African Journal of Biotechnology Vol. 7 (3), pp. 211-216, 5 February, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Production of gastrodin through biotransformation of p-hydroxybenzyl alcohol using hairy root cultures of *Datura tatula* L.

C.X. Peng[1], Gong J.S.[2]*, Zhang X.F.[2], M. Zhang[3] and Zheng S.Q.[4]

[1]Faculty of Modern Agricultural Engineering, Kunming University of Science and Technology, Kunming 650224, P.R.China.
[2]Faculty of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, P.R.China.
[3]Faculty of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming 650201, P.R.China.
[4]Institute of Material Medical, Kunming Pharmaceutical Corp, Kunming 650100, P.R.China.

Accepted 6 December, 2007

Inoculation of leaf sections of *Datura tatula* L. plants with A4 strain of *Agrobacterium rhizogenes*, induced transformed roots. The rol C (root loci) gene of TL region of *A. rhizogenes* was detected by PCR analyses in hair roots. The conversion of exogenous p-hydroxybenzyl alcohol into p-hydroxy-methyl-phenol-β-D-glucoside (gastrodin) was studied using hairy root cultures of *D. tatula* L. The chemical structure of the synthesized gastrodin was identified on the basis of spectral analysis and chemical evidence. The procedure of conversion of p-hydroxybenzyl alcohol into gastrodin by *D. tatula* L. hairy root cultures was established. The synthesized gastrodin was isolated from the ferment liquor and identified by spectral analysis. The biotransformation of exogenous p-hydroxybenzyl alcohol to gastrodin using hairy root cultures of *D. tatula* L. is a promising approach.

Key words: *Datura tatula* L., hairy root, p-hydroxybenzyl alcohol, gastrodin, biotransformation.

INTRODUCTION

Gastrodia elata Bl. is one of the notable folk medicines in China (Zhao et al., 1999). The steamed and dried roots of this plant were commonly used as a folk medicine known as 'Tianma'. It is considered to have very beneficial properties and is used as an anticonvulsant and a sedative (Heihachiro et al., 1981; Sun et al., 2004). Gastrodin is the main active constituent in *G. elata* Bl. and is considered to have advantageous properties such as anti-inflammatory, anti-convulsion, analgesic and anti-anoxemia. It plays a role in immunity enhancement in organisms and blood vessel expansion, and acts as scavenger of free radical (Zhou et al., 1979). It was discovered and synthesized by Zhou Jun at the end of 1970s (Zhou et al., 1980). However, the production of gastrodin by chemical synthesis is extremely difficult. Therefore, the development a new method for the synthesis of gastrodin is important.

The technology of biotransformation that is applied in the synthesis of medicines has high practical value and theoretical implications. It plays an important role in an extremely active research area (Achmad et al., 1999). Hairy root system arising from inoculation with *Agrobacterium rhizogenes* became popular in the last decade as a system to produce secondary metabolites synthesized in plant roots (Toivonen, 1993; Palazón et al., 1997). Hairy roots result from the transfer of genes located on the root-inducing plasmid Ri to plant cells and their expression therein (White and Nester, 1980). Two sets of pRi genes are involved in the root induction process: aux genes located in the TR region and the rol (root loci) genes of TL region (Jouanin, 1984). Opines are synthesized by plant transformed cells and are only used by *Agrobacterium* as a source of nitrogen and carbon. Transformed root clones of *Atropa, Datura, Du*boisia and *Hyoscyamus* species have all been established for tropane alkaloid production (Boitel-Conti et al., 2000). Most remarkable developments of scale-up in large ves-

*Corresponding author. E-mail: gong199@163.com. Tel: +86-871-5228661.

sels have been in the cultivation of Panax ginseng hairy root biomass in a 20-ton cultivation tank (Scheidegger, 1990).

