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Optimization of Medium Composition for Antibacterial Substance Production by *Aspergillus niger* xj using Response Surface Methodology

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To investigate the optimal medium composition for antibacterial substance production by *Aspergillus niger* xj in liquid-state fermentation (LSF). The disc diffusion method was used to assay the production. Statistical methodologies, including the Plackett-Burman design (PBD) and the central composite design (CCD), were employed to investigate the individual crucial component of the medium that significantly affected the production. The optimum values of the critical components for the maximum antibacterial substance production were obtained as follows: x_1($MgSO_4$)=-0.3417 (0.2982 g/L), x_2(Tween-80)=-0.28878 (0.242245 ml/L) and the predicted diameter of the inhibitory zone value was 27.39847 mm. Using the optimized condition, the diameter of inhibitory zone reached 27.74±3.07 mm. By using PBD and CCD, we determined the optimal composition for antibacterial substance production by *Aspergillus niger* xj in LSF.

Key words: Antibacterial substance, Optimization of medium composition, Plackett-Burman design (PBD), Response surface methodology (RSM), *Aspergillus niger* xj.

Aspergillus niger is a widely used host organism for the industrial production of food processing enzymes and metabolites such as organic acids or antibiotics (Sauer *et al*. 2008; Tevz *et al*. 2010; Dashtban *et al*. 2011; Frisvad *et al*. 2011). There are few reports concerned with the optimization of cultural conditions for the production of antibacterial substance by *Aspergillus niger*.

Fractionation of the extract of *Aspergillus niger* IFB-E003, an endophyte in *Cyndon dactylon*, yielded four known compounds, namely, naphtho-γ-pyrones rubrofusarin B, fonsecinone A, asperpyrone B and aurasperone A, which were further investigated biologically. The 4 naphtho-γ-pyrones exhibited growth inhibitions against the 5 test microbes with MICs ranging in between 1.9 and 31.2 ug/ml (Song *et al*. 2004). The antibacterial substance in the fermentation broth from *Aspergillus niger* xj had varying degrees of inhibitory activity towards 5 pathogenic fungi species, namely, *Pythium Pringsheim*, *Fusarium solan*, *Trichoderma viride*, *Colletotrichum orbiculare* and *Penicillium citrinum*. Among these, the inhibitory activity against *Fusarium solan* was the highest, with the diameter of the inhibitory zone reaching 45.11±0.21 mm (LI Zhu *et al*.2007).Six new

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alkylitaconic acids, designated tensyuic acids A to F, were isolated from the culture broth of *Aspergillus niger* FKI-2342 by a solvent extraction, silica gel column chromatography and HPLC. Their structures were elucidated by spectroscopic analysis including UV, NMR, and MS. They are all alkylitaconic acid derivatives. Only the tesyuic acid C showed moderate antimicrobial activity against *Bacillus subtilis* (Yoko H *et al*.2007). Three flavonoids lupinifolin, 8-methoxy-7,3',4'-trihydroxyflavone, and 7,8,3',4'-tetrahydroxyflavone, a triterpenoid lupeol as well as 4 sterols β-sitosterone, stigmasta-5,22-dien-3-one, β-sitosterol, and stigmasterol were isolated from *Albizia myriophylla* wood. The antibacterial activity of these compounds against *Streptococcus mutans* ATCC 25175 was performed using broth microdilution method. All compounds exhibited antibacterial activity against *S. mutans* with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) ranging from 1–256 and 2–256 ug/ml, respectively (Joycharat, *et al*. 2013).

There are some advantages of using statistical methods, over the one-factor-at-a-time classical method. A statistical design enables easy selection of important parameters from a large number of factors and explains the interactions between important variables. A number of statistical experimental designs have been used for optimizing fermentation variables. The Plackett–Burman design (Plackett and Burman 1946) is a well known and widely used statistical technique for the screening and selection of the most significant culture variables, while the central composite design (CCD) (Box and Wilson, 1951) provides important information regarding the optimum level of each variable along with its interactions with other variables and their effects on product yield (Pardeep and Satyanarayana 2006).

