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SHORT COMMUNICATION

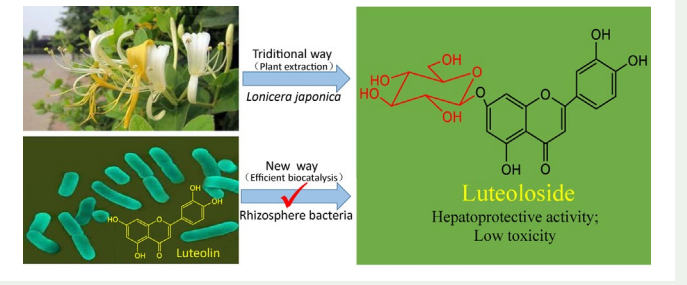
Bioactive luteoloside produced by *Myroides odoratimimus*, solvent-tolerant bacterium form the rhizosphere of *Lonicera japonica*

Y. X. Zhao, J. J. Wang, L. Zhang, S. Zhang[#], S. L. Su, J. A. Duan[#], Z. Z. Yao and S. K. Xu

Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Jiangsu Key Laboratory for High Technology Research of TCM Formulae, State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine, Nanjing, PR China

ABSTRACT

Luteoloside (luteolin-7-O-glucoside), the biomarker of *Lonicera japonica*, was efficiently bio-synthetized from its cheaper precursor luteolin. The structure of luteoloside was characterized by LC-MS and NMR analyses. Compared to the significant inhibitory effect of luteolin on human hepatocyte cell line LO2 at high doses, luteoloside did not show obvious cytotoxic effects at any test dose. Moreover, luteoloside exhibited obvious promotive effects on human hepatocyte cells, suggesting a potential application in hepatoprotective therapies.



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Myroides odoratimimus; rhizosphere bacteria; *Lonicera japonica*; biotransformation; luteoloside; hepatocytotoxicity

1. Introduction

Lonicera japonica, 'Jinyinhua' in Chinese, is an important herbal plant that has been widely used in Chinese medicine for thousands of years. This important herb is used to treat various diseases, such as severe acute respiratory syndromes, H1N1 influenza, and hand-foot-and-mouth disease (Yuan et al. 2014). Luteoloside as well as chlorogenic acid is the biomarker used by the Chinese Pharmacopoeia for evaluating the quality of *Lonicera japonica*. Pharmacological experiments have shown that luteoloside has a spectrum of biological

CONTACT S. Zhang zhangsci@njucm.edu.cn; J. A. Duan dja@njucm.edu.cn
[#]Current address: 138 Xianlin Road, College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China.
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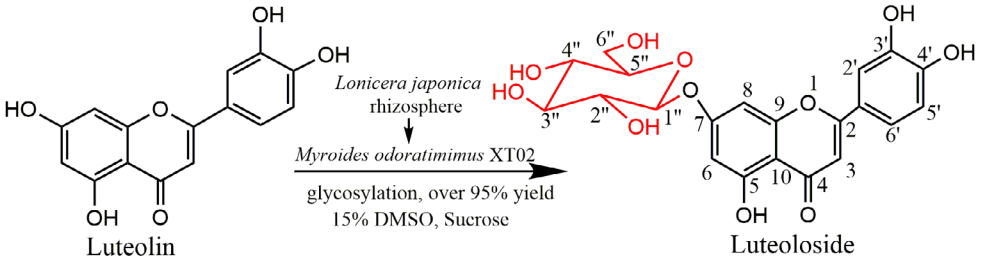

Figure 1. Efficient biosynthesis of luteoloside by *Myroides odoratimimus* XT02 from the rhizosphere of *Lonicera japonica*.

activities, particularly anti-inflammatory (Francisco et al. 2014) and hepatoprotective properties (Zheng et al. 2004).

To obtain plenty of luteoloside, a number of methods have been developed, such as plant extraction (He et al. 2012; Wang et al. 2017) and aqueous biocatalysis from cheaper precursors (Hyung Ko et al. 2006; Palmeri et al. 2017), but it is still difficult to satisfy the commercial demand for luteoloside for pharmaceutical production. Glycosylation of flavonoid aglycones in aqueous miscible organic media has long been proven to be an ideal way to both enhance value and reduce cytotoxicity (Wu et al. 2013; Chu et al. 2014). In this study, we reported an efficient biosynthesis of luteoloside by the newly isolated *Myroides odoratimimus* XT02 from the rhizosphere of *Lonicera japonica*. The hepatocytotoxicities of luteoloside and its aglycone luteolin were also investigated.

