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Research Article

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Optimization of the bioconversion conditions for GAMA production

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ABSTRACT

We identified Aspergillus .sp IS254 from licorice, which can convert glycyrrhizic acid (GL) into single glucuronic acid glycyrrhetinic acid (GAMA). Based on the study, the optimized fermentation medium contained 1.19g GL, 2g $NaNO_3$, 0.7g $MgSO_4$, 1g K_2HPO_4 and 0.01g $FeSO_4$ in 1L fermentation liquid. In addition, the best pH was 5.5, and the inoculum amount was 10 ml. Cultures were incubated at 28℃ for 6 days. In the end, the transformation rate could reach 35.72%.

Key words: Glycyrrhizic Acid; Biotransformation; Strain screening; fermentation conditions

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INTRODUCTION

1 The experimental materials

1.1 Instruments and reagents

The 1100 series high performance liquid chromatograph was purchased from Agilent Co., LTD of America
98% GL was from Tianzhirun Biological Technology Co., LTD of Shaanxi
Both 99.5% standard sample of GL and 99.9% standard sample of GA were from Hengyuanqitian Institute of Chemical Technology of Beijing

75% Comparison of samples to GAMG was given by Professor Chun Li from Beijing Institute of Technology

1.2 Strain

Aspergillus.sp IS254 was selected from the microbial chamber of University of Science and Technology Liaoning

1.3 Culture medium

The solid culture medium was made of 2% agar，0.2% $NaNO_3$，0.1% K_2HPO_4，0.05% $MgSO_4$，0.001% $FeSO_4$ and 50% water extract of Glycyrrhizin（licorice：water=1：10）
The seed medium contained 30% potato juice，1% water extract of Glycyrrhizin，1g glucose and 1g peptone，made in 100mL water. The medium was in natural pH.
The modified *Czapek* liquid culture medium contained 1.2g of 98% GL，1.5g $NaNO_3$，1g K_2HPO_4，0.5g $MgSO_4$ and 0.01g $FeSO_4$.

1.4 Test conditions

The ratio of acetonitrile to water for the mobile phase was 70：30（v/v）. The flow rate for the test was 1.00 ml/min. The column temperature was 25℃, ultraviolet wavelength was 254nm, and the sample size was 10 μL. The standard curves of GL and GAMG were created based on the following equations:
The regression equation for GL: y=0.2308x-6.2082，R2=0.9987；

The regression equation for GAMA: y=0.1694x+20.421，R2=0.99943.

METHODS

2.1 Microbial screening and purification of licorice

The licorice was inoculated on the surface of the solid culture medium, and incubated at 25℃for 5 day. The efficient conversion strain was isolated and purified. The full length of18S rDNA was determined by the molecular biology method (Figure 2.1(?)).

TAGTTATAAGCACTTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGATAGTACC
TTACTACATGGATACCTGTGGTAATTCTAGAGCTAATACATGCTGAAAACCTCGACTTCGGAAGGGGTGT
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GGTGGCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACAT
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TACGGGGCTCTTTTGGGTCTCGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGAACAATTGGAG
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AGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGAAAGCATTCGCCAAGGATGTTTTCATTAATC
AGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGAC
TAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTG
GGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCC
TGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAATAAGGATTGACAGATTGA
GAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAA
TTGCGATAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCAATTTGCGGGCCGCTGGCTTCTTA
GGGGACTATCGGTTCCCA

Fig.2.1 PCR amplification products of *Aspergillus niger* IS254

The results showed that the homology of 18S rDNA sequence in IS254 with *Aspergillus sp* (CPCC480386) was 99%. The phylogenetic tree generated by IS254 and other related strains was as shown in Figure 2.2.

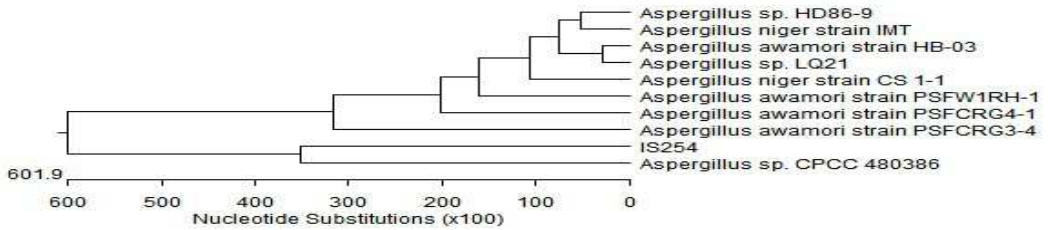


Fig.2.2 The phylogenetic tree of IS254 and other related strains based on their 18S rDNAs

The results showed that the identified IS254 strain was at the same branch as *Aspergillus sp* (CPCC480386).

