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ORIGINAL PAPER

Statistical optimization of medium components for avilamycin production by *Streptomyces viridochromogenes* Tü57-1 using response surface methodology

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Abstract A fermentation medium for avilamycin production by *Streptomyces viridochromogenes* Tü57-1 has been optimized. Important components and their concentrations were investigated using fractional factorial design and Box–Behnken Design. The results showed that soybean flour, soluble starch, $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ are important for avilamycin production. A polynomial model related to medium components and avilamycin yield had been established. A high coefficient of determination ($R^2 = 0.92$) was obtained that indicated good agreement between the experimental and predicted values of avilamycin yield. Student's *T*-test of each coefficient showed that all the linear and quadratic terms had significant effect ($P > |T| < 0.05$) on avilamycin yield. The significance of tested components was related to $MgSO_4 \cdot 7H_2O$ (0.37 g/L), $CaCl_2 \cdot 2H_2O$ (0.39 g/L), soybean flour (21.97 g/L) and soluble starch (37.22 g/L). The yield of avilamycin reached 88.33 ± 0.94 mg/L ($p < 0.05$) that was 2.8-fold the initial yield.

Keywords *Streptomyces viridochromogenes* · Avilamycin · Statistical optimization · Response surface methodology · Fractional factorial design

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Avilamycin, belongs to the oligosaccharide (orthosomycin) group of antibiotics, is a potent antibiotic with excellent activity against Gram-positive bacteria [1]. Presently it is used for growth promotion of swine and poultry [2, 3]. Avilamycin and other important members of the orthosomycins contain a dichloroisoeverninic acid moiety and one or more orthoester linkages that associate with carbohydrate residues [1].

Avilamycin can be produced by *Streptomyces viridochromogenes* NRRL2860, Tü57 and their mutants [4]. It has been shown that avilamycin can improve average daily gain and feed efficiency of pigs during early growth and finishing phases of swine and broiler production [5]. Avilamycin is approved of growth promotion in many countries and its market prospect is immense [6]. In order to increase avilamycin production, *S. viridochromogenes* Tü57 induced by nitrogen ion-beam implantation and a high-yield avilamycin-producing *S. viridochromogenes* Tü57-1 was selected. However, the mycelium of *S. viridochromogenes* Tü57-1 clustered in the fermentation medium reported [4, 7], and the avilamycin potential throughput of *S. viridochromogenes* Tü57-1 could not fully been bring into play. So, it is necessary to optimize the medium components for a high yield of avilamycin by *S. viridochromogenes* Tü57-1.

Optimal medium is not always obtainable by the traditional one-factor-at-a-time optimization strategy because of potential interactions among medium components. Moreover, following the conventional strategy, large numbers of experiments are required for identifying the optimal levels of all medium components [8–13]. The timesaving benefits of response surface methodology (RSM) have been indicated in some cases of optimizing medium components [10–18].

The aim of this work is to optimize the medium in order to maximize avilamycin production by *S. viridochromogenes* Tü57-1 and to reduce the cost of medium. This work was based on a statistics experimental design. The following optimization experiments are performed: (a) multiple nitrogen sources and multiple carbon sources were screening; (b) the medium components that significantly affect avilamycin production were elucidated using a fractional factorial design; (c) these significant ingredients were optimized by Box–Behnken design (BB).

Materials and methods

Strains and growth conditions

S. viridochromogenes Tü57-1 was a mutant of *S. viridochromogenes Tü57* obtained by nitrogen ion-beam injection [19]. The nitrogen ion-beam implantation dose was 5×10^{15} N^+/cm^2 with a survival rate of 26.3%. *S. viridochromogenes* Tü57 was kindly provided by Andreas Bechthold from Pharmazeutisches Institute, Pharmazeutische Biologie, Universitat Tübingen. The strain was maintained in the commercial HA medium containing 10 g/L malt extract, 4 g/L yeast extract, 4 g/L glucose, 20 g/L agar, and 0.1 g/L $CaCl_2$ pH 7.2 [7].

