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# Glycosylation of luteolin in hydrophilic organic solvents and structure–antioxidant relationships of luteolin glycosides†

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An effective approach was developed to biotransform luteolin glycosides in hydrophilic organic solvents. *Bacillus cereus* A46 cells showed high activity and stability in 5–20% (v/v) DMSO with 90–98% conversion rates of luteolin glycosides. Five glycosides of luteolin 7-*O*- $\beta$ -glucoside, luteolin 4'-*O*- $\beta$ -glucoside, luteolin 3'-*O*- $\beta$ -glucoside, luteolin 7,3'-di-*O*- $\beta$ -glucoside and luteolin 7,4'-di-*O*- $\beta$ -glucoside were obtained. The addition of DMSO greatly promoted the solubility of luteolin and further regulated the formation of the main products from five luteolin glycosides to luteolin 7-*O*- $\beta$ -glucoside (931.2  $\mu$ M). Fourteen flavonoids and anthraquinones were used as tentative substrates. Glycosylation positions were located at the C-7, C-3' or C4' hydroxyl groups of flavonoids and C-5 hydroxyl group of anthraquinones. The 3',4'-dihydroxy arrangement played the key role for the antioxidant activity of luteolin.

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## 1. Introduction

Luteolin (3',4',5,7-tetrahydroxyflavone) is a natural flavonoid widely found in plants. It is reported to have anti-inflammatory,<sup>1</sup> antioxidant,<sup>2</sup> anti-tumor,<sup>3</sup> anti-tuberculosis immunity,<sup>4</sup> and neuroprotective properties.<sup>5</sup> Although luteolin itself exhibits various biological activities with valuable medicinal applications, several drawbacks have been reported including poor aqueous solubility and low bioavailability.<sup>6,7</sup> Glycosylation serves to enhance the solubility and bioactivity of flavonoid aglycones. The biosynthesis of flavonoid-*O*-glycosides in plants is mainly mediated by glycosyltransferases.<sup>8</sup> Some luteolin *O*-glycosides have been isolated from various plants, including luteolin 3'-*O*-glucoside, luteolin 4'-*O*-glucoside,<sup>9</sup> luteolin 7-*O*-glucoside, luteolin 7,4'-di-*O*-glucoside,<sup>10,11</sup> luteolin-7,3'-di-*O*-glucoside.<sup>12</sup> Among of them, luteolin 4'-*O*-glucoside and 7-*O*-glucoside have been confirmed to be cytotoxic activity against NCI-H929, U266, and OPM2 cell lines.<sup>13</sup> However, further bioactivity study of luteolin glucosides is hampered by its low in planta abundance.<sup>9–12</sup> Thus, chemical synthesis or biotransformation approach supplies potential alternatives to obtain sufficient luteolin glucosides.

Compared with chemical glycosylation, which relies on a heavy dependence on protecting-group mechanisms that

introduce many additional steps, biotransformation provides a direct regioselective glycosylation method to construct glycosidic bonds.<sup>14</sup> For instance, glycosyltransferase (*XcGT-2*) from *Xanthomonas campestris* was reported regiospecifically glycosylated the 3'-hydroxyl group of luteolin.<sup>15</sup> UDP-glycosyltransferase (*RUGT-5*) from rice was recorded glycosylated the 7- and 4'-hydroxyl groups of luteolin.<sup>16</sup> However, enzyme reaction in aqueous phase is not efficient with lower yields due to the solubility issue of the substrate. The conversion rate of luteolin 7-*O*-glycoside by UDP-glycosyltransferase (*BcGT-1*) from *Bacillus cereus* was only 40.9%.<sup>17</sup> Furthermore, the availability of glycosyltransferase has been constrained by need to provide activated sugar as donor for the glycosylation.<sup>15–17</sup>

Fortunately, enzymatic glycosylation in organic solvents using directly oligosaccharides as donors supply industrially attractive advantages, such as improving the solubility of hydrophobic substrates, reversing of the thermodynamic equilibrium of hydrolysis reactions, and lowering cost process.<sup>18,19</sup> Herein, we investigated the glycosylation of luteolin in hydrophilic organic solvents using the biotransformation approach, whereas five luteolin glycosides were enzymatically biosynthesized. Furthermore, the structure–antioxidant relationships of luteolin glycosides were also studied.