The aim of this work was to use statistical methods to optimize the fermentation medium compositions for the improvement of antibacterial substance production by *A. niger* xj. PBD was used to identify the medium components which had significant effects on antibacterial substance production. CCD was then employed to optimize the factors that had a significant influence on antibacterial substance production.

MATERIALS AND METHODS

Microorganism and Culture Maintenance

The organism used in this study were *Aspergillus niger* xj and *Agrobacterium tumefaciens* T-37. *A. niger* xj was isolated by The Institute of Fungi Resource, GuiZhou University, and preserved in the China Center for Type Culture Collection (Address: WuHan University WuHan China). Accession Number: CCTCCNO:M206021. The strain was grown on potato dextrose agar (PDA) slants at 28°C for 5d and then stored at 4°C. Spore suspension was prepared by suspending the spores from a PDA slant by adding sterile distilled water to give a final spore count of 1×10^6 spores/ml. *Agrobacterium tumefaciens* T-37 was grown on Luria-Bertan (LB) slants at 37°C for 20h. The bacterium suspension was prepared by the bacterium from a LB slant by adding sterile distilled water to give a final bacterium density of 2×10^8 cfu/ml.

Media

The PDA medium consisted of the following: 200ml potato extract (200g potato was extracted in 1L boiled water for 30min and filtered with cotton gauzes. After the extraction, the required distilled water was added to offset the evaporated water.), 20g of glucose, 20g of agar and pH7.0. The LB medium consisted of the following: tryptone 1%, NaCl 0.5%, yeast extract1%, and pH 7.2.

Liquid-state Fermentation

The antibacterial substance production by *A. niger* xj was conducted in 250ml Erlenmeyer flask with 50ml of the production medium. The production medium which sterilized by autoclaving at 121°C for 30min was inoculated with 10% *v/v* of inoculum, and the Erlenmeyer flask was incubated in an orbital shaker at 150 rpm and 28°C for cultivation period of 6d. Inoculum was inoculated with 2% *v/v* of spore suspension at 150 rpm and 28°C for cultivation 3d. To assess the effects of medium ingredients on the production of antibacterial substance by using PBD and CCD, the composition of the nutrient solution varied according to the experimental designs.

Antibacterial Substance Production Assay

The antibacterial substance production using the disc diffusion method was performed

against *Agrobacterium tumefaciens* T-37 (Vijay C V 2011). Adding 200uL bacterium suspension and 10mL LB medium (cool down to 50!) to a sterilized culture dish. 20uL fermentation broth which was put under the UV for 30min to kill *A. niger* xj was placed onto a sterile disc (6 mm) and put it into the culture dish. The zone of inhibition surrounding the disc on a bacterial plate was recorded in three replicates by inhibiting the plate of *Agrobacterium tumefaciens* T-37 at 37°C for 36h.

One-Factor-at-a-Time Classical Method

The following factors were investigated for the optimization of medium components, which include effect of different nutritional factors such as carbon source (maltose, olive oil, citric acid, cane sugar, dextrin,glucose; 3% concentration (w/v)), nitrogen source (bran, soybean meal, $NaNO_3$, casein, yeast extract, tryptone, peptone; 0.3% concentration (w/v)), concentration of carbon source (10, 20 30, 40, 50, 60 g/L), concentration of nitrogen source (2.50, 3.00, 3.75, 5.00, 7.50 g/L), concentration of $MgSO_4$ (0.25, 0.50, 1.00, 2.00, 4.00 g/L), concentration of K_2HPO_4 (0.5, 1.0, 2.0, 3.0, 4.0 g/L), concentration of growth factor of the yeast extract (0%, 0.05%, 0.1%, 0.2%, 0.4%), and concentration of surface active agent of PEG 6000 (0%, 0.05%, 0.1%, 0.3%) or Tween-80 (0%, 0.05%, 0.1%, 0.3%).