Important components of medium

Corn flour contains 82.3% starch, 8.9% crude protein and 2.3% crude lipid; sweet potato starch contains 90.3% starch, 2.2% crude protein; soybean flour contains 73.2% crude protein, 11.3% crude lipid and 11.7% ash; fish flour is 63.5% crude protein, 8.8% crude lipid and 19.2% ash; silkworm pupa flour contains 64.2% crude protein, 3.8% crude lipid and 8.1% ash; maize slurry contains 6.3% reducing sugar, 12.3% mineral, 5.2% ammonia, and 1.8% amido nitrogen. All of the above data were based on dry material basis. The moisture content sucrose, maltose, soluble starch, peptone and beef extract were in the order of 8.2, 6.8, 10.3, 11.5, 10.2 and 8.4%.

Cultivation and production of avilamycin by *S. viridochromogenes* Tü57-1

Five milliliters of sterile saline solution (0.85%) was added to the agar slant of HA medium. The spores were scraped and the suspension of spores was then filtered using eight layers of sterile cheesecloth. Then the spores suspension was adjusted to 10^7 to 10^8 cfu/mL. A 1.5 mL of the spore suspension was transferred into 250 mL Erlenmeyer flask containing 50 mL of seed medium (10 g/L malt extract, 8 g/L yeast extract, 10 g/L glucose, and 0.1 g/L $CaCl_2$) and incubated at 28 °C for 24 h at 180 rpm. This procedure was then used as the standard inoculums preparation throughout this study.

The composition of the production media varied according to experimental design. After the pH was adjusted to 7.2 using 20% NaOH solution, all media were sterilized at 121 °C for 15 min. An amount of 2.5 mL of the standard inoculum was transferred into each flask containing 50 mL production medium. Cultivations were conducted at 28 °C for 72 h at 180 rpm on an orbital shake. Cells were harvested by centrifugation at 6000 rpm for 15 min, and broken up by ultrasonic and suspended in acetone (1:10, w/v). After 6 h, the supernatant was collected by centrifugation at 6000 rpm for 15 min and was used for HPLC assay. All experiments were carried out in triplicate.

HPLC-UV analysis

Avilamycin analysis was carried out by HPLC using a reversed-phase column (Waters 5C18-ms-II, 4.6 mm × 250 mm) and a gradient with acetonitrile in 0.2% mono ammonium phosphate solution (pH 3.0, and a flow rate of 2 mL/min). The detection was carried out at the wavelength of 214 nm. Detection and spectral characterization of peaks were accomplished with a 486-UV detector and M32 software (Waters) [20].

Optimization of nitrogen sources and carbon sources

Glucose and yeast extract have been reported to be optimal carbon and nitrogen sources for avilamycin production by Tü57-1 [21], but maximum avilamycin yield was only 31.5 mg/L. In order to increase the yield of avilamycin, nitrogen and carbon sources were optimized in this study. Sucrose, maltose, mannitol, glycerol, corn flour, soluble starch, sweet potato starch, bean oil, and cotton oil were selected as potential carbon sources to be compared all at concentration of 20 g/L (db) using 20 g/L glucose as control. As potential nitrogen sources, medium containing 16 g/L (db) soybean flour, silkworm pupa flour, fish flour, peptone, maize slurry, beef extract, carbamide, NH_4SO_4 and $NaNO_3$ were compared against a control medium containing 16 g/L yeast extract. All results were obtained from triplicate experiments.

Fractional factorial design (FFD)

FFD is an experimental design method for two-level and by far the most popular fractional design in engineering at least. It is suitable for optimizing multitudinous factors, since it makes it possible to pick up the relevant factors from a long list [8].

