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*Full Length Research Paper*

# Medium optimization for acarbose production by *Actinoplanes* sp. A56 using the response surface methodology

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**In order to improve the production of acarbose, the fermentation medium of acarbose-producing strain *Actinoplanes* sp. A56 was optimized in this paper. Firstly, fractional factorial design was employed to investigate the influences of glucose, maltose, corn steep liquor, soy bean flour and monosodium glutamate on acarbose production, and the results revealed that maltose and corn steep liquor had significant and positive effects. Then, the two significant factors used the method of the steepest ascent to reach the yield plateau of acarbose. To explore the best condition obtained by the steepest method, the concentrations of maltose and corn steep liquor were further optimized with central composite design and response surface analysis. As a result, the acarbose production was increased from 837 to 1043 mg/L by using this optimization strategy.**

**Key words:** Acarbose production, medium optimization, *Actinoplanes* sp. A56, response surface methodology.

## INTRODUCTION

Acarbose is a pseudo-oligosaccharide, which acts as a competitive α-glucosidase inhibitor. Because it is hardly digestible and generally have no detectable toxicity (Wehmeier and Piepersberg, 2004), acarbose has widely been used in the therapy of type II diabetes (non-insulin-dependent), which could better control blood sugar contents of patients after meals (Hanefeld et al., 2008; Kihara et al., 1997; Hanefeld, 2007).

Acarbose belongs to the member of the C7N-aminocyclitol family of natural products (Mahmud, 2003), which is produced by fermentation of *Actinoplanes* sp., and the enzymology of acarbose metabolism in *Actinoplanes* sp. SE50 has been elucidated (Drepper and Pape, 1996; Drepper et al., 1996; Goeke et al., 1996). According to its structure, acarbose consists of the moieties of acarviose and maltose. Acarviose is the core unit of acarbose, which is mainly responsible for inhibition of α-glucosidase. The maltosyl unit of acarbose is derived mostly directly from maltose (Lee et al., 1997). The linkage between acarviose and maltose in acarbose is established by an acarviosyl transferase (Hemker et al., 2001).

Many strategies have been employed to improve the production of acarbose (Beunink et al., 1997; Choi and Shin, 2003). However, to date, little information is available on the optimization of fermentation medium on acarbose biosynthesis. We previously reported the isolation and identification of *Actinomycetes* sp. A56, which could produce acarbose (Cheng et al., 2008). In the present work, the fermentation medium of *Actinoplanes* sp. A56 was optimized for acarbose production by using response surface methodology.

## MATERIALS AND METHODS

### Microorganism and media

*Actinoplanes* sp. A56 (Cheng et al., 2008) was used for acarbose production, which was maintained on agar slant containing (g/L): glucose, 20; peptone, 5; KCl 0.5; $K_2HPO_4$ 1.0; $MgSO_4$ 0.5; and agar, 2.0 (pH: 7.0).

Inoculum medium was composed of (g/L): glucose, 30; soy bean flour, 40; $KH_2PO_4$, 1.0; $MgSO_4$, 1.0; and $CaCO_3$, 20. The pH was adjusted to 7.0 with 1 N NaOH prior to sterilization.

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**Abbreviations: RSM,** Response surface methodology; **HPLC,** high performance liquid chromatography; **X1,** glucose; **X2,** maltose; **X3,** corn steep liquor; **X4,** soy bean flour; **X5,** monosodium glutamate.

**Table 1.** Levels and actual values of factors for fractional factorial design.

| Factors | Levels | | | | |
|---|---|---|---|---|---|
| | –2 | –1 | 0 | +1 | +2 |
| Glucose, $X_1$ (g/L) | 10 | 20 | 30 | 40 | 50 |
| Maltose, $X_2$ (g/ L) | 10 | 20 | 30 | 40 | 10 |
| Corn steep liquor, $X_3$ (g/ L) | 6 | 8 | 10 | 12 | 14 |
| Soy bean flour, $X_4$ (g/L) | 10 | 15 | 20 | 25 | 30 |
| Monosodium glutamate, $X_5$ (g/L) | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 |

The basic medium contained the following ingredients (g/L): glucose, 30; maltose, 30; corn steep liquor, 10; soy bean flour, 20; monosodium glutamate, 1.0; $CaCl_2$ , 2.0; $FeCl_3$, 0.5; $K_2HPO_4$ 1.0; and $CaCO_3$ 2.5. The pH was adjusted to 7.2 with 1 N NaOH before autoclaving.

