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Research on Biotransformation Media of baicalin to Baicalein by White-rot Fungi

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Abstract

Baicalin, an important effective component in *Radix Scutellariae*, possesses extensive pharmacological activities and enjoys extensive application in drugs. However, it cannot be easily absorbed by humans. As the aglycone of baicalin, baicalein is more pharmacologically effective and better bio-available than baicalin, but less concentrated in *Radix Scutellariae*. Taking *White-rot Fungi* as the biotransformation microorganism, this paper carries out the biotransformation of baicalin and optimizes the carbon and nitrogen sources in the biotransformation media. Generally, the best biotransformation media of baicalin by *White-rot Fungi* was optimized as the combination of 0.0017% arginine, 0.0017% valine, 0.0017% glutamic acid, 0.0017% alanine, 0.0017% histidine, 0.0017% tyrosine and 0.1% urea, 0.6% KH_2PO_4, 0.1% $CaCl_2$, and 0.1% $MgSO_4$ at the pH of 5.5. The combination can achieve the biotransformation efficiency of 89.8%. If there is no carbon source but the substrate baicalin, the biotransformation of baicalin would proceed well, even better than using corncob, bran, glucose, dissoluble starch as carbon source. The best nitrogen source was the combination of 0.1% urea and six amino acids. Through comprehensive consideration of efficiency and cost, 0.1% urea was the most applicable inorganic nitrogen source for the biotransformation of baicalin by *White-rot Fungi*.

Key words

Baicalin, Biotransformation media, *White-rot Fungi*, β-glucuronidase.

1. Introduction

Thanks to the high biological activities in the root, *Scutellaria baicalensis* is amongst the 50 fundamental herbs used in traditional Chinese medicine. The main effective components in *Scutellaria baicalensis* include baicalin and its aglycone baicalein. The two components have been extensively applied in the pharmaceutical industry due to such properties of anti-inflammatory effect, toxin resistance, fever relief and liver protection [9, 14, 3, 12, 11, 13]. In general, *Scutellaria baicalensis* is rich in baicalin and poor in baicalein. However, baicalin can hardly be adsorbed by human digestive tract, while baicalein boasts good pharmacological effects and high bio-availbility [12, 11, 13, 4, 10, 1]. Thus, it is very meaningful to convert baicalin to baicalein.

The conversion is possible by biological, chemical and physical means. Compared with chemical and physical methods, biotransformation is an enzyme-catalyzed process with mild conditions, simple operation, strong effect, high selectivity and environmental-friendliness. There have been many successful cases of microbial biotransformation. For instance, rutin was bio-transformed by fungi (*Streptomyces griseus* and *Aspergillus niger*) with an efficiency of 54% [16, 2]. The microbial transformations of artemisinin was made possible by the alga *Cunninghamella echinulata* and the fungus *Aspergillus niger* [18]. The limonene was transformed into other compounds by a number of microorganisms (e.g. fungi, bacteria, yeast) [5, 15].

Both baicalin and baicalein can be extracted *Scutellaria baicalensis*. The conversion from baicalin into baicalein is accompanied by hydrolysis [6]. In the microbial biotransformation of baicalin, the most important enzyme is undoubtedly *β*-glucuronidase. In reference [8], the activating condition of *β*-glucuronidase in the bacterium *Lactobacillus delbrueckii Rh2* is optimized and adopted in the biotransformation of baicalin.

Despite the frequent use in biotransformation processes, enzyme-rich mycetes were seldom mentioned in previous literature on the biotransformation from baicalin to baicalein. Over 1,000 years, *White-rot fungi* has been safety utilized in the production of food, feed, kojic acid and wine. It is a strain with complex enzymes, including protease, amylase, glucoamylase, cellulase, phytase, and the important enzyme of β-glucuronidase. Gao *et al.* studied the fermentation conditions of *White-rot fungi* producing β-glucuronidase, and obtained the media with a good yield [17]. In this research, the author explored the effects of different carbon and nitrogen sources in the biotransformation media on the biotransformation of baicalin by *White-rot fungi* and optimized the carbon and nitrogen sources to enhance the biotransformation efficiency.