In the process of medium optimization, 12 factors (as listed in Table 1,2) were tested on the basis of reported results [4, 7, 21]. Each factor was tested at both high (+1) and low (–1) concentrations (Table 2). The experimental design protocol was contrived based on ''Statistical Analysis System Version 8.0'' (SAS 8.0). The 1/256 fraction of fractional factorial designs was selected (Table 3). All experiments were performed all in triplicate. Avilamycin was listed as the response variable. Student's *T*-test was carried out using SAS 8.0, and the results of the *T*-test were shown in Table 4 and Fig. 1. Four components, namely soybean flour (X_2), soluble starch (X_4), $MgSO_4{\cdot}7H_2O$ (X_5), and $CaCl_2{\cdot}2H_2O$ (X_{10}), all with probabilities $Pr > |T| < 0.1$ were selected for further optimization by Box–Behnken.

Optimization by response surface methodology (RSM)

Further medium optimization by RSM was focused only on the four important components ($Pr > |T| < 0.1$). The rest of the positive factors were kept at the low level and negative factors were removed. Box–Behnken in SAS 8.0 was used to optimize the concentration of the four factors selected by FFD. Each factor was tested at three levels (Table 5). This part of the study included 27 experiments (Table 6); all experiments were performed in triplicate. Avilamycin production, as the response variable was analyzed using SAS 8.0.

Table 1 Screening the optimal multiple nitrogen and carbon sources

Carbon sources	Avilmycin (mg/L)	Nitrogen sources	Avilmycin (mg/L)
Glucose (Control 1)	31.5 ± 0.6	Yeast extract (Control 2)	40.5 ± 0.8
Sucrose	28.3 ± 0.8	Soybean flour	49.8 ± 0.9
Maltose	30.5 ± 0.7	Silkworm pupa flour	48.6 ± 0.8
Mannitol	34.2 ± 1.1	Fish flour	47.3 ± 1.0
Glycerol	36.1 ± 0.8	Peptone	36.3 ± 0.8
Corn flour	33.5 ± 0.8	Maize slurry	38.4 ± 0.6
Soluble starch	39.3 ± 0.8	Beef extract	37.6 ± 1.0
Sweet potato starch	35.6 ± 0.7	Carbamide	40.5 ± 0.9
Bean oil	37.0 ± 0.6	NH4SO4	39.5 ± 1.0
Cotton oil	37.2 ± 0.7	NaNO3	43.2 ± 0.6

Each value represents the mean ± standard deviations, $p < 0.05$

Table 2 Concentration ranges of variables taken for fractional factorial design

Factor	Code value	
	Low level (–1)	High level (1)
X_1: yeast extract (g/L)	8	16
X_2: soybean flour (g/L)	8	32
X_3: glucose (g/L)	10	20
X_4: soluble starch (g/L)	10	40
X_5: $MgSO_4{\cdot}7H_2O$ (g/L)	0.1	0.5
X_6: $FeSO_4{\cdot}7H_2O$ (g/L)	0.01	0.05
X_7: $ZnSO_4{\cdot}H_2O$ (g/L)	0.05	0.20
X_8: KH_2PO_4 (g/l)	0.10	0.50
X_9: $MnCl_2{\cdot}4H_2O$ (g/L)	0.02	0.1
X_{10}: $CaCl_2{\cdot}2H_2O$ (g/L)	0.1	0.4
X_{11}: $CoCl_2{\cdot}6HO_2$(g/L)	0.001	0.01
X_{12}: L-valine (g/L)	0.5	1.5

Results

Optimization of nitrogen sources and carbon sources

Table 1 lists the effects of various carbon sources on avilamycin production. Soluble starch demonstrated the most profound effect, raising final concentration to 39.3 ± 0.8 mg/L ($p < 0.05$). Generally, the synthesis of secondary metabolisms is restrained to a certain extent by the readily available carbon sources [22]. The effects of different nitrogen sources in comparison to that of yeast extract on avilamycin production are also listed in Table 1. The soybean flour, silkworm pupa flour and fish flour showed obvious influence on avilamycin production with a maximum final concentration of 49.8 ± 1.0 mg/L ($p < 0.05$) in medium of soybean flour. The yeast extract and the soybean flour were used as the nitrogen source in further optimization.