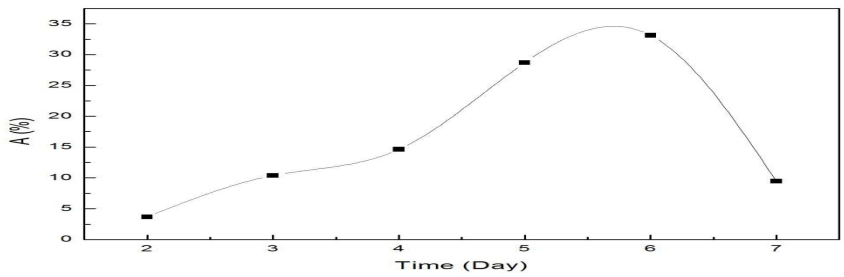


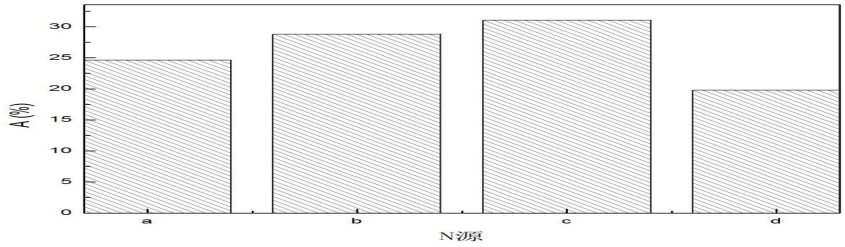
Fig. 2.3 Relationship between the conversion rate and training time

2.2 Optimization of fermentation conditions

1**.** The influence of training time on biotransformation.

100ml of the modified Czapek compounded liquid culture medium was added into each flask. 10ml *Aspergillus.sp IS254* liquid was inoculated into the medium and cultured in a constant-temperature oscillation incubator at 25□ with the speed of 160r/min. The resulted conversion rate was shown in Figure 2.3, and the highest rate was observed at Day 6.

2. Influence of different inorganic nitrogen source on biotransformation.
According to equivalent nitrogen given ability, 10ml *Aspergillus.sp* IS254 liquid was inoculated into the modified Czapek compounded liquid culture medium with the nitrogen source of $(NH_4)_2SO_4$, NH_4NO_3, $NANO_3$ and NH_4CL. It was then incubated in a constant-temperature oscillation incubator at 25□ with the speed of 160r/min for 6 days. The resulted conversion rate was shown in Figure 2.4. The medium with $NaNO_3$ gave the highest conversion rate.



Note: a is $(NH_4)_2SO_4$， b is NH_4NO_3， c is ,NANO3 and d is NH4CL
Fig. 2.4 Relationship between the conversion rate and different inorganic nitrogen source

3. The influence of $NaNO_3$ on biotransformation.
0.1g, 0.15g, 0.2g, 0.25g and 0.3g of $NaNO_3$ was used as the nitrogen source and added into 100 ml culture medium per conical flask, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.5. The most suitable condition was 0.2g of $NaNO_3$.

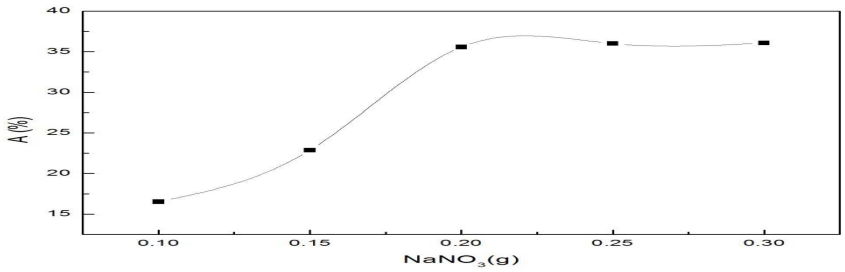


Fig.2.5 Relationship between the conversion rate and different amount of $NaNO_3$

4. The influence of $MgSO_4$ on biotransformation
0.01g, 0.03g, 0.05g, 0.07gand 0.09g of $MgSO_4$ was used to prepare culture medium, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.6. The medium with 0.07g of $MgSO_4$ gave the highest conversion rate. s.

