Chapter 5
Microbial Modifications of Flavonols

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Abstract Development of microbial cell factories via application of synthetic biology, protein engineering for metabolic engineering has revolutionized the maximum use of microbial consortium for biosynthesis and structural alteration of valuable flavonoids. From a single enzyme expression to complex metabolic pathway, it has been possible to manipulate strains of *Escherichia coli*, *Saccharomyces cerevisiae*, *Streptomyces*, and *Bacillus* for target-based modification of compounds to industrial level in laboratory. Biotransformation, a biotechnological approach, can be applied to structurally modify and generate library of natural products such as flavonoid derivatives.

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This chapter highlights the significance of engineered new molecules and biotransformation approaches used to generate flavonoids by the use of microbial platforms. Basically, *E. coli* has been engineered by expressing secondary metabolites post modifying enzymes, glycosyltransferases, *O*-methyl transferases, and prenyltransferases, in particular to generate the natural and nonnatural flavonol derivatives. Indigenously present cytoplasmic cofactors, coenzymes, and donor substrates are utilized by such enzymes for target-based chemical modifications. Engineering the central carbon flux pathway to enhance the flow of carbon toward target donor substrates and cofactors such as nucleotide diphosphate (NDP)-sugars, S-adenosyl methionine, dimethylallyl pyrophosphate, and other cofactors which enhanced the cytoplasmic pool while maximizing the biotransformation efficiency for level up production are discussed. Moreover, heterologous expression of different pathway genes from different organisms and engineering of glycosyltransferases and *O*-methyl transferases into bacterial host does help to generate nonnatural flavonol glycosides.

5.1 Introduction

Flavonols are a group of phytochemicals that are widely available in plant-based foods and vegetables such as onions, broccoli, kale, apples, and tea. Structurally they are 3-phenolic ringed compounds of a flavonoid class having 3-hydroxyflavone backbone in C_6-C_3-C_6 carbon framework. Quercetin, kaempferol, myricetin, fisetin, and morin are the most ubiquitous flavonols studied for various biological activities (Fig. 5.1a). They are derived from the phenylpropanoid pathway through a common unit of naringenin chalcone converted into naringenin and further modified to flavonol backbones by the action of flavanone 3-hydroxylase (Fig. 5.1b) (Zhang and Liu 2015). *Arabidopsis thaliana* is a model plant for flavonoid biosynthesis where 35 molecules of flavonols are found among 54 flavonoid molecules (Saito et al. 2013). The structural diversity to the flavonoid core occurs after post biosynthesis modifications such as glycosylation, hydrogenation, hydroxylation, methylation, prenylation, etc. during biosynthesis which also alters their biological significances. Recent biotechnological techniques and tools have harnessed these natural modification steps of plant secondary metabolites into microbial platforms generating various natural and nonnatural scaffolds (Pandey et al. 2016a).

5.2 Significance of Flavonol and Their Microbial Modified Derivatives

Astragalin (kaempferol 3-*O*-glucoside), afzelin (kaempferol 3-*O*-rhamnoside), kaempferol 7-*O*-rhamnoside, kaempferitrin (kaempferol 3, 7-*O*-dirhamnoside), and kaempferide (kaempferol 4′-*O*-methoxide) are the commonly known kaempferol derivatives synthesized expressing various regiospecific glycosyltransferases and *O*-methyltransferases in microbial hosts (Simkhada et al. 2010; Pei et al.