Plackett–Burman Experimental Design

There are a range of factors that need to be tested for their importance in antibacterial substance production. PBD is a useful tool to screen 'n' variables ('n"3' actual factors and '3' invented variables for estimating errors) in just 'n+1' experiments (Plackett and Burman, 1946), which will reduce the enormous total number of experiments in comparison with full factorial designs which require 2^N (N denotes the number of factors) experiments. PBD is a preliminary optimization technique, which tests only two levels of each medium component, it cannot provide the optimal quantity of each component required in the medium. This technique, however, provides indications of how each component tends to affect enzyme production (Yu *et al*. 1997).Twelve experiments based on the Plackett–Burman experimental design were generated for the nine factors. The variables with confidence level greater than 90% were considered to have significant influence on antibacterial substance production. The main effect was calculated as the difference between the average of measurements made at the high level setting (+1) and the average of measurements observed at low level setting (-1) of each factor (Abdel-Fattah and Olama 2002).

Central Composite Experimental Design and Optimization by Response Surface Methodology

Response surface methodology, using a central composite design, was adopted for the augmentation of antibacterial substance production *A. niger* xj. The significant variables $MgSO_4$ and Tween-80, were assessed at five coded levels. The central composite design experiment was designed using the SAS software package, version 9.2. The variables were coded according to the following equation 1:

$$x_i=(X_i-X_c)/\Sigma X_i \qquad ...(1)$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_c is the real value of an independent variable at the center point, and $\gamma\%X_i$ is the step change value. An orthogonal 2^2 factorial central composite experimental design with six star points (α=1.41421) and five replicates at the center resulting in a total of 13 experiments was used to optimize the variables. (Jian-Zhong *et al*. 2003) These parameters were tested at five levels, coded "1.41421, "1, 0, +1, and +1.41421 for lowest, low, middle, high, and highest concentration, respectively. The experimental range with the levels of independent variables and experimental plan is shown in Table 5 for parameter optimization. The relationship between dependent and independent variables is explained by the following second-degree polynomial Equation 2 :

$$Y=\beta_0+\Sigma\beta_i x_i+\Sigma\beta_{ii} x_i^2+\Sigma\beta_{ij} x_i x_j \qquad ...(2)$$

where Y is the predicted response,Σ_0 is the offset term, Σ_i is the coefficient linear effect, Σ_{ii} is the coefficient squared effect, and Σ_{ij} is the coefficient of interaction effect (Xu *et al*. 2008; Aravindan Rajendran and Viruthagiri Thangavelu 2012).

Statistical analysis

Quantification of antibacterial substance production was carried out in triplicate experiments and the mean values were given. The SAS software

statistical program package was used for regression analysis of the data obtained and to estimate the coefficients of the regression equation. The goodness of fit of the regression model obtained is given by the multiple correlation coefficients R and by the coefficient of determination R^2. The response surface plots are used to describe the individual and cumulative effects of the variables as well as the mutual interactions between the variables on the dependent variable (antibacterial substance production). The polynomial equation was maximized by a constraint search procedure using the SAS software (version 9.2) to obtain the optimal levels of the independent variables and the predicted maximum value.

RESULTS

Results One-Factor-at-a-Time experiment

"d" stand for the diameter of inhibitory zone. The results of One-Factor-at-a-Time experiment were in fig.1. From this figure, we can get the best single factors that were maltose(30.00 g/L), bran(7.50 g/L), $MgSO_4$ (0.25 g/L), K_2HPO_4 (1.0 g/L), yeast extract (1.00 g/L), and Tween-80(0.1%), respectively.