Hairy roots also produce valuable compounds by biotransformations, such as scopolamine (Hashimoto and Yamada, 1983; Yoshioka et al., 1989; Subroto et al., 1996), quinine (Hay et al., 1986), nicotine (Walton and Belshaw, 1988), anabasine (Walton et al., 1988), hyoscyamine (Yoshioka et al., 1989), sugar esters (Furuya et al., 1989), digitoxin (Kawaguchi et al., 1990), glucosides (Asada et al., 1993), stilbene quinines (Flores et al., 1994), glycosylated phenolic compounds (Ushiyama and Furuya, 1989) and so on. It was reported that *Datura* cell culture was capable of glucosylating exogenous phenolic compounds (Mamoru et al., 1988). In our pervious study, the cell suspension cultures of *D. tatula* L and *D. stramonium* had the specific capabilities to glucosylate p-hydroxybenzaldehyde and p-hydroxybenzyl alcohol to gastrodin (Gong et al., 2006). However, there are few detailed studies on the production of gastrodin via biotransformation by plant hairy root suspension cultures.

This article reports the hairy root of *D. tatula* L arising from inoculation with *A. rhizogenes* and the results of the study on the specific capabilities of *D. tatula* L. hairy root cultures to glucosylate p-hydroxybenzyl alcohol.

MATERIALS AND METHODS

Hairy root cultures

Hairy roots of *D. tatula* L. was obtained by transformation with *A. rhizogenes* A4. The transformed roots developed at wound sites were excised and cultured individually for 1.5 months on solid, half-strength MS medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, 500 mg/L cefalothin sodium and 30mg/L kanamin. The genetic transformation rate was calculated as follows: Effectiveness of agroinfection (%) = (total number of explants produced root / total number of explants infected *A. rhizogenes*) ×100%

Genomic DNA extraction and analysis

Total DNA was isolated from hairy root culture clones by using cetyl trimethyl ammonium bromide (CTAB) method (Pharmacia Biotech). PCR analysis was performed using the pre-formulated, pre-dispensed single-dose reaction beads ``Ready to Go™'' (Pharmacia Biotech). The complete PCR mixture contained 200 ng of total DNA, 12.5 pmol/ml of each oligonucleotide primer, 200 µM dNTPs, 1.5 U Taq polymerase, and buffer supplied by the enzyme manufacturer (1/10 V) in a total volume of 25 µl. The used oligonucleotide primers that would anneal to the rolC gene, produced a DNA fragment of 534 bp. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1.5 min, with a final extension at 72°C for 5 min. The PCR reaction mixtures (10 µl) were then loaded directly onto 1.5% agarose gel for electrophoretic analysis. A 100 Base-Pair Ladder (Pharmacia Biotech) was used as a molecular weight marker for the PCR- amplified double-stranded DNA fragment.

Opine analysis

Opine analysis was performed by High Voltage Paper Electrophoresis according to a modification of the method described by (Petit et al., 1983). Samples were immersed in distilled water and then incubated at 100°C for 10 min. After a brief centrifugation (13,000 g for 5 min), supernatant was vacuum-evaporated at 40°C in order to yield a dry plant extract. This extract was then resuspended in distilled water and kept frozen until analyzed. One to 6 ml of plant extracts were spotted on high quality chromatography paper and allowed to dry. Electrophoresis was performed in 1.1 M acetic acid/0.7 M formic acid (pH 1.9) at 50 ± 100 V/cm. Electrophoretograms were then dried in a stream of hot air and processed with the staining solutions to detect the presence of opines as described by (Dessaux and Petit, 1994).

Biotransformation

p-Hydroxybenzyl alcohol were purchased from Sigma Company (USA). Chemosynthetic gastrodin was a gift from Kunming Pharmaceutical Corp. The exogenous substrate p-hydroxybenzyl alcohol was dissolved in distilled water and then diluted to a 10.0 mg/mL stock solution before use. The subcultivated suspension hairy roots (fresh weight 3 g) were cultured in 150 mL flasks, each containing 50 mL of MS/2 liquid medium (pH 5.8) supplemented 0.1 mg/L salicylic acid and maintained at 25°C on a rotary shaker (100 r/min). After 7 d of incubation, the stock solution was added to the flasks, and the flasks were incubated for 25 d.