## 2. Materials and methods

### 2.1 Materials and reagents

Flavonoids and emodin were purchased from Zelang (Jiangsu, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma (St. Louis, MO, USA). Phycion, catenarin, erythroglaucin, skyrin, emodin bianthrone and emodin-catenarin bianthrone were isolated from our laboratory.<sup>20,21</sup> Methanol

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used for HPLC analysis was of chromatographic grade from Sinopharm (Shanghai, China). All other chemicals were of analytical grade and purchased from Sunshine (Nanjing, China).

## 2.2 Strain and culture condition

*Bacillus cereus* A46 was isolated by the previous methods and stored as 15% glycerol stocks at  $-80\text{ }^{\circ}\text{C}$ .<sup>18</sup> Bacterial identification was based on the 16S rDNA sequence, and identified as *Bacillus cereus*. The strain A46 was incubated in liquid culture medium containing ( $\text{g L}^{-1}$ ): sucrose 20.0, peptone 10.0,  $\text{KH}_2\text{PO}_4$  2.0,  $\text{CaCl}_2$  0.6, adjusted to pH 7.5. Cultivation was conducted at  $30\text{ }^{\circ}\text{C}$ , 200 rpm. After 12 h incubation, the cultured cells were harvested by centrifugation at  $8500 \times g$  and  $4\text{ }^{\circ}\text{C}$  for 20 min.

## 2.3 Glycosylation of luteolin

The glycosylation reactions (360 mL) were conducted in  $1/15\text{ M}$   $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$  buffer (pH 6.47) containing 2% (w/v) harvested wet cells, 20% (w/v) sucrose,  $970\text{ }\mu\text{M}$  luteolin and 20% (v/v) dimethyl sulfoxide (DMSO). The reaction mixture was conducted at  $30\text{ }^{\circ}\text{C}$  with shaking at 200 rpm for 48 h.

## 2.4 Analysis, isolation and identification of products

In general, products were analyzed by reversed-phase liquid chromatography on Agilent 1260 HPLC, using a XBridge C18 column ( $5\text{ }\mu\text{m}$ ,  $4.6 \times 250\text{ mm}$ ). The HPLC analysis method was performed using the following gradient: 0.0–13.0 min, 10–100% B; 13.0–20.0 min, 100% B; 20.0–20.1 min, 100–10% B; 20.1–23.0 min, equilibration with 10% B. The mobile phase consisted of solvents A (water) and B (methanol). The column was operated with a constant flow rate of  $0.8\text{ mL min}^{-1}$  at  $30\text{ }^{\circ}\text{C}$  and a sample injection volume of  $20\text{ }\mu\text{L}$ . The liquid chromatography hyphenated with the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was run on an Agilent 6546 Accurate-Mass Q-TOF LC/MS instrument with a Poroshell 120 EC-C18 column ( $2.7\text{ }\mu\text{m}$ ,  $4.5 \times 50\text{ mm}$ ). The LC-HR/MS analysis of products was performed using the “A + B” mobile phase as specified below: 0.0–13.0 min, 10–100% B. The column was operated with a constant flow rate of  $0.3\text{ mL min}^{-1}$  at  $30\text{ }^{\circ}\text{C}$ . Glycosylation products were purified by semipreparative HPLC on an Agilent Technologies 1260 Infinity LC System (Agilent, California, USA) by an Agilent Eclipse XDB-C18 column ( $5\text{ }\mu\text{m}$ ,  $9.4 \times 250\text{ mm}$ ) at a flow rate of  $2.0\text{ mL min}^{-1}$ . For structure elucidation, NMR spectra were recorded on a Bruker AV-500 NMR spectrometer (Bruker, Fällanden, Switzerland). Samples were dissolved in  $\text{DMSO}-d_6$  at room temperature with tetramethylsilane ( $\text{Me}_4\text{Si}$ ) as the chemical shift reference.