2. Results and discussion

2.1. Establishment of efficient biotransformation system

Efficient glycosylation of luteolin was carried out in aqueous miscible organic media by the *Lonicera japonica* rhizosphere strain *M. odoratimimus* XT02 (Figure 1). Retention times of HPLC spectrograms for luteolin and its derivative were 18.7 min and 14.9 min, respectively (Figure S1). In 15% dimethyl sulfoxide (DMSO, v/v) reaction system for 15 h, over 95% of luteolin (1.5 g /L) was converted to the product, which was much higher than that in the water phase (approximately 8% yield of 0.2 g/L luteolin in 15 h). DMSO, considered as a green solvent and catalyst, is usually used as the solvent or cosolvent for the biotransformation of natural products (Cao et al. 2017). However, organic solvents, especially with a log *P* value below 0 (DMSO has a log *P* value of -1.35), are extremely toxic to enzymes as well as microbes (Zhang et al. 2013). Thus, the use of solvent tolerant strains is considered to be a key successful factor for efficient biotransformation in natural product research.

2.2. Purification and characterization of the bio-transformed product

The high resolution ESI-MS gave a molecular ion $[M - H]^-$ at m/z 447.0219 (Figure S2), suggesting an empirical molecular formular of $C_{21}H_{20}O_{11}$. The m/z of the product was 162 (equal to the mass of one hexose) higher than that of luteolin, presumably corresponding to a glycosyl luteolin derivative. Since the ^1H NMR and ^{13}C NMR results (Figure S3) were identical to those of an authentic luteolin in the literature (Wang et al. 2017), the product was

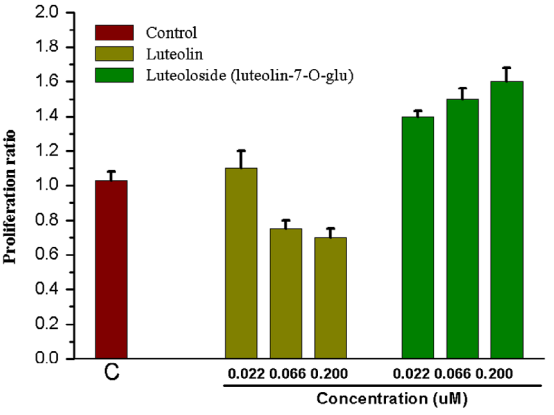

Figure 2. Effects of luteoloside and luteolin on the proliferation of human hepatocyte cell line LO2.

identified as luteoloside, which was further confirmed by HMBC and HSQC correlations (Figure S4). Although the glycosylation of luteolin by plant cells (El Riachy et al. 2011) and UDP-glucosyltransferase (Hyung Ko et al. 2006; Palmeri et al. 2017) have been elaborated, our report is the first demonstration of the efficient glycosylation of luteolin by bacteria.

2.3. Hepatocytotoxicity tests of luteolin and luteoloside

The effects of luteolin and luteoloside on the proliferation of human hepatocyte cell line LO2 were evaluated by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. As shown in Figure 2, higher doses of luteolin (0.067 μM and 0.200 μM) exhibited significant inhibitory effects on human hepatocyte cell line LO2 proliferation in a dose-dependent manner, while its glycosides showed the opposite effect.

Luteolin is known to have a protective effect on liver cells, and could be a promising auxiliary drug for the treatment of liver cancer (Chei et al. 2017). However, fewer hydrophilic groups leads to a low bioavailability which limits its clinical application (Ross and Kasum 2002). Moreover, according to previous reports (Galati and O'Brien 2004), higher dose of flavonoids may cause an increased toxicity. Some studies have found that glycosyl can increase the bioavailability of flavonoids (Hyung Ko et al. 2006; Ajish et al. 2015; Palmeri et al. 2017). However, there are few reports on the toxic effects of the flavonoid glycoside compared to its aglycone. In this study, we found that luteoloside did not show obvious cytotoxic effect compared to luteolin at a higher dose. Moreover, luteoloside exhibited obvious promotive effect on human hepatocyte cells.