RT-HPLC

The standard sample was dissolved in deionized water to make a 1.0 mg/mL solution. The liquid chromatograph (Agilent 1100 Series) was equipped with lichrospher RP-18 (7.8 mm×150 mm, Waters). Eluent, acetonitrile/deionized water = 10/90; flow rate, 1.0 mL/min; detector, Hitachi Model L-400 UV Detector/OD220nm; injection volume, 5 µl; the record and retention time, 20 min. The efficiency of glucosylation of p-hydroxybenzyl alcohol was calculated on the basis of the equation: (%) = m/n, where m is the amount of gastrodin (mol) and n is the amount of p-hydroxybenzyl alcohol (mol).

Extraction and isolation

After a few days of incubation, the hairy root cultures added with p-hydroxybenzyl alcohol were filtered. The 1000-mL filtrate (containing 105 mg of gastrodin) was extracted with chloroform (filtrate/chloroform = 1:1) five times, and the residual water liquor was extracted with butyl alcohol (filtrate/butyl alcohol = 1:1) another five times. All extractions extracted by butyl alcohol were collected and then concentrated under reduced pressure. The extract (containing 88 mg of gastrodin) obtained using butyl alcohol extraction from the total 1000 mL cultures was chromatographed over a silica gel (GF-254, 160–200 mesh) column (Φ 5 cm × 50 cm), starting with chloroform (100 %) and ethyl acetate-benzene (1:8) elution followed by the elution of methanol-ethyl acetate (1:9). All the fractions (containing 71 mg of gastrodin) were concentrated and chromatographed over the RP-18 column with methanol-distilled water (10, 20, 40, 60, and 100 % methanol) elution. The elution of 10% (methanol distilled water) was collected and concentrated under reduced pressure. The residual (containing 63 mg of gastrodin) was chromatographed over the Sephadex LH-20 column twice with chloroform-methanol (1:1) elution and methanoldistilled water (1:1) elution. The elution of methanol- distilled water (1:1) was collected and concentrated under reduced pressure. The residual

Table 1. Effect of infection time on the genetic transformation rate (X±SD, n=4).

Infection time (min)	Genetic transformation rate (%)	
	Stem	Leaf
1	15.5±1.64	19.4±3.49
2	34.6±3.32	39.5±3.89
5	58.4±7.80	65.3±3.25
10	52.1±3.22	45.8±3.16
20	10.3±1.99	2.5±0.95

Table 2. Effect of quadratic infection (5 min - 5 min) on the genetic transformation rate (X±SD, n=4).

Explants	Genetic transformation rate (%)	
	5 min first time	5 min – 5 min (quadratic time)
Leaf	29.5±4.33	95.0±2.43
Stem	25.7±2.85	90.5±2.09

58 mg (containing 53 mg of gastrodin) was dissolved in distilled water and further separated by RT-HPLC (RP18, 10 μm, 19 mm×150 mm). The purified gastrodin (49 mg) was used for identification and analysis.

Structure analysis

Structural elucidation of gastrodin was on the basis of spectrum data. 1H NMR spectra were recorded in CD3OD using a Bruker AM-400 Spectrometer. 13C NMR spectra were recorded in CD3OD using a Bruker AM-100 Spectrometer. MS spectra were obtained using a VG-Autospec-3000 mass Spectrometer. The optical rotation value was measured using a JASCO-20 Polarimeter.