## 2.5 Evaluation of antioxidant activity

The effects of luteolin and its five glycosides on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were estimated.<sup>19</sup> Briefly,  $150\text{ }\mu\text{L}$  of various concentration samples (5, 10, 20, 40, 80 and  $160\text{ }\mu\text{M}$ ) were mixed with  $150\text{ }\mu\text{L}$  of  $150\text{ }\mu\text{M}$  DPPH solution. After being incubated at  $37\text{ }^{\circ}\text{C}$  for 30 min, the absorbance of the mixture was measured at  $517\text{ nm}$ . The free radical

scavenging activity of the reaction solution was calculated as a percentage of DPPH discolouration using the following equation: radical scavenging activity (%) =  $(1 - A_{\text{sample}}/A_{\text{control}}) \times 100$ , where  $A_{\text{sample}}$  is the absorbance of the solution when the sample has been added at a particular level, and  $A_{\text{control}}$  is the absorbance of the DPPH solution. The results were expressed as mean values  $\pm$  standard deviations ( $n = 8$ ).

# 3. Results and discussion

## 3.1 Biotransformation and identification of luteolin glycosides

*Bacillus cereus* A46 was used to biotransformation of the luteolin glycosides. The glycosylation reaction mixture of this strain showed five product peaks (compounds 2–6) with reference to the luteolin (1) (Fig. 1A), which were further analysed by LC-HR/MS. The HR-ESI mass analysis of the reaction mixture showed the ion peaks 2, 3 and 4  $[\text{M} + \text{H}]^+$  at  $m/z$  449.1167, 449.1168 and 449.1170, correspondingly (calculated for  $\text{C}_{21}\text{H}_{21}\text{O}_{11}$ , 449.1084) (Fig. 1B). Compared with luteolin (1), the compound 2, 3 or 4 has an additional formula  $\text{C}_6\text{H}_{10}\text{O}_5$ , suggesting that these compounds could be the luteolin monosaccharide derivatives. The comparison analysis between the  $^1\text{H}$ -NMR data of luteolin (1) and compound 2 revealed that the high field peak  $\delta$  4.87 (d,  $J = 7.2\text{ Hz}$ ) ( $\delta$  4.88 (d,  $J = 7.3\text{ Hz}$ ) for 3,  $\delta$  5.08 (d,  $J = 7.4\text{ Hz}$ ) for 4) was the anomeric sugar proton signal (Table 1 and Fig. S1–S4†). The resonance of H-1'' at  $\delta$  4.87, 4.88 or 5.08 as a double with  $J_{1,2} > 7.0\text{ Hz}$  indicates that compound 2, 3, or 4 is the sugar  $\beta$ -anomer. The  $^1\text{H}$ -NMR spectra of compounds 2–4 showed similarities to those of previous reported compounds luteolin 3'-O- $\beta$ -glucoside, luteolin 4'-O- $\beta$ -glucoside and luteolin 7-O- $\beta$ -glucoside.<sup>10,22,23</sup> Therefore, the chemical structure of compound 2 was elucidated as luteolin 3'-O- $\beta$ -glucoside,<sup>22,23</sup> 3 was elucidated as luteolin 4'-O- $\beta$ -glucoside<sup>23</sup> and 4 was elucidated as luteolin 7-O- $\beta$ -glucoside (Fig. 1C).<sup>10,22</sup> Furthermore, the mass spectrum of compounds 5 and 6 showed the ion peaks  $[\text{M} + \text{H}]^+$  at  $m/z$  611.1726 and 611.1728, correspondingly (calculated for  $\text{C}_{27}\text{H}_{31}\text{O}_{16}$ , 611.1612) (Fig. 1B). Compared with luteolin, the compound 5 or 6 has an additional formula  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ , suggesting that compounds 5 and 6 may be the luteolin disaccharide derivatives. The  $^1\text{H}$ -NMR spectra of compound 5 showed the presence of two anomeric sugar protons at  $\delta$  5.01 (d, 7.6 Hz) and  $\delta$  5.08 (d, 7.5 Hz) (Table 1, Fig. S5 and S6†). The  $^1\text{H}$ -NMR spectra of compound 5 showed similarities to those of the known compounds luteolin di-O-glucoside.<sup>24</sup> Hence, compound 5 was established as luteolin 7,3'-di-O- $\beta$ -glucoside.<sup>24</sup> Moreover, two anomeric sugar protons appear at  $\delta$  4.89 (d, 7.3 Hz) and  $\delta$  5.09 (d, 7.4 Hz) for compound 6 (Table 1), which was elucidated as luteolin 7,4'-di-O- $\beta$ -glucoside (Fig. 1C).<sup>23</sup>

