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Research Article

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Optimizing the conditions for adenosine extraction from mycelia of *Cordyceps gunnii* using genetic algorithm

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ABSTRACT

The study was performed to optimize the adenosine extraction conditions from the mycelium of Cordyceps gunnii by artificial neural network coupled with genetic algorithm. With the extraction rate of adenosine as index, the critical factors selected for the investigation were extracting temperature, extracting time and solid-liquid radio. The results obtained by the application of GA-ANN were more reliable since better statistical parameters were obtained. The optimum extraction procedure was as follow: extracting time 2.88h, extracting temperature 46.7℃, solid-liquid ratio 1:53 g·mL-1. The extraction rate of adenosine under this condition could reach the highest value of 4.93 mg g-1 with one time extraction.

Key words: Adenosine, Cordyceps gunnii, Artificial neural network, Genetic algorithm

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INTRODUCTION

Cordyceps gunnii (berk.) Berk, a major entomogenous fungus, belongs to the Ascomycota, Pyrenomycetes, Sphaeriales, Clavicipitaceae and parasites on the larvae of Hepialidae. It is also well known as the Chinese rare caterpillar fungus and has similar pharmacological activities to the famous Chinese traditional medicine Cordyceps sinensis[1-2]. Many important secondary metabolic products were found in Cordyceps gunnii mycelia including adenosine, cordycepin, cordycepic acid and anti-ultraviolet (UV) radiation constituents[3-4]. Consequently, it has received special attention for medicinal purpose due to its various physiological constituents.Artificial neural network (ANN) is a non-linear computational model based on biological neural networks[5-7]. It simulates the human brain learning process by mathematically modeling the network structure of interconnected node cells. ANN possesses good predictive capability for complex and non-linear systems. There were several literatures demonstrated that the predictive accuracy of ANN models were superior to RSM model using the same experiment design. However, ANN is known as a black box modeling approach. The relationships between the variables and responses are not described by specification of suitable fitting function in ANN models. The effects of factors on response values and the interaction effects among the factors cannot be studied by ANN model[8-9]. Genetic algorithm (GA) is usually suggested to search for optimum solutions in non-linear systems. It mimics the principles of biological evolution, namely “survival-of-the-fittest” and “random exchange of data during propagation” followed by biologically evolving species[10]. In present study, sequential statistical methods including BBD, ANN and GA were successfully applied to optimize the extraction conditions for maximizing the extraction rate of adenosine from Cordyceps gunnii mycelium. The predictive capability of ANN model was much more satisfied than BBD model in this complex system[11].

EXPERIMENTAL SECTION

Materials

The strain of Cordyceps gunnii Mycelia was given by Jilin Tonghua Yongcang Pharmaceutical Co., Ltd. The

Cordyceps gunnii Mycelia was maintained in cuvette with 20% glycerol modified PDA medium at -80°C. The modified PDA medium composed of (g·L-1): glucose 20, peptone 20, KH2PO4·5H2O 3.0, MgSO4·7H2O 3.0 and was autoclaved at 121°C for 30 minutes. The maintained strain was inoculated to 100 mL sterile modified PDA medium in Erlenmeyer flask (250 mL) incubated at 26°C in rotary shaker at 150 r/min for 4 d. Then, the culture was inoculated to Erlenmeyer flask (250 mL) containing 100 mL sterile modified PDA medium, which was incubated at 26°C in rotary shaker at 150 r/m for 4 d, and the seed culture had been prepared. After submerged culture of this strain, the mycelium was harvested by centrifugation for 8min at 5000 r/min to separate it from the liquid medium. After repeated washing with distilled water, the mycelial pellets were lyophilized for this experiment.

The Extraction of Adenosine from Cordyceps Gunnii Mycelium

The adenosine was extracted by warm water at various extracting temperature, extracting time and solid-liquid ratio from the powder of lyophilized mycelium. After this extraction, the solution was centrifuged at 10000 rpm for 10 min to obtain the crude adenosine solution.

Determination Method

The adenosine contents in mycelium were determined by using a Shimadzu HPLC system with tow LC-6AD pumps and SPD-A UV-vis detector (Shimadzu, Kyoto Japan). 20 μL of each sample was injected into the separation column (3.9×250 mm; Agilent ZORBAX SB C-18, 4 μm) in a mobile phase of 85% methanol and 15% PBS (pH 6.5). The column temperature was 35°C and the detection wavelength was 260 nm.

Experimental Design

In this paper, we followed by the use of Box-Behnken design, multi-quadratic regression (MQR), artificial neural network linked with genetic algorithm (ANN-GA) to optimization of the extracting process for the extraction ratio of adenosine.

