Symposium

Screening and bioconversion of glycyrrhizin of *Glycyrrhiza glabra* root extract to 18β-glycyrrhetinic acid by different microbial strains

Abstract

Objective: The objective of the present study is to perform screening of different microorganisms (7 bacteria and 14 fungi) for conversion of glycyrrhizin (GL) to 18β-glycyrrhetinic acid (GA). *Penicillium chrysogenum* produced the highest concentration of β-glucuronidase enzyme (61 U/mL) and produced GA of 52 μg/mL while *E. coli* produced the highest β-glucuronidase of 376 U/mL with GA concentration of 2.1 μg/mL. **Materials and Methods:** Submerged and solid state biotransformation of GL was carried out. To 9.0 mL of bacterial supernatant, 1.0 mL 0.2% w/v of aqueous *Glycyrrhiza glabra* root extract was added and incubated at 37°C for 24 h. β-glucuronidase activity was measured and high-performance liquid chromatography analysis was carried out. **Results and Discussion:** Induced-*Escherichia coli* produces 2.1 μg/mL of GA with an enzyme activity of 376 U/mL which shows that the enzyme has a potential biotransformation capability. *Rhizopus oryzae* and *P. chrysogenum* have the potential ability to biotransform GL to GA with 2.6 μg/mL and 61 μg/mL of GA with enzyme activity of 569 U/mL and 61 U/mL, respectively. **Conclusions:** *G. glabra* roots containing GL can be hydrolyzed by microbial β-glucuronidase enzyme under sub-merged fermentation (SmF). β-glucuronidase, an enzyme of *E. coli*, was found to be the best microbial source of enzyme which biocatalyzed the reaction than fungal strain under SmF.

Key words:
18β-glycyrrhetinic acid, β-glucuronidase, biotransformation, screening

Introduction

Glycyrrhiza glabra root, commonly known as sweet root, is widely used in the Indian traditional system of medicine. The active component of *G. glabra* root is glycyrrhizin (GL),[1] and it is composed of one molecule of 18β-glycyrrhetinic acid (GA) and two molecules of glucuronic acid.[2] It inhibits cyclooxygenase activity and prostaglandin formation, all factors in the inflammatory process by inhibiting phospholipase A_2 activity.[3,4] GL is potentially valuable for HIV therapy.[5,6] GA is absorbed into the systemic circulation and produces pharmacological action. Further, it is metabolized to 3 β-D-(monoglucuronyl) 18β-GA in the liver and excreted out from the body in the urine.[7] The biological activity of GA is 20 times more than GL.[8] High concentration of GL or GA in serum resulted severe adverse reaction. Therefore, scientists are now trying to produce GA from GL by biocatalysis and by bioprocess under *in vitro* means.

The objective of this study was to investigate the concentration of GL and GA present in fermented *G. glabra* root extract by high-performance liquid chromatography method.

Access this article online	
Website: www.ddtjournal.org	Quick Response Code
DOI: 10.4103/2394-6555.180170	

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How to cite this article: Ahmad M, Jalaluddin, Ali M, Panda BP. Screening and bioconversion of glycyrrhizin of *Glycyrrhiza glabra* root extract to 18β-glycyrrhetinic acid by different microbial strains. Drug Dev Ther 2016;7:69-72.

Materials and Methods

Plant material, microbes, and chemicals

The roots of *G. glabra* were collected from the Global Herbs, New Delhi, and authenticated. Different bacterial strains and fungal strains were isolated and identified. All the chemicals, reagents, and microbiological medium were obtained from Hi-media, Mumbai, India.

Biotransformation of glycyrrhizin to glycyrrhetinic acid

Submerged biotransformation of GL was carried out in 250 mL Erlenmeyer flasks. Nutrient broth was prepared, and pH was adjusted to 7.2. A loop full of different bacterial cultures was inoculated and incubated for 2 days at 37°C at 170 rpm. Bacterial broth was filtered, pH was adjusted to seven, and centrifuged at 3000 rpm. To 9.0 mL of supernatant, 1.0 mL 0.2% w/v of aqueous *G. glabra* extract was added and incubated at 37°C for 24 h.[9] The β-glucuronidase activity was analyzed at 0 h. The reaction mixture was analyzed for bio-converted product (GA) during and after biotransformation reaction.