### Culture conditions

All the cultures were grown in 250-mL Erlenmeyer flasks containing 30 ml media. Inoculum medium was inoculated with cells from fresh slant, and cultivated at 28℃ on a rotary shaker at 180 rpm for 48 h. The seed culture was then transferred to fermentation medium with 10% inoculum, and incubated at 28℃ on a rotary shaker at 180 rpm for 144 h.

### Experimental design and statistical analysis

Fractional factorial design was firstly used to identify the medium components that significantly influence acarbose production by *Actinoplanes* sp. A56. The results of the fractional factorial design were regressed to obtain a first order polynomial, and the significance of the regression coefficients was checked by Student *t*-test. Based on the above statistical analysis of fractional factorial design, the significant factors used steepest ascent path to reach the yield plateau. To efficiently explore the best condition obtained by the steepest method, the central composite design was performed, and the optimal concentrations of medium components for the acarbose production by *Actinoplanes* sp. A56 were determined by response surface methodology.

All experimental designs and statistical analyses were carried out by using the software DPS (Data Processing System) v. 7.05 (Tang and Feng, 2007).

### Quantification of acarbose in the broth

Acarbose concentration in fermentation broth was determined by HPLC. Broth sample (1.0 ml) was extracted for 30 min with 4.0 ml of ethanol. The mixture was then filtrated, and the resulting upper aqueous phase was injected into HPLC system (Agilent HP1100). $NH_2$ column (4.6 × 250 mm, 5 μm) was employed for HPLC analysis with a flow rate of 2.0 ml/min and a wavelength of 210 nm. The mobile phase was acetonitrile/phosphate buffer (0.6 g of $KH_2PO_4$ and 0.48 g of $Na_2HPO_4$ in distilled water) = 70/30 (v/v).

## RESULTS

## Fractional factorial design

According to the preliminary results obtained under mono-factor experiments, the following five components of the fermentation medium were optimized for further enhancing acarbose production by *Actinoplanes* sp. A56: glucose ($X_1$), maltose ($X_2$), corn steep liquor ($X_3$), soy bean flour ($X_4$) and monosodium glutamate ($X_5$). In the first optimization step, the above five components were evaluated by using fractional factorial design, and each factors were coded with five different levels (–2, –1, 0, +1, +2), as shown in Table 1. Based on the experimental design carried out by software DPS v. 7.05, a total number of 30 runs were required in the fractional factorial design (Table 2), in which four replicates were conducted at the center point. The results of fractional factorial design were also given in Table 2.

The analysis of the variation for the fractional factorial design was summarized in Table 3. From the results of regression analysis, it is obvious that the significant terms (significant at the 0.01 level) were $X_2$ (maltose) and $X_3$ (corn steep liquor). A linear model for acarbose yield (*Y*) could be obtained from the regression results of fractional factorial experiment:

$$Y \text{ (mg/L)} = 531.5333+1.0417X_1+3.8750X_2+13.9583X_3+0.4833X_4-10.4167X_5 \quad (1)$$

In Eq.-(1), $X_1$, $X_2$, $X_3$, $X_4$ and $X_5$ were the actual concentrations of glucose, maltose, corn steep liquor, soy bean flour and monosodium glutamate in fermentation medium, respectively. The statistical significance of the model was checked by the Student *t*-test. As shown in Table 3, the model was highly significant ($P < 0.0001$) and $R^2 = 0.7294$.

