PRODUCTION OF ANGKAK THROUGH CO-CULTURE OF MONASCUS PURPUREUS AND MONASCUS RUBER

Bibhu Prasad Panda¹, Saleem Javed², Mohd Ali¹

¹Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India-110062; ²Molecular Biology and Biotechnology Laboratory, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India-110062.

Submitted: August 12, 2008; Returned to authors for corrections: March 30, 2009; Approved: February 19, 2010.

ABSTRACT

Angkak (red mold rice, red yeast rice, Chinese red rice) is a traditional Chinese medicine produced by solid-state fermentation of cooked non-glutinous rice with Monascus species. The secondary metabolite of Monascus species, monacolin K /lovastatin, has been proven to lower blood lipid levels. In this study, a co-culture of Monascus purpureus MTCC 369 and Monascus ruber MTCC 1880 was used for angkak production. Four medium parameters screened by Plackett-Burman design were optimized by response surface methodology for highest lovastatin production in angkak during solid-state fermentation by the co-culture. Maximum lovastatin production of 2.84 mg g⁻¹ was predicted in solid medium containing 20 g rice and 40 ml liquid nutrients medium (malt extract 9.68 g l⁻¹, dextrose 38.90 g l⁻¹, MnSO₄·H₂O 1.96 g l⁻¹, and MgSO₄·7H₂O 0.730 g l⁻¹) by point prediction tool of Design Expert 7.1 software (Statease Inc. USA).

Key words: Angkak. Co-culture. Monascus purpureus. Monascus ruber. Lovastatin. Response surface methodology

INTRODUCTION

Angkak (red mold rice, red yeast rice, Chinese red rice) is a traditional Chinese medicine produced by solid-state fermentation of cooked non-glutinous rice (Oryzae sativa L. Gramineae) with Monascus purpureus, M. ruber, M. anka and M. pilosus (3, 6, 9, 14). The angkak has long been recognized as a folk medicine for improving food digestion and blood circulation and for treatment of muscle bruising and dysentery. The manufacturing process for angkak and its therapeutic applications are well documented in the ancient Chinese pharmacopoeia (Ben-Taso-Gum-Mu) (9, 12). Recent chemical investigation and clinical observation show that angkak contains different secondary metabolites: lovastatin (HMG Co A reductatse inhibitor), also known as monacolin K, which lowers blood lipid levels in animal models and in humans, γ-aminobutyric acid (GABA) which has blood pressure lowering effects, dimerumic acid which is antioxidant, and monascin which has anti-inflammatory effects (6,10,11,24).

A previous study on red mold rice production by Monascus species under monoculture conditions showed that secondary metabolites production is greatly affected by fermentation medium, cultivation conditions and types of Monascus species used in the fermentation process (1, 5, 13, 21, 23). Increasing concentration of lovastatin in angkak was a prime area of research in order to produce high quality bio-
nutraceutical or health functional food (4, 11, 22). During process optimization, screening or selection of important parameters influencing fermentation productivity is initially carried out by barrowing method or by Plackett-Burman design, and finally the screened parameters are optimized using different advanced statistical techniques (3, 7, 8, 15). Response surface methodology (RSM) is a widely used technique for optimization of fermentation process parameters. RSM based on Box-Behnken’s design has some advantages over other designs like central composite and full factorial design. RSM requires less experiment runs, and is suitable for multiple factor experiments, search for relationships between factors, and for finding the most suitable condition and prediction of response (8, 19).

In nature, solid-state fermentation (SSF) is carried out by mixed or co-cultures of different fungal species. The co-culture of fungi during fermentation may provide help for better biomass and secondary metabolites production. There are several reports of co-culture of fungal species, and mixed cultures have been found to enhance enzyme and organic acid production (2, 16). However, no reports are available on production of lovastatin (monacolin K) by co-culture of different Monascus species under SSF condition. Therefore, the objective of this study was to produce finest quality angkak containing maximum amount of lovastatin by co-culture of two Monascus species.

Two filamentous fungi, Monascus purpureus MTCC 369 and Monascus ruber MTCC 1880, were used together as inoculum for production of angkak. The effects of medium parameters on lovastatin production were studied and screened by Plackett-Burman design (PBD) and their optimum levels were determined by response surface methodology (RSM).

**MATERIALS AND METHODS**

**Chemicals and raw materials**

Pure lovastatin was a gift from Ranbaxy Laboratories (New Delhi, India). HPLC grade acetonitrile was purchased from Merck (Bombay, India). Microbiological media and chemicals were purchased from Himedia Laboratories (Bombay, India) and long nonglutinous rice was purchased from a local market of New Delhi, India.