## 3.2 Efficient glycosylation in hydrophilic organic solvents

The hydrophilic solvents such as methanol, ethanol, 2-propanol, DMSO and DMF were employed to promote the solubility of hydrophobic acceptors. The solubility of luteolin in this

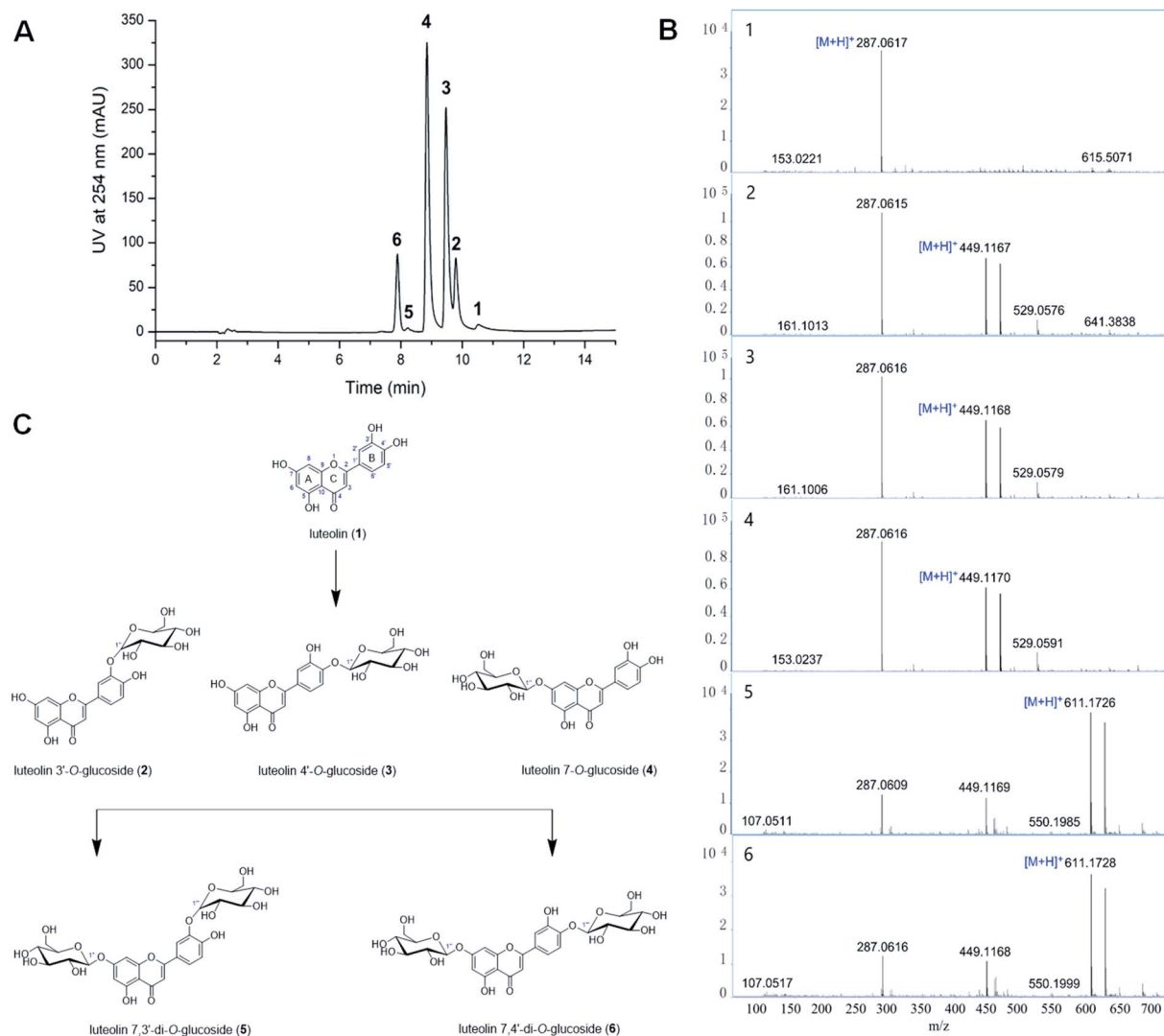


Fig. 1 Biotransformation of luteolin glycosides by *Bacillus cereus* A46. (A) HPLC profiling of biotransformation for luteolin glycosides by A46 cells. (B) The LC-HR/MS analysis of the reaction products. (C) The chemical structures of luteolin and luteolin glycosides.

reaction system was improved about 4.0 times than in aqueous solution. Strikingly, highly efficient glycosylation of luteolin was obtained in the hydrophilic solvents by *Bacillus cereus* A46 (Fig. 2). The A46 cell shows high activity and stability in 20% (v/v) DMSO and DMF with 90–96% conversion rates of luteolin glycosides, however methanol and 2-propanol have solvent denaturing effect for A46 cells resulting less 80% conversion rates (Fig. 2A).

Significantly, the participation of DMSO not only obviously increased the solubility of luteolin, but also displayed the regulation for the products of luteolin glycosylation. The main products changed from five luteolin glucosides (luteolin 7-*O*- $\beta$ -glucoside, luteolin 