RESULTS AND DISCUSSION

Hairy root cultures of *Datura tatula* L

All tested leaf segments and stems of *D. tatula* L inoculated with *A. rhizogenes* appeared transformed roots after 4 weeks of culture, while *Datura* metel transformed roots began to appear 6 weeks after the infection with *Agrobacterium* (Moyano et al., 1999). The genetic transformation rate depended on the different *Agrobacterium* and explants. The results show that 58.05% of segments infected with *A. rhizogenes* A4 developed transformed roots, while only 30.05% of *A. rhizogenes* A1601 at the same conditions. As shown in (Table 1), the genetic transformation rate was highest for 5 min infection time and it was remarkably enhanced using quadratic infection (5 - 5 min) (as shown in (Table 2), where the genetic transformation rate was over 90%. The time of producing transformed roots was also reduced to 2 - 3 weeks. This suggests that the explants infected first for 5 min may produce the anti-stress reaction and restrain the rolC gene expression. But as the infected explant was infected again, this restriction may be destroyed and the rolC gene expression may be enhanced .

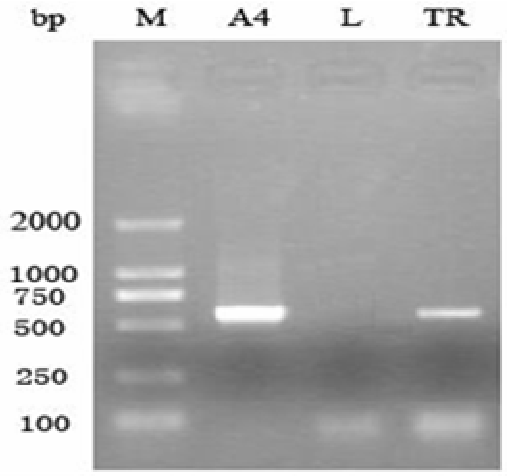


Figure 1. PCR detection of ro1C gene in transformed hairy root. **M**, DNA marker; **A4**, A4 strain of *Agrobacterium rhizogenes*, **L**, Leaf of *Datura tatula* L.; **TR**, hairy root of *Datura tatula* L

Fragments of *Agrobacterium* T-DNA integrated into the transformed root genome

Hairy roots result from the transfer of genes located on the root-inducing plasmid Ri to plant cells and their expression therein (White and Nester, 1980). Two sets of pRi genes are involved in the root induction process: aux genes located in the TR region and the rol (root loci) genes of TL region (Jouanin, 1984). The rolC gene located in the segment TL of the T-DNA of *A. rhizogenes*, which plays a significant role in developing hairy root syndrome, was detected by PCR analysis. As shown in Figure 1, the fragments of *Agrobacterium* T-DNA had already integrated into the transformed root genome. Randomly selected root lines were analyzed in order to investigate opine presence in transformed roots. Manopine was also detected.

Morphology and growth of transformed root cultures established

In order to establish root line cultures, root tips appearing at the wound sites of leaf segments were picked off and separately cultured in hormone-free solid medium for successive subcultures of 4 weeks. As previously mentioned, the transformed roots were grown on MS/2 medium. When cultured on solid medium, transformed roots grew actively and showed two different morphologies. Most of the transformed root cultures showed the characteristic traits of hairy roots (Figure 2a) described

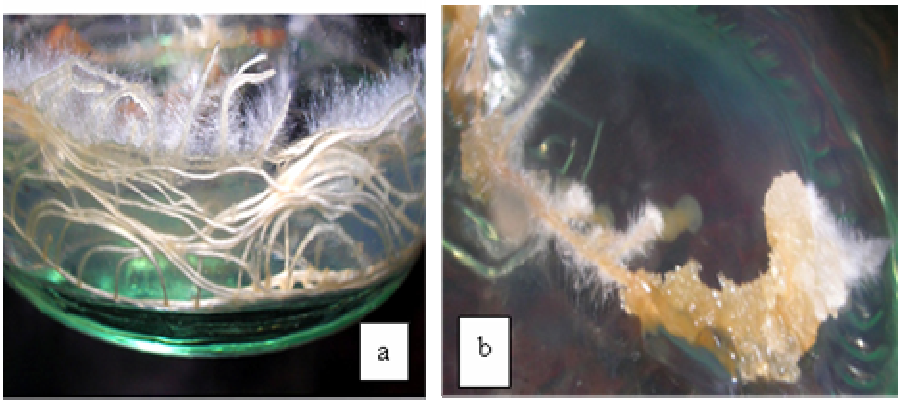


Figure 2. Seven-week-old *D. tatula* L. transformed root cultures on MS/2 solid medium. (a) Root exhibiting hairy root morphology. (b) Root exhibiting callus-like morphology.