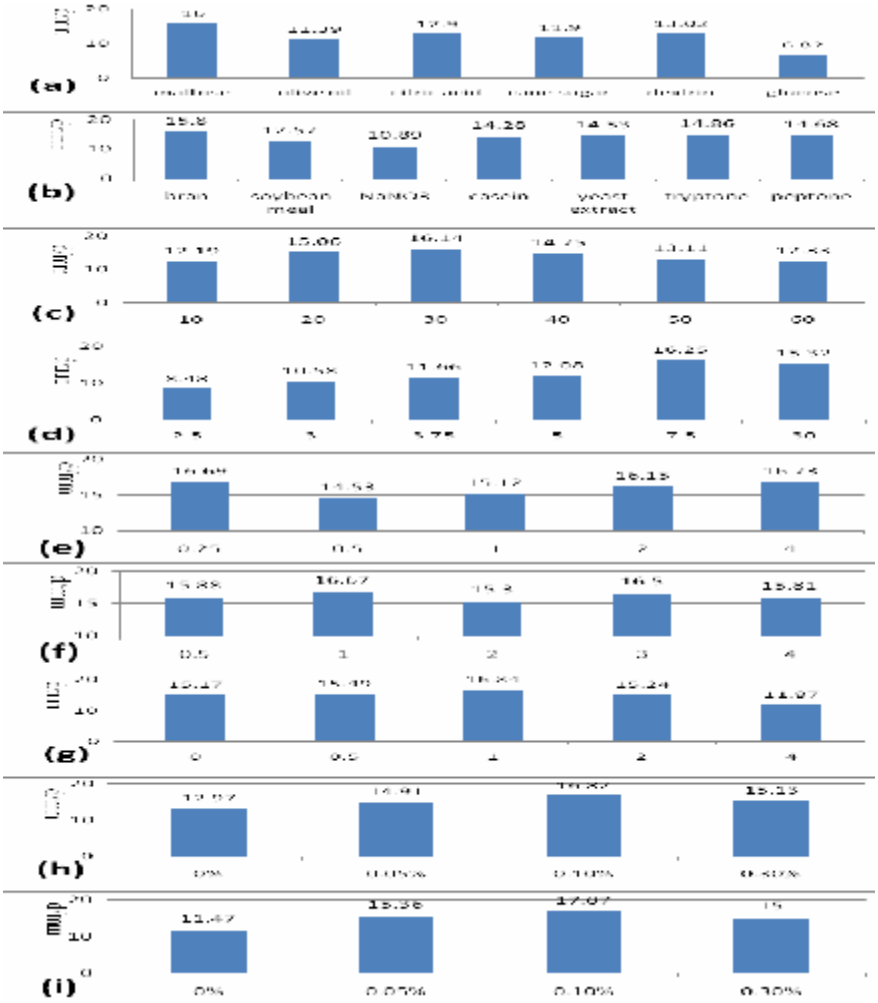

Fig.1. The effect of carbon sources, nitrogen source, carbon source concentrations, nitrogen source concentrations, $MgSO_4$ concentrations, K_2HPO_4 concentrations, yeast extract concentrations, PEG 6000 concentrations, and Tween-80 concentrations on xj antibacterial substance production are (a), (b), (c), (d), (e), (f), (g), (h), and (i), respectively

PBD and response values

Plackett–Burman experiments (Table 1) highlighted the importance of optimizing culture variables in attaining higher antibacterial substance production. PBD for 9 factors, namely, maltose, bran, yeast extract, K_2HPO_4, $MgSO_4$, $CaCl_2$, Trace elements solution ($FeSO_4 \cdot 7H_2O$ 0.5%, $MnSO_4 \cdot H_2O$ 0.16%, $ZnSO_4 \cdot 7H_2O$ 0.14%, $CoCl_2 \cdot 6H_2O$ 0.37%), Tween-80 and KCl, made a total of 12 experimental treatments. The PBD factors and response values are listed in Table 1. Table 2 shows test factors and the rank of significance. The significant variables were $CaCl_2$, $MgSO_4$ and Tween-80.

Outcome of steepest ascent experiment

Based on the results from PBD, $MgSO_4$, $CaCl_2$ and Tween-80 were selected for the further evaluation of their effects on antibacterial substance production by steepest ascent experiment. The results were in the Table 3. From this experiment, the center point is $CaCl_2$ (0 g/L), $MgSO_4$ (0.305 g/L) and Tween-80 (0.30 g/L).

Outcome of CCD experiment

CCD is a very useful tool for determining the optimal level of medium constituents and their interaction. Based on the results from steepest ascent experiment, $MgSO_4$ and Tween-80 were selected for the further evaluation of their effects on antibacterial substance production by CCD.

