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Efficient glucosylation of flavonoids by organic solvent-tolerant *Staphylococcus saprophyticus* CQ16 in aqueous hydrophilic media

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ABSTRACT

Glucosylation of flavonoids improves their bioavaibility and pharmacological properties. An organic solvent-tolerant bacterium *Staphylococcus saprophyticus* CQ16 was newly isolated and was found to glucosylate daidzein. Strikingly, the polar solvent 15% DMSO significantly improved the glucosylation of daidzein with 3.5 times yield, and glucosylation was further improved with the supplemental co-solvents. The most effective glucosylation of daidzein to daidzein-7-*O*-glucoside catalyzed by whole cells of strain CQ16 was achieved with a molar yield of 90% in a system with addition of 15% DMSO and 0.5% butyl acetate. The conversion process produced very few by-products, and therefore simplified purification of the glycoside product. The glucosyltransferase from strain CQ16 showed broad substrate specificity to the various flavonoids as well as flavonoid analogs, nonetheless an exquisite regioselectivity of the C-7 hydroxyl group of flavonoids. It would be substantive benefits for exploiting the new candidates with higher bioavailability for pharmaceuticals.

1. Introduction

Flavonoids have beneficial health effects and therapeutic potential, particularly in disease prevention [1–3]. Daidzein is an isoflavone found in numerous plants, especially in soybean. It has received much attention for its antioxidant, antibacterial, and anti-inflammatory activity in human health [4–7]. However, pharmacological exploitation of daidzein is limited by its low solubility and bioavailability. Glycosylation of lipophilic small molecules, such as flavonoids and steroids, is one of the predominant strategies by which the bioactivity of these compounds is modulated in living organisms. However, chemical glycosidation has relatively low yield of the final glycoside and requires glycosyl activation and multiple steps of protection/deprotection to control region- and stereoselectivity [8–10]. Enzymatic glycosylation is of especial interest because the enzymes use unprotected aglycones and their catalytic activity is chemo-, region-, and enantioselective [11,14]. There are a few of studies on the glycosylation of daidzein. Due to the multi-glycosidases in plant or fungi cells, complex by-products and relatively low yields restricted the efficient preparation of daidzein glycosides. Such as, the plant *Eucalyptus perriniana* [12] could glycosylate daidzein to daidzein-7-*O*-glucopyranoside (daidzein-7-*O*-glucoside, yield 39%) and daidzein-7-*O*-gentiobioside (yield 6%). The fungi *Mortierella isabellina* ATCC 38063 [13] also could convert daidzein to daidzein-4′-rhamnopyranoside with a yield lower than 10%.

We report the first example of glucosylation of daidzein catalyzed by bacterium. The efficient glucosylation of daidzein in aqueous hydrophilic media was achieved using the newly isolated organic solvent-tolerant *Staphylococcus saprophyticus* CQ16. The remarkable effects of aqueous hydrophilic solvent and co-solvents on the glucosylation efficiency were elucidated. Especially, the broad substrate selectivity of glucosyltransferase in strain CQ16 was also discussed.

2. Materials and methods

2.1. Chemicals

Daidzein, flavonoids were purchased from Nanjing Zelang Medical Technology, Nanjing, China (>98% purity). All chemicals used were of analytical grade. Solvents used in high-performance liquid chromatography (HPLC) were HPLC grade.

2.2. Quantitative analysis and identification of glycosylation product

The glycosylation product was analyzed using HPLC as described in Li et al. [14] under the following conditions: column, discovery

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octadecylsilyl silica gel (ODS) (250 × 4.6 mm, 5 μm); flow rate, 1.0 mL min^{-1}; with UV detection at 254 nm.

The glycosylation product was purified through a porous resin column (Macroporous Adsorption Resin AB-8) and eluted with the mixture (methanol–water, 40%, v/v). The purified product was identified by mass spectrometry (Voyager-DE MALDI-TOF MS, USA) and NMR (Bruker AV-500 spectrometer, Switzerland) analysis. All NMR spectra were obtained in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard.

2.3. Screening and isolation of solvent-tolerant bacteria with glycosylation activity to daidzein

Organic solvent-tolerant bacteria were screened from soil samples which area contaminated with crude oil and chemicals. The screening medium (g/L) containing: sucrose, 3; peptone, 5; $MgSO_4$, 0.25; KH_2PO_4, 1.0; the initial pH was adjusted to 7.0 with 2 M NaOH. DMSO was added to the medium at the concentrations of 20%. Cultivations were incubated at 30 °C and 200 rpm for 24 h, and acclimated in screening medium for three times, and then spread on Luriu-Bertani plates. The isolated colony was incubated in screening medium for 24 h, the harvested cell was suspended in the reaction mixture containing 0.20 mM of daidzein, 30 mM of sucrose, and 5% DMSO, and incubated at 30 °C and 200 rpm for 48 h. The supernatants of reaction mixtures were analyzed by HPLC for daidzein glycoside.

The isolated strain was identified by the Microlog Microbial Identification System (Biolog Automated Micro Station System, USA) and 16S rDNA sequence analysis.