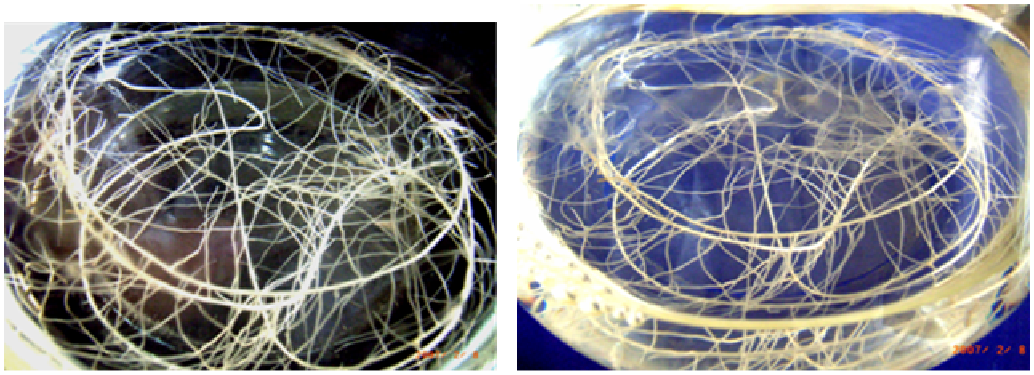


Figure 3. Highly branched hairy root cultures of *Datura tatula* L in liquid medium.

previously by David et al. (1984) (fast growing, highly branched and plagiotropic). On the contrary, the remaining root lines showed the denominated callus-like morphology (Figure 2b), characterized for its high capacity to differentiate and produce callus tissues in the hormone-free culture medium. Root morphology was maintained for successive subcultures. At the same time, we found that the hairy roots had many branches in liquid medium and also grow fast (Figure 3).

Biotransformation of exogenous p-hydroxybenzyl alcohol by *D. tatula* L. hairy root suspension cultures

The hairy root suspension was prepared in a 150-mL flask containing 50 mL of MS/2 liquid medium supplemented with 30.0 g/L sucrose and 0.1 mg/L salicylic acid at 23 ± 1°C. A total of 1.0 mL of p-hydroxybenzyl alcohol solution (10 mg) was added into the root suspension cultures, and a flask without the substrate addition was treated as the control. p-Hydroxybenzyl alcohol was administered to root suspension cultures for 25 d incubation under specified conditions. After incubation, the root cultures were filtered under vacuum and the roots were crushed and extracted with methanol. The root extracts were identified and analyzed by RT-HPLC. The RT-HPLC (Figure 4) showed that a new peak (Compound I) ap-