Table 1. Experimental design and results of Plackett-Burman design

Run No.	A	B	C	D	E	F	G	H	I	the diameter of inhibitory zone (mm) [a]
1	1(37.5)	-1(7.5)	1(1.25)	-1(0.75)	-1(0.2)	-1(0)	1(2)	1(2)	1(0.5)	13.69
2	1(37.5)	1(15)	-1(1)	1(1)	-1(0.2)	-1(0)	-1(0)	1(2)	1(0.5)	14.03
3	-1(30)	1(15)	1(1.25)	-1(0.75)	1(0.25)	-1(0)	-1(0)	-1(1)	1(0.5)	18.20
4	1(37.5)	-1(7.5)	1(1.25)	1(1)	-1(0.2)	1(3)	-1(0)	-1(1)	-1(0.4)	10.40
5	1(37.5)	1(15)	-1(1)	1(1)	1(0.25)	-1(0)	1(2)	-1(1)	-1(0.4)	17.66
6	1(37.5)	1(15)	1(1.25)	-1(0.75)	1(0.25)	1(3)	-1(0)	1(2)	-1(0.4)	10.43
7	-1(30)	1(15)	1(1.25)	1(1)	-1(0.2)	1(3)	1(2)	-1(1)	1(0.5)	11.08
8	-1(30)	-1(7.5)	1(1.25)	1(1)	1(0.25)	-1(0)	1(2)	1(2)	-1(0.4)	16.91
9	-1(30)	-1(7.5)	-1(1)	1(1)	1(0.25)	1(3)	-1(0)	1(2)	1(0.5)	12.16
10	1(37.5)	-1(7.5)	-1(1)	-1(0.75)	1(0.25)	1(3)	1(2)	-1(1)	1(0.5)	12.49
11	-1(30)	1(15)	-1(1)	-1(0.75)	-1(0.2)	1(3)	1(2)	1(2)	-1(0.4)	9.85
12	-1(30)	-1(7.5)	-1(1)	-1(0.75)	-1(0.2)	-1(0)	-1(0)	-1(1)	-1(0.4)	15.71

A maltose (g/L), B bran (g/L), C yeast extract (g/L), D K_2HPO_4 (g/L), E $MgSO_4$ (g/L), F $CaCl_2$ (g/L), G Trace element solution, H Tween-80 (ml/L), I KCl (g/L).

[a] Data are means of triplicate measurements.

Table 2. Statistical analysis of Plackett–Burman design showing main effect, t values and p values

Variable	Main effect	t value	p value	Ranking
A	-0.8683	-4.2209	0.0518	4
B	-0.0167	-0.0810	0.9428	9
C	-0.1983	-0.9641	0.4367	6
D	0.31	1.5069	0.2708	5
E	2.1817	10.6048	0.0088	2
F	-4.9667	-24.1423	0.0017	1
G	0.1242	0.60356	0.6075	7
H	-1.4117	-6.8619	0.0206	3
I	0.1125	0.5468	0.6393	8

A maltose (g/L), B bran (g/L), C yeast extract (g/L), D K_2HPO_4 (g/L), E $MgSO_4$ (g/L), F $CaCl_2$ (g/L), G Trace element solution, H Tween-80 (ml/L), I KCl (g/L)

And the less significant variables were kept constant during the optimization of significant variables by response surface methodology. The less significant parameters kept constant were maltose, 30 g/L; bran, 7.5 g/L ; yeast extract, 1 g/L; K_2HPO_4, 1 g/L; Trace elements solution, 2 ml/L; and KCl 0.4 g/L. For RSM analysis based on the CCD, 13 experiments were carried out and their response values with different combinations of two factors are demonstrated in Table 4. From Table 4 it can be seen that the centre points were set up at runs of 9, 10, 11, 12, 13 and the maximum diameter of inhibitory zone (27.90 mm) was achieved at the centre points. The minimum diameter of inhibitory zone (18.18 mm) was detected in run No.6.

Through multiple regression analysis, we found that the polynomial equation can explain antibacterial substance production regardless of the significance of coefficients:

$$Y=27.088-1.764832x_1-0.062048x_2-3.045252x_1^2+1.095x_1x_2-0.755249x_2^2$$

Table 3. The design and results of the steepest ascent experiment

Trial No.	$CaCl_2$ (g/L)	$MgSO_4$ (g/L)	Tween-80(ml/L)	d (mm)
1	1.5	0.225	1.50	11.70±0.46
2	1.3	0.235	1.35	13.02±0.44
3	1.1	0.245	1.20	18.30±0.54
4	0.9	0.255	1.05	18.60±0.63
5	0.7	0.265	0.90	21.57±0.57
6	0.5	0.275	0.75	22.43±1.89
7	0.3	0.285	0.60	23.42±0.49
8	0.1	0.295	0.45	23.56±0.68
9	0	0.305	0.30	24.99±1.12
10	0	0.315	0.15	23.59±0.66
11	0	0.325	0	23.10±1.27
12	0	0.335	0	22.17±1.50

