Scholars Journal of Economics, Business and Management OPEN ACCESS
Abbreviated Key Title: Sch J Econ Bus Manag
ISSN 2348-8875 (Print) | ISSN 2348-5302 (Online)
Journal homepage: https://saspublishers.com/journal/sjebm/home

Screening and Preliminary Identification of Ginsenoside Transformedstrains in Ginseng Rhizosphere Soil

JIA Guiyan[1], PAN Fang[2], MA Yixuan[1], LI Jingying[2], WANG Weifeng[2], GE Wenzhong[1], ZHOU Yan[1], WANG Yanhong[1*]

[1]College of Life Science and Technology, HeiLongJiang BaYi Agricultural University, Daqing 163319, China
[2]Daqing Oilfield General Hospital, Daqing 163319, China

DOI: 10.36347/sjebm.2019.v06i07.002 | **Received:** 13.07.2019 | **Accepted:** 22.07.2019 | **Published:** 30.07.2019

***Corresponding author:** Wang Yanhong

Abstract **Review Article**

In this study, strains with transforming ginsenoside activity were screened from fresh ginseng rhizosphere soil in changbai mountain, huai hua, ji 'an and yulin town, tonghua city, jilin province, China.Single colony wasscreened by tablet marking method with ginseng stem leaf total ginsenoside as substrates for the liquid fermentation. Chromatography and thin-layer chromatography were used for preliminarily determine conversion activity, ultraviolet spectrophotometry determined the ginseng total ginsenoside of conversion rate. PCR and DNA sequencing technology were for identify the best strain of active species. Finally, 16 strains were isolated from the rhizosphere soil of fresh ginseng, among which the code C4 strain had the best transformation activity, which increased the content of ginseng stem and leaf ginsenoside by 28.7%. The phylogenetic tree was constructed by neighbor-joining method, it belongs to *Trichoderma*.

Keywords: Ginsenoside, Transformation, TLC, Extraction.

INTRODUCTION

Panax ginseng C. A. Mey is a kind of the genus Panax, which is a traditional and precious Chinese traditional medicine in China [1]. Modern pharmacological studies have shown thatGinsenoside is the main active component of ginsenoside, which has the functions of anti-fatigue, anti-aging, anti-tumor molecular diffusion, anti-inflammation, anti-oxidation, regulating the central nervous system of the brain, and improving the immunity of the body [2-4]. Because of the low content of natural ginsenosides and the long planting life and high cost of medicinal materials, the conversion of ginsenosides by chemical method, enzymatic hydrolysis method and microbial methodhas become a research hotspot [4].

Microbial transformation of ginsenosides is essentially an enzyme produced by microorganisms using their own cell metabolism to hydrolyze glycoside bonds and obtain ginsenosides through enzymatic reaction [5, 6]. This method has the advantages of mild reaction conditions, high conversion efficiency, simple and convenient experimental operation and no pollution in the whole process [7, 8]. Studies have shown that rare ginsenosides, which are transformed by microbial fermentation, are more easily absorbed by the body through the mucous membrane on the intestinal tract and improving the efficacy [9-11]. Literature shows that the method of screening the active strains of transformed ginsenosides by using the soil near the root system of ginseng is convenient and simple, and the possibility of obtaining the active strains is high [12-15]. So this experiment used flat line from ginseng root in the soil screening active strains, ginseng total ginsenosides as the substrate liquid fermentation for transformation, preliminary determined by thin layer chromatography, the absorbance value of fermentation was determined by UV spectrophotometry to investigate the transformation of total ginsenosides. At last, phylogenetic trees were constructed by neighbor-joining methodto identify the active strains.

MATERIALS AND REAGENTS

Fresh ginseng with soil was purchased in changbai mountain of jilin province, fusong county of baishan city, ji 'an city and yulin town of tonghua city (all five years of ginseng).The substrates used in the experiment, total ginsenosides of ginseng stems and leaves, were purchased from the biomedical research and development center of liaoning province.Ginsenoside Re standard products were purchased from hefei bomei biotechnology company.