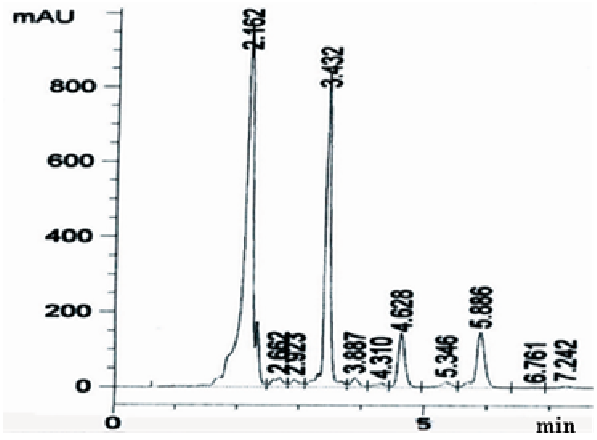
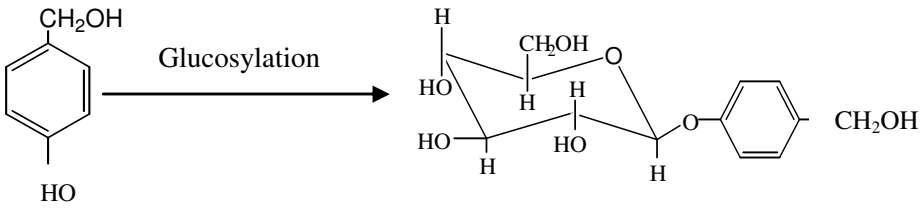


Figure 4. HPLC of gastrodin through biotransformation of exogenous *p*-hydroxybenzyl alcohol by suspension culture of hair root of *Datura tatula* L. (3.432 min - gastrodin; 5.886 min - exogenous *p*-hydroxybenzyl alcohol).



p-hydroxybenzyl alcohol *p*-hydroxy-methyl-phenol-β-D-glucoside

Figure 5. Biotransformation of exogenous p-hydroxybenzyl alcohol to gastrodin using *D. tatula* L. hairy root suspension cultures.

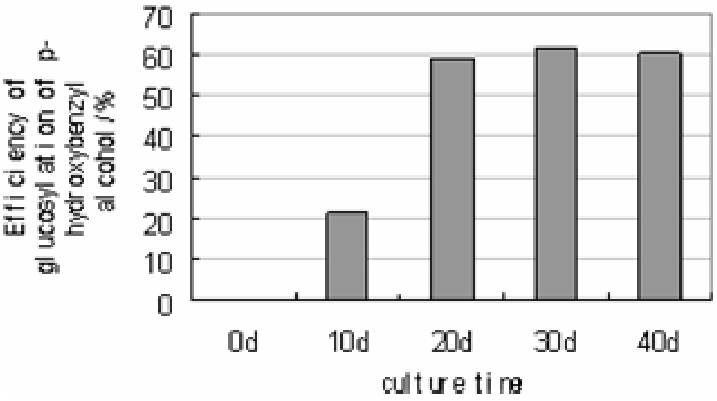


Figure 6. Time-course of glucosylation of exogenous p-hydroxybenzyl alcohol by root suspension cultures of *D. tatula* L.

peared when compared with that of the control after an additional 25 d of incubation.

Compound (I) had similar retention time as those for gastrodin analyzed by RT-HPLC. The structures of (I) was chemically and physically identified as p-hydroxy-methyl-phenol-β-D-glucoside (gastrodin), which is a derivative of p-hydroxybenzyl alcohol. p-Hydroxy-methyl-phenol-β-D-glucoside (gastrodin) (I), $C_{13}H_{18}O_7$, 1H NMR (CD3OD, 400 MHz): 7.26 (d, J = 8.6) H3,5, 7.06 (d, J = 8.6) H2,6 , 4.89 (d, J = 9.7, H-1′), 4.52 (2H, s), 3.89 (dd, J = 1.9, 12.0, H-6′), 3.69 (dd, J = 4.9, 12.0, H-6′), 3.29–3.45 (4H, H-2′~H-5′) or (m, 4H); 13C NMR (100 MHz, CD3OD): 136.6 (C1), 129.4 (C2, 6), 117.7 (C3, 5), 158.4(C4), 64.8 (C7), 102.4 (C-1′), 74.9 (C-2′), 78.1 (C-3′), 71.3 (C-4′), 77.9 (C-5′), 62.5 (C-6′). FAB– MS m/z: 285[M-H]–, 377[M +Gly-H]–, 469[M + 2Gly-H]–, 561[M + 3Gly-H]–, [α]D23–76.9 (in 0.4 g/100 mL water solution) was consistent with the previously reported [α]D 15 –66.4 (in 0.65 g/100 mL water solution) (Feng et al., 1979). The examination of the chemical structure and the analysis of RT-HPLC and TLC confirmed that *D. tatula* L. root cultures used for biotransformation were capable of converting exogenous p-hydroxybenzyl alcohol into gastrodin. The possible pathway of biotransformation is shown in (Figure 5).

