.290E−04	0.06	0.8183
$(NH_4)_2SO_4$ (K)	0.020	1	0.020	2.19	0.1992
$MgSO_4{\cdot}7H_2O$ (L)	0.180	1	0.180	19.83	0.0067**
KH_2PO_4 (N)	1.640E−03	1	1.640E−03	0.18	0.6876
$NaNO_3$ (O)	0.013	1	0.013	1.49	0.2765
Residual	0.045	5	9.024E−03		
Total	0.880	15			

*Statistically significant at 95% of probability level ($P<0.05$)

**Statistically significant at 99% of probability level ($P<0.01$)

To approach the vicinity of the optimization, a first-order model as a function of these ten nutrient variables in terms of coded variables is given below:

$$\begin{aligned}\text{Pristinamycins}(\text{g/L}) = {} & 0.580 - 0.190 \times A - 0.052 \times B + 8.375\text{E} - 03 \times D - 0.022 \\ & \times E + 0.016 \times G + 5.750\text{E} - 03 \times H + 0.035 \times K + 0.110 \\ & \times L + 0.010 \times N - 0.029 \times O \end{aligned} \quad (4)$$

The coefficient of each variable in Eq. 4 represents the strength of the effect of this variable on pristinamycins production. The coefficient of determination (R^2) of the model was 0.9489, which indicated that only about 5.11% of the total variations were not explained by the model.

Equation 4 again showed that *L* ($MgSO_4{\cdot}7H_2O$) and *A* (soluble starch) variables played the most important effect. Positive coefficient for *L* ($MgSO_4{\cdot}7H_2O$) indicated a linear effect to increase pristinamycins production, which could be explained by the capacity of Mg^{++} to activate some enzymes and to promote the antibiotics bound on the mycelia to release into the broth [34]. While negative coefficient for *A* (soluble starch) was observed to decrease pristinamycins production in a linear effect, which could be explained by the catabolite repression from higher soluble starch concentration.

The Steepest Ascent Method

The first-order model, Eq. 4, was used to approximate the true response surface in a small region of the tested range. The experiments were started from the zero level in the Plackett–Burman design. To move away from the base point (no. 1 in Table 4) along the path of the steepest ascent, we move −4 and +0.2g/L in soluble starch and $MgSO_4{\cdot}7H_2O$ directions, respectively. The design and results of the experiments are shown in Table 4. The yield of pristinamycins increased along the path from nos. 1 to 7 reaching the peak of 1.22g/L and then decreased from nos. 7 to 9, indicating the optimal level was close to that in no. 7. In this experiment, the yield of pristinamycins had remarkably improved, implying the

Table 4 Experimental results along the path of the steepest ascent.

	Soluble starch (g/L)	$MgSO_4 \cdot 7H_2O$ (g/L)	Pristinamycins (g/L)
(1) Base point (zero level in the Plackett–Burman design)	50	4.4	
(2) Origin step unit (concentration range of unity level)	10	0.88	
(3) Slope (estimated coefficient ratio from Eq. 4)	−0.19	+0.11	
(4) Corresponding concentration range (2)×(3)	−1.9	+0.0968	
(5) Step unit (4)×2[a]	−3.8	+0.1936	
(6) New step unit used in this method	−4	+0.2	
(7) Experiment			
No. 1	50	4.4	0.64
No. 2	46	4.6	0.69
No. 3	42	4.8	0.77
No. 4	38	5.0	0.85
No. 5	34	5.2	0.98
No. 6	28	5.4	1.04
No. 7	24	5.6	1.22
No. 8	20	5.8	1.17
No. 9	16	6.0	0.80

[a] 2 was a factor determined by experiment based on process knowledge and was appropriate in this experiment.

steepest ascent method was an effective technique for approaching the optimal level. Nevertheless, the optimum values of the two variables are still unknown and need to be determined by the subsequent CCD.

Central Composite Design

Experiments were carried out to arrive at an optimal combination of soluble starch and $MgSO_4 \cdot 7H_2O$ by a CCD with five different coded levels (Table 5). The concentrations of the two nutrients in no. 7 from the steepest ascent method were taken as a center point, which was considered as zero. The full experimental design, along with the observed and predicted responses, is presented in Table 6. By applying multiple regression analysis on the observed data, a second-order polynomial model as a function of soluble starch (X_1) and $MgSO_4 \cdot 7H_2O$ (X_2) in terms of coded forms is given below:

$$\text{Pristinamycins(g/L)} = 1.220 - 0.190 \times X_1 + 0.100 \times X_2 + 0.017 \times X_1 \times X_2 - 0.110 \times X_1^2 - 0.088 \times X_2^2 \quad (5)$$

The ANOVA for the model (Table 7) demonstrated that Eq. 5 was a highly significant model, as was shown from the F test with a very low probability value of 0.0020. Among

Table 5 Levels and codes of the variables for central composite design.