4'-*O*- $\beta$ -glucoside, luteolin 3'-*O*- $\beta$ -glucoside, luteolin 7,3'-di-*O*- $\beta$ -glucoside and luteolin 7,4'-di-*O*- $\beta$ -glucoside) to luteolin 7-*O*- $\beta$ -glucoside with increasing DMSO concentration (Fig. 2C). This regulation of the formation of main products depends the balance between the concentration of organic solvents and the catalytic activity of A46 cell. As shown

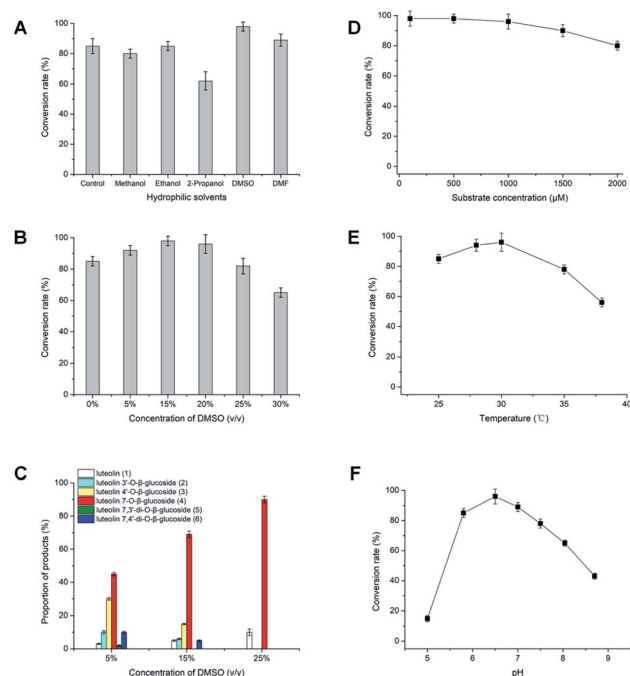
in Fig. 2B and C, A46 cell displays highly catalytic activity in 5–15% (v/v) DMSO with 90–98% conversion rates and multi-glucoside products. However, the activity and stability of A46 cell began to reduce in 25–30% (v/v) DMSO with less 80% conversion rate. Luteolin 7-*O*- $\beta$ -glucoside was the first glucoside products, followed by luteolin 3'-*O*- $\beta$ -glucoside, luteolin 4'-*O*- $\beta$ -glucoside and luteolin 7,4' or 3'-di-*O*- $\beta$ -glucoside catalyzed by A46 cell (Fig. 1C and 2C). The multi-glucoside products changed to luteolin 7-*O*- $\beta$ -glucoside in 5–25% (v/v) DMSO mainly due to the reduction of catalytic activity. Furthermore, the optimization of luteolin glycosylation process was conducted shown in Fig. 2D–F. The efficient glycosylation was achieved in 30 °C, pH 6.47 and 970  $\mu$ M substrate concentration.