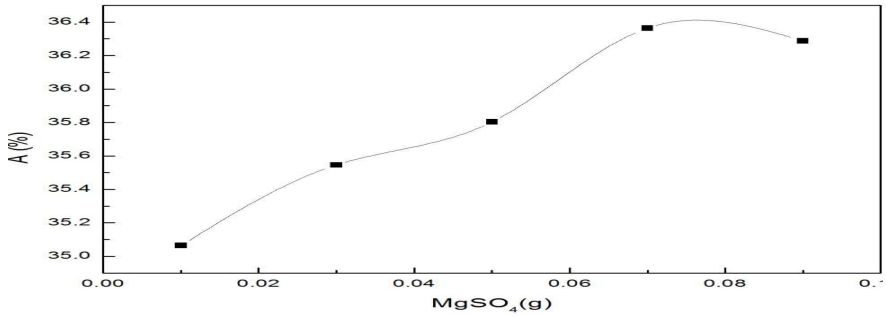


Fig. 2.6 Relationship between the conversion rate and addition of $MgSO_4$

5. The influence of K_2HPO_4 on biotransformation
0.05g, 0.1g, 0.15g, 0.2g and 0.25g of K_2HPO_4, was used to prepare culture medium, respectively, and cultures were incubated for 6 days. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.7. The most suitable condition was 0.1g of K_2HPO_4

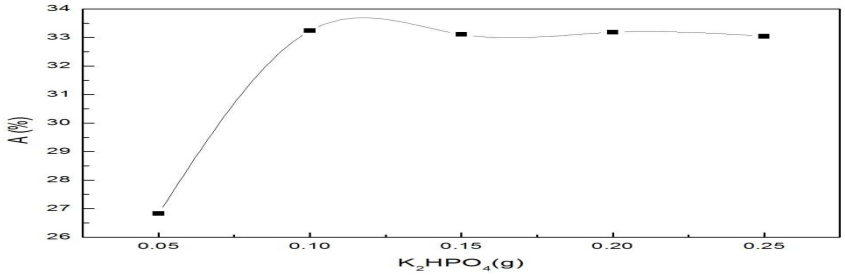


Fig.2.7 Relationship between the conversion rate and addition of K_2HPO_4

6. The influence of the substrate concentration on biotransformation

1g/L, 1.25g/L, 1.5g/L, 1.75/Land 2g/L of GL was used to prepare culture medium, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.8. Based on the results, 1.25g/L of GL was selected to be right condition for the following experiment.

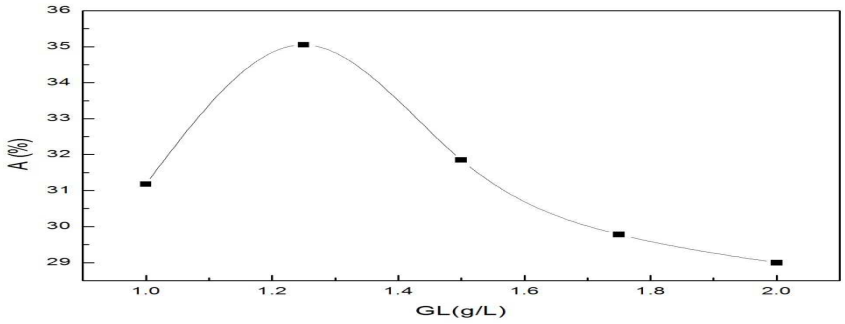


Fig. 2.8 Relationship between the conversion rate and different concentration of GL

7. Optimization of temperature

The tested temperature was 24℃, 26℃, 28℃, 30℃ and 32℃, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.9. The most suitable temperature was 28℃.