Nutrient AGAR (Beijing Macao star biotechnology company), Yeast genomic DNA extraction kit (tiangen biochemical technology (Beijing) company, Silica gel panel GF254 (Qingdao Marine chemical plant branch), All other reagents were analytical pure.

METHODS

Medium

PDA medium 200 g potato, add 700 mL distilled water, boil for 20 min, filter with gauze, add 20 g agar powder and 20 g glucose in the filtrate, heat and dissolve, and finally add distilled water to a volume of 1000 mL.

Nutrient agar medium Peptone 10 g, beef extract 3 g, sodium chloride 5 g, agar powder 20 g, 1000 mL distilled water.

R_2A agar medium peptone 0.25 g, yeast extract0.5 g, soluble starch 0.5 g, peptone 0.25 g, acid hydrolyzed casein 0.5 g, potassium phosphate 0.3 g, magnesium sulfate 0.1 g, sodium pyruvate 0.3 g, glucose 0.5 g, agar powder 12 g, 1000 mL distilled water.

YM agar medium yeast extract 3 g, wheat extract 3 g, peptone 5 g, glucose 10 g, 20 g agar powder, 1000 mL distilled water.

PDB medium potato 200 g, add 700 mL distilled water, boil for 20 min, filter with gauze, add 20 g glucose in the filtrate, and finally add distilled water to a volume of 1000 mL [16].

All above medium, agar powder should be heated and dissolved first, and then other components should be added successively. The pH of the culture solution needs to be controlled at about 7, so sodium hydroxide concentration needs 1 mol/L.

Microbial Isolation of Rhizosphere Soil of Fresh Ginseng

Samples of fresh ginseng rhizosphere soil from four producing areas of changbai mountain, fusong wanliang, baishan city, ji 'an city and yulin town, tonghua city were taken and marked. They were placed in an electrothermal thermostatic air drying box at 40°C- 50°C and dried for 2-3 hours. 1 g of dried soil was taken respectively, and 10 mL of sterile distilled water was added. The soil samples were diluted from 10^{-1} to 10^{-5}.0.1 mL of diluent was used for evenly coating the PDA medium containing kanamycin (50 ug/mL), Nutrient agar medium, R2A agarmedium, YM agarmedium all containing nystatin (50 ug/mL). Under the condition of 30℃, each medium was inverted and cultured in the incubator for 72 hours [16, 17].

Fermental cultivation

The strains in the purified plate culture medium were rinsed with 2 mL sterile distilled water to make the spore suspension, which was added to the PDB culture medium containing total ginsenosides, the content of total ginsenosides was 0.4 mg/mL, and the strain free PDB medium with the same concentration and volume of total ginsenosides solution was used as the control, It was shaken for 11 d at 30°C and 120 rpm /min. After 48 h, samples were sampled every 24 h, and the reaction was terminated by 1:1 extraction with saturated butanol with water. The supernatant was centrifuged at 6000 rpm /min for 10 min.

Screening of active strains by TLC

Quantitative absorption of control and test solution was conducted on the same thin layer chromatography (GF254, 100×100 mm, silica gel plate). Blow dry solvent, otherwise affect the development and color effect. The developing agent (trichloromethane: methanol = 3:1, prepared before use) shall be taken out after the specified distance and dried in the fume hood. Spray 10% sulfuric acid ethanol solution and heat it evenly at 110°C for 5~10 mins to make the spots clear. Under daylight and ultraviolet light (365 nm), the transformation of ginsenosides components in the sample after microbial transformation was determined by comparing the spots displayed by ginsenosides with the control.