Independent variable	Coded levels of variable				
	−1.41	−1	0	+1	+1.41
Soluble starch (g/L)	18.3	20	24	28	29.7
$MgSO_4 \cdot 7H_2O$ (g/L)	5.32	5.4	5.6	5.8	5.88

Table 6 Experimental results of central composite design.

Run	Soluble starch	$MgSO_4 \cdot 7H_2O$	Pristinamycins (g/L)	
			Observed	Predicted
1	−1	−1	1.19	1.13
2	−1	+1	1.20	1.29
3	+1	−1	0.78	0.71
4	+1	+1	0.86	0.94
5	−1.41	0	1.28	1.26
6	+1.41	0	0.72	0.72
7	0	−1.41	0.80	0.91
8	0	+1.41	1.31	1.19
9	0	0	1.22	1.22
10	0	0	1.20	1.22
11	0	0	1.23	1.22
12	0	0	1.21	1.22
13	0	0	1.23	1.22

model terms, X_1, X_2, X_1^2, and X_2^2 were all significant with a probability of no less than 95%. The interaction between X_1 and X_2, however, had no significant influence on pristinamycins production because of the value of "X_1 X_2 Prob > F" more than 0.5. The goodness of a model can be checked by the determination coefficient (R^2) and the adjusted determination coefficient (Adj R^2). In this model, R^2 was 0.9024, implying that the sample variation of 90.24% for pristinamycins production was attributed to the independent variables. The value of Adj R^2 was 0.8327, so it was also high to advocate for the significance of the model. "Adequate precision" measures the signal to noise ratio. And a ratio greater than 4 is desirable. In this case, the value of adequate precision was 9.770, indicating an adequate signal. At the same time, a relatively lower value of the coefficient of variation (8.06) indicated a better precision and reliability of the experiments carried out. The close agreement between the experimental results and the theoretical values predicted by the model equation (Table 6) indicates it is reasonable to use the regression model to analyze the trends in the responses.

Table 7 Analysis of variance for the experimental results of central composite design.

Source	Effect	Standard Error	Sum of squares	Degree of freedom	Mean square	F value	Prob>F
X_1—soluble starch	−0.190	0.031	0.300	1	0.300	38.05	0.0005**
X_2—$MgSO_4 \cdot 7H_2O$	0.100	0.031	0.080	1	0.080	10.27	0.0150*
X_1 X_2	0.017	0.044	1.122E−03	1	1.122E−03	0.14	0.7155
X_1^2	−0.110	0.033	0.090	1	0.090	11.56	0.0114*
X_2^2	−0.088	0.033	0.052	1	0.052	6.65	0.0366*
Model			0.500	5	0.100	12.94	0.0020**
Residual			0.055	7	7.790E−03		
Total			0.560	12			

*Statistically significant at 95% of probability level (P<0.05)

**Statistically significant at 99% of probability level (P<0.01)

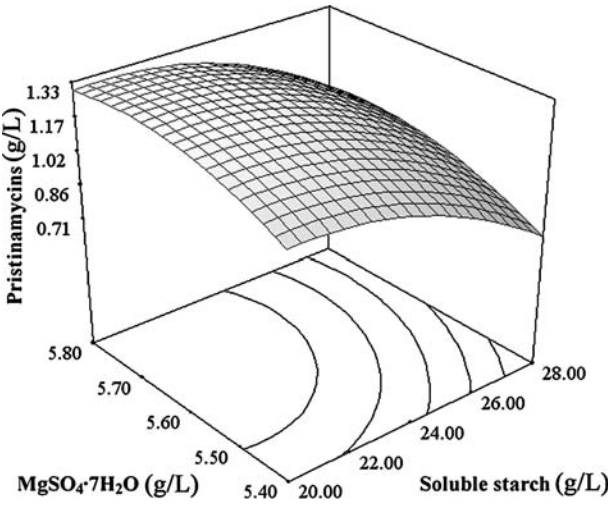

Fig. 1 Response surface plot of pristinamycins production expressed as a function of soluble starch vs $MgSO_4 \cdot 7H_2O$

The response surface showing the effect of soluble starch and $MgSO_4 \cdot 7H_2O$ is presented in Fig. 1. It is evident that decreasing the concentration of soluble starch and increasing the concentration of $MgSO_4 \cdot 7H_2O$ could enhance the yield of pristinamycins. Figure 1 indicates that the maximum yield could be obtained in the range of soluble starch, 20–22g/L, and $MgSO_4 \cdot 7H_2O$, 5.7–5.8g/L. The estimated surface does not have a unique optimum in this case, and from canonical and ridge analyses of the regression model, the predicted maximum yield of 1.32g/L could be obtained under several candidate levels provided by the software. In consideration of some economic factors, we finally set the level of X_1 (soluble starch) to be 20.95g/L and X_2 ($MgSO_4 \cdot 7H_2O$) to be 5.67g/L, respectively.