2. Material and Method

2.1 Microorganisms and Chemicals

The *White-rot fungi* was separated, identified and stored in our lab. *Radix Scutellariae*, the root of *Scutellaria baicalensis*, was purchased from a pharmacy in Zhanjiang, China. The baicalin and baicalein (purity>98%, HPLC-grade) were both purchased from National Pharmaceutical Engineering Centre for Solid Preparation in Chinese Herbal Medicine, Nanchang. The amino acids (e.g. leucine, cysteine, histidine, aspartic acid, glycine, alanine, tyrosine, valine, proline, cystine, lysine, methionine, arginine, and glutamic acid) were all purchased from Sangon Biotech (Shanghai) Co., Ltd. The reagents like methanol, H_3PO_4, KH_2PO_4, $CaCl_2$, $MgSO_4$ and urea were all analytically pure. The corncob and soybean powder were bought from a market in Zhanjiang.

2.2 Biotransformation Media

Three media were employed for the activation, intermediate cultivation of *White-rot fungi* and the biotransformation of baicalin. According to our previous study [7], integrated potato slant culture medium and liquid integrated potato medium were prepared separately for the activation and intermediate cultivation. The biotransformation media was constructed in reference to the media reported by Gao et al. [17]

2.3 Extraction, Purification and Identification of Baicalin

The baicalin was extracted from *Radix Scutellariae*, purified, and then identified by thin-layer chromatography (TLC), as described in our previous study [7].

2.4 Determination of Baicalein in the Media

As mentioned in our previous research [7], the baicalein was determined by high-performance liquid chromatography (HPLC). First, the baicalein methanol solution (100 μg /mL) was prepared as stock solution. Then, the stock solution was diluted to 10, 20, 30, 40 and 50 μg /mL, respectively. Each diluted solution (20 μL) of baicalein was measured by the HPLC. The standard curve was drawn and the regression equation was obtained according to the peak area of each concentration of baicalein.

2.5 Activation of *White-rot fungi* and Biotransformation of Baicalin

The *White-rot fungi* was activated in integrated potato slant culture medium and then transferred to liquid integrated potato medium for intermediate cultivation. Next, 1mL liquid integrated potato medium containing cultured *White-rot fungi* was added into 250 mL of the biotransformation media. After that, 0.2g baicalin was added into the biotransformation media and maintained for 3 days in a shaker. At the end of the 3-day period, the biotransformation media (5 mL) was extracted with ether (2 mL×4) and the organic layers were combined. The solution was evaporated to dryness to obtain a yellow solid. The solid was later dissolved in methanol (10 mL) and determined by the HPLC against the baicalein standard.

The conversion rate of baicalin was calculated bythe following formula.

$$\text{Conversion rate}(\%) = \frac{n_{(\text{balcalein})}}{n_{(\text{balcalin})}}$$

2.6 Optimization of Carbon and Nitrogen Sources

The carbon and nitrogen sources in the biotransformation media were optimized based on the basic media of 0.6% KH_2PO_4, 0.1% $CaCl_2$, and 0.1% $MgSO_4$.

To optimize the carbon sources, 0.1% peptone was employed as the nitrogen source in the biotransformation media. The carbon sources of the media were selected as corncob, bran, glucose, and dissoluble starch. Apart from changing the corncob concentration, the effect of carbon sources was investigated by altering the carbon species, such as 2% bran, 1% bran and 1% corncob, 2% glucose, and 2% dissoluble starch, and even eliminating all the carbon sources.