Fractional factorial design

In order to search the optimal medium component for avilamycin production, experiments were conducted by the application of fractional factorial design using avilamycin concentration as the response variable (Table 3). The results from the Student's *T*-test and the probabilities of $Pr > |T|$ are shown in Table 4, while the prediction profiles of each factor are displayed in Fig. 1. The *T*-test values of four factors ($ZnSO_4{\cdot}H_2O$,

Table 3 Fractional factorial designs

Trial	X_1 (g/L)	X_2 (g/L)	X_3 (g/L)	X_4 (g/L)	X_5 (g/L)	X_6 (g/L)	X_7 (g/L)	X_8 (g/L)	X_9 (g/L)	X_{10} (g/L)	X_{11} (g/L)	X_{12} (g/L)	Avilamycin production (mg/L)
1	–1	–1	–1	–1	1	–1	–1	1	–1	1	1	–1	49.2
2	1	–1	–1	–1	–1	–1	1	1	1	1	–1	1	47.3
3	–1	1	–1	–1	–1	1	–1	1	1	–1	1	1	44.9
4	1	1	–1	–1	1	1	1	1	–1	–1	–1	–1	55.3
5	–1	–1	1	–1	–1	1	1	–1	–1	1	1	1	48.3
6	1	–1	1	–1	1	1	–1	–1	1	1	–1	–1	68.6
7	–1	1	1	–1	1	–1	1	–1	1	–1	1	–1	62.8
8	1	1	1	–1	–1	–1	–1	–1	–1	–1	–1	1	58.3
9	–1	–1	–1	1	–1	1	1	–1	1	–1	–1	–1	58.5
10	1	–1	–1	1	1	1	–1	–1	–1	–1	1	1	60.6
11	–1	1	–1	1	1	–1	1	–1	–1	1	–1	1	62.1
12	1	1	–1	1	–1	–1	–1	–1	1	1	1	–1	70.8
13	–1	–1	1	1	1	–1	–1	1	1	–1	–1	1	50.3
14	1	–1	1	1	–1	–1	1	1	–1	–1	1	–1	40.5
15	–1	1	1	1	–1	1	–1	1	–1	1	–1	–1	60.6
16	1	1	1	1	1	1	1	1	1	1	1	1	65.6

Table 4 *T*-value and the probability of $Pr > |T|$

Name	T	$Pr > \|T\|$
X_1: yeast extract (g/L)	2.080179	0.128981
X_2: soybean flour (g/L)	3.920073	0.029524
X_3: glucose (g/L)	0.432512	0.694575
X_4: soluble starch (g/L)	2.35479	0.099871
X_5: $MgSO_4{\cdot}7H_2O$ (g/L)	3.10997	0.052883
X_6: $FeSO_4{\cdot}7H_2O$ (g/L)	1.448573	0.243297
X_7: $ZnSO_4{\cdot}H_2O$ (g/L)	–1.57215	0.213962
X_8: KH_2PO_4 (g/l)	–5.23821	0.013542
X_9: $MnCl_2{\cdot}4H_2O$ (g/L)	2.327329	0.102397
X_{10}: $CaCl_2{\cdot}2H_2O$ (g/L)	2.835359	0.065898
X_{11}: $CoCl_2{\cdot}6HO_2$(g/L)	–1.25635	0.29792
X_{12}: L-valine (g/L)	–1.98406	0.141497

KH_2PO_4, $CoCl_2{\cdot}6HO_2$, L-valine) were negative, and the avilamycin yield was reduced when these factors were included in the medium. Yeast extracts, glucose, $FeSO_4{\cdot}7H_2O$ and $MnCl_2{\cdot}4H_2O$ had positive influence on avilamycin production, but the influence was not significant according to the probabilities ($Pr > |T| > 0.1$). So the four factors were maintained at low concentrations. Factors of positive influence on avilamycin production and of higher probabilities ($Pr > |T| < 0.1$), including soybean flour, soluble starch, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$ were selected for further optimization by RSM.