**Microorganisms**

Cultures of Monascus purpureus MTCC 369 and Monascus ruber MTCC 1880 were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Fungal cultures were maintained routinely on potato dextrose agar (PDA) medium and subcultured in every 30 d interval (3, 19).

**Preparation of mixed seed cultures**

Spore suspensions of M. purpureus and M. ruber were prepared separately from actively growing slants in sterile water and diluted to a concentration 5.7 \times 10^³ spores ml⁻¹. Spore counting was carried out using a hemocytometer. Spore suspension (15% v/v) (7.5ml) of M. purpureus was inoculated to conical flasks containing 50ml basal medium (100g dextrose, 10g peptone, 2g KNO₃, 2g NH₄H₂PO₄, 0.5g MgSO₄·7H₂O, 0.1g CaCl₂ in 1000 ml distilled water; adjusted to pH 6.0) and incubated at 30°C for 48 h in a shaker incubator at 110 rpm (19,21). For preparation of Monascus ruber seed culture, 15% spore suspension (7.5ml) of M. ruber was inoculated to conical flasks containing 50ml of potato dextrose broth (PDB), and incubated at 30°C for 4 days with shaking at 150 rpm (3). Finally the two seed cultures of M. purpureus and M. ruber were mixed at a ratio of 1:1.

**Solid -State Fermentation (SSF)**

Long grain, non-glutinous rice was purchased from a local market of New Delhi, India and used as substrate for angkak production under solid-state culture. Initially 20 g of pre-soaked rice was transferred to a 250 ml conical flask to which 40 ml of distilled water containing different nutrients (Table 1) was added. The pH of the medium was adjusted to 6.0 with 0.1M HCl or NaOH and autoclaved for 20min at 121°C. The cooled rice-based medium was inoculated with 5ml of the mixed seed cultures of M. purpureus and M. ruber, incubated for 14 days at 30°C and 70% relative humidity (21).
Table 1. Concentrations of variables of liquid medium in Plackett-Burman design for solid-state fermentation.

| Designation | Variable      | Low level (-) Per liter | High level (+) Per liter |
|-------------|---------------|-------------------------|--------------------------|
| X₁          | Peptone       | 5.0g                    | 15.0g                    |
| X₂          | Glucose       | 80g                     | 160g                     |
| X₃          | Glycerin      | 16ml                    | 32ml                     |
| X₄          | NaCl          | 2.0g                    | 8.0g                     |
| X₅          | NH₄Cl         | 2.0g                    | 8.0g                     |
| D₁          | Dummy 1       | -                       | -                        |
| X₆          | MgSO₄·7H₂O    | 0.1g                    | 0.9g                     |
| X₇          | CaCl₂·2H₂O    | 0.0g                    | 0.6g                     |
| X₈          | MnSO₄·H₂O     | 0.0g                    | 1.5g                     |
| X₉          | Malt extract  | 2.0g                    | 8.0g                     |
| D₂          | Dummy 2       | -                       | -                        |

Extraction of lovastatin from angkak

Angkak (1g) was suspended in 5 ml ethyl acetate and kept in a shaker incubator at 180 rpm and 70°C for 1.5 h. The mixture was centrifuged at 3000g for 8 min and 10 ml of 1% (v/v) trifluoroacetic acid was added to 1ml of the supernatant for lactonization of the lovastatin. The mixture was concentrated at 80°C, mixed with 1ml with acetonitrile and filtered through 0.45μm filter for HPLC (High Performance Liquid Chromatography) analysis (21).

Quantitative analysis of lovastatin

For HPLC analysis the procedure of Samiee et al. for HPLC, with slight modification, was used. Lovastatin concentration was estimated by HPLC (SHIMADZU, Japan) using a 250 mm x 4.6 mm ID Lichrospher® 100 C₁₈ column containing 5μm sized particles and a 20μl loop injector. The mobile phase was acetonitrile:water acidified to the concentration 0.1% (v/v) with ortho-phosphoric acid (65:35 v/v). The flow rate was 1.5 ml min⁻¹. Detection was carried out by a UV-detector at 235 nm (18, 19).

Screening of nutrient parameters

Peptone, glucose, glycerine, NaCl, NH₄Cl, MgSO₄·7H₂O, CaCl₂·2H₂O, malt extract, and MnSO₄·H₂O were the nine medium constituents selected for study. The selection of nutrients was done by barrowing methodology (8, 15). The Plackett-Burman experimental design for eleven variables (Table 2) with nine nutritional components (X₁ to X₉) (independent variables) and two dummy variables (D₁ and D₂) were used to evaluate the relative importance of the nutrients for high quality angkak production with high quantity of monacolin K (lovastatin). For each nutrient variable two different concentrations [high (+) and a low (-)] were tested (Table 2). Influence of medium variables on lovastatin production was calculated according to standard analysis procedure of Plackett-Burman experimental design (17).