Determination of Conversion Rate of Active Strain

Preparation of standard curve for determination of total ginsenosides in ginseng. Taken ginsenoside Re standard solution (0.5 mg/mL) 0.1 mL, 0.15 mL, 0.2 mL, 0.25 mL, 0.3 mL, 0.35 mL respectively and steam it dry in the water bath at 60°C.Add 0.2 mL 5% vanilla-glacial acetic acid and 0.8 mL perchloric acid solution.Heat it in water bath at 60°C for 15 min then cool, add 5mL glacial acetic acid solution, shake well and take the corresponding reagent as contral. The absorbance was measured at 540 nm. The standard curve was drawn by taking the sample quantity of standard ginsenoside Re as the horizontal coordinate and the absorbance as the vertical coordinate [19, 20].

Identification of Active Strains

Morphological of strain

The strain with the best activity was screened and cultured on PDA medium for 5 days, and its morphological characteristics were observed.

Extraction of DNA from Active Strains

Genomic DNA extraction kit was used to extract the DNA of the active strain. Lyticase and sorbitol buffer were prepared with 0.1m sodium phosphate buffer [21, 22].

The specific methods are as follows: Take an appropriate amount of fungus liquid culture solution,

then centrifuged at 12,000 rpm/min for 1 min. Discard the supernatant and get the pure bacteria, add 600 μL sorbitol buffer and 50 U soluble enzyme, mixing and waiting for an appropriate time. At 30°C, the supernatant was discarded by centrifuge at 4000 rpm for 10 min. Add 200 μL GA buffer solution and 220 μL GB buffer solution to the precipitation, mix evenly, and leave it at 70°C for 10 min, the solution becomes transparent. Add 220 μL anhydrous ethanol to the obtained mixture, mix well and form flocculent precipitate. The solution and precipitate were put into CB3 and centrifuged at 12,000 rpm for 30 secs and then discard the waste liquid. Add buffer GD 500 μL and bleach PW 600 μL to adsorption column CB3 (anhydrous ethanol is added before GD and PW are used), centrifuge 30 sec at the same speed, discard the waste liquid and put it back into the collection tube. Put the adsorption column back into the collection tube, centrifuge for 2 min, and discard the waste liquid. The adsorption column was lowered at room temperature for several minutes, and the residual liquid was completely dried. After the adsorption column was placed into the new centrifuge tube, the eluent buffer TE (100 μL) was dropped vertically into the middle of the membrane and placed at room temperature for several minutes. The solution was centrifuged at the same speed for 2 min, and all the solutions were collected into the tube.

PCR amplification and sequence analysis

After the genomic DNA was tested, 1 μL was used as template for PCR amplification. A universal primer for fungal 18S rDNA：ITS1：TCCGATGGTGGCGC；ITS4：TCCTCCGCTTATTGATATGC.PCR amplification reaction parameters: predenaturation at 94°C for 4 min, denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min, 30 cycles, extension at 72°C for 10 min, and preservation at 4°C. PCR products were detected by 1.5% agarose gel electrophoresis. The target gene recovered by cutting gum was connected with pMD19-T vector, and the linked products were transferred to *Escherichia coli* JM109 receptive cells for culture. A single colony was selected for PCR detection, and the cloned strain with positive results was sent to shengggong biological engineering (Shanghai)company for sequencing.The measured ITS sequences were submitted to GenBank database of NCBI, and BLASTn software was used to search for similar sequences, Clustal X (V 1.83) software was used for multi-sequence matching analysis of high-homologous sequences, and neighbor-j method in MEGA 4.0 software was used to construct phylogenetic trees with 1000 times of self-expansion.

RESULTS

Determination of Culture Medium and Purification of Bacteria

PDA medium, nutrient agar medium, R2A agar medium and YM agar medium were compared in the process of strain screening and culture experiment, and PDA medium was finally selected in the purification process. PDA medium is more suitable for the cultivation of fungi, and the components are simple, economical and avoid complicated preparation process. And in the process of active microbial screening, rhizosphere soil single colony strain screening strains obtained in most of fungi, bacteria, almost did not get, can be purchased fresh ginseng rhizosphere bacteria in soil is less, may also link operation error, improper conditions lead to strains of death does not fully grown, late can expanding the scope of the sieve sample continue to study.