Optimization by RSM

In order to observe the combined effect, experiments were designed by RSM (Table 6), according to the results obtained via FFD. Avilamycin production (i.e. the response variable) was used to obtain the following experiential model through multiple regression analysis:

$$\begin{aligned} Y = {} & 87.9 + 1.71667 \times X_2 - 1.466667 \times X_4 + 2.191667 \\ & \times X_5 + 1.708333 \times X_{10} - 5.1725 \times X_2 \times X_2 \\ & + 0.225 \times X_2 \times X_4 - 0.275 \times X_2 \times X_5 + 0.15 \\ & \times X_2 \times X_{10} - 2.1625 \times X_4 \times X_4 + 0.05 \times X_4 \times X_5 \\ & + 3.785 \times X_4 \times X_{10} - 6.25 \times X_5 \times X_5 + 2.2 \\ & \times X_5 \times X_{10} - 2.4 \times X_{10} \times X_{10} \end{aligned}$$

(polynomial 1)

The statistical significance of this model was verified by variance analysis (ANOVA) using SAS 8.0.

As listed in Table 7, the high *F*-value and the very low probability ($Pr > F < 0.05$) indicated that the experimental model was in good agreement with the experimental results. The ANOVA showed that the linear, quadratic and cross product terms of the polynomial model were much significant ($Pr > F < 0.05$). The high *F*-value and the low probability ($Pr > F < 0.05$) of the cross product terms suggested that there were obvious interactions among the four factors. The coefficient of determination ($R^2 = 0.92$) in the experimental model suggested a good agreement between experimental results and their predictions [10]. The precision and reliability of the experiments were confirmed by the relatively low value of variation coefficient (CV = 4.55%) [23].

The significance of each coefficient in the experimental model was determined by *T*-value and the probability of $Pr > |T|$ using SAS 8.0. The results were

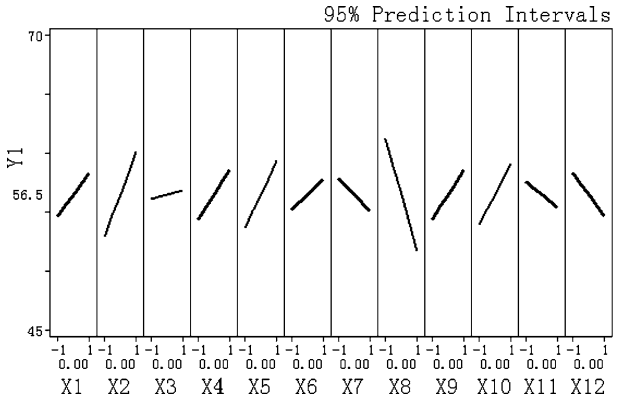

Fig. 1 Prediction profiler of each factor. X_1: yeast extract (g/L); X_2: soybean flour (g/L); X_3: glucose (g/L); X_4: soluble starch (g/L); X_5: $MgSO_4{\cdot}7H_2O$ (g/L); X_6: $FeSO_4{\cdot}7H_2O$ (g/L); X_7: $ZnSO_4{\cdot}H_2O$ (g/L); X_8: KH_2PO_4 (g/L); X_9: $MnCl_2{\cdot}4H_2O$ (g/L); X_{10}: $CaCl_2{\cdot}2H_2O$ (g/L); X_{11}: $CoCl_2{\cdot}6HO_2$ (g/L); X_{12}: L-valine (g/L). Y_1: avilamycin production (mg/L)