The carbon source with the highest biotransformation efficiency was selected to optimize the nitrogen sources of the media. First, the author evaluated the impact of nitrogen sources (peptone, yeast extract, soybean powder, urea, NH_4NO_3, $(NH_4)_2SO_4$) fixed at 0.1% and the effect of no nitrogen source. In this way, the best nitrogen source was obtained to figure out the best concentration for the biotransformation. Then, the author explored the effect of single amino acid (leucine, histidine, glycine, tyrosine, proline, cysteine, lysine, arginine, etc.) at the concentration of 0.01%. Finally, the author measured how the biotransformation of baicalin was influenced by the combinations with different levels of the above mentioned amino acids, and the combinations of amino acid mixtures with different concentrations of urea.

3. Results and Discussion

3.1Measurement of Baicalein

The HPLC measurement of baicalein demonstrates a good linear relationship between the peak area and the concentration of baicalein in the range of 10~50 μg/mL. The regression equation was y=58.68x-23.77, and the R^2 was 0.99977.

3.2 Optimization of Carbon Sources

(1) Effects of corncob

Corncob has been widely considered as the best carbon source in biotransformation of isoflavone in *White-rot fungi* [17]. In this research, the author examined the effects of different weight/volume (w/v) concentrations (0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% respectively) of corncob on the biotransformation of baicalin. The results show that corncob is not a good carbon resource in the biotransformation of baicalin in *White-rot fungi*, which differs from the biotransformation process of common isoflavones. The highest efficiency of 37.1% appeared when there was no corncob in the media (Figure 1). Other types of carbon sources were applied to improve the biotransformation efficiency.

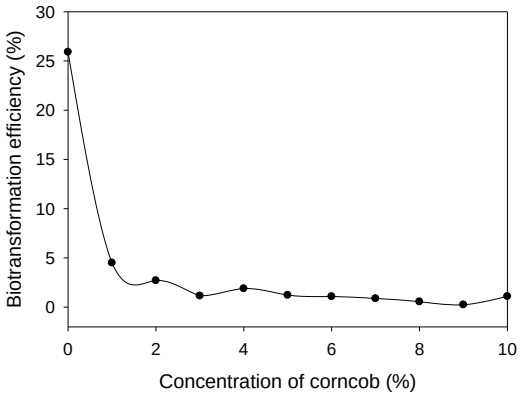


Fig.1. The Effects of Corncob with Different Concentrations on the Biotransformation of Baicalin

(2) Effects of carbon sources

The author explored how the biotransformation of baicalin by *White-rot fungi* was affected by 2 w/v % carbon sources including bran, glucose, dissoluble starch, the combination of bran (50%) and corncob (50%), and even no carbon source. In general, the biotransformation efficiency of baicalin ranged from 1.1% to 40.8% (Figure 2), and peaked in the case of no carbon source. This means the best carbon resource is baicalin itself, not external carbon source. Therefore, the media with no carbon source was adopted for the optimization below.

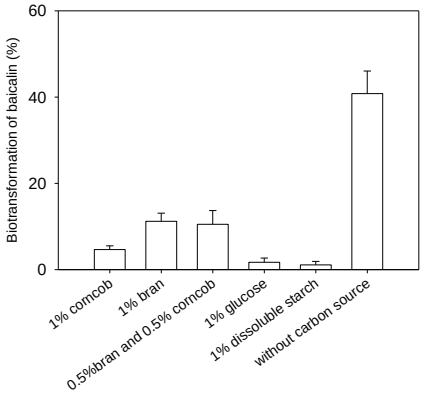


Fig.2. The Effects of Different Carbon Source on the Biotransformation of Baicalin

3.3 Optimization of Nitrogen Sources

The nitrogen sources in the biotransformation media was optimized under the conditions of 0.6% KH_2PO_4, 0.1% $CaCl_2$, 0.1% $MgSO_4$, no external carbon source, and 0.1% nitrogen sources.

(1) Effects of inorganic nitrogen sources

This subsection evaluates the effects of 0.1% peptone, yeast extract, soybean powder, urea, NH_4NO_3, $(NH_4)_2SO_4$, and no nitrogen source on the biotransformation of baicalin by *White-rot fungi*. As can be seen from Figure 3, the biotransformation efficiency of baicalin varied from 2.0% to 40.9%. With a biotransformation efficiency from 31.2% to 40.9%, peptone, yeast extract, urea and soybean powder were good nitrogen sources for the biotransformation of baicalin by *White-rot fungi*. Among them, urea stood out as the best nitrogen source.