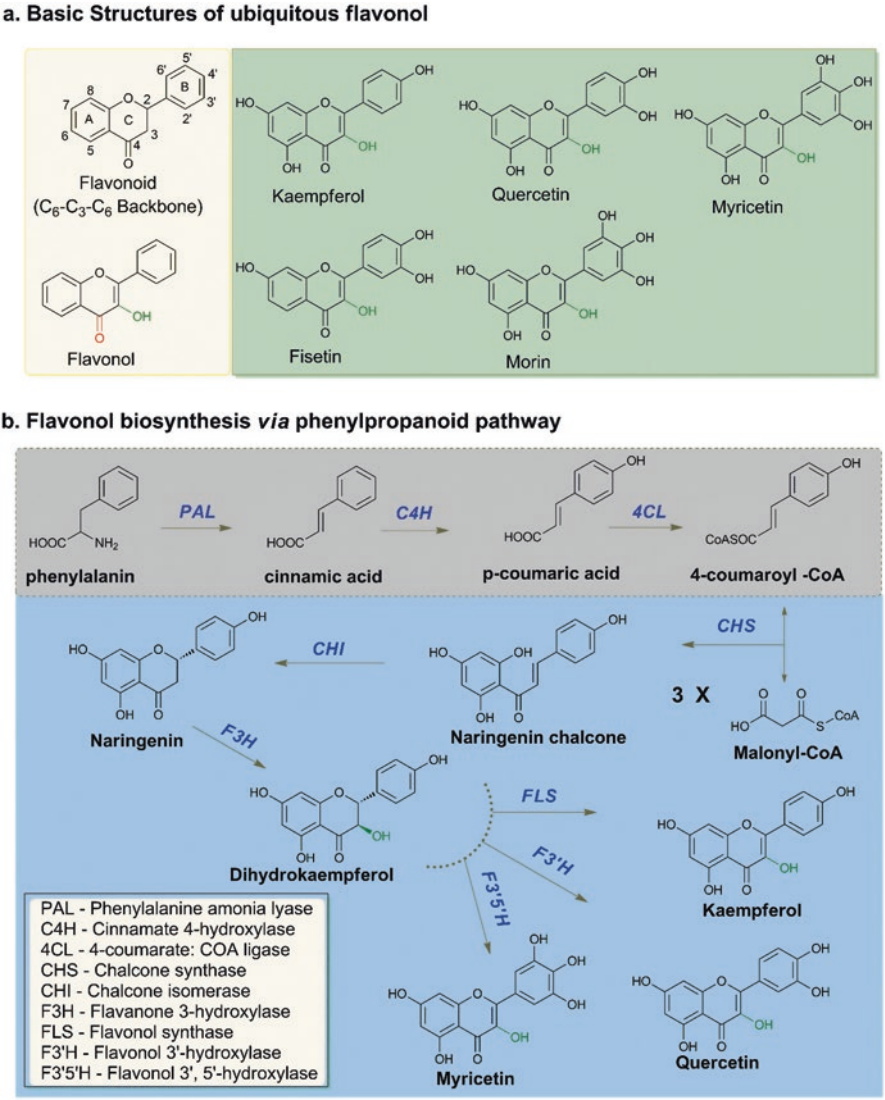

Fig. 5.1 (**a**) Figure showing the basic flavonoid backbone and flavonol representation including structures of major flavonols. (**b**) Biosynthesis pathway of flavonols via phenylpropanoid route involves series of enzyme with respective functions

2016). Various biological significances have been reported of each compound. Astragalin possesses cardioprotective (Qu et al. 2016), antiproliferative (Li et al. 2017), and anti-inflammatory (Ma et al. 2015; Zhang et al. 2017) effects. Afzelin has antibacterial effect against *Pseudomonas aeruginosa* (Lee et al. 2014) and has DNA-protective, anti-inflammatory, and UV-absorbing antioxidant activity (Shin et al. 2013). Kaempferitrin prevents bone loss (Ma et al. 2015) while exhibiting

antidepressant (Cassani et al. 2014) and antidiabetic effects (Da Silva et al. 2014; Jorge et al. 2004). Kaempferide is also a naturally occurring flavonol derivative which also has anticancer potential (Nath et al. 2015; Marnon et al. 2015). Myricetin is a flavonol having various therapeutic values (Phillips et al. 2011; Xue et al. 2015; Mondal et al. 2016; Buchter et al. 2015) including anti-HIV (Pasetto et al. 2014), mitochondrial activating agent (Jung et al. 2017), and attenuates gastric acid secretion, thereby inhibiting H^+, K^+-ATPase that functions as a proton pump in gastric parental cells (Miyazaki et al. 2018).

