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Short communication

Biosynthesis of amino deoxy-sugar-conjugated flavonol glycosides by engineered *Escherichia coli*

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ABSTRACT

Escherichia coli BL21(DE3) was engineered to divert the flow of carbon flux from glucose-1-phosphate to thymidine diphosphate 4-keto 4,6-dideoxy-D-glucose (dTKDG), an intermediate of various dTDP-sugars. Glucose phosphate isomerase (*pgi*), glucose-6-phosphate dehydrogenase (*zwf*) and uridylyltransferase (galU) genes were deleted while two additional genes, dTDP-D-glucose synthase (*tgs*) and dTDP-D-glucose 4,6-dehydratase (*dh*), were overexpressed to produce a pool of dTKDG in the cell cytosol. The flow of dTKDG was further diverted to dTDP-D-viosamine, dTDP 4-amino 4,6-dideoxy-D-galactose, and dTDP 3-amino 3,6-dideoxy-D-galactose sugars using sugar aminotransferases (*gerB*, *wecE*, and *fdtB*, respectively) from different sources. These sugar moieties were transferred to the 3-hydroxyl position of quercetin and kaempferol with the help of *Arabidopsis thaliana* glycosyltransferase (ArGT3). As a result, 4-amino 4,6-dideoxy-D-galactose and 3-amino 3,6-dideoxy-D-galactose sugars conjugated to quercetin and kaempferol were biosynthesized successfully from exogenously supplemented quercetin and kaempferol. However, no D-viosamine conjugated kaempferol or quercetin derivatives were produced during the biotransformation. All the synthesized glycosides are novel unnatural compounds, which could have potent biological activities.

1. Introduction

A huge number of natural products derived from microbial sources are glycosylated and are in clinical practice for the treatment of various diseases. Many but not all of these glycosylated molecules contain amino deoxy-sugars. For examples, doxorubicin, staurosporine, vancomycin, calicheamicin, amphotericin B, tylosin, and erythromycin contains various kinds of amino deoxy-sugars in their structures (Fig. 1). These sugars contribute to the biological activities of metabolites [1]. Moreover, the amino sugars are also part of the structures of proteins and lipids in cells and are involved in energy metabolism, the generation of biosynthetic resources, the maintenance of structural integrity, and cellular pathogenesis [2]. Unlike microbial secondary metabolites [3], plant-derived natural products contain simple sugars, such as D-glucose, D-galactose, D-glucuronic acid, L-rhamnose, D-xylose, and D-arabinose. However, few plant- derived molecules are found conjugated with simple amino sugars, like D-*N*-acetyl glucosamine, D-glucosamine, and D-4-deoxy-4-formaido-L-arabinose [4].

Previous studies showed that engineering of sugar moieties in both plant and microbial secondary metabolites had profound effects on their biological, physico-chemical, and pharmacokinetic properties [5]. Thus, exchanging the microbial glycone part with an aglycone from plant-derived secondary metabolites is an interesting approach for the generation of novel biologically potent compounds. Several approaches, such as (i) chemical syntheses [6,7], (ii) chemo-enzymatic approaches [8], (iii) enzymatic syntheses [9], and (iv) combinatorial biosynthesis [10,11] using microbial cells, are being used to conjugate different kinds of sugar units to natural products. Chemical synthesis of glycosides of plant secondary metabolites requires multiple steps and process is not eco-friendly whereas chemo-enzymatic and enzymatic syntheses need expensive cofactors as well as sugar donors along with purified enzymes. Enzymatic synthesis of sugar donors requires multiple steps, expensive cofactors, purified enzymes while purification process is tedious and final product yield is very low. Thus, these approaches are not cost effective and difficult to apply in industry for scale-up. Combinatorial biosyntheses of plant glycosides by engineering microbial cells (*e.g.*,

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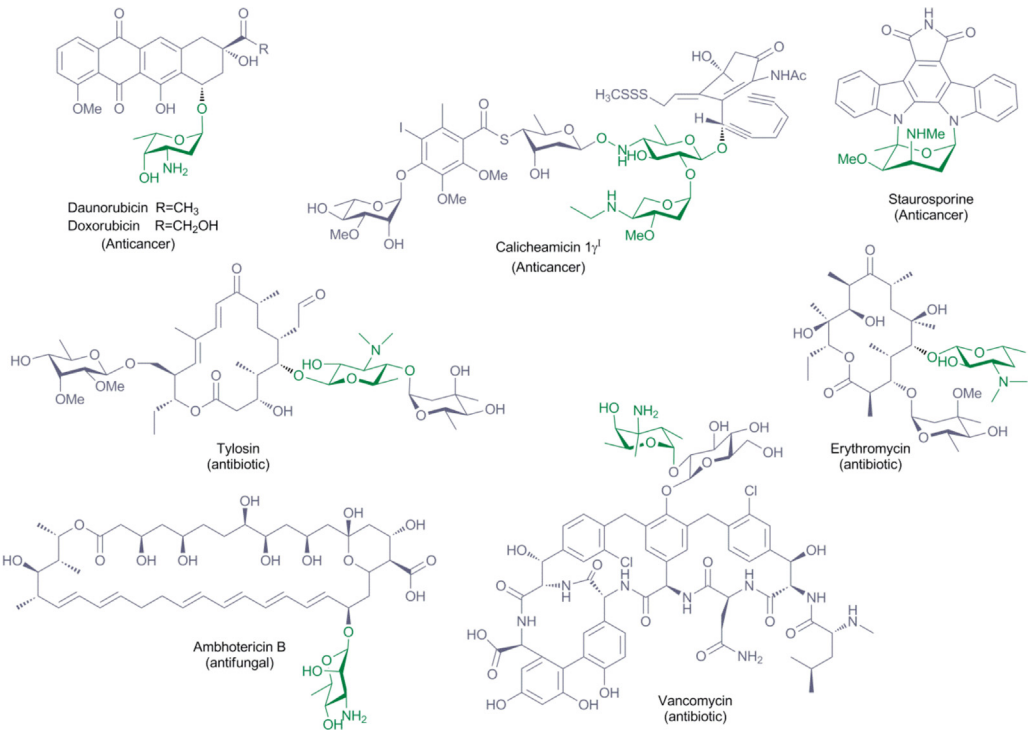