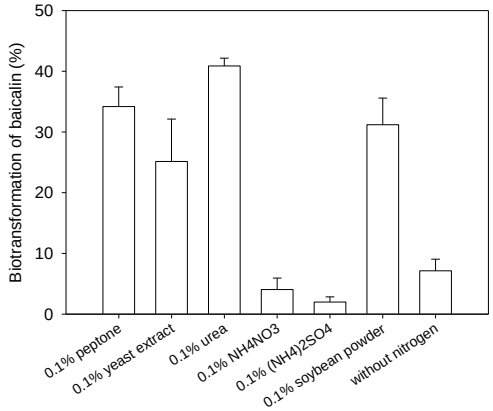


Fig.3. The Effects of Different Nitrogen Source on the Biotransformation of Baicalin

(2) Effects of different levels of urea

Since urea was proved as the best nitrogen resource for the biotransformation of baicalin, the author determined the impact of urea at four different levels (0.01%, 0.1%, 0.5% and 1%) on the

biotransformation of baicalin was determined (Figure 4). According to Figure 4, the biotransformation efficiency first increased and then decreased with the growing concentration of urea. It is measured by the HPLC that baicalin was almost completely bio-transformed when the concentration of urea exceeded 0.5%. By contrast, the amount of baicalein declined with the increase in the concentration of urea. From the existing literature, baicalein was prone to decomposition and oxidation. Hence, high urea concentration accelerated the decomposition and oxidation of baicalein. To improve the yield of baicalein, it is necessary to strike a balance between the conversion efficiency of baicalin and the protection of baicalein. Therefore, 0.1% and 0.01% were selected as the urea concentrations for the subsequent optimization processes.

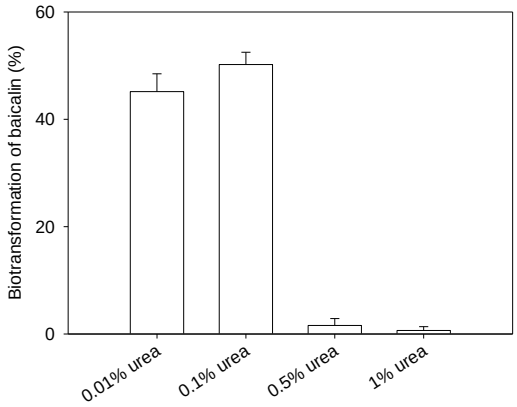


Fig.4. The Effects of Different Levels of Urea on the Biotransformation of Baicalin

(3) Effects of organic nitrogen source - amino acids

In this subsection, the author examined the effects of 14 species of amino acids on the biotransformation of baicalin (Figure 5). The amino acids include leucine, cysteine, histidine, aminosuccinic acid, glycine, alanine, tyrosine, valine, proline, cystine, lysine, methionine, arginine, and glutamic acid. When an individual amino acid was used as the nitrogen source, the biotransformation efficiency of baicalin fluctuated between 9.4% and 27.4%, which is below that of 0.01% urea (45.2%). Relatively speaking, histidine, alanine, tyrosine, valine, arginine, and glutamic acid displayed better effects in the biotransformation of baicalin. Thus, these amino acids were combined with urea to obtain the optimal nitrogen source.