To verify the predicted result, the experiment was performed under the optimal levels of soluble starch and $MgSO_4 \cdot 7H_2O$ stated above, and the final yield of pristinamycins reached 1.30 ± 0.04g/L (n = 3), which was coincident with the predicted value, indicating that the model was adequate to predict the yield of pristinamycins. The average yield of 1.30g/L obtained under the optimized medium in submerged fermentation by *S. pristinaespiralis* was about twice that before optimization (0.60–0.62g/L), and it is the highest yield reported in literature to date.

Time Course of Pristinamycins Fermentation

After optimization of medium components, scale-up studies were carried out in a 5-L laboratory bioreactor. The time course of pristinamycins fermentation under the optimized medium by *S. pristinaespiralis* CGMCC 0957 is plotted in Fig. 2. The dried cell weight increased rapidly in the first 38 h of cultivation because of biomass formation, and it maintained 27–29 g/L from 38 to 58 h, then decreased sharply after 58 h. Compared with the previous report [28], the stationary phase was longer, which was beneficial for antibiotic production. The production of pristinamycins suddenly increased after 35 h of cultivation. The maximum yield of 1.01 g/L in the bioreactor was attained in 49 h as compared to that of 65–70 h in shake flasks. A similar reduction in fermentation time in the bioreactor was also observed during the fermentation of some enzymes [25]. Therefore, it is clearly suggested that pristinamycins production was not associated with the mycelia growth, and it

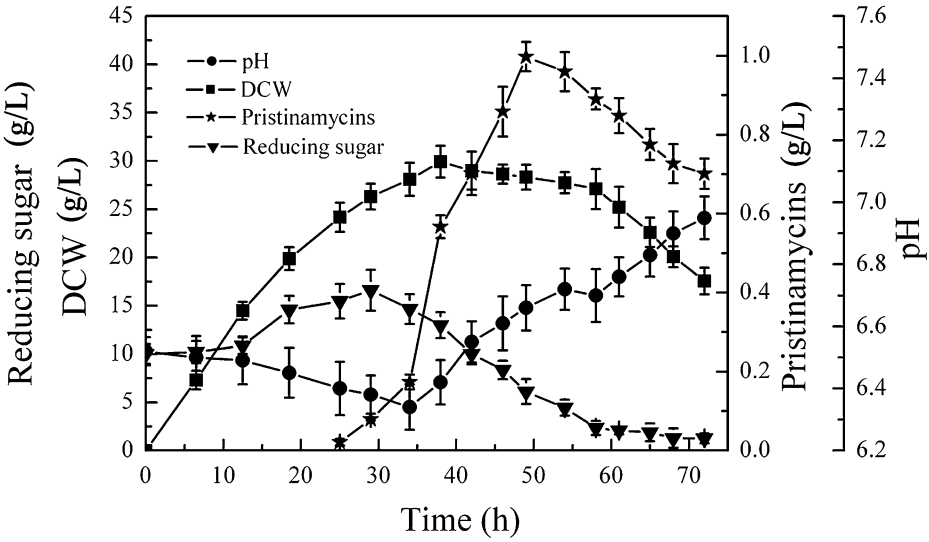

Fig. 2 Time course of pristinamycins fermentation in a 5-L bioreactor under the optimized medium by *Streptomyces pristinaespiralis* CGMCC 0957. *Error bars* were given showing standard deviations for $n=3$

might be a typical example of a growth-disassociated product. On the other hand, because of the degradation of pritinamycins by enzymes, a limitation of the antibiotic production was observed after reaching the maximum, which was in agreement with the previous report [35]. The reducing sugar concentration slowly increased in the first 29 h and decreased thereafter until to 1.29 g/L. This could be attributed to the gradual release of reducing sugars from starch and other saccharides in the natural nitrogen sources during the exponential-growth phase. In this uncontrolled pH fermentation process, the pH value had gradually increased since the stationary phase, which should be further studied.

Conclusion

The production medium of pristinamycins fermentation by *S. pristinaespiralis* CGMCC 0957 was optimized by using the statistical methodologies based on the Plackett–Burman design, the steepest ascent method, and CCD, and the maximum yield of pristinamycins in the shake flask could reach 1.30 g/L, which was almost a 100% increase compared with that before optimization and much higher than the reported yield of about 100 mg/L [14]. The great improvement of the yield may be attributed to both the optimum medium and the overproducing strain. The yield of pristinamycins in the 5-L bioreactor was 1.01 g/L, which was 22% lower than that in the shake flasks, presumably resulting from the different condition of aeration and shear stress between the bioreactor and shake flasks. At present, the research on the scale-up of pristinamycins fermentation is under way.

Statistical experimental designs appear to be a powerful tool for optimization of medium nutrients to improve the pristinamycins production. Considering that few reports focused on optimization of medium nutrients or fermentation process parameters for antibiotic production using statistical experimental methodologies, this study could also serve as a guide for optimization of antibiotic fermentation.

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