Fig. 1. Selected structures of amino deoxy-sugars bearing microbial secondary metabolites. The amino-sugars are highlighted in green color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Escherichia coli, yeast, *Streptomyces*) is one of the widely accepted approaches because of the ease of production by simple fermentation. Plant polyphenols, particularly the flavonoid groups of compounds, have been modified by glycosylation at different positions with various sugars by engineering the nucleotide diphosphate (NDP)-sugar pathways in *E. coli* cells. Flavonoids belong to a large group of plant-based phenolic bioactive natural products having anti-oxidant, anti-inflammatory, anti-cancer, anti-estrogenic, and anti-diabetic properties [12–15]. Selected flavonoid glycosides produced by reconstructing sugar pathways in *E. coli* include kaempferol 3-*O*-glucoside (astragalin) [16], naringenin 7-*O*-xyloside [17], quercetin 3-*O*-xyloside [18], quercetin 3-*O*-rhamoside, kaempferol 3-*O*-rhamnoside, quercetin 3-*O*-alloside [19], quercetin 3-*O*-6-deoxytaloside [20], quercetin 3-*O*-arabinoside [21], quercetin 3-*O*-*N*-acetylglucosamine [22] and quercetin 3-*O*-glucoside-7-*O*-rhamnoside [23]. The cytoplasmic pool of these sugars has been elevated either by editing the NDP-sugar pathway genes in the genome or by introducing NDP-sugars pathway genes along with a glycosyltransferase (GT).

In this study, we developed a background strain of engineered *E. coli* BL21(DE3) in which glucose phosphate isomerase (*pgi*), glucose-6-phosphate dehydrogenase (*zwf*), and uridylyltransferase (*galU*) genes were deleted from the genome. The disruption of these genes diverted the carbon flux from glucose to dTKDG *via* G-1-P and dTDP-glucose. Finally, a cytoplasmic pool of three dTDP-amino deoxy-sugars (dTDP-D-viosamine (4-amino 4,6-dideoxy-D-glucose), dTDP-4-amino 4,6-dideoxy-D-galactose, and dTDP-3-amino 3,6-dideoxy-D-galactose) was expected to be produced by downstream modifications of dTKDG by consecutive actions of aminotransferase enzymes. In conjunction with an *Arabidopsis thaliana* GT (*ArGt-3*), these recombinant *E. coli* biotransformed exogenously supplemented flavonols to novel amino deoxy-sugars conjugated derivatives (Fig. 2A).

2. Materials and methods

2.1. Bacterial strains, plasmids, cultured conditions and chemicals

All strains, vectors and plasmids used in this study are listed in Table S2. *E. coli* XL1 Blue was used for plasmid cloning and propagation while *E. coli* BL21(DE3) was used for flavonol modification. Vectors pET-28a(+), CDFDuet-1, pET-32a(+), and pETDuet-1 (Novagen) were used for cloning and subcloning. A Red helper plasmid pKD46, plasmid pKD3 for amplification of chloramphenicol resistant marker and plasmid pCP20 to remove antibiotic marker were used to carry out FLP–FRT mediated gene knockout in *E. coli* BL21(DE3) [24]. All DNA manipulations were carried out by following standard protocols [25].

E. coli strains were grown in Luria–Bertani (LB) broth or on an agar plate supplemented with the appropriate amount of antibiotics (ampicillin 100 μg/mL, streptomycin 50 μg/mL and kanamycin 35 μg/mL) when necessary, for the selection or maintenance of the plasmids. Authentic kaempferol and quercetin were purchased from Sigma–Aldrich Co.

2.2. Solvents and buffer

1 M ice-cold formic acid was saturated with 1-butanol was prepared. 1 L of 1 M triethyl ammonium acetate (TEAA) buffer was prepared by mixing 138.6 mL of triethylamine (Et_3N) and 56 mL of glacial acetic acid dissolved in 750 mL of water. pH was maintained to 7.0 by acetic acid and final volume was adjusted to 1 L. 80% (v/v) acetonitrile (ACN) in 0.1% trifluroacetic acid (TFA), 25% (v/v) ACN in water, 50 mM TEAA buffer was prepared from 1 M TEAA buffer dilution. 25% (v/v) ACN containing 50 mM TEAA buffer was separately prepared for elution of nucleotide sugars.