Table 5 Levels of response surface methodology

Factor	Code value		
	–1	0	1
X_2: soybean flour (g/L)	8	20	32
X_4: soluble starch (g/L)	15	30	45
X_5: $MgSO_4{\cdot}7H_2O$ (g/L)	0.1	0.3	0.5
X_{10}: $CaCl_2{\cdot}2H_2O$ (g/L)	0.1	0.25	0.4

Soybean flour (g/L), X_2:ξ_2; soluble starch (g/L), X_4:ξ_4; $MgSO_4{\cdot}7H_2O$ (g/L), X_5:ξ_5; $CaCl_2{\cdot}2H_2O$ (g/L), X_{10}:ξ_{10}. Code value: $X_2 = (\xi_2 - 20)/12$; $X_4 = (\xi_4 - 30)/15$; $X_5 = (\xi_5 - 0.3)/0.2$; $X_{10} = (\xi_{10} - 0.25)/0.15$

listed in Table 8. A high *T*-test value and a low probability indicated a high significance [12]. Student's *T*-test of each coefficient of the model showed all four linear and four quadratic terms have significant effects ($P > |T| < 0.05$) on avilamycin production. The interactive effect of $X_4 \times X_{10}$ and $X_5 \times X_{10}$ was also significant, while the interactive effect of any other two factors was found to be not significant. Comparison among the value of each coefficient in the experimental model also revealed descending significances of the four factors from $MgSO_4{\cdot}7H_2O$ through $CaCl_2{\cdot}2H_2O$ and soybean flour to soluble starch.

The effect of these four important medium components on avilamycin production by *S. viridochromogenes* Tü57-1 was further analyzed using 3D response surface plots, which were the graphical representations of the regression model. By simulating the experimental results using the empirical model, these plots (Fig. 2) efficiently identified the optimum values of the variables. From these plots, it was convenient to understand the interactions between any two factors and to locate their optimum levels. When avilamycin production was observed as a response variable to the interaction of soybean flour and soluble starch as variables and rest of the parameters were at central points, it was observed that there was an enhancement in avilamycin production at soybean flour and soluble starch concentrations between minimum to central levels (Fig. 2a). However, avilamycin yield decreased when increased soybean flour and soluble starch concentrations beyond this limit. So, maximal avilamycin production could be obtained at optimal-value of soybean flour and soluble starch. The same course of the rest of medium components (Fig. 2b–f) indicated there were the optimal value of each medium component. Therefore, the experimental model had a stationary point, and the predictive avilamycin yield was the maximal value in the stationary point.

The predictive maximal avilamycin production and the coded value of each factor were obtained by canonical analysis of response surface using SAS. The coded values of the four factors, soybean flour, soluble starch, $MgSO_4{\cdot}7H_2O$ and $CaCl_2{\cdot}2H_2O$, was found to be 0.16, 0.48, 0.33, and 0.90, respectively, and the predictive avilamycin production was 88.82 mg/L.

When translating these coded values, the concentration of soybean flour, soluble starch, $MgSO_4{\cdot}7H_2O$ and $CaCl_2{\cdot}2H_2O$ was calculated as 21.97 g/L, 37.22 g/L, 0.37 g/L and 0.39 g/L, respectively. Validation experiments were carried out in triplicate in shake flask culture, the result (88.33 ± 0.94 mg/L, $p < 0.05$) indicated that the experimental model could be applied to predict avilamycin production.