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) has been extensively studied for its various biological significances. Due to the fact of bioavailability, poor aqueous solubility, and the rapid body clearance, several derivatives of quercetin have been proposed and used to intense research for potentially improved properties in clinical applications. Recently, some of the flavonols and their derivatives caught the research attentions due to highly promising biological activities. Quercetin 3-*O*-*β*-D-glucoside was studied against Ebola viral infection (Qiu et al. 2016); isoquercitrin was reported as a strong antiviral agent against African historical and Asian epidemic strains of Zika virus tested over human hepatoma, epithelial, and neuroblastoma cell lines (Gaudry et al. 2018). Polyhydroxy flavonols (quercetin and myricetin) were used as promising inhibitor of CatB (cathepsin B, a cysteine protease involved in tumor progression that represents a potential therapeutic target in cancer) (Ramalho et al. 2015), other flavonols are studied to have antiparasitic activity against *Trypanosoma brucei* (Borsari et al. 2016), while tamarixetin was studied as strong antioxidant (Lemmens et al. 2014; Moalin et al. 2012) and tamarixetin 3-*O*-*β*-D-glucoside as a potential anti-ulcer (Yadav et al. 2017) molecule. Other post-modified flavonols such as rhamnetin (7-*O*-methyl quercetin) and isorhamnetin (3′-*O*-methyl quercetin) are known to have better bioavailability and anti-inflammatory effect than its aglycon (Lee et al. 2011; Jnawali et al. 2014). Hyperoside (quercetin 3-*O*-galactoside) inhibits the proliferation and stimulation of osteogenic differentiation of human osteosarcoma cells (Zhang et al. 2014). It protects against hypoxia/reoxygenation during cardiomyocytes injury (Xiao et al. 2017). Isorhamnetin 3-*O*-galactoside was found to have greater anticoagulant and profibrinolytic effect compared to hyperoside (Ku et al. 2013). Quercetin (quercetin 3-*O*-rhamnoside) and guajavarin (quercetin 3-*O*-arabinoside) have shown better cytotoxic and antiviral activity compared to ribavirin (dos Santos et al. 2014). Quercetin 7-*O*-rhamnoside has shown considerable antiviral activity during early stage of porcine epidemic diarrhea virus (Choi et al. 2009; Song et al. 2011). Quercetin 3-*O*-*α*-L-rhamnoside was found to protect against snake venom isolated from a plant *Euphorbia hirta* (Gopi et al. 2016). Quercetin 3-*O*-xyloside was recently presented as a new immunostimulator agent (Lee et al. 2016). Rhamnazin (3′, 7-*O*-dimethyl quercetin) has been introduced as a novel angiogenesis inhibitor with potential antitumor efficacy (Yu et al. 2015; Philchenkov and Zavelevych 2015). Beside anticancer and antioxidant activities, a glycoside derivative isorhamnetin 3-*O*-glucuronide has been extensively studied which is suggested as a valuable therapeutic agent for inflammation-related pathological illnesses (Park et al. 2016). Morin was found to be a novel inhibitor of glycogen synthase kinase 3β (GSK3β)

by reducing tau pathology in Alzheimer's disease condition (Gong et al. 2011). Fisetin is another flavonol (5-deoxy quercetin) with potential biological activities including memory enhancer (Maher et al. 2006), neuroprotective effect (Ahmad et al. 2017), and anti-Alzheimer's (Currais et al. 2014; Kim et al. 2016). Fisetin and myricetin were studied for antimalarial activities and were found to have dual inhibition function against falcipain-2 and plasmepsin II, thereby proving chance to development as antimalarial drug (Jin et al. 2014). The significance of flavonol derivatives discussed here is tabulated in Table 5.1. Although numerous derivatives of myricetin have been reported, microbial post-modified derivatives, myricetin 3-*O*-glucoside (Parajuli et al. 2015) and myricetin 3-*O*-rhamnoside (Thuan et al. 2013; Parajuli et al. 2015), are limited. So far microbial modified flavonol derivatives are presented in Figs. 5.2 and 5.3.

5.3 Current Approaches for Microbial Flavonol Modifications

Biotransformation is an alternative and cost-effective strategy to produce various natural and nonnatural flavonoid derivatives based on the simple enzymatic modification. The most promising biotechnological technique applied nowadays is in vivo whole-cell biotransformation. The major microbial post modification platforms for glycosylation, methylation, hydroxylation, and prenylation are generated in microbial hosts (*Escherichia coli*, *Saccharomyces cerevisiae*, *Streptomyces* strains, fungal mycelia) by overexpressing various secondary metabolites post-modifying enzymes including glycosyltransferases, *O*-methyl transferases, cytochrome P450s, and prenyltransferases. Application of these enzymes to modify the structures of natural flavonoids to improve their physicochemical and biological properties has been of a great scientific and industrial interest due to their large availability, low cost, and wide substrate spectra. Besides the single genetic manipulations and one step reaction, total biosynthetic pathways of flavonoids are copied and heterologously expressed into desired host bacteria for the biosynthesis and modification from simple and low-cost precursor using various biotechnological tools (Kaneko et al. 2003; Malla et al. 2012; Stahlhut et al. 2015).

Biotransformation is considered to be the most explored techniques in flavonol modifications used by current scientists to achieve target products even in industrial scale. Since the microbial indigenous primary metabolites such as cofactors (ATP, S-adenosyl L-methionine, NDP-sugars), amino acid and coenzyme (pyridoxal-5′-phosphate), nucleotide diphosphate sugars as sugar donor substrate are utilized by post-modifying enzymes like methyltransferase and glycosyltransferases for chemical modifications in exogenously supplied flavonols as acceptor substrates (Fig. 5.3).