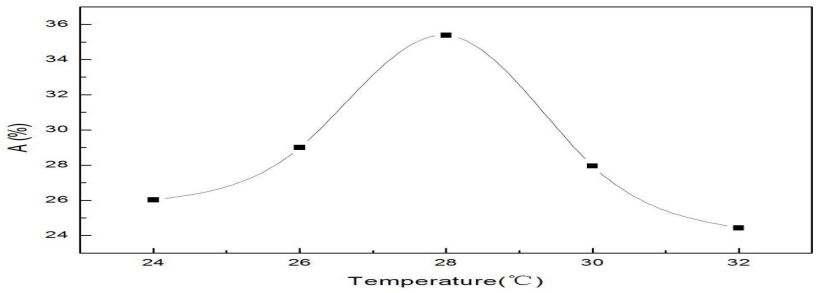


Fig.2.9 Relationship between the conversion rate and temperature

8. Optimization of pH

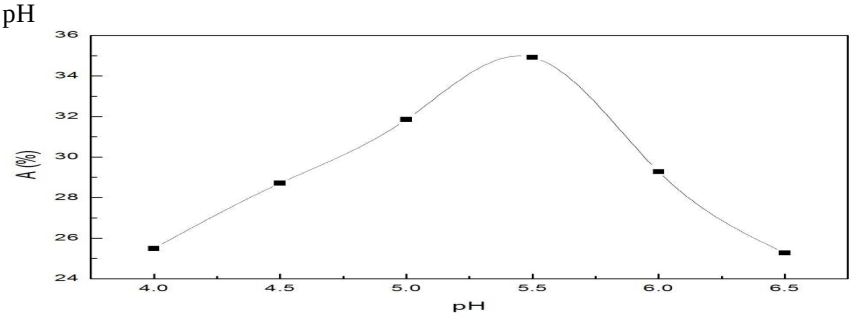


Fig. 2.10 Relationship between the conversion rate and pH

The tested pH conditions were 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.10. The most suitable pH was 5.5.

9. Optimization of inoculation amount

5mL, 10mL, 15mL, 20mL and 25mL of culture liquid was inoculated into the prepared medium, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.11. The best inoculation amount was 10 ml according to the results.

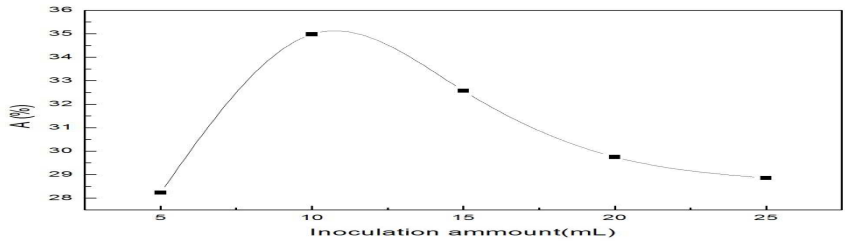


Fig.2.11 Relationship between the conversion rate and inoculum amount

10. The influence of peptone on biotransformation

0g, 0.05g, 0.1g, 0.15g and 0.2g of peptone was used to prepare culture medium, respectively. The other conditions for tested medium were the same. The resulted conversion rate was shown in Figure 2.12. 0.1g of peptone gave the highest conversion rate.

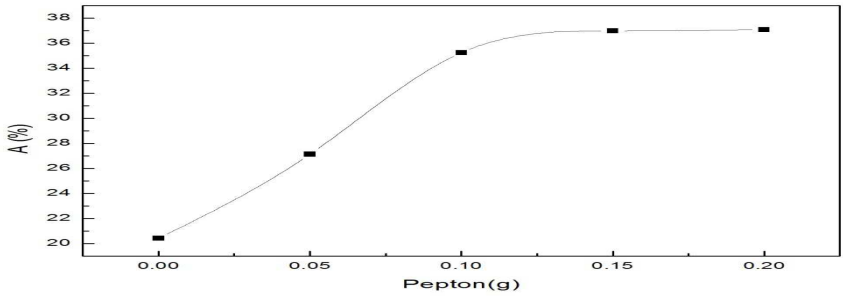


Fig. 2.12 Relationship between the conversion rate and peptone

CONCLUSION

We identified efficient *Aspergillus.sp* IS254, which converted GL into GAMG. In the present study, we determined the optimum conditions. The best amount of GL, $NaNO_3$, $MgSO_4$ and K_2HPO added into the 100ml fermentation liquid medium was 1.25g/L, 0.2g, 0.07g and 0.1g, respectively. The most suitable pH was 5.5, and the inoculation amount was better to be 10 ml. The best conversion rate was obtained at 28℃ with 0.1g peptone in the medium. After 6-days incubation, the transformation rate could be as high as 35.72%.

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