After repeated plate marking, cultivation and purification, a total of 38 single bacterial strains were screened from the soil. After morphological observation, they were preliminarily merged into 16 strains, among which C1 and C3 were yellow-white strains, C2 was white-white mycelial strains, and C4 was green-green strains. Y5 was cyan strain, Y6 was mycelial gray strain, F7 and F8 were light green strains, and F9, F10 and F11 were green strains. J12 and J13 are blue-green strains, J14 and J15 are white mycelium strains, and J16 is yellowish white strain (C represents changbai mountain, Y represents yulin town, F represents fusong county, and J represents ji 'an city).

Analysis of Active Strains by TLC

After the fermentation liquid was cultured in a full-temperature oscillator for 48 h, samples were sampled every 24 h, the reaction was terminated by butanol extraction, and the changes of ginsenoside content in the control and test samples were detected by TLC. Eight strains of fermentation liquid were preliminarily analyzed by TLC to compare their spots with the blank control, and the spots were big and dark in color. It was preliminarily determined that these strains had the ability to transform and improve the content of total ginsenosides, and were the target active strains. The spots of C2, C3, C4 and F8 strains were obviously larger and darker than those of the control. It was preliminarily determined that this strain had the activity of transforming and improving the content of ginsenosides in ginseng monomer. Partial TLC results are shown in figure 1. (strains numbered 1-16, 3 parallel experiments numbered A, B, C; Empty: culture medium of ginsenoside without bacterial strain).

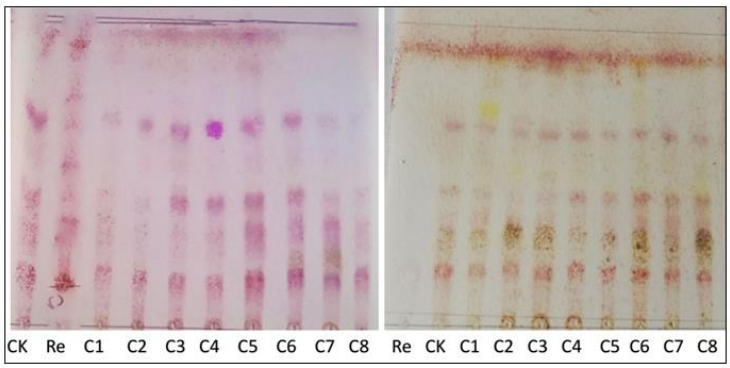

Fig-1: TLC chromatographic note of the fermentation liquid of the active strain

Note-blank control C1、C2、C3、C4、Y5、Y6、F7、F8, the number corresponds to the number on the graph one by one

Determination of Conversion Rate of Active Strain

The regression equation of the standard curve for the determination of total ginsengin content was obtained as follows: Y=4.2624X+0.0269, r=0.9998, with good linearity within the range of 0.05-0.175 mg. The absorbance value of the supernatant was determined by UV spectrophotometry, and the conversion rate of each strain to the total ginsenosides was calculated. The culture days were taken as the horizontal coordinate, and the conversion rate of each strain as the vertical coordinate, as shown in Figure-2.

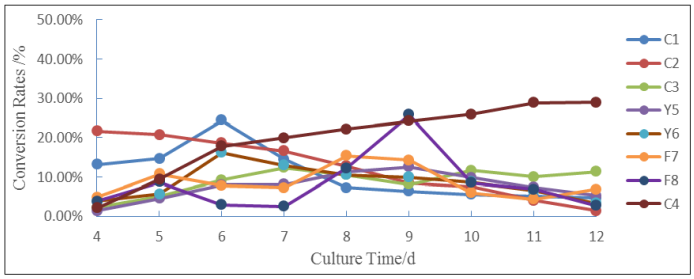

Fig-2: Transformation of active strains

The above results show that the active strains identified by TLC can improve the content of total ginsenosides in ginseng to some extent, among which C4 conversion rate of the strains isolated from the rhizosphere soil of ginseng in changbai mountain, jilin province slowly increases, and the highest conversion rate of total ginsenosides is 28.92%. The liquid fermentation was used to transform total ginsenosides of the strain C4, and the transformation was shown in Figure-3. The final conversion rate was 28.7%.