Table 5.1 Lists of flavonol derivatives studied for their specific significance are tabulated with references

Flavonol derivatives	Significance of flavonols	References
Quercetin 3-*O*-*β*-D-glucoside	Ebola viral infection	Qiu et al. (2016)
Isoquercitrin	Zika virus infection	Gaudry et al. (2018)
Quercetin	Anticancer/inhibitor of cathepsin B (CatB)	Ramalho et al. (2015)
Tamarixetin	Antioxidant	Lemmens et al. (2014) and Moalin et al. (2012)
Tamarixetin 3-*O*-*β*-D-glucoside	Anti-ulcer	Yadav et al. (2017)
Rhamnetin	Anti-inflammatory	Lee et al. (2011) and Jnawali et al. (2014)
Isorhamnetin	Anti-inflammatory	Lee et al. (2011) and Jnawali et al. (2014)
Hyperoside	Against hypoxia/reoxygenation	Xiao et al. (2017)
Isorhamnetin 3-*O*-galactoside	Anticoagulant and profibrinolytic effect	Ku et al. (2013)
Quercetin and guajavarin	Better cytotoxic and antiviral activity	dos Santos et al. (2014)
Quercetin 7-*O*-rhamnoside	Antiviral for porcine epidemic diarrhea virus	Choi et al. (2009) and Song et al. (2011)
Quercetin 3-*O*-*α*-L-rhamnoside	Antivenom	Gopi et al. (2016)
Quercetin 3-*O*-xyloside	Immunostimulator agent	Lee et al. (2016)
Rhamnazin	Angiogenesis inhibitor	Yu et al. (2015) and Philchenkov and Zavelevych (2015)
Isorhamnetin 3-*O*-glucuronide	Therapeutic agent for pathological illness	Park et al. (2016)
Astragalin	Cardioprotective, antiproliferative; anti-inflammatory	Qu et al. (2016), Li et al. (2017), Ma et al. (2015), and Zhang et al. (2017)
Afzelin	Antibacterial, DNA-protective, anti-inflammatory and UV-absorbing antioxidant	Lee et al. (2014) and Shin et al. (2013)
Kaempferitrin	Prevents bone loss, antidepressant, antidiabetic effects	Ma et al. (2015), Cassani et al. (2014), Da Silva et al. (2014), and Jorge et al. (2004)
Kaempferide	Anticancer	Nath et al. (2015)
Myricetin	Anticancer/inhibitor of cathepsin B (CatB), antimalarial; anti-HIV-1, mitochondrial activating agent	Ramalho et al. (2015), Jin et al. (2014), Pasetto et al. (2014), Jose et al. (2016), and Jung et al. (2017)
Fisetin	Memory enhancer, neuroprotective effect, anti-Alzheimer's; antimalarial	Maher et al. (2006), Ahmad et al. (2017), Currais et al. (2014), Kim et al. (2016), and Jin et al. (2014)
Morin	Inhibitor of glycogen synthase kinase 3β	Gong et al. (2011)