Table 5. Analysis of variance for the response of the diameter of inhibitory zone

Source	df	F-value	P>F
Model	5	22.03063	0.000
Linear	2	14.43896	0.003
Quadratic	2	37.86182	0.000
Cross Product	1	5.551592	0.051

where Y is the response value. In current experiment, Y value is the diameter of inhibitory zone (mm). x_1 and x_2 represent the coded levels of $MgSO_4$ and Tween-80, respectively.

The statistical significance of the regression model was checked by F-test, and the analysis of variance for the response surface quadratic model is shown in Table 5. The model was highly significant, as manifested by the F-value and the probability value [(Ptotal model>F) =0.000]. The linear terms, quadratic terms and cross terms were all statistically significant based on the F-value.

Table 4. The central composite design matrix of independent variables used in response surface methodology (RSM) with corresponding experimental

Trial No.	$MgSO_4$ x_1	Tween -80 x_2	The diameter of inhibitory zone (mm) [a]
1	-1(0.285)	-1(0.1)	27.05
2	-1(0.285)	1(0.5)	24.93
3	1(0.325)	-1(0.1)	20.82
4	1(0.325)	1(0.5)	23.08
5	-1.41421 (0.277)	0(0.3)	22.45
6	1.41421 (0.333)	0(0.3)	18.18
7	0(0.305)	-1.41421 (0.02)	25.12
8	0(0.305)	1.41421 (0.58)	24.67
9	0(0.305)	0(0.3)	26.24
10	0(0.305)	0(0.3)	26.62
11	0(0.305)	0(0.3)	27.35
12	0(0.305)	0(0.3)	27.33
13	0(0.305)	0(0.3)	27.90

The coded values of the significant variables are given in parenthesis x_1 $MgSO_4$ (g/L), x_2 Tween-80 (ml/L)
[a] Data are means of triplicates.

Table 6. Results of the regression analysis of the CCD

Term	Estimate	Std Err	t	Pr > |t|
x_1	-1.76483	0.328617	-5.37048	0.0010
x_2	-0.06205	0.328617	-0.18882	0.8556
x_1^2	-3.04525	0.352403	-8.64138	<.0001
x_1x_2	1.095	0.464735	2.356182	0.0506
x_2^2	-0.75525	0.352403	-2.14314	0.0693

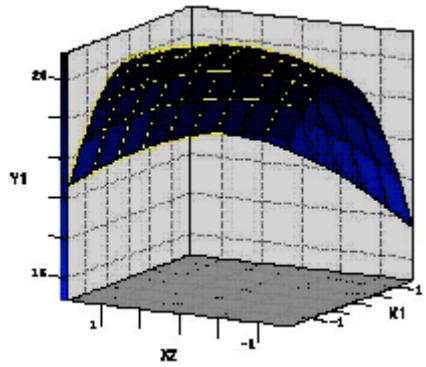

Fig.2. Response surface for antibacterial substance production by *A. niger*. The interaction between $MgSO_4$ and Tween-80(Y1: the diameter of inhibitory zone ;x1: $MgSO_4$;x2 Tween-80)

The Student's *t*-distribution and the corresponding *P*-value, along with the parameter, are given in Table 6. The *P*-values are used as a tool to check the significance of each coefficient, which will help to explain the pattern of mutual interactions between the best variables. The parameter coefficient and the corresponding *P*-value suggested that, among the independent variables, x_1 ($MgSO_4$) and x_2 (Tween-80) have a significant effect on antibacterial substance production.

The 3D response surfaces plots were employed to determine the interaction of the basal medium components and the optimum levels that have the most significant effect on antibacterial substance production. Fig.2 illustrated the relationship between the response and the experimental data. Fig.2 describes the effects of $MgSO_4$ and Tween-80 on antibacterial substance production.