Efficiently production of the luteolin glucosides was attained in 20% (v/v) DMSO solvent system, which gave a final conversion rate of 96%. This resulted in 931.2  $\mu$ M (417.2 mg L<sup>-1</sup>) of luteolin 7-*O*- $\beta$ -glucoside. The substrate



Table 1  $^1\text{H}$  NMR (500 MHz) spectral data of luteolin and glucosides in DMSO- $d_6$ 

$\delta_{\text{H}}$ ( $\delta$ , Hz)		Luteolin		Luteolin 3'-O- $\beta$ -glucoside (2)		Luteolin 4'-O- $\beta$ -glucoside (3)		Luteolin 7-O- $\beta$ -glucoside (4)		Luteolin 7,3'-di-O- $\beta$ -glucoside (5)		Luteolin 7,4'-di-O- $\beta$ -glucoside (6)	
Position	(1)	Luteolin		Luteolin 3'-O- $\beta$ -glucoside (2)		Luteolin 4'-O- $\beta$ -glucoside (3)		Luteolin 7-O- $\beta$ -glucoside (4)		Luteolin 7,3'-di-O- $\beta$ -glucoside (5)		Luteolin 7,4'-di-O- $\beta$ -glucoside (6)	
3	6.67 (s)	6.77 (s)		6.77 (s)		6.82 (s)		6.74 (s)		6.79 (s)		6.92 (s)	
5-OH	12.97 (s)	12.98 (s)		12.98 (s)		12.91 (s)		13.07 (s)		12.96 (s)		12.96 (s)	
6	6.19 (s)	6.16 (s)		6.16 (s)		6.19 (d, $J = 1.9$ Hz)		6.44 (d, $J = 2.1$ Hz)		6.44 (d, $J = 2.1$ Hz)		6.45 (d, $J = 2.0$ Hz)	
7-OH	10.81 (s)	6.50 (s)		6.50 (s)		6.49 (d, $J = 1.9$ Hz)		6.79 (d, $J = 1.9$ Hz)		6.75 (s)		6.86 (d, $J = 2.0$ Hz)	
8	6.44 (s)	7.63 (m)		7.63 (m)		7.49 (d, $J = 2.1$ Hz)		7.43 (m)		7.42 (d, $J = 1.7$ Hz)		7.54 (d, $J = 2.0$ Hz)	
2'	7.40 (d, $J = 2.6$ Hz)												
3'-OH	9.47 (s)												
4'-OH	9.92 (s)												
5'	6.89 (d, $J = 8.2$ Hz)	6.93 (d, $J = 8.5$ Hz)		6.93 (d, $J = 8.5$ Hz)		7.24 (d, $J = 8.6$ Hz)		6.89 (d, $J = 8.2$ Hz)		6.90 (d, $J = 8.3$ Hz)		7.26 (d, $J = 8.6$ Hz)	
6'	7.42 (m)	7.76 (m)		7.76 (m)		7.51 (dd, $J = 2.2, 8.5$ Hz)		7.44 (dd, $J = 2.0, 8.4$ Hz)		7.44 (dd, $J = 2.1, 8.3$ Hz)		7.56 (dd, $J = 2.1, 8.6$ Hz)	
1''		4.87 (d, $J = 7.2$ Hz)		4.87 (d, $J = 7.2$ Hz)		4.88 (d, $J = 7.3$ Hz)		5.08 (d, $J = 7.4$ Hz)		5.08 (d, $J = 7.5$ Hz)		5.09 (d, $J = 7.4$ Hz)	
1'''										5.01 (d, $J = 7.6$ Hz)		4.89 (d, $J = 7.3$ Hz)	