2.4. Optimization of culture media for high activity of glucosyltransferase in strain CQ16

The effects of various carbon sources (10 g/L of glycerol, glucose, fructose, lactose, sucrose, maltose, dextrin, and soluble starch), various nitrogen sources (10 g/L of beef extract, yeast extract, peptone, tryptone, urea, and ammonium sulfate) on the glucosyltransferase activity of strain CQ16 were investigated. Various kinds of metal ions (KH_2PO_4, $MgSO_4$, $CaCl_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$), initial pH (from 5.0 to 9.0) of the medium, and cultivation conditions were also optimized. At the indicated time, the culture was sampled and whole cells were used for catalytic transformation of daidzein.

2.5. Effect of hydrophilic solvents on glycosylation of daidzein by strain CQ16

The reaction conditions, such as pH (70 mM Na_2HPO_4/NaH_2PO_4 buffer), temperature, and sugar (30 mM) donor (sucrose, lactose, maltose, glucose, dextrin, and soluble starch) supplement of surfactant or organic solvents, were investigated for efficient glycosylation.

The effect of hydrophilic solvents (methanol, ethanol, acetonitrile, acetone, DMF, and DMSO) and concentration of DMSO on the yield of glucosylated daidzein was studied. The effect of various co-solvents (n-heptane, n-octane, ethanol, isopropanol, acetone, ethyl acetate and butyl acetate) on the yield of glucosylated daidzein in the presence of 15% DMSO was also investigated. The reaction was carried out at 30 °C and 200 rpm for 48 h, and stopped by adding methanol up to 80% (v/v). The supernatant of the reaction mixture was analyzed by HPLC.

2.6. Substrate specificity of glucosyltransferase in strain CQ16

For specificity of the substrates, the reactions were conducted under the optimized conditions, with a concentration of 0.8 mM different flavonoids. The time course of glucosylation daidzein was determined in a reaction mixture containing 15% DMSO and 0.5% buthyl acetate system under the optimized conditions.

3. Results and discussion

3.1. Screening and identification of orangic solvent-tolerant bacteria able to glycosylate daidzein

In general, cyclohexane and toluene were widely used in the screening of solvent tolerant microbes [15–18]. For efficient glycosylation, the polar solvents, such as DMSO and DMF, were required to satisfy hydrophilic sugar donors and hydrophobic acceptors to overcome the poor solubility of natural products. Therefore, the screening of water-miscible solvent tolerant strains is considered to be the key success factor for efficient glycosylation. In this study, DMSO (20%, v/v) was added to the medium right at the beginning of soil inoculation. About 90 strains from 260 soil samples were obtained on the basis of their DMSO tolerance. Among them, two strains were found to be capable of converting daidzein into daidzein glycoside. Most efficient Strain CQ16 was Gram positive, catalase negative. The 16S rRNA sequence (accession No. JX276379) from the isolated strain CQ16 was 100% and 99.9% similar to that of *S. saprophyticus* ATCC 15305 and *S. saprophyticus* CU20 respectively. Combined with the result from the biolog automated microbiology identification system (SIM = 0.45, 16 h), the strain CQ16 was identified as *S. saprophyticus*, and deposited in CCTCC (Wuhan, China) with an accession number of CCTCC M 2012099. Although the glycosylation of daidzein by fungi and plant cells have been elaborated [11–13], our report is the first demonstration of efficient glycosylation of daidzein by bacteria.

3.2. Preparation and identification of the daidzein glycosylation product

The glycosylation product was separated by column adsorption chromatography (about 12 g dry resin was used to absorb the products in 1 L of the reaction mixture). The purified product gave a main ion peak at *m/z* 417.09 $[M+H]^+$ in a positive TOF-MS-ES spectrum and an ion fragment (daidzein) at *m/z* 255.06$[M+H]^+$. Since the *m/z* of product was 162 (equal to the mass of one hexose) larger than the *m/z* of daidzein, corresponding to the glycosyl derivative. The NMR spectroscopic characterization of the product was identical to that of an authentic daidzein-7-*O*-glucoside reported in references [13,14].

3.3. Optimization of medium and sugar donors, reaction conditions for glucosylation by strain CQ16

The effects of various substances added to the mineral salts medium on the growth and activity of the glucosyltransferase of strain CQ16 were investigated (data not shown). Glycerol, sucrose, and lactose were efficient carbon sources for cell growth, while sucrose could further improve the activity of glucosyltransferase. Yeast extract was the optimal nitrogen source both for cell growth and glucosyltransferase activity. The glucosylation activity of the whole cells was enhanced markedly during the exponential growth phase and reached maximal at the late of exponential phase. The optimized medium had the composition (g/L): sucrose, 10; yeast extract, 10; KH_2PO_4, 0.6; $MgSO_4$, 1.0; pH 7.5. The cultivation was carried out at 30 °C, 200 rpm. With the optimized medium, the glucosylation yield was about 5.6 times higher than in the screening medium.

Sucrose was the best sugar donor for daidzein glucosylation. Glucose, lactose, and maltose could be used as the donor for glucosylation of daidzein with relative yields of 70%, 38% and 14%, respectively. The optimized reaction conditions were