The prediction from response optimizer analysis gives a maximum level of the diameter of inhibitory zone as 27.39847 mm in the medium containing x_1(MgSO4) =-0.3417 (0.2982 g/L), x_2(Tween-80) =-0.28878 (0.242245 ml/L). Verification of the predicted values was conducted by using optimal conditions which including maltose 30 g/L, bran 7.5 g/L, yeast extract 1.0 g/L, K_2HPO_4 1.0 g/L, $MgSO_4$ 0.2982 g/L , Trace elements solution 2.00 ml/L, Tween-80 0.24 ml/L and KCl 0.5 g/L in fermentation. The practical corresponding response was 27.74±3.07 mm (Data are means of triplicates), which corroborated the validity and the effectiveness of the current model and increased 12.8 mm compared with the one (14.90±0.96 mm) before optimization (the liquid PDA fermentation medium).

DISCUSSION

Through the One-Factor-at-a-Time experiment, the best single factor as follow: maltose 30.00 g/L, bran 7.50 g/L, $MgSO_4$ 0.1 g/L, K_2HPO_4 1.00 g/L, yeast extract 1.00 g/L and Tween-80 0.1%. Besides, $CaCl_2$, Trace elements solution and KCl were added into the further experiment. The reliability of $CaCl_2$, $MgSO_4$, and Tween-80 reached 90%, suggesting a significant effect on antibacterial substance production. As 0 g/L (coded value, -1) of $CaCl_2$ was used in Plackett–Burman Experimental, so $MgSO_4$ and Tween-80 were included in the next CCD optimization. Magnesium is required by all fungi and has a variety of regulatory functions. Magnesium stimulates the sporulation, perhaps through increased adenosine triphosphate metabolism and nucleic acid synthesis (Sandip B. Bankar *et al*.2009).The data from the analysis of variance showed that the model was well fitted to the experimental data. The goodness of fit was manifested by the determination coefficient (R^2). In this case the R^2 value of 94.02% indicated that the response model can explain 94.02% of the total variations. In general, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation (Haaland, 1989). The value of the adjusted determination coefficient ($R_{Adj}{}^2$= 89.76%) was also high enough to indicate the significance of the model. From Fig.2 it can be seen that the yield of antibacterial substance production increased gradually while the Tween-80 concentration increased at a high level of $MgSO_4$. With the increase in the concentration of $MgSO_4$, the antibacterial substance production steadily increased at a high concentration of Na_2CO_3. This observation was consistent with the results demonstrated in Table 6, which suggest a positive interaction of $MgSO_4$ and Tween-80.

The antibacterial substance production by *A. niger* xj was optimized by using response analysis, which was found to be an efficient tool. From PBD experiments, $CaCl_2$, $MgSO_4$, and Tween-80 were shown to be critical components for antibacterial substance production by *A. niger* xj.

The CCD experiment estimated the optimum values of the critical components for maximum antibacterial substance production. Under the following conditions: maltose 30 g/L, bran 7.5 g/L, yeast extract 1.0 g/L, K_2HPO_4 1.0 g/L, $MgSO_4$ 0.2982 g/L , Trace elements solution 2.00 ml/L, Tween-80 0.24 ml/L and KCl 0.5 g/L. In the current experiment, the diameter of inhibitory zone reached 27.74±3.07 mm after 6d of fermentation at 150 rpm and 28°C in the shake flask experiment, which accorded with the predicted value.

In the current work, we determined the optimal fermentation medium composition for antibacterial substance production by *Aspergillus niger* xj and laid the foundation for the research of some aspects such as separation and purification of the active substances, the antibacterial mechanism, and provided information for *Aspergillus niger* in the exploition and effectiveness of various antibacterial drugs.