RESULTS AND DISCUSSION

Response Surface Methodology

RSM was used in this experiment to study the effects of solid-liquid ratio, extracting temperature, and extracting time. In this experimental design, three coded levels for each variable were selected. The independent variables and representative coded and uncoded levels were given in Table 1. Fifteen experimental runs were carried out in present work. The experimental data were shown in Table 2. A regression analysis was carried out to attain a mathematical model that better described the relation among indepen ent variables and the studied response. The mathematical model was presented as follow:

$$Y=4.6937+0.1110X1+0.0802X2+0.0816X3–0.1627X1X1+0.0856X1X2+0.0401X1X3–0.1654X2X2–0.0535X2X3–0.2055X3X3$$

Table 1. Independent variables and their levels in the Box-behnken design

Independent variables	Coded values		
	-1	0	1
extracting time (*X1*)/h	1.5	2.5	3.5
extracting temperature (*X2*)/ °C	25	40	55
solid-liquid ratio (*X3*)/ g·mL-1	1:30	1:45	1:60

Table 2. THE DESIGN MATRIX AND THE RESULTS OF BBD

Num	*X1*	*X2*	*X3*	*Y*
1	-1	-1	0	4.1944
2	-1	1	0	4.3014
3	1	-1	0	4.2586
4	1	1	0	4.708
5	0	-1	-1	4.1623
6	0	-1	1	4.4405
7	0	1	-1	4.3121
8	0	1	1	4.3763
9	-1	0	-1	4.1837
10	1	0	-1	4.3121
11	-1	0	1	4.2586
12	1	0	1	4.5475
13	0	0	0	4.6973
14	0	0	0	4.7187
15	0	0	0	4.6652

Table 3. THE STATISTICAL RESULTS OF MQR MODEL

Sources	DF	SS	MS	F	*P>F*
X1	1	0.0986	0.0986	16.6294	0.0096
X2	1	0.0515	0.0515	8.6901	0.0320
X3	1	0.0532	0.0532	8.9821	0.0302
X1X1	1	0.0978	0.0978	16.4919	0.0097
X1X2	1	0.0293	0.0293	4.94367	0.0768
X1X3	1	0.0064	0.0064	1.0863	0.3450
X2X2	1	0.1010	0.1010	17.0386	0.0091
X2X3	1	0.0114	0.0114	1.9311	0.2233
X3X3	1	0.1560	0.1560	26.3080	0.0037
Model	9	0.5589	0.0621	10.4753	0.0093
Error	5	0.0296	0.0059		
Total	14	0.5886		*R2*	0.9496

The R2 of the model was 0.9496 and the root mean square error (RMSE) was 0.0770. The results were shown in table 3 and the effects of individual variables and the mutual effects between the variables were learnt from these results. As can be seen in Table 3, the effects of extracting time (X1), extracting temperature (X2), and solid-liquid ratio (X3) were extremely significant. The effects of interaction among other factors were insignificant. Partial derivative method was employed to search for the maximum value in model. The optimum condition was found as follows: extracting time 2.95h, extracting temperature 44.9°C, solid-liquid ratio 1:48 g·mL-1. Then the highest extraction rate of adenosine of 4.74 mg·g-1 predicted by the model could be obtained.

Artificial Neural Network and Genetic Algorithm

ANN a nonlinear modeling method was also employed to model the data of BBD for more accurate prediction and pursuing the true maximum yield rate in the test regions.

The BBD data were divided into three sets. 2 of them were used as prediction set, 2 of them were used as test set and 11 of them were used as calibration set. The extraction rate was used as output data. The coded variables were used as input data. And then, the ANN model was developed. The ANN model was optimized by selecting the suitable number of hidden nodes. The most suitable number of hidden nodes with the maximum Da was 7, as the Fig.1. The R2 of the optimized ANN model was 0.9814 which indicating that the fit of this model was very satisfied. The root mean square error of test set (RMSET) and the root mean square error of prediction set (RMSEP) were 0.0054 and 0.0128. These results indicated that the predictive capability of the ANN model was satisfied.

While the ANN model was developed, genetic algorithm (GA) was employed to search for the optimum medium which was as follow: extracting time 2.88h, extracting temperature 46.7°C, solid-liquid ratio 1:53 g·mL-1. Then the highest extraction rate of adenosine of 4.93 mg·g-1 predicted by the model could be obtained. And it was increased 4.01% from that obtained by MQR model.

The validation experiments with these optimum conditions were implemented in triplicate and the average extraction rate of adenosine was 4.84 mg·g-1. The relative error between expected value and exprimental value was 1.82%.

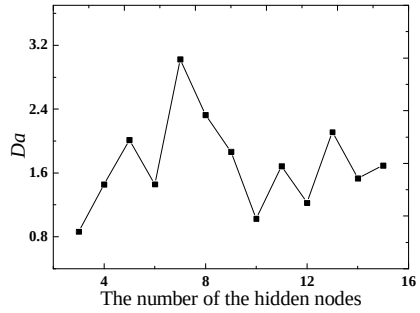

Fig. 1 The effect of the number of hidden nodes on *Da*

CONCLUSION

In the case exposed here, ANN provided more reliable results than RSM for defining the formulation of an improved extraction condition of adenosine. Optimum conditions (extracting time 2.88h, extracting temperature 46.7°C, solid-liquid ratio 1:53 g·mL-1) for extraction procedure were identified at last. The extraction rate of adenosine

under this condition could reach the highest value of 4.93 mg·g-1 with one time extraction.

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