Table 2. Plackett-Burman experimental design of 12 trials for eleven variables (9 nutrients + 2 dummy) and observed concentration of lovastatin in angkak samples.

| Trial | X₁ | X₂ | X₃ | X₄ | X₅ | D₁ | X₆ | X₇ | X₈ | X | D₂ | Mean lovastatin (mg g⁻¹) |
|-------|----|----|----|----|----|----|----|----|----|---|----|--------------------------|
| 1     | +  | +  | -  | +  | +  | -  | -  | -  | -  | + | -  | 1.060                    |
| 2     | -  | +  | +  | -  | +  | +  | -  | -  | -  | - | +  | 0.563                    |
| 3     | +  | -  | +  | +  | -  | +  | +  | -  | -  | - | -  | 0.764                    |
| 4     | -  | +  | -  | +  | +  | -  | +  | +  | -  | - | -  | 0.450                    |
| 5     | -  | -  | +  | -  | +  | +  | +  | +  | -  | - | -  | 1.880                    |
| 6     | -  | -  | -  | +  | +  | +  | -  | +  | +  | - | -  | 1.990                    |
| 7     | +  | +  | -  | -  | -  | +  | -  | +  | +  | - | -  | 1.780                    |
| 8     | +  | +  | -  | -  | +  | +  | -  | +  | -  | - | -  | 0.764                    |
| 9     | +  | +  | -  | -  | -  | +  | +  | -  | +  | - | -  | 1.760                    |
| 10    | -  | +  | +  | +  | -  | -  | +  | -  | +  | - | -  | 1.080                    |
| 11    | +  | -  | +  | +  | +  | -  | -  | +  | -  | - | -  | 0.984                    |
| 12    | -  | -  | -  | -  | -  | -  | -  | -  | -  | - | -  | 0.534                    |
Response surface methodology experimental design

In Plackett-Burman experimental design, various carbon, nitrogen and micronutrients were evaluated for their influence in the production of lovastatin during solid-state fermentation by mixed cultures of \textit{M. purpureus} and \textit{M. ruber}. Preliminary data of Plackett-Burman experimental design indicated that lovastatin production was greatly influenced by malt extract, dextrose, MnSO\(_4\).H\(_2\)O and MgSO\(_4\).7H\(_2\)O (Table 3). Therefore these four medium parameters were chosen for further optimization by response surface methodology (3, 19, 20). The various levels of nutrients are summarized in Table 4. An experimental design of 29 runs containing 3 central points (Table 5) was made according to Box-Behnken’s response surface design for selected four parameters using Design Expert 7.1 software of Statease Inc. USA. The response was measured in terms of lovastatin concentration in produced angkak. An optimum value of the factors for maximum production of lovastatin was determined by the point prediction tool of the software.

| Designation | Variable | \(\Sigma H\) | \(\Sigma L\) | Mean square | Effect | F Value | % Contribution | P value |
|-------------|----------|------------|------------|------------|--------|---------|----------------|---------|
| X\(_1\) | Peptone | 7.112 | 6.497 | 0.034 | 0.102 | 1.214 | 0.968 |
| X\(_2\) | Dextrose | 5.677 | 7.932 | 0.421 | -0.375 | 15.035 | 11.900 |
| X\(_3\) | Glycerine | 7.031 | 6.578 | 0.016 | 0.075 | 0.571 | 0.454 |
| X\(_4\) | NaCl | 6.328 | 7.281 | 0.076 | -0.158 | 2.714 | 0.216 |
| X\(_5\) | NH\(_4\)Cl | 6.717 | 6.892 | 0.002 | -0.029 | 0.071 | 0.061 |
| X\(_6\) | MnSO\(_4\).7H\(_2\)O | 7.307 | 6.302 | 0.085 | 0.167 | 3.035 | 0.240 |
| X\(_7\) | CaCl\(_2\).2H\(_2\)O | 6.718 | 6.891 | 0.002 | -0.288 | 0.071 | 0.076 |
| X\(_8\) | MnSO\(_4\).H\(_2\)O | 7.828 | 5.781 | 0.345 | 0.341 | 12.321 | 0.971 |
| X\(_9\) | Malt extract | 9.550 | 4.059 | 2.510 | 0.915 | 89.642 | 70.700 |