3. Conclusions

This study reported a rhizosphere bacterium isolated from *Lonicera japonica*, which showed efficient glycosylation activity for the production of luteoloside, a biomarker of *Lonicera japonica*. Compared to its aglycone, luteloside not only showed lower hepatotoxicity, but also exhibited promotive effect on human hepatocyte cells. In this context, the efficient production of luteoloside and its application in hepatoprotective drugs could be an exciting prospect.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Ajish KR, Antu KA, Riya MP, Preetharani MR, Raghu KG, Dhanya BP, Radhakrishnan KV. 2015. Studies on α-glucosidase, aldose reductase and glycation inhibitory properties of sesquiterpenes and flavonoids of *Zingiber zerumbet* Smith. Nat Prod Res. 29(10):947–952.

Cao SL, Deng X, Xu P, Huang ZX, Zhou J, Li XH, Lou WY. 2017. Highly efficient enzymatic acylation of dihydromyricetin by the immobilized lipase with deep eutectic solvents as cosolvent. J Agricult Food Chem. 65(10):2084–2088.

Chei S, Hwang JH, Lee YJ, Koh EJ, Choi J, Song JH, Lee BY. 2017. Luteolin prevents palmitic acid-induced hepatic steatosis by regulating ER stress in HepG2. FASEB J. 31(1):972–23.

Chu J, Wu X, Li B, He B. 2014. Efficient glucosylation of flavonoids by organic solvent-tolerant *Staphylococcus saprophyticus* CQ16 in aqueous hydrophilic media. J Mol Catal B: Enzym. 99:8–13.

El Riachy M, Priego-Capote F, León L, Rallo L, de Castro L, Dolores M. 2011. Biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. Eur J Lipid Sci Technol. 113(6):692–707.

Francisco V, Figueirinha A, Costa G, Liberal J, Lopes MC, García-Rodríguez C, Batista MT. 2014. Chemical characterization and anti-inflammatory activity of luteolin glycosides isolated from lemongrass. J Funct Foods. 10:436–443.

Galati G, O'Brien PJ. 2004. Serial review: flavonoids and isoflavones (phytoestrogens): absorption, metabolism, and bioactivity. Free Radical Biol Med. 37:287–303.

He XZ, Jiang JH, Xu XN, Yu CY, He DX. 2012. Optimization of ultrasonic-assisted extraction of chlorogenic acid and luteoloside from *Chrysanthemum indicum* L. Guangzhou Chem Ind. 5:005.

Hyung Ko J, Gyu Kim B, Joong-Hoon A. 2006. Glycosylation of flavonoids with a glycosyltransferase from *Bacillus cereus*. FEMS Microbiol Lett. 258(2):263–268.

Palmeri R, Restuccia C, Monteleone JI, Sperlinga E, Siracusa L, Serafini M, Spagna G. 2017. Bioactivity improvement of olea europaea leaf extract biotransformed by *Wickerhamomyces anomalus* enzymes. Plant Food Human Nutr. 1–8.

Ross JA, Kasum CM. 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. Ann Rev Nutr. 22(1):19–34.

Wang J, Geng S, Wang B, Shao Q, Fang Y, Wei Y. 2017. Magnetic nanoparticles and high-speed countercurrent chromatography coupled in-line and using the same solvent system for separation of quercetin-3-O-rutinoside, luteoloside and astragalin from a *Miknia micrantha* extract. J Chromatograp A. doi:10.1016/j.chroma.2017.05.062.

Wu X, Chu J, Wu B, Zhang S, He B. 2013. An efficient novel glycosylation of flavonoid by β-fructosidase resistant to hydrophilic organic solvents. Biores Technol. 129:659–662.

Yuan Y, Wang Z, Jiang C, Wang X, Huang L. 2014. Exploiting genes and functional diversity of chlorogenic acid and luteolin biosyntheses in *Lonicera japonica* and their substitutes. Gene. 534(2):408–416.

Zhang S, Zhou Z, Yao Z, He B. 2013. Efficient production of skimmin and 6′-succinylskimmin from umbelliferone by organic solvent-tolerant *Bacillus licheniformis* ZSP01 using nitrogen sources regulation strategy. Biochem Eng J. 71:105–110.

Zheng QS, Sun XL, Li G, Song M, Wang CH. 2004. Protective effects of luteolin-7-glucoside against liver injury caused by carbon tetrachloride in rats. Die Pharmazie-An Int J Pharm Sci. 59(4):286–288.