Time course of glucosylation of p-hydroxybenzyl alcohol by *D. tatula* L. hairy root suspension cultures

The glucosyltransferase activity was influenced by the stage of cell growth and other important factors. According to the literature (Takayuki and Toshifumi, 1990), the activity of glucosyltransferase is maximum at the exponential growth phase of the plant cells. If exogenous substrate was added, particularly to this stage of plant cells, it would be efficiently converted to the corresponding glucoside. In order to reveal the time-course of glucosylation of p-hydroxybenzyl alcohol by hairy root suspension cultures of *D. tatula* L., 1 mL of substrate solution was administered to the each 100 mL flask containing 30 mL root (ca. 4 g) cultures on the 30-day old cultures with a total amount of 10 mg substrate. After incubation of the culture, the gastrodin was extracted from the root cultures at different times, and its content was analyzed by RT-HPLC. As shown in (Figure 6), after first 10 d incubation, the yield of gastrodin was not increased considerably, but the yield of gastrodin in-

creased dramatically and can reach approximately 59.75% after 20 d of incubation. Therefore, the efficiency of glucosylation of p-hydroxybenzyl alcohol was enhanced.

Conclusion

In general, gastrodin is mainly found in *G. elata* Bl. and other plants cannot largely accumulate gastrodin. In the present paper, the production of gastrodin through biotransformation of exogenous p-hydroxybenzyl alcohol by hairy root suspension cultures of *D. tatula* L. was successful obtained. The efficiency of glucosylation of p-hydroxybenzyl alcohol can reach approximately 59.75% after 20 d of incubation. The results also have elucidated the possible biotransformation pathways of producing gastrodin from exogenous p-hydroxybenzyl alcohol using *D. tatula* L. root suspension cultures.

ACKNOWLEDGEMENT

The work was supported by grants from the Found of Natural Science of Yunnan Province (Projects number 2004C0034M).

REFERENCES

Achmad S, Evi R, Gunawan I (1999). Biotransformation of O- and p-aminobenzoic acids and N-acetyl p-aminobenzoic acid by cell suspension cultures of *Solanum mammosum*. Phytochemistry.51: 615-620.

Asada Y, Saito H, Yoshikawa T, Sakamoto K, Furuya T (1993). Biotransformation of 18b-glycyrrhetinic acid by ginseng hairy root culture. Phytochemicals 34: 1049-1452.

Boitel-Conti M, Laberche JC, Lanoue A, Ducrocq C, Sangwang-Norreel BS (2000). Influence of feeding precursors on tropane alkaloid production during an abiotic stress in *Datura innoxia* transformed roots. Plant Cell, Tissue Organ Cult. 60: 131-137.

Dessaux Y, Petit A (1994). Opines as screenable for plant transformation. Plant Mol. Biol Manual. C3: 1-12.

Feng XZ, Chen YW, Yang JS (1979). Studies on constitutes of tian-ma (*Gastrodia elata* Bl.). Acta Chem. Sinica. 37: 175-181.

Flores HE, Dai YR, Freyer AJ, Michaels PJ (1994). Biotransformation of butylated hydroxytoluene in hairy root cultures. Plant Physiol. Biochem. 32: 511-519.

Furuya T, Ushiyama M, Asada Y, Yoshikawa T (1989). Biotransformation of 2-phenylpropionic acid in root culture of Panax ginseng. Phytochemicals. 28: 483-487.