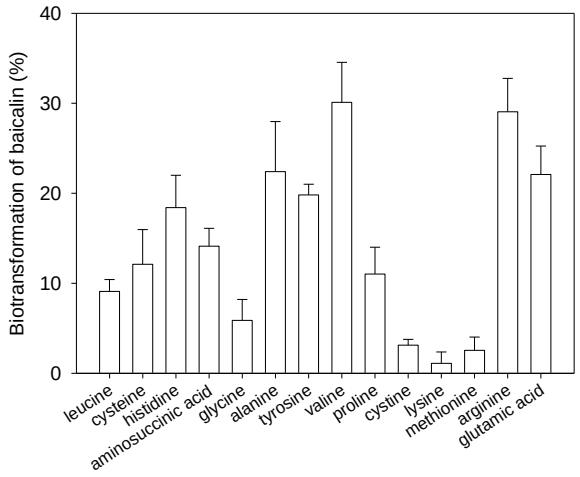
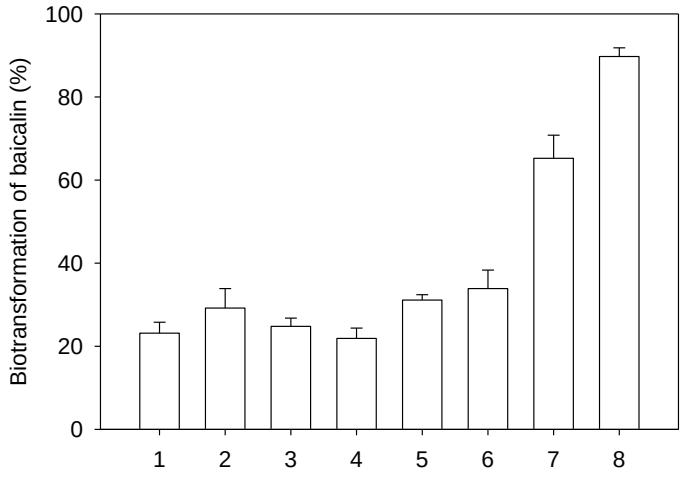


Fig.5. The Effects of Different Amino Acid on the Biotransformation of Baicalin

(4) Effects of different combinations of urea and amino acids

This subsection discusses the baicalin biotransformation effects of different combinations of urea and amino acids. The 0.1% and 0.01% urea were separately combined with the six effective amino acids (histidine, alanine, tyrosine, valine, arginine, and glutamic acid) to test the exact effects. As shown in Figure 6, the biotransformation of baicalin fell between 21.9% and 89.8% under these biotransformation conditions, and the best nitrogen source was combined of 0.0017% arginine, 0.0017% valine, 0.0017% glutamic acid, 0.0017% alanine, 0.0017% histidine, 0.0017% tyrosine and 0.1% urea.



1、0.005%arginine+0.005%histidine
2、0.005%valine+0.005%tyrosine
3、0.005%valine+0.005%alanine
4、0.005%tyrosine+0.005%alanine
5、0.0033%valine+0.0033%alanine+0.0033%tyrosine
6、0.0017%arginine+0.0017%valine+0.0017%glutamic acid+0.0017%alanine+0.0017%histidine+0.0017%tyrosine
7、0.0017%arginine+0.0017%valine+0.0017%glutamic acid+0.0017%alanine+0.0017%histidine+0.0017%tyrosine+0.01%urea
8、0.0017%arginine+0.0017%valine+0.0017%glutamic acid+0.0017%alanine+0.0017%histidine+0.0017%tyrosine+0.1%urea

Fig.6.

Conclusions

After the optimization of the carbon and nitrogen sources in the biotransformation media, the biotransformation efficiency of baicalin was elevated to 89.8%. The best biotransformation media was optimized as 0.0017% arginine, 0.0017% valine, 0.0017% glutamic acid, 0.0017% alanine, 0.0017% histidine, 0.0017% tyrosine, 0.1% urea, 0.6% KH_2PO_4, 0.1% $CaCl_2$, and 0.1% $MgSO_4$ at the pH of 5.5, without any external carbon source.

It is concluded that the combination of inorganic and organic nitrogen sources can facilitate the biotransformation of baicalin, and inorganic nitrogen has a greater function in the biotransformation than organic amino acids. Due to the high cost of amino acids, 0.1% urea should be the most applicable nitrogen source for the biotransformation of baicalin by *White-rot fungi*.

Acknowledgments

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