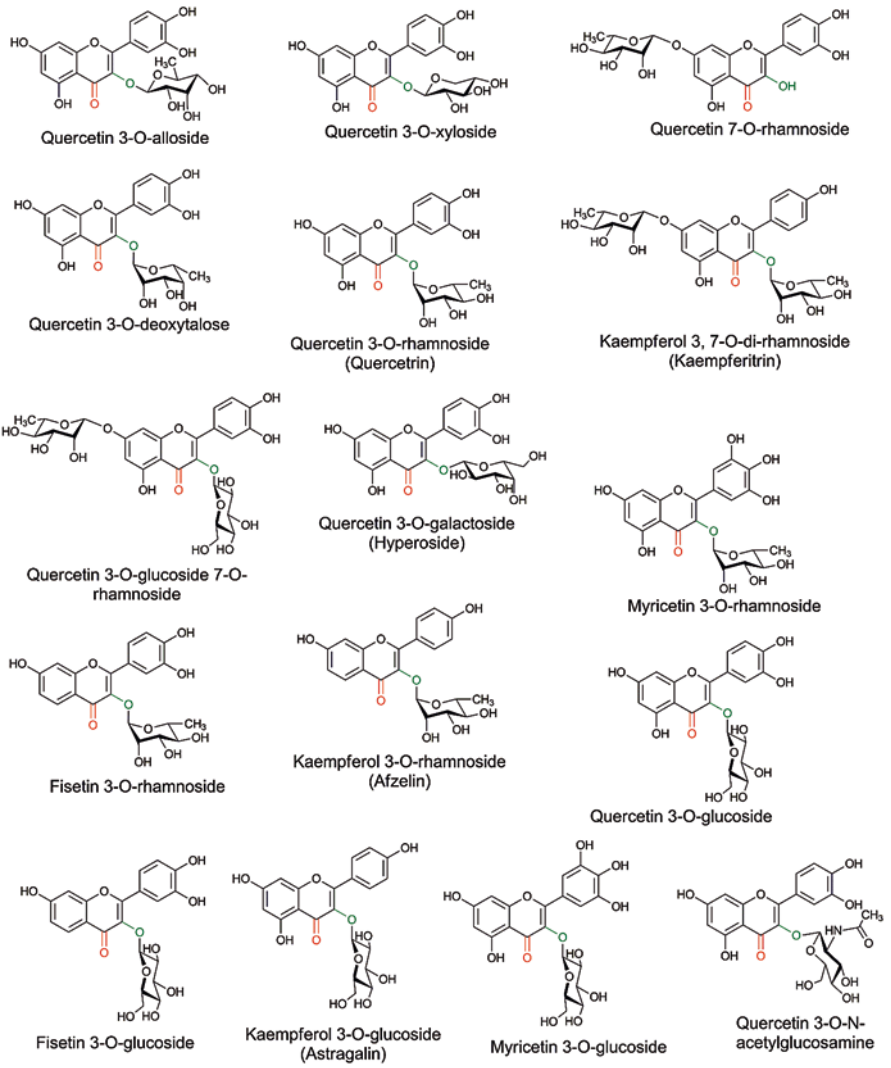

Fig. 5.2 Flavonol glycoside structures synthesized from microbial modification using various glycosyltransferases

5.3.1 Glycosylation

Glycosylation is a common post-modification step involved at the later stage during biosynthesis of natural products in plants. Glycosyltransferase mediates the biochemical reaction to form glycoside bonds via transfer of an activated nucleotide diphosphate sugar to an acceptor molecule. Flavonoids are usually present in their

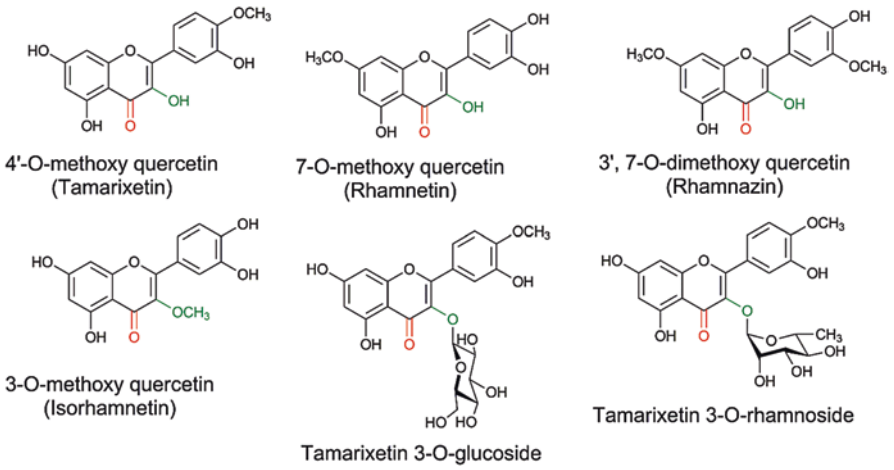

Fig. 5.3 Flavonol *O*-methoxide structures synthesized from microbial modification using various *O*-methyltransferases

O- or *C*-glycosides in plants. Various biological activities are associated with the types of sugar moieties attached to flavonoids including their physical nature like solubility and stability (Plaza et al. 2014). Most common glycosylation modification in flavonols takes place in 3-hydroxyl and 7-hydroxy position. Either simply overexpression of glycosyltransferases or nucleotide diphosphate sugar (NDP-sugar) biosynthetic pathways (Fig. 5.4) including glycosyltransferases are engineered in *E. coli* for regiospecific biotransformation of flavonols. Novel quercetin glycoside quercetin 3-*O*-(6-deoxytalose) including quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside was reported by engineering *E. coli* glycolysis pathway and expression of *tll* (encoding dTDP-6-deoxy-L-lyxo-hexulose reductase, i.e., dTDP-talose synthase) and AtUGT78D1 from *Arabidopsis thaliana* (Yoon et al. 2012). UDP-xylose pathway enzymes phosphoglucomutase (*nfa44530*) from *Nocardia farcinica*, glucose-1-phosphate uridylyltransferase (*galU*) from *E. coli K-12*, and UDP-glucose dehydrogenase (*calS8*) and UDP-glucuronic acid decarboxylase (*calS9*) from *Micromonospora echinospora* spp. *calichensis* were overexpressed in multiple vector along with *Arabidopsis thaliana* glycosyltransferase (*ArGt-3*) to biotransform quercetin into quercetin 3-*O*-xyloside in *E. coli* host (Pandey et al. 2013). In the same year, improved production of myricetin 3-*O*-rhamnoside was reported in *E. coli* mutant expressing *ArGt-3* (Thuan et al. 2013). The *E. coli* mutant strain was generated disrupting glucose-6-phosphate utilizing pathway genes: glucose phosphate isomerase (*pgi*), glucose-6-phosphate 1-dehydrogenase (*zwf*), and UDP-α-D-glucose hydrolase (*ushA*) (Pandey et al. 2013). An improved production of quercetin 3-*O*-xyloside was reported by Han et al. (2014) by overexpressing UDP-xylose synthase (*uxs*), UDP-glucose 6-dehydrogenase (*ugd*), and AtUGT78D3 from *A. thaliana* in a UDP-4-amino-4-deoxy-L-arabinose (*L-Ara4N*) formyltransferase/