Beta-glucuronidase enzyme assay

The β-glucuronidase activity was measured at 37°C by mixing cultured broth with 3 mM phenolphthalein-β-d-glucuronic acid buffered with 75 mM potassium phosphate buffer with 1.0% bovine serum albumin at a pH of 6.8.[10]

Extraction of glycyrrhetinic acid and glycyrrhizin

The extraction of GL and GA from fermented broth was carried out under biphasic condition.[11] Methanol was used for extraction of GL and GA from the fermented mass.

Chromatographic condition and analysis of glycyrrhetinic acid and glycyrrhizin

The chromatography was carried out by RP C_{18} column, the mobile phase consists of methanol:water (85:15, v/v) at a flow rate of 1.0 mL/min with run time of 10 min. Ultraviolet detection was carried out at 254 nm.[12]

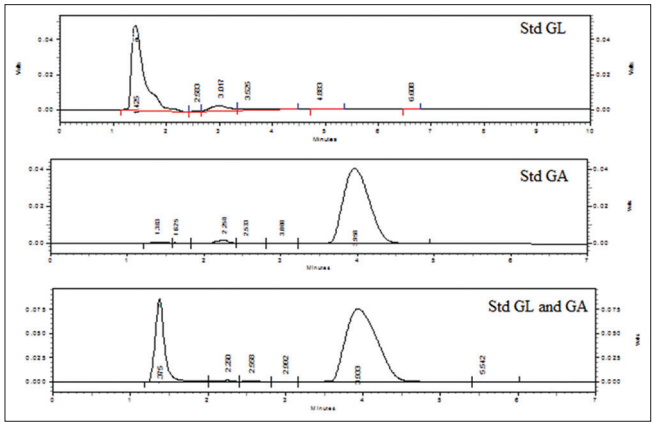

Figure 1: High-performance liquid chromatography chromatograms of standard glycyrrhizin (75 μg/mL) and standard glycyrrhetinic acid (75 μg/mL) and their mixture (75 μg/mL)

Results and Discussion

Isolation of bacterial and fungal strain

Different bacterial stains were isolated and identified as *Bacillus subtilis*, *Bacillus megaterium*, *Lactobacillus acidophilus*, and *Escherichia coli*. All the bacterial strains were tested for bioconversion of GL to GA under submerged fermentation (SmF). Among fungal strains, *Penicillium chrysogenum* MPBL1 and *Rhizopus oryzae* MPBL2 were identified. The chromatograms of standard GL and GA are shown in Figures 1-3.

Bioconversion of glycyrrhizin to glycyrrhetinic acid with enzyme activity under submerged fermentation