Discussion

Each strain or its mutant has its own requirement of special conditions for maximum antibiotic production. Medium compositions have profound effects on antibiotic production [22]. Medium optimization by a traditional one-factor-at-a-time optimization strategy does leads to a substantial increase in avilamycin production, however, this strategy is not only inconvenient and time consuming, but also ignored potential interactions among medium components [8–13]. FFD is suitable for optimizing multitudinous factors because it makes it possible to pick up the important components from a long list. The function of the statistical strategy using RSM for medium optimization was to find out the optimal medium components in important components and to establish the relationship between more than one variable and a given response [10–18]. In this study, the crucial factors (e.g., soybean flour, soluble

Table 6 Response surface methodologies

Trial	X_2 Soybean flour	X_4 Soluble starch	X_5 $MgSO_4 \cdot 7H_2O$	X_{10} $CaCl_2 \cdot 2H_2O$	Avilamycin production (mg/L)
1	–1	–1	0	0	80.3
2	–1	1	0	0	76.4
3	1	–1	0	0	81.5
4	1	1	0	0	78.5
5	0	0	–1	–1	75.7
6	0	0	–1	1	76.2
7	0	0	1	–1	76.2
8	0	0	1	1	85.5
9	–1	0	0	–1	76.2
10	–1	0	0	1	80.8
11	1	0	0	–1	79.7
12	1	0	0	1	84.9
13	0	–1	–1	0	77.5
14	0	–1	1	0	83.3
15	0	1	–1	0	76.8
16	0	1	1	0	82.8
17	–1	0	–1	0	72.3
18	–1	0	1	0	75.2
19	1	0	–1	0	77.7
20	1	0	1	0	79.5
21	0	–1	0	–1	89.6
22	0	–1	0	1	82.3
23	0	1	0	–1	77.1
24	0	1	0	1	85.3
25	0	0	0	0	88.3
26	0	0	0	0	87.6
27	0	0	0	0	87.8

Table 7 The ANOVA results of the experiential model

Source	DF	SS	MS	*F*-value	Probability (Pr) > *F*
Model	14	526.8175	37.62982	10.32624	0.000125
Linear	4	153.8383	38.45958	10.55394	0.000667
Quadratic	4	292.9517	73.23792	20.09768	0.0001
Cross product	6	80.0275	13.33792	3.660143	0.026589
Error	12	43.72917	3.644097		
Total	26	570.5467			

R-square (R^2) = 0.92; coefficient of variation (CV) = 4.55

Table 8 Student's *T*-test of the experimental model

Term	Estimate	SE	T	Pr > |*T*|
X_2	1.7166667	0.551067	3.115169	0.008935
X_4	–1.466667	0.551067	–2.6615	0.020734
X_5	2.1916667	0.551067	3.977133	0.001836
X_{10}	1.7083333	0.551067	3.100047	0.009189
$X_2 \times X_2$	–5.7125	0.8266	–6.91084	0.0001
$X_2 \times X_4$	0.225	0.954476	0.235731	0.817616
$X_2 \times X_5$	–0.275	0.954476	–0.28812	0.778174
$X_2 \times X_{10}$	0.15	0.954476	0.157154	0.877736
$X_4 \times X_4$	–2.1625	0.8266	–2.61614	0.022545
$X_4 \times X_5$	0.05	0.954476	0.052385	0.959084
$X_4 \times X_{10}$	3.875	0.954476	4.059819	0.001582
$X_5 \times X_5$	–6.25	0.8266	–7.56109	0.0001
$X_5 \times X_{10}$	2.2	0.954476	2.30493	0.03984
$X_{10} \times X_{10}$	–2.4	0.8266	–2.90346	0.013241

starch, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$) for avilamycin production were attained by FFD and analyzed using SAS 8.0. Box–Behnken design was successfully applied to optimize the medium composition. The optimal concentration (soybean flour, 21.97 g/L, soluble starch 37.22 g/L, $MgSO_4 \cdot 7H_2O$ 0.37 g/L and $CaCl_2 \cdot 2H_2O$ 0.39 g/L) of the crucial components for avilamycin production was then determined. Avilamycin production in the optimal medium reached 2.8-fold of that in the initial medium by *S. viridochromogenes* Tü57-1. Another significant achievements of this study are the selection of readily available medium components, increased avilamycin yield and a reduced cost of medium. An empirical model (polynomial 1) was established for the description of the relationships