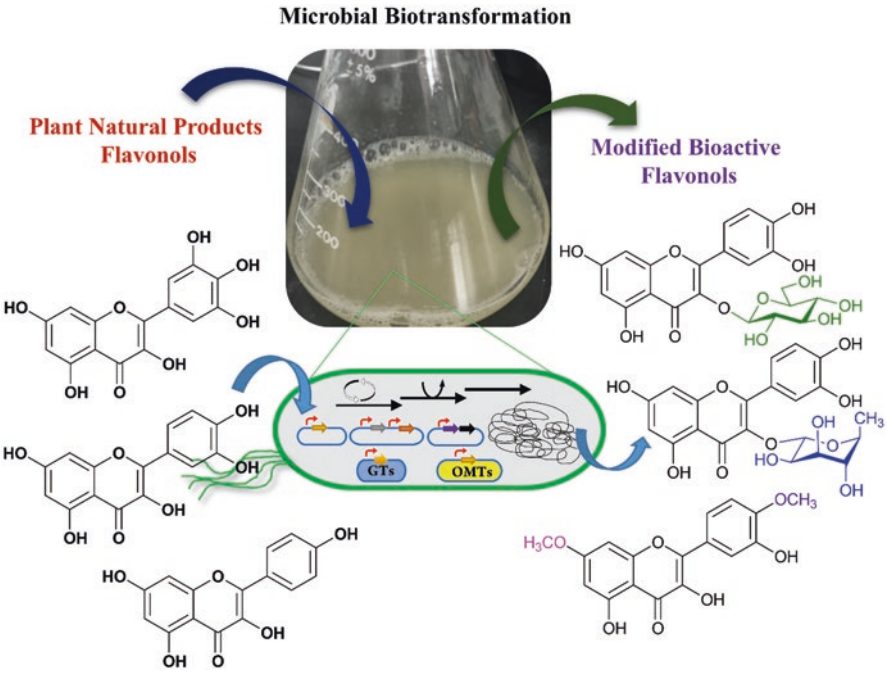

Fig. 5.4 Representation of a simple microbial biotransformation of flavonols to modified bioactive molecules in engineered *E. coli*. The modified products are glycosides and *O*-methoxides in common

UDP-glucuronic acid C-4″-decarboxylase (*arnA*) deleted *E. coli* mutant. Similarly, *ArGt-3* was also used along with TDP-glucose synthase (*Tgs*) from *Thermus caldophilus* GK24, TDP-glucose 4,6-dehydratase (*DH*) from *Salmonella typhimurium* LT2, TDP-4-keto-6-deoxyglucose 3,5-epimrase (*epi*), and TDP-glucose 4-ketoreductase (*Kr*) from *Streptomyces antibioticius*Tu99 to synthesize quercetin 3-*O*-rhamnoside and kaempferol 3-*O*-rhamnoside whereas enzymes Tgs and DH along with TDP-hexose 3-epimerase (*GerF*) and TDP-4-keto-6-deoxyglucose reductase (*GerK*) from *Streptomyces* sp. KCTC 0041BP to synthesize quercetin 3-*O*-alloside (Simkhada et al. 2010).

In our recent report, different flavonols were modified into their natural and non-natural glycosides (Parajuli et al. 2015; Pandey et al. 2015). We constructed sugar cassettes assembling UDP-glucose and TDP-rhamnose pathway-specific enzymes and inserted into *E. coli* strain to biotransform different flavonols (fisetin, quercetin, kaempferol, and myricetin) into respective glycosides efficiently (Parajuli et al. 2015). In the same year, an expanded in vivo glycosylation platform was generated in *E. coli* W for efficient galactosylation catalyzed by galactosyltransferase (F3GT) from *Petunia hybrid* and rhamnosylation catalyzed by rhamnosyltransferase (*RhaGT*) from *A. thaliana* using a cheap source of sugar as sucrose to increase the

pool of UDP-galactose and UDP-rhamnose for biosynthesis of 3-*O*-galactoside and 3-*O*-rhamnoside of various flavonols: quercetin, kaempferol, fisetin, morin, and myricetin (De Bruyn et al. 2015).