E. coli produces 2.1 μg/mL of GA with an enzyme activity of 376 U/mL [Table 1]. The chromatograms of all the bacterial

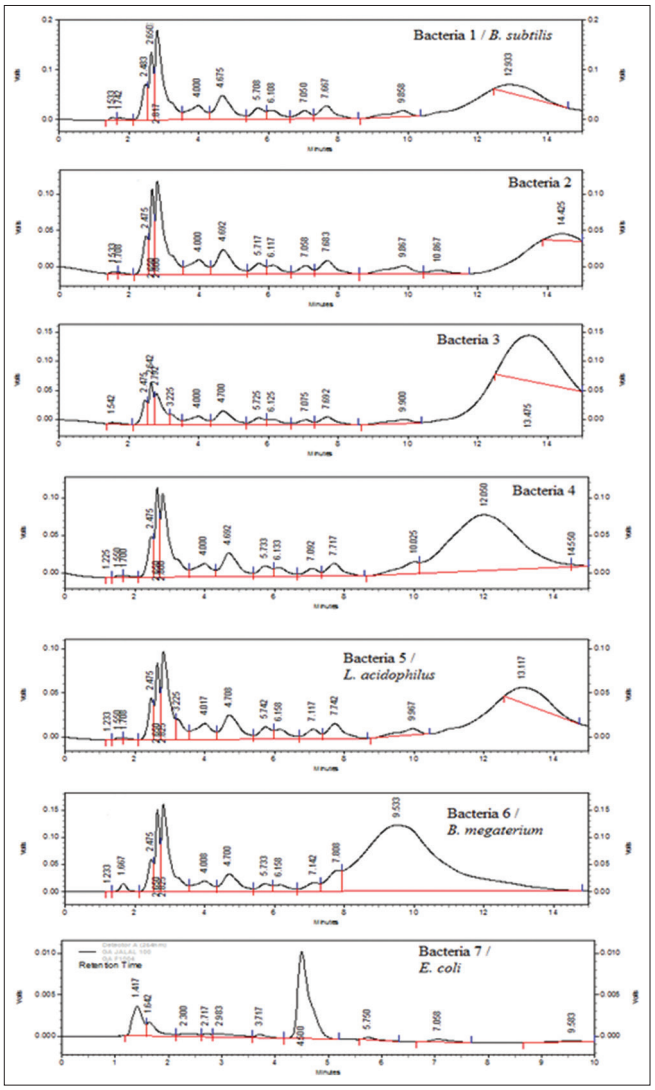

Figure 2: High-performance liquid chromatography chromatograms of produced glycyrrhetinic acid in different bacterial (*Bacillus subtilis*, unidentified *B2*, unidentified *B3*, unidentified *B4*, *Lactobacillus acidophilus*, *Bacillus megaterium*, and *Escherichia coli*) fermented broth