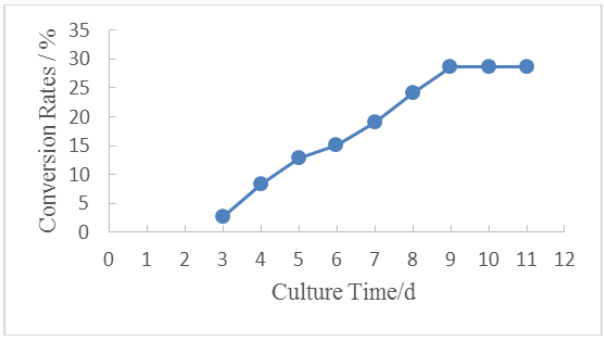

Fig-3: Transformation of C4 strain

Identification of Active Strains

Morphological observation

On the colony diameter of the active strain was nearly 5 cm, the center of the colony surface was dark green villus, the back was dark green, the edges were irregular and light yellow wavy, the center was slightly raised, and the surrounding formed concentric ring.

The ITS sequence of active strain C4

The sequence of active strain C4 was 596bp, BLASTn analysis shows that this sequence has a high similarity to the ITS sequences of *Trichoderma* fungi, and the closest similarity to the ITS sequences of the strains *Trichoderma lonlogic achiatum* and *Trichoderma* sp (Figure-4).

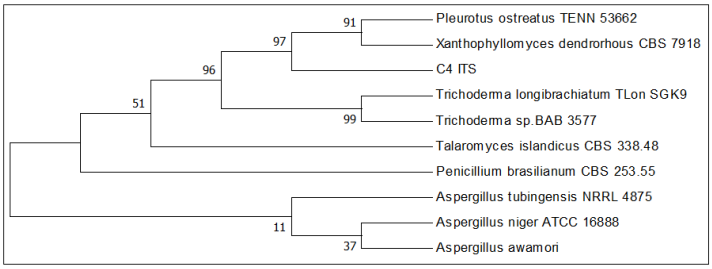

Fig-4: Phylogenetic tree of C4 strainby neighbor-joining method

CONCLUSION

In this study, 8 strains of fungi were isolated from the rhizosphere microorganisms of fresh ginseng soil. TLC method was used to preliminarily identify its conversion activity and UV spectrophotometry was used to determine its conversion ability. One strain had a conversion rate of 28.7% and was identified as *Trichoea* fungus by DNA sequencing.

Through DNA extraction and sequencing, the species and genus of the strain were determined, which lay a foundation for further optimization of its liquid culture conditions and higher conversion rate.

ACKNOWLEDGEMENTS

This work was supported by Heilongjiang bayi agricultural university intramural cultivation fund (XZR2017-12), DA QIN Gguiding science and technology planning project (zd-2016-144), Innovative training program for college students (201810223005).