Fig. 2 Effects of multi-factors on the glycosylation of luteolin catalyzed by *Bacillus cereus* A46.

concentration (970  $\mu\text{M}$ ) of luteolin in 20% (v/v) DMSO solvent was over tenfold than other reports, such as glycosylation of luteolin (30.0  $\mu\text{M}$ ) with glycosyltransferase (*XcGT-2*) from *X. campestris*<sup>15</sup> and *trans*-glycosylation of luteolin (70.0  $\mu\text{M}$ ) by UDP-glycosyltransferase (*RUGT-5* and *BcGT-1*).<sup>16,17</sup> The high concentration and simple products of luteolin glucosides (mainly luteolin 7-O- $\beta$ -glucoside) have a great facility for separating and achieving products in large-scale process, which supply convenience for further bioactivity study of luteolin glucosides.

### 3.3 Antioxidant activities of luteolin glucosides

The role of 3'-OH and 4'-OH groups in the B ring on DPPH radical scavenging potential of luteolin glucosides was studied. As shown in Table 2, luteolin ( $\text{IC}_{50}$  20.2  $\mu\text{M}$ ) and luteolin 7-O- $\beta$ -glucoside ( $\text{IC}_{50}$  21.2  $\mu\text{M}$ ) showed higher antioxidant activities in comparison with luteolin 3' or 4'-mono glucosides and 7,3' or 7,4'-diglucosides ( $\text{IC}_{50} > 100$   $\mu\text{M}$ ). Both luteolin and luteolin-7-O- $\beta$ -glucoside contain a 3',4'-dihydroxy structure in the B ring. The results showed clear differences of antioxidant activities between these luteolin glucosides and suggest that the B ring 3',4'-dihydroxy group may be particularly important to their activities in this system. These structure-activity relationships are consistent with the observations by Tournaire<sup>25</sup> and Nagai.<sup>26</sup> Who both demonstrated that the presence of the O-dihydroxy group in the B ring is essential for  $^1\text{O}_2$  quenching in chemical systems.<sup>25,26</sup> Clearly, the presence of the 3',4'-dihydroxy arrangement in this biological system is also important for the superior capacity of luteolin on DPPH

Table 2 DPPH radical-scavenging activities of luteolin and glucosides

Compounds	Free OH	IC <sub>50</sub> (μM)
Luteolin	5, 7, 3', 4'	20.2
Luteolin 3'-O-β-glucoside	5, 7, 4'	>100
Luteolin 4'-O-β-glucoside	5, 7, 3'	>100
Luteolin 7-O-β-glucoside	5, 3', 4'	21.2
Luteolin 7,3'-di-O-β-glucoside	5, 4'	>100
Luteolin 7,4'-di-O-β-glucoside	5, 3'	>100

radical scavenging activities, and this appears to relate to the formation of a more stable *ortho*-hydroxyl phenoxyl radical and an intramolecular hydrogen bond in the process of oxidation<sup>27</sup> or increase the rate of H-atom transfer to radicals.<sup>28</sup>