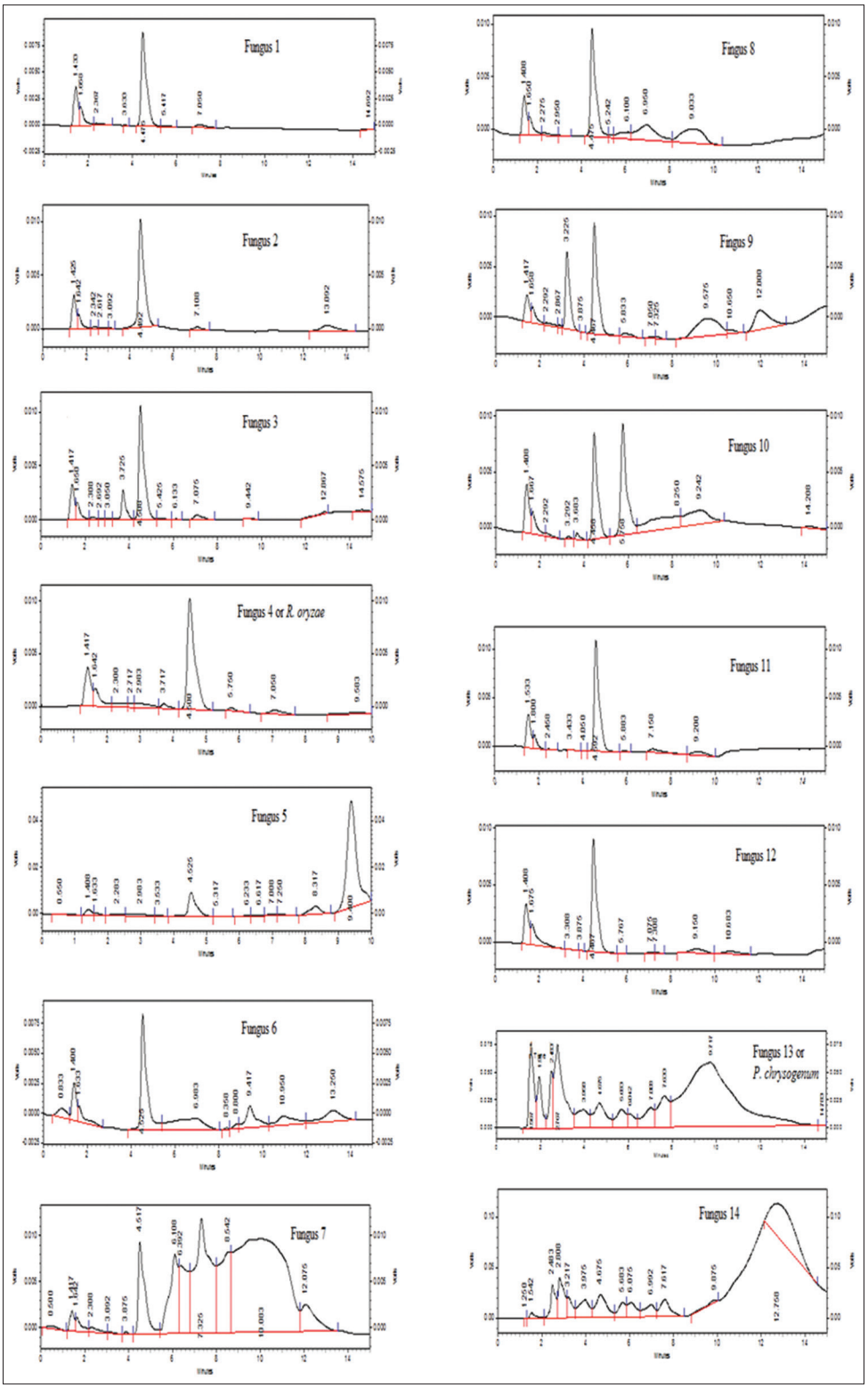

Figure 3: High-performance liquid chromatography chromatograms of produced glycyrrhetinic acid in different fungal (unidentified F1, unidentified F2, unidentified F3, *Rhizopus oryzae*, unidentified F5, unidentified F6, unidentified F7, unidentified F8, unidentified F9, unidentified F10, unidentified F11, unidentified F13, *Penicillium Chrysogenum*, and unidentified F14) fermented broth

fermented samples were shown in Figure 1. Higher enzyme activity was observed without the presence of inducer for bacteria as well as fungi. This may be due to the presence of water extract of drug as it also has antimicrobial action, which inhibits the growth of microorganism and inhibits the production of enzyme. Enzyme activity of

Table 1: Concentration of glycyrrhizin, glycyrrhetinic acid, and enzyme activity under submerged biotransformation process by different bacterial strains

Bacterial strains	GL (μg/mL)	GA (μg/mL)	Enzyme activity (U/mL)
Bacillus subtilis	150	36	623
B2	147	52	123
B3	245	23	7.6
B4	157	25	438
Lactobacillus acidophilus	128	252	53
Bacillus megaterium	122	65	38
Escherichia coli	181	2.1	376
Control	190	0.0	7.6

GL – Glycyrrhizin; GA – Glycyrrhetinic acid

Table 2: Concentration of glycyrrhizin, glycyrrhetinic acid, and enzyme activity under submerged biotransformation by different fungal strains

Fungal strains	GL (μg/mL)	GA (μg/mL)	Enzyme activity (U/mL)
F1	150	0.0	53
F2	181	2.1	38
F3	174	2.1	623
Rhizopus oryzae	135	2.4	569
F5	171	2.6	30
F6	163	0.0	7
F7	144	2.2	23
F8	62	2.1	84
F9	271	2.2	130
F10	86	0.0	23
F11	136	0.0	707
F12	20	0.0	23
Penicillium chrysogenum	65	52	61
F14	130	20	261
Control	190	0.0	7.6

GL – Glycyrrhizin; GA – Glycyrrhetinic acid

bacteria was much more than fungal strain under SmF due to higher water activity. Some bacterial strains were found to have good enzyme activity but were not capable of biotransforming GL to GA, which may be due to bioconversion of GL into unknown molecules or lack of selectivity of bond.