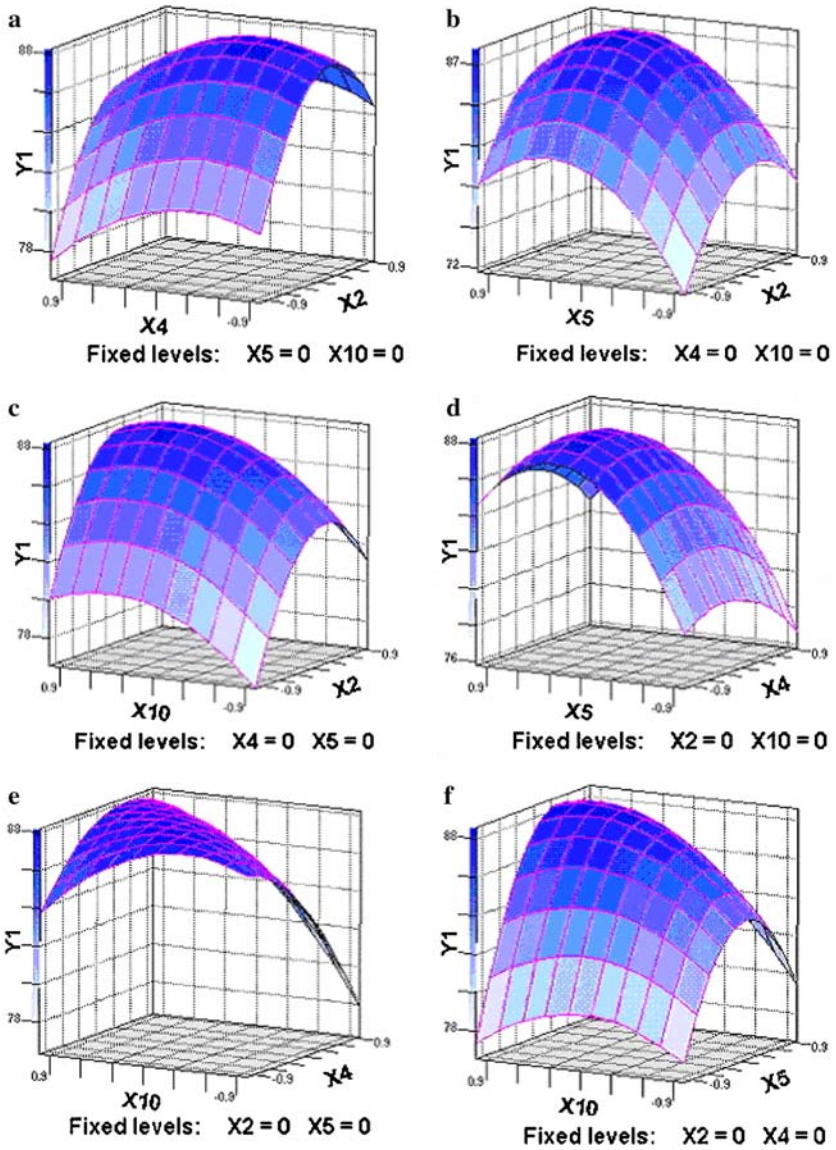

Fig. 2 Response surface plots of soybean flour (X_2) and soluble starch (X_4) with rest of two parameters at central points (**a**), soybean flour and $MgSO_4 \cdot 7H_2O$ (X_5) with rest of two parameters at central points (**b**), soybean flour and $CaCl_2 \cdot 2H_2O$ (X_{10}) with rest of two parameters at central points (**c**), soluble starch and $MgSO_4 \cdot 7H_2O$ with rest of two parameters at central points (**d**), soluble starch and $CaCl_2 \cdot 2H_2O$ with rest of two parameters at central points (**e**), $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ with rest of two parameters at central points (**f**). Y_1: avilamycin production (mg/L)

between the medium components and avilamycin production using statistical analysis system.

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