Table 4. Levels of nutrient parameters used in Box-Behnken’s response surface design

| Nutrient parameter | Levels |
|-------------------|--------|
| (g l\(^{-1}\)) | -1 | 0 | +1 |
| Malt extract | 07.00 | 10.00 | 13.00 |
| Dextrose | 20.00 | 40.00 | 60.00 |
| MnSO\(_4\).H\(_2\)O | 01.00 | 01.50 | 02.00 |
| MgSO\(_4\).7H\(_2\)O | 00.50 | 01.00 | 01.50 |
Table 5. Box-Behnken’s design with actual and predicted lovastatin concentrations

| Run | Malt extract (g l⁻¹) Code A | Dextrose (g l⁻¹) Code B | MnSO₄·H₂O (g l⁻¹) Code C | MgSO₄·7H₂O (g l⁻¹) Code D | Lovastatin (mg g⁻¹) |
|-----|-----------------------------|-------------------------|--------------------------|---------------------------|--------------------|
|     | Actual                      | Predicted               | Actual                   | Predicted                 |                    |
| 1   | 7                           | 20                      | 1.5                      | 1                         | 1.58               | 1.44               |
| 2   | 13                          | 20                      | 1.5                      | 1                         | 0.612              | 0.27               |
| 3   | 7                           | 60                      | 1.5                      | 1                         | 0.756              | 0.784              |
| 4   | 13                          | 60                      | 1.5                      | 1                         | 0.986              | 0.812              |
| 5   | 10                          | 40                      | 1                        | 0.5                       | 2.68               | 2.27               |
| 6   | 10                          | 40                      | 2                        | 0.5                       | 2.45               | 1.93               |
| 7   | 10                          | 40                      | 1                        | 1.5                       | 0.764              | 0.966              |
| 8   | 10                          | 40                      | 2                        | 1.5                       | 0.875              | 0.97               |
| 9   | 7                           | 40                      | 1.5                      | 0.5                       | 2.58               | 2.49               |
| 10  | 13                          | 40                      | 1.5                      | 0.5                       | 0.765              | 1.01               |
| 11  | 7                           | 40                      | 1.5                      | 1.5                       | 0.657              | 0.446              |
| 12  | 13                          | 40                      | 1.5                      | 1.5                       | 0.654              | 0.78               |
| 13  | 10                          | 20                      | 1                        | 1                         | 0.435              | 0.668              |
| 14  | 10                          | 60                      | 1                        | 1                         | 1.78               | 1.86               |
| 15  | 10                          | 20                      | 2                        | 1                         | 1.78               | 1.75               |
| 16  | 10                          | 60                      | 2                        | 1                         | 0.645              | 0.447              |
| 17  | 7                           | 40                      | 1                        | 1                         | 1.76               | 1.78               |
| 18  | 13                          | 40                      | 1                        | 1                         | 0.784              | 0.667              |
| 19  | 7                           | 40                      | 2                        | 1                         | 0.674              | 1.07               |
| 20  | 13                          | 40                      | 2                        | 1                         | 0.783              | 1.04               |
| 21  | 10                          | 20                      | 1.5                      | 0.5                       | 1.56               | 1.95               |
| 22  | 10                          | 60                      | 1.5                      | 0.5                       | 1.24               | 1.62               |
| 23  | 10                          | 20                      | 1.5                      | 1.5                       | 0.645              | 0.543              |
| 24  | 10                          | 60                      | 1.5                      | 1.5                       | 0.873              | 0.763              |
| 25  | 10                          | 40                      | 1.5                      | 1                         | 2.65               | 2.63               |
| 26  | 10                          | 40                      | 1.5                      | 1                         | 2.56               | 2.63               |
| 27  | 10                          | 40                      | 1.5                      | 1                         | 2.67               | 2.63               |
| 28  | 10                          | 40                      | 1.5                      | 1                         | 2.65               | 2.63               |
| 29  | 10                          | 40                      | 1.5                      | 1                         | 2.63               | 2.63               |
RESULTS

Among the nine nutrient components used in the Plackett-Burman Experimental Design, malt extract, dextrose, MnSO$_4$.H$_2$O, and MgSO$_4$.7H$_2$O had contributed to a large extent for lovastatin production. Peptone, glycerine, CaCl$_2$.2H$_2$O, and NH$_4$Cl had little impact, while NaCl contributed moderately in production of lovastatin in angkak under the co-culture system.