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REFERENCES

1. Abdel-Fattah, Y. R., Olama, Z. A., L-asparaginase production by *Pseudomonas aeruginosa* in solid-state culture: evaluation and optimization of culture conditions using factorial designs. *Process Biochem,* 2002; **38** : 115-122.
2. Aravindan Rajendran , Viruthagiri Thangavelu., Optimization and Modeling of Process Parameters for Lipase Production by *Bacillus brevis*. *Food Bioprocess Technol*, 2012; **5**: 310-322.
3. Box, G., Wilson, K.B., On the experimental attainment of optimum conditions. *J Roy Stat,* 1951; **13**(1):1-45.
4. Dashtban M, Buchkowski R, Qin W, Effect of different carbon sources on cellulase production by *Hypocrea jecorina* (*Trichoderma reesei*) strains. *Int J Biochem Mol Biol.* 2011; **2**: 274-286
5. Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA, Nielsen KF , Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. 2011; PLoS One 6: e23496
6. Haaland, P.D. (Ed.), Separating Signals from the Noise. *In*: Experimental Design in Biotechnology. Marcel Dekker, New York, 1989: 61-83
7. Jian-Zhong, L., Li-Ping, W., Qian-Ling, Z., Hong Xu, Liang-Nian, Optimization of glucose oxidase production by *Aspergillus niger* in a benchtop bioreactor using response surface methodology. *World J Microbiol Biotechnol,* 2003; **19**: 317-323.
8. LI Zhu, GE Yong- yi, CHEN Qing, ZHOU Li-hong, LIU Wu- juan, Antifungal Activity and Stability of *Aspergillus niger* xj Fermentation Broth. *Food Res Dev*, 2011; **32**(7): 141-143.
9. N. Joycharat, S. Thammavong, S. Limsuwan, S. Homlaead, S.P. Voravuthikunchai, B.E. Yingyongnarongkul, S. Dej-Adisai, S. Subhadhirasakul, Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. *Arch Pharm Res,* 2013 [doi:10.1007/s12272-013-0085-7]
10. Pardeep, K., Satyanarayana,T., Optimization of culture variables for improving glucoamylase production by alginateentrapped *Thermomucor indicae-seudaticae* using statistical methods. *Bioresour Technol,* 2006; **98**: 1252-1259.
11. Plackett, R.L., Burman, J.P., The design of optimum multifactorial experiments. *Biometrika,* 1946; **33**(4):305-325 [doi:10.1093/biomet/33.4.305]
12. Sandip B. Bankar, Mahesh V. Bule ,Rekha S. Singhal, Laxmi Ananthanarayan., Optimization of *Aspergillus niger* Fermentation for the Production of Glucose Oxidase. *Food Bioprocess Technol*, 2009; **2**: 344-352
13. Sauer M, Porro D, Mattanovich D, Branduardi P, Microbial production of organic acids: expanding the markets. *Trends Biotechnol,* 2008; **26**: 100-108.
14. Tevz G, Bencina M, Legisa M, Enhancing itaconic acid production by *Aspergillus terreus*. *Appl Microbiol Biotechnol,* 2010; **87**: 1657-1664.
15. Vijay C V, Emil Lobkovsky, Alan C G Santosh K G,Satya P, Piperine production by endophytic fungus *Periconia* sp. isolated from *Piper longum*

L. *J Antibiotic*, 2011; **64**: 427-431.

16. Xu Yao-xing, Li Yan-li, Xu Shao-chun, Liu Yong, Wang Xin, Tang Jiang-wu., Improvement of xylanase production by *Aspergillus niger* XY-1 using response surface methodology for optimizing the medium composition. *J Zhejiang Univ Sci B*, 2008; **9**(7):558-566. doi: 10.1631/jzus.B0820038.
17. Y.C. Song, H. Li, Y.H. Ye, C.Y. Shan, Y.M. Yang, R.X. Tan, Endophytic naphthopyrone metabolites are co-inhibitors of xanthine oxidase, SW1116 cell and some microbial growths. *FEMS Microbiol Lett*, 2004; 241:67-72.
18. Yoko HASEGAWA, Takashi FUKUDA, Keiichi HAGIMORI, Hiroshi TOMODA,and Satoshi OMURA, Tensyuic Acids, New Antibiotics Produced by *Aspergillus niger* FKI-2342. *Chem Pharm Bull*, 2007; **55**(9): 1338-1341.
19. Yu, X., Hallett, S. G., Sheppard, J., Watson, A. K., Application of the Plackett Burman experimental design to evaluate nutritional requirements for the production of *Colletotrichum coccodes* spores. *Appl Microbiol Biotechnol*, 1997; **47**: 301-305.