Similarly, *E. coli* BL21 (DE3)/Δ*pgi*Δ*zwf*Δ*galU* mutant was engineered expressing enzymes (*tgs*, *dh*, *epi*, and *kr*) to distract the flow of carbon flux toward thymidine diphosphate 4-keto-4,6-dideoxy-D-glucose (dTKDG) along with sugar amino-transferases: 4-aminotransferase (*gerB*) from *Streptomyces* sp. GERI-155 to generate dTDP-D-viosamine pool, 4-aminotransferase (*wecE*) from *E. coli* K-12 to generate pool of dTDP-4-amino 4,6-dideoxy-D-galactose, and two genes for dTDP-3-amino 3,6-dideoxy-D-galactose (*fdtA* and *fdtB*) from *Aneurinibacillus thermoaerophilus* L420–91 (DSM 10154). Here also an *Arabidopsis* glycosyltransferase, ArGt-3, catalyzed to transfer these unnatural sugars to generate nonnatural quercetin and kaempferol derivatives through microbial biotransformation. In the meantime, novel fisetin glycosides were also produced in engineered *E. coli* host conjugating various amino sugars at 3-hydroxyl position of fisetin (Pandey et al. 2016b). Microbial modifications of quercetin into other glycosides as quercetin 3-*O*-4-deoxy-4-formamido-L-arabnose, quercetin 3-*O*-*N*-acetylglucosamine, quercetin 3-*O*-arabinoside, quercetin 3-*O*-6-deoxytaloside, and quercetin 3-*O*-glucuronide are covered in recent review in detail (Pandey et al. 2016a). We recently testified the microbial synthesis of tamarixetin glucoside at significant yield in *E. coli* for the first time (Parajuli et al. 2018). In previous years, without engineering sugar pathways and microbes, glycosyltransferases were simply overexpressed to modify flavonols into glycosides. Quercetin 3-*O*-glucoside, quercetin 7-*O*-glucoside, quercetin 3′-*O*-glucoside, quercetin 4′-*O*-glucoside, kaempferol 3-*O*-glucoside, and isorhamnetin 3-*O*-glucoside have been reported (Lim et al. 2004; Kim et al. 2006a, 2010).

5.3.2 Methylation

Methylation is another common post-modification after the biosynthesis of secondary metabolites. Hydroxyl, carbon, or nitrogen atoms present in terminal positions are decorated by methyl groups to signify the chemical structures of secondary metabolites. Especially S-adenosyl-L-methionine (SAM)-dependent *O*-methyltransferases catalyze methylation to plant flavonols. Very few of the SAM-dependent microbial origin *O*-methyltransferases are characterized to methylate plant flavonols. Microbial C-methyl derivatives of flavonols have not yet been reported. However, plant-originated *O*-methyltransferase has been functionally expressed in microbial hosts for the modification of different flavonols in respective hydroxyl positons. SOMT-2 originated from *Glycine max* overexpressed in *E. coli* biotransformed quercetin into 4′-*O*-methoxy quercetin (Kim et al. 2005a). The same group considered co-expression of two regiospecific *O*-methyltransferases

ROMT-9 and SOMT-2 from rice in *E. coli* and produced 3′-*O*-methylated and the 3′,4′-*O*-dimethylated quercetin derivatives (Kim et al. 2005b). In the following year, 7-*O*-methylated derivatives of quercetin, kaempferol, and isorhamnetin were produced using *Poplar*-originated *O*-methyltransferase: POMT-7 (Kim et al. 2006b). Similar co-expression method was applied to biotransform quercetin into quercetin 3′-*O*-methylquercetin, 3′,4′-*O*-dimethylquercetin, 7,3′-*O*-dimethyl quercetin, and 7,3′,4′-*O*-trimethylquercetin, respectively, using ROMT-9 and POMT-7 by Kim et al. (2008). A putative *O*-methyltransferase, SIOMT3, from tomato was isolated and overexpressed into *E. coli* and the transgenic *E. coli* efficiently modified quercetin, myricetin, and laricitrin into methoxide derivatives (Lee et al. 2017). Fusion of two regiospecific3′-*O*-methyltransferases (*SIOMT3*) from tomato and 7-*O*-methyltransferase (*OsNOMT*) from rice was reported recently to biotransform quercetin into rhamnazin efficiently (Lee et al. 2017).