The predicted and experimental lovastatin concentrations obtained from Box-Behnken’s response surface design in each run are show in Table 5. Results were analyzed using the software Design Expert 7.1 and fitted into a multiple nonlinear regression model, resulting in the following equation for lovastatin production:

Lovastatin (mg g$^{-1}$) = $+2.63-0.285A-0.0275B-0.0832C-0.568D+0.299AB+0.272AC +0.452AD -0.622BC +0.137BD +0.0853CD -0.924A^2 -0.884B^2 -0.569C^2 -0.529D^2$

This model resulted in six response graphs and a few representative surface graphs with contours of nonlinear regression model for lovastatin production, shown in Fig. 1a, 1b, 1c and 1d. Analysis of variance of regression is summarized in Table 6. The optimum values of medium parameters for maximum lovastatin production were malt extract 9.68 g l$^{-1}$, dextrose 38.90 g l$^{-1}$, MnSO$_4$.H$_2$O 1.96 g l$^{-1}$, and MgSO$_4$.7H$_2$O 0.730 g l$^{-1}$. These values predicted 2.84 mg g$^{-1}$ of lovastatin production in angkak under co-culture of M. purpureus and M. ruber. These optimized values of nutrient parameters were validated by solid-state fermentation and an average 2.75 mg g$^{-1}$ of lovastatin production in angkak was obtained, indicating a 96.83 % validity of the predicted model.

| Table 6. Analysis of variance of the calculated model for lovastatin production |
|-------------------------------------------------|
| **Regression**                                  | 17.8 |
| Sum of squares                                  | 14   |
| df                                              | 1.27 |
| Mean squares                                    | 12.3 |
| $F$ Value                                       | <0.0001 |
| **Residual**                                    | 1.45 |
| Sum of squares                                  | 14   |
| df                                              | 0.103 |
| Mean squares                                    | 81.4 |
| $P$ Value                                       | 0.000354 |
| Correlation coefficient ($r^2$)                 | 0.925 |
| Coefficient of variation (CV %)                 | 22.5 |
| Adequate precision value                        | 10.2 (> 4) |

![Graphs](image_url)
DISCUSSION

Plackett-Burman experimental design and response surface methodology are powerful tools for screening and optimization of medium parameters for lovastatin production under solid-state fermentation by co-culture of *M. purpureus* and *M. ruber*.

Malt extract, dextrose, MnSO$_4$.H$_2$O, and MgSO$_4$.7H$_2$O influenced negatively lovastatin production. The model equation indicated that all medium parameters except dextrose and MnSO$_4$.H$_2$O interacted positively with each other in lovastatin production. The simultaneous effects of two medium parameters on lovastatin production while the other two parameters were kept at optimized levels was depicted in the contours and surface graphs. As shown in Figure 1, reducing malt extract, dextrose, MgSO$_4$.7H$_2$O concentrations below the original search levels and increasing MnSO$_4$.H$_2$O concentration above the original levels stimulated production of lovastatin under co-culture of *M. purpureus* and *M. ruber*.

Co-culture of the two *Monascus* species resulted in production of 2.75 mg of lovastatin per gram of optimized solid medium in angkak, which is much higher then the lovastatin or monacolin K concentration obtained under mono culture of *Monascus pilosus* M12-69, *Monascus purpureus* NTU 601, 301 or *Monascus purpureus* BCRC 31499, 31504, 31530, 31540, 32966, 32807, 32808, 32809 on different substrates (4). However the lovastatin / monacolin K production in dioscorea by *Monascus purpureus* NTU 601 was found to be equivalent to lovastatin produced by co-culture of *Monascus* species in rice (11). This suggests that co-culture or mixed cultures of different *Monascus* species might result in high quantity of lovastatin or monacolin K in angkak.

REFERENCES

1. Babitha, S.; Soccol, C.R.; Pandey, A. (2007). Effect of stress on growth, pigment production and morphology of *Monascus* sp. in solid cultures. *J. Basic Microbiol.*, 47(2), 118-126.
2. Banerjee, R.; Mukherjee, G.; Patra, K.C. (2005). Microbial
transformation of tannin–rich substrate to gallic acid through co culture method. Bioresource Technol. 96(8), 949-953.

3. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chen, F.; Hu, X. (2005). Study on red fermented rice with high concentration of monacolin K and low concentration of citrinin. Int J. Food Microbiol. 103(3), 331-337.

5. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.

4. Chang, Y.N.; Huang, J.C.; Lee, C.C.; Shih, I.L.; Tzeng, Y.M. (2002). Use of response surface methodology to optimize by Monascus ruber. Enzyme Microb. Tech. 30(7), 889-894.