## The path of the steepest ascent

The direction of steepest ascent could be determined by Equation (1) and regression results. Coefficients of maltose ($X_2$) and corn steep liquor ($X_3$) in the linear model [Equation (1)] were significant and positive, which demonstrated that increasing the concentrations of maltose and corn steep liquor would have positive effects on acarbose production. Therefore, in order to improve acarbose yield, the concentrations of maltose and corn steep liquor should be increased. One basal increment of the concentrations of maltose and corn steep liquor was

**Table 2.** Experimental design and results of fractional fractional design.

| Run | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | Acarbose concentration (mg/L) |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 | 897 |
| 2 | 1 | 1 | 1 | –1 | –1 | 868 |
| 3 | 1 | 1 | –1 | 1 | –1 | 876 |
| 4 | 1 | 1 | –1 | –1 | 1 | 844 |
| 5 | 1 | –1 | 1 | 1 | –1 | 819 |
| 6 | 1 | –1 | 1 | –1 | 1 | 784 |
| 7 | 1 | –1 | –1 | 1 | 1 | 745 |
| 8 | 1 | –1 | –1 | –1 | –1 | 738 |
| 9 | –1 | 1 | 1 | 1 | –1 | 847 |
| 10 | –1 | 1 | 1 | –1 | 1 | 854 |
| 11 | –1 | 1 | –1 | 1 | 1 | 754 |
| 12 | –1 | 1 | –1 | –1 | –1 | 782 |
| 13 | –1 | –1 | 1 | 1 | 1 | 776 |
| 14 | –1 | –1 | 1 | –1 | –1 | 826 |
| 15 | –1 | –1 | –1 | 1 | –1 | 743 |
| 16 | –1 | –1 | –1 | –1 | 1 | 755 |
| 17 | –2 | 0 | 0 | 0 | 0 | 849 |
| 18 | 2 | 0 | 0 | 0 | 0 | 857 |
| 19 | 0 | –2 | 0 | 0 | 0 | 717 |
| 20 | 0 | 2 | 0 | 0 | 0 | 914 |
| 21 | 0 | 0 | –2 | 0 | 0 | 747 |
| 22 | 0 | 0 | 2 | 0 | 0 | 865 |
| 23 | 0 | 0 | 0 | –2 | 0 | 798 |
| 24 | 0 | 0 | 0 | 2 | 0 | 824 |
| 25 | 0 | 0 | 0 | 0 | –2 | 844 |
| 26 | 0 | 0 | 0 | 0 | 2 | 864 |
| 27 | 0 | 0 | 0 | 0 | 0 | 847 |
| 28 | 0 | 0 | 0 | 0 | 0 | 839 |
| 29 | 0 | 0 | 0 | 0 | 0 | 819 |
| 30 | 0 | 0 | 0 | 0 | 0 | 844 |

**Table 3.** Results of the regression analysis of the fractional fractional design.

| Term | Coefficients | t value | Significant level |
|---|---|---|---|
| Intercept | 531.5333 | 9.478 | <0.0001 ** |
| $X_1$ | 1.0417 | 1.710 | 0.100084 |
| $X_2$ | 3.8750 | 6.363 | <0.0001 ** |
| $X_3$ | 13.9583 | 4.584 | <0.0001 ** |
| $X_4$ | 0.4833 | 0.397 | 0.695007 |
| $X_5$ | –10.4167 | –0.342 | 0.735263 |

$R^2$ = 0.7294, F = 12.94, Prob>p = 0.00001.
** Meant significant at the 0.01 level.

set at 5 and 2 g/L each time, respectively, while the concentrations of the other components (glucose, soy bean flour and monosodium glutamate) were fixed at zero level. The experimental design and results of the steepest ascent was shown in Table 4. From Table 4, the maximal acarbose yield (1013 mg/L) was obtained at the seventh step. Therefore, 50 g/L of maltose and 18g/L of corn steep liquor were chosen as the central point for the