Gong JS, Ma WP, Pu JX, Xu SG, Zheng SQ, Xiao CJ (2006). Production of gastrodin through biotransformation of p-hydroxybenzaldehyde using cell suspension cultures of *Datura tatula* L. Chin. J. Biotech. 22(5): 800–804.

Hashimoto T, Yamada Y (1983). Scopolamine production in suspension cultures and redifferentiated roots of *Hyoscyamus niger*. J. Med. Plant Res. 47: 195-199.

Hay CA, Anderson LA, Roberts MF, Phillipson JD (1986). *In vitro* cultures of Cinchona species. Plant Cell Rep., 5: 1-4.

Heihachiro T, Itiro Y, Kazuo YII HK (1981). Studies on the constituents of *Gastrodia elata* Blume. Chem Pharm Bull. 29: 55-62.

Jouanin L (1984). Restriction map of an agropine-type Ri-plasmid and its homologies with Ti-plasmids. Plasmid 12: 91-102.

Kawaguchi K, Hirotani M, Yoshikawa T, Furuya T (1990). Biotransformation of Digitoxigenin by Ginseng Hairy Root Cultures. Phytochemistry 29(3): 837.

Mamoru T, Yasuko U, Mitsuko O, Shigeo T (1988). Glucosylation of phenolic compounds by plant cell cultures. Phytochemistry 3 (27): 809-813.

Moyano E, Fornalé S, Palazó J, Cusidó RM, Bonll M, Morales C, Piñol MT (1999). Effect of *Agrobacterium rhizogenes* T-DNA on alkaloid production in Solanaceae plants. Phytochemistry. 52: 1287-1292.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Palazón J, Piñol MT, Cusidó RM, Morales C, Bonfill M (1997). Application of transformed root technology to the production of bioactive metabolites. Recent Res. Dev. Plant Physiol. 1: 125-143.

Petit A, David C, Dahl GA, Ellis JG, Guyon P, Casse-Delbart F, Tempe ÂJ (1983). Further extension of the opine concept: plasmids in *Agrobacterium rhizogenes* cooperate for opine degradation. Mol. Gen. Genet.190: 204-214.

Scheidegger A (1990). Plant biotechnology goes commercial in Japan. Trends Biotechnol. 8:197-198.

Subroto MA, Kwok K, Hamill JD, Doran PM (1996). Coculture of genetically transformed roots and shoots for synthesis, translocation and biotransformation of secondary metabolites. Biotechnol Bioeng. 49: 481-494.

Sun XF, Wang W, Wang DQ, Du FG (2004). Research progress of neuroprotective mechanisms of *Gastrodia elata* and its preparation. China J. Chin. Mater. Med. 29: 292-295.

Toivonen L (1993). Utilization of hairy root cultures for production of secondary metabolites. Biotechnol. Prog. 9: 12-20.

Ushiyama M, Furuya T (1989). Glycosylation of phenolic compounds by root culture of Panax ginseng. Phytochemistry. 28(11): 3009-3013.

White FF, Nester EW (1980). Hairy root: plasmid encodes virulence in Agrobacterium rhizogenes. J. Bacteriol. 141: 1134-1141.

Yoshioka K, Yamagata H, Ito H, Deno Y, Fujita Y, Yamada Y (1989). Effects of exogenous polyamines on tropane alkaloid production by a root culture of Duboisia myoporoides. Planta Med. 55: 523-524.

Zhao YK, Cao QE, Xiang YQ, Hu ZD (1999). Identification and determination of active components in *Gastrodia elata* Bl. by capillary electrophoresis. J. Chromatogr. 849: 277-283.

Zhou J, Yang YB, Yang CR (1979). Chemical study on gastrodin and related compounds, separation and identification of chemical constitutes of *Gastrodia elata* Bl. Acta Chem. Sinica (in Chinese). 37: 183-189.

Zhou J, Yang YB, Yang CR (1980). Chemical study on gastrodin and related compounds, the synthese of gastrodin and related compounds. Acta Chem. Sinica (in Chinese). 38: 162-165.