REFERENCES

1. Colzani M, Altomare A, Caliendo M, Aldini G, Righetti PG, Fasoli E. The secrets of Oriental panacea: Panax ginseng. Journal of proteomics. 2016 Jan 1;130:150-159.
2. Lei W, Ying W. Inhibitory effects of ginsenoside rd on apoptosis of human glioma U251 cells in vitro. Toxicon-Oxford. 2019 Jan 1;158(1):S74.
3. Kim K. Effect of ginseng and ginsenosides on melanogenesis and their mechanism of action. Journal of ginseng research. 2015 Jan 1;39(1):1-6.
4. Zhang L, Virgous C, Si H. Ginseng and obesity: observations and understanding in cultured cells, animals and humans. The Journal of nutritional biochemistry. 2017 Jun 1;44:1-10.
5. Li SS, Jin YP, Yao CL, Wang YP. Research achievements on structures and activities of polysaccharides from Panax ginseng. Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica. 2014 Dec;39(24):4709-4715.
6. Ding S, Chunying L, Longquan X. Optimization of Conditions for Catalytic Conversion of Ginsenosides to Rare Saponins. Journal of Xuzhou Institute of Technology (Natural Sciences Edition), 2018, 33(4):49-54.
7. Li X, Zang P, Zhang LX, Gao YG, Li P, Hao JX, Wang YX. Research Progress on Ginsenoside CK Production by Microbial Transformation [J]. Food Science. 2012, 33(11):323-327.
8. Hongjun N, Peng W, Guane Y. Application of Microbial Transformation in Research of Chinese Medicine. Chinese Journal of Experimental Traditional Medical Formulae, 2013, 19(18): 346-349.
9. Xiuli W, Cheng L, Jing C. Identification of the active strain of ginsenoside Re_. Journal of Anhui Agricultural Sciences. 2011, 39(36): 22321-22322.
10. Ćwikowska M, Pruchnik FP, Starosta R, Chojnacki H, Wilczok A, Ułaszewski S. Dinuclear Rh (II) complexes with one polypyridyl ligand, structure, properties and antitumor activity. Inorganica Chimica Acta. 2010 Aug 10;363(11):240-2408.
11. Gao J, Xu W, Fang Q, Liang F, Jin R, Wu D, Tai G, Zhou Y. Efficient biotransformation for preparation of pharmaceutically active ginsenoside compound K by Penicillium oxalicum sp. 68. Annals of microbiology. 2013 Mar 1;63(1):139-49.
12. Lunpeng W, Longlv B, Chunfeng H. Microbiological Transformation of Ginsenoside Rb1 into C-K.Ginseng Research, 2016, 28(2): 7-11.
13. Kohda H, Tanaka O. Enzymic hydrolysis of ginseng saponins and their related glycosides. Yakugaku Zasshi, 1975, 95(2):246.
14. Yosioka I, Sugawara T, Imai K, KITAGAWA I. Soil bacterial hydrolysis leading to genuine aglycone. V. On ginsenosides-Rb1, Rb2, and Rc of the ginseng root saponins. Chemical and Pharmaceutical Bulletin. 1972 Nov 25;20(11):2418-21.
15. Meili C, Longlv B, Lunpeng W. Transformation of ginsenoside Rb1 to Rg3 by microorganisms from soil. Ginseng Research, 2014,(2): 43-44.
16. Yao Y, Huo Y, Zhou W, Ye J, Chu M, Zhu S, Jiang H. Screening of β-glucosidase-highly-producing strains and optimization of their fermentation conditions. Acta Agriculturae Jiangxi. 2018;30(3):97-101.
17. Huijuan D, Lingli C, Lianhai S. Effect of edible fungus fermentation liquid on growth and biofilm formation of Candida albicans in vitro. China Brewing, 2016, 35(01): 86-89.
18. [18] Changsha Institute of Microbiology. Isolation, purification and Preservation of bacteria. Pesticide Industry, 1970, (Z3): 63-65.
19. Lianghua C, Qin Q, Hanshen Z. Determination of

ginsenosides Re in Yanyan granules by UV spectrophotometry. Journal of Guangxi University of Chinese Medicine, 2016, 19(02): 68-70.

20. Ma L, Liu S, Xu NS, Jiang YQ, Song FR, Liu ZQ. Interactions of ginsenosides with DNA duplexes: A study by electrospray ionization mass spectrometry and UV absorption spectroscopy. Chinese Chemical Letters. 2014 Aug 1;25(8):1179-1184.
21. Haiyun C, Lei Z, Muge Y. A rapid and efficient extraction method of fungus DNA for PCR. Chinese Journal of Mycology, 2017, 12(06): 359-361, 367.
22. Xiaolan T, Junli F, Wang Y. A rapid and efficient DNA extraction method and its application in the detection of Staphylococcus aureus. Food Science and Technology, 2019, 449(02): 344-350.