**Table 4.** Experimental design and results of the steepest ascent.

| Run | $X_1$ (Maltose, g/L) | $X_3$ (Corn steep liquor, g/L) | Acarbose concentration (mg/L) |
|---|---|---|---|
| 1 | 20 | 6 | 789 |
| 2 | 25 | 8 | 813 |
| 3 | 30 | 10 | 827 |
| 4 | 35 | 12 | 842 |
| 5 | 40 | 14 | 897 |
| 6 | 45 | 16 | 952 |
| 7 | 50 | 18 | 1013 |
| 8 | 55 | 20 | 985 |

**Table 5.** Design and results of central composite design.

| Run | Maltose ($X_2$) | | Corn steep liquor ($X_3$) | | Acarbose concentration (mg/L) |
|---|---|---|---|---|---|
| | Level | Value (g/L) | Level | Value (g/L) | |
| 1 | +1 | 60 | +1 | 21 | 1031 |
| 2 | +1 | 60 | –1 | 15 | 1017 |
| 3 | –1 | 40 | +1 | 21 | 998 |
| 4 | –1 | 40 | –1 | 15 | 867 |
| 5 | –1.414 | 35.9 | 0 | 18 | 914 |
| 6 | +1.414 | 64.1 | 0 | 18 | 1023 |
| 7 | 0 | 50 | –1.414 | 13.77 | 951 |
| 8 | 0 | 50 | +1.414 | 22.23 | 1004 |
| 9 | 0 | 50 | 0 | 18 | 1017 |
| 10 | 0 | 50 | 0 | 18 | 1003 |
| 11 | 0 | 50 | 0 | 18 | 1022 |
| 12 | 0 | 50 | 0 | 18 | 1006 |
| 13 | 0 | 50 | 0 | 18 | 1016 |

**Table 6.** Results of the regression analysis of central composite design.

| Term | Coefficients | t value | Significant level |
|---|---|---|---|
| Intercept | –1353 | –4.85 | 0.001856 ** |
| $X_2$ | 42.673 | 7.26 | 0.000168 ** |
| $X_3$ | 123.401 | 5.87 | 0.000620 ** |
| $X_2$*$X_2$ | –0.209 | –4.54 | 0.002667 ** |
| $X_3$*$X_3$ | –1.819 | –3.56 | 0.009262 ** |
| X2*$X_3$ | –0.975 | –4.84 | 0.001878 ** |

$R^2$ = 0.9648, F = 38.35, Prob>p = 6.103E-05.
** Meant significant at the 0.01 level.

further experiments of central composite design.

## Central composite design

The optimal concentration of medium components was determined using a central composite design with the two variables, maltose and corn steep liquor. The coded levels of the two factors and the experimental results were presented in Table 5, and the results of the regression analysis were summarized in Table 6.

From the results of regression analysis, the significant terms (significant at the 0.01 level) were as follows: $X_2$, $X_3$, $X_2^2$, $X_3^2$ and $X_2$*$X_3$. A second-order polynomial model

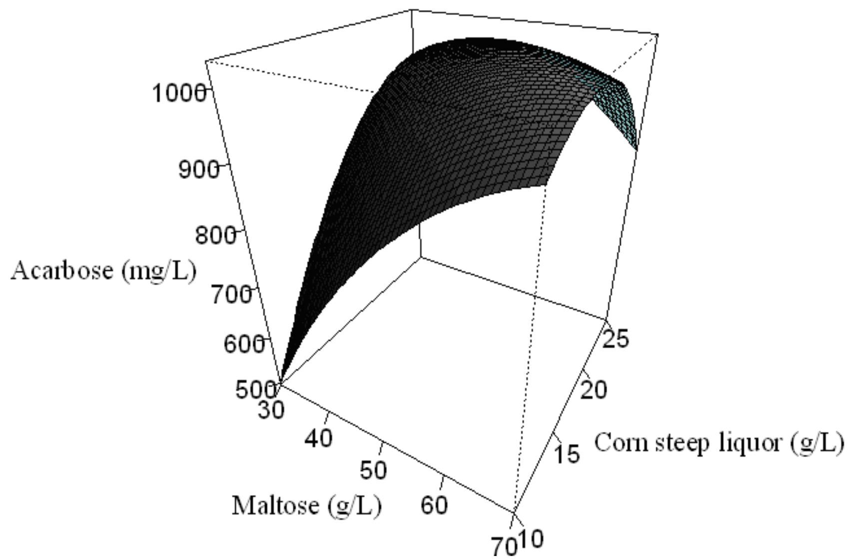


**Figure 1.** The three-dimensional presentation of the response surface for the concentrations of maltose and corn steep liquor on acarbose yield.