### 3.4 Substrate specificity for glycosylation by *Bacillus cereus* A46

The specificity for glucosyl acceptor catalyzed by A46 cell was investigated using flavonoids and anthraquinones. All seven flavonoids contain a 7-OH group in the A ring or 3'-OH/4'-OH group in the B ring (Fig. 3 and S7–S12†), could be glycosylated by A46 cells. The results showed broad substrate specificity of *B. cereus* A46, which made us interested in studying other structural types of substrates. Seven anthraquinone derivatives were used as substrate, A46 cells were found to convert emodin, catenarin, skyrin, emodin bianthrone and emodin-catenarin bianthrone to glycosylated forms (Fig. 3 and S13–S27†). When the 5-OH group of anthraquinones was replaced by a methyl group, such as physcion and erythroglaucon, these anthraquinones could not be glycosylated by A46 cells. The results showed

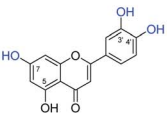
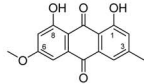
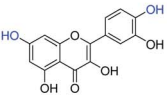
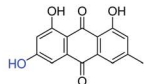
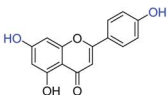
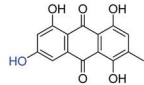
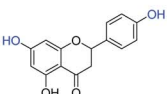
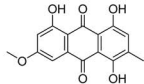
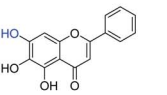
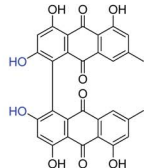
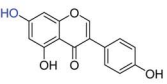
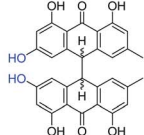
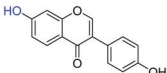
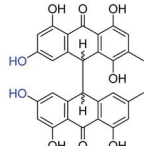
Flavonoids <sup>1</sup>	Structure	Relative conversion rate (%) <sup>2</sup>	Anthraquinones <sup>1</sup>	Structure	Relative conversion rate (%) <sup>2</sup>
luteolin		100	physcion		ND
quercetin		62	emodin		65
apigenin		58	catenarin		48
naringenin		95	erythroglaucon		ND
baicalein		98	skyrin		45
genistein		99	emodin bianthrone		38
daidzein		42	emodin-catenarin bianthrone		32

Fig. 3 Glycosylation specificity of flavonoids and anthraquinones by A46 cells. <sup>1</sup>The glycosylation reactions (100 mL) were conducted in 1/15 M Na<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> buffer (pH 6.47) containing 2% (w/v) harvested wet cells, 20% (w/v) sucrose, 100 μM substrate and 20% (v/v) DMSO. The reaction mixture was conducted at 30 °C for 24 h. <sup>2</sup>Relative conversion rate was calculated on the bases of the remaining acceptor.





that *B. cereus* A46 cells have exquisite regioselectivity for the hydroxyl group at C-5 of anthraquinones. The glycosylation catalysed by *B. cereus* A46 with broad substrate specificity will be beneficial to exploit the new candidate pharmaceuticals.

## 4. Conclusions

In summary, *Bacillus cereus* A46 cells showed high stability and activity in 20% methanol, ethanol, DMSO and DMF. High conversion rate (96%) of luteolin glycosides and regulated preparation of luteolin 7-O- $\beta$ -glucoside were achieved in organic solvents system. A46 cells could efficiently transfer glucose to specific hydroxyl group (the 7<sup>th</sup> position in the A ring and 3' or 4'-position in the B ring) of the flavone structure and 5-OH group of anthraquinones. DPPH radical-scavenging activities of luteolin glucosides confirmed that the presence of the 3',4'-dihydroxy arrangement is important for the superior antioxidant capacity of luteolin.

## Author contributions

X. M. Wu conceived and supervised the project. T. T. Xu designed and performed experiments, and wrote the manuscript. All authors contributed to the data analysis and manuscript preparation.

## Conflicts of interest

All authors declare no conflicts of interest.

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