R. oryzae and *P. chrysogenum* have higher capability to biotransform GL to GA. The concentration of 2.6 μg/mL and 61 μg/mL of GA with enzyme activity of 569 U/mL and 61 U/mL was produced by *R. oryzae* and *P. chrysogenum*, respectively [Table 2]. The chromatograms of all the bacterial fermented samples were shown in Figure 2. Some fungal strains were found to have good enzyme activity, but they do not biotransform GL to GA. Moreover, biotransformation under submerged condition showed that bacterial strains were having higher capability to biotransform GL to GA than fungal strains, since bacterial cells grow faster in the presence of large volume of water, but fungal cells require solid support to grow.

Conclusion

Twenty-one microbial strains (bacterial and fungal) were screened for biotransforming GL to GA in *G. glabra* root. *E. coli* was found to be the best source of enzyme (β-glucuronidase) for biotransformation among all bacterial strains. It has been found that bacterial strains produce higher amount of β-glucuronidase enzyme than fungal strain during SmF.

Acknowledgment

The authors are highly thankful to Bioactive Natural Product Laboratory, Jamia Hamdard, New Delhi, for their kind support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Farag MA, Porzel A, Wessjohann LA. Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques. Phytochemistry 2012;76:60-72.
2. Hennell JR, Lee S, Khoo CS, Gray MJ, Bensoussan A. The determination of glycyrrhizic acid in *Glycyrrhiza uralensis* Fisch. ex DC. (Zhi Gan Cao) root and the dried aqueous extract by LC-DAD. J Pharm Biomed Anal 2008;47:494-500.
3. Ohuchi K, Tsurufuji A. A study of the anti-inflammatory mechanism of glycyrrhizin. Mino Med Rev 1982;27:188-93.
4. Okimasu E, Moromizato Y, Watanabe S, Sasaki J, Shiraishi N, Morimoto YM, *et al.* Inhibition of phospholipase A2 and platelet aggregation by glycyrrhizin, an antiinflammation drug. Acta Med Okayama 1983;37:385-91.
5. Numazaki K, Umetsu M, Chiba S. Effect of glycyrrhizin in children with liver dysfunction associated with cytomegalovirus infection. Tohoku J Exp Med 1994;172:147-53.
6. Shabani L, Ehsanpour AA, Asghari G, Emami J. Glycyrrhizin production by *in-vitro* cultured *Glycyrrhiza glabra* elicited by methyl jasmonate and salicylic acid. Russ J Plant Physiol 2009;56:621-6.
7. Kerstens MN, Guillaume CP, Wolthers BG, Dullaart RP. Gas chromatographic-mass spectrometric analysis of urinary glycyrrhetinic acid: An aid in diagnosing liquorice abuse. J Intern Med 1999;246:539-47.
8. Lu DQ, Hui L, Yan D, Ping-Kai O. Biocatalytic properties of a novel crude glycyrrhizin hydrolase from the liver of the domestic duck. J Mol Catal B Enzym 2006;43:148-52.
9. Morana A, Lazzaro AD, Lerina ID, Ponzone C, Rosa MD. Enzymatic conversion of 18-β-glycyrrhetinic acid from *Glycyrrhiza glabra* L. Biotechnol Lett 2002;24:1907-11.
10. Combie J, Blake JW, Nugent TE, Tobin T. Morphine glucuronide hydrolysis: Superiority of beta-glucuronidase from patella vulgata. Clin Chem 1982;28:83-6.
11. Siracusa L, Saija A, Cristani M, Cimino F, D'Arrigo M, Trombetta D, *et al.* Phytocomplexes from liquorice (*Glycyrrhiza glabra* L.) leaves – chemical characterization and evaluation of their antioxidant, anti-genotoxic and anti-inflammatory activity. Fitoterapia 2011;82:546-56.
12. Wang J, Sun Q, Gao P, Wang JF, Xu C. Bioconversion of glycyrrhizinic acid in liquorice into 18β-glycyrrhetinic acid by *Aspergillus parasiticus* speare BGB. Appl Biochem Microbiol 2010;46:421-66.