for predicting acarbose yield (*Y*) was obtained:

$$Y \text{ (mg/L)} = -1353+42.673X_2+123.401X_3-0.209X_2^2-1.819X_3^2-0.975X_2X_3 \quad (2)$$

In Equation (2), $X_2$ and $X_3$ were the actual concentrations of maltose and corn steep liquor, respectively. This regression model for acarbose production was highly significant ($P < 0.0001$) with a satisfactory value of determination coefficient ($R^2 = 0.9648$). Therefore, the Student *t*-test of regression demonstrated that the second-order model was adequate for the results obtained in experiments.

The optimal concentration of maltose and corn steep liquor for acarbose production was determined by response surface method, as shown in Figure 1. From equations derived by differentiation of Equation (2), the maximum point of the model could be obtained, which was 61.25 g/L of maltose and 17.50 g/L of corn steep liquor. The maximum response predicted from the model was 1033.81 mg/L. To confirm the predicted optimum, four replicated experiments were further performed at optimal point of maltose and corn steep liquor. The average acarbose yield obtained in the experiments with optimal medium was 1043.16 mg/L, which was close to the optimum value predicted by the model. The good correlation between the experimental and predicted results demonstrated that the second-order model was a valid model for predicting acarbose production.

As a result, the final composition of the optimal medium for *Actinoplanes* sp. A56 fermentation was as follow (g/L): glucose, 30; maltose, 61.25; corn steep liquor, 17.50; soy bean flour, 20; monosodium glutamate, 1.0; $CaCl_2$ , 2.0; $FeCl_3$, 0.5; $K_2HPO_4$ 1.0; and $CaCO_3$ 2.5. By using this optimization strategy, the acarbose yield obtained under the optimal medium was increased from 837 to 1043 mg/L, with 24.61% higher than that obtained with the original medium recipe.

## DISCUSSION

The results showed that maltose had significant and positive effects on acarbose production by *Actinoplanes* sp. A56. Due to maltose directly incorporated into acarbose (Lee et al., 1997), the level of maltose in culture broth probably plays an important role in acarbose biosynthesis. Choi and Shin (2003) investigated the effect of maltose on acarbose production in *Actinoplanes* sp. CKD485-16, and the results revealed that maltose concentrations should be maintained at high levels during cultivation to obtain high acarbose yields. In this paper, the maltose concentration in the optimal medium was increased from 30.00 to 61.25 g/L, which was consistent with the results obtained by Choi and Shin (2003).

Corn steep liquor is an excellent source of nitrogen for most microorganisms (Shah and Cheryan, 1995; Silveira et al., 2001). Furthermore, corn steep liquor is a major by-product of the corn wet-milling industry, which has been widely and successfully used as a low-cost medium for a variety of fermentations, e.g., the production of solvents, antibiotics, and enzymes (De Azeredo et al., 2006). Our results revealed that corn steep liquor was another significant and positive factor for acarbose production. Therefore, corn steep liquor can be used as the inexpensive nitrogen source for large-scale production of acarbose by *Actinoplanes* sp. A56.

Response surface methodology (RSM) is a useful statistical technique for the investigation and optimization of complex processes (Zhuang et al., 2006), which has widely and successfully been employed for the optimization of the medium components (Liu et al., 2004; Parra et al., 2005; Tang et al., 2004). In this paper, the fermentation medium of acarbose-producing strain *Actinoplanes* sp. A56 was optimized by using this optimization strategy. As a result, the acarbose yield obtained under the optimal medium was increased from 837 to 1043 mg/L, which also proved that response surface methodology was a valuable tool in optimizing medium.

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