Plant *O*-methyltransferases are regiospecific. However, there are few reports of using *Streptomyces*-derived *O*-methyl transferases for biotransformation of selective flavonols. SaOMT-2 from *S. avermitilis* MA-4680 and SpOMT2284 from *S. peucetius* ATCC27952 were explored for flavonoids methylation where SaOMT-2 biotransformed kaempferol, quercetin, and isorhamnetin into their methoxides regiospecifically and SpOMT2284 catalyzed *O*-methylation over quercetin and rutin non-regiospecifically (Kim et al. 2006c; Koirala et al. 2014). We have recently characterized *O*-methyltransferase (*GerMIII*) from *Streptomyces* sp. KCTC 0041BP to regioselectively produce 4′-*O*-methoxides of quercetin, myricetin, fisetin, and quercetin 3-*O*-glucoside, respectively (Darsandhari et al. 2018). Microbial and plant source *O*-methyltransferases are tabulated in Table 5.2.

5.3.3 Hydroxylation

Hydroxylation is an important post modification for the diversification of plant secondary metabolites. They are biosynthesized through phenylpropanoid metabolic pathway where flavonols, in particular quercetin, myricetin, morin, and fisetin, are different hydroxylated skeleton of kaempferol. However, very limited studies have been reported producing hydroxylated derivatives of flavonols expressing hydroxylases (CYP P450 mono-oxygenase) in microbial platform. But through microbial transformation of flavonols, hydroxylated derivatives were detected and characterized from the culture media in preparative scale. Hosny et al. (2001) reported the hydroxylation of fisetin and quercetin through the biotransformation via *S. griseus.* Those hydroxylated products were subsequently *O*-methylated into geraldol and 3, 7, 3′-trihydroxy-4′-methoxyflavone in case of fisetin and isorhamnetin and dillenetin, 3, 5, 7-trihydroxy-3′-4′-dimethoxyflavone, in case of quercetin.

Table 5.2 *O*-methyltransferases from microbial and plant sources used for post-modification of flavonols

S. No.	O-Methyltransferase (organism source)	Products catalyzed by O-methyltransferases	References
Microbial O-methyltransferase			
1	SpOMT2884 (*Streptomyces peucetius*)	*O*-Methylation on quercetin, rutin,	Koirala et al. (2014) and Chiang et al. (2015)
2	SaOMT5 (*Streptomyces avermitilis*)	*O*-Methylation of quercetin	Yoon et al. (2010)
3	ScOMT1 (*Streptomyces coelicolor* A3(2))	*O*-Methylated products were isorhamnetin, tamarixetin, fisetin methoxide, gossypetin	Yoon et al. (2005)
4	SaOMT-2 *Streptomyces avermitilis*	*O*-Methylation of kaempferol and quercetin	Kim et al. (2006c)
5	SpnK (*Saccharopolyspora spinosa*)	4′-*O*-Methoxy quercetin 3-*O*-glucoside	Parajuli et al. (2018)
Plant O-methyltransferase			
1	SOMT-2 *Glycine max*	*O*-Methylated quercetin	Kim et al. (2005a)
2	ROMT-9 and SOMT-2 *Rice* and *Glycine max*	*O*-Methylated quercetin	Kim et al. (2005b)
3	POMT-7 Poplar, *Populus deltoides*	*O*-Methylated kaempferol, quercetin	Kim et al. (2006b)
4	SIOMT3 *Tomato*	*O*-Methylated quercetin, rhamnetin	Lee et al. (2017)
5	OsNOMT *Rice*	*O*-Methylated kaempferol, quercetin, isorhamnetin	Lee et al. (2017)
6	CdFOMT5 *Citrus depressa*	*O*-Methylated quercetin	Itoh et al. (2016)

Second column shows products catalyzed by particular *O*-methyltransferase from first column

5.3.4 Prenylation

Prenylated flavonoids are uncommon, and they are characterized by the presence of lipophilic prenyl (5-carbon) chain, dimethylallyl or geranyl chain (10-carbon), or farnesyl (15-carbon). No reports have been found to generate prenylated flavonols through microbial transformation in particular although few prenylated derivatives of flavonoids reported have been reviewed in Pandey et al. 2016b (Fig. 5.5).

5.4 Conclusion

Apart from multifaceted therapeutic applications, flavonol and its derivatives have long been explored for potential nutritional values since they are particularly abundant in daily consumable vegetables, fruits, nuts, red wine, green tea, etc. Microbial modification of flavonols has been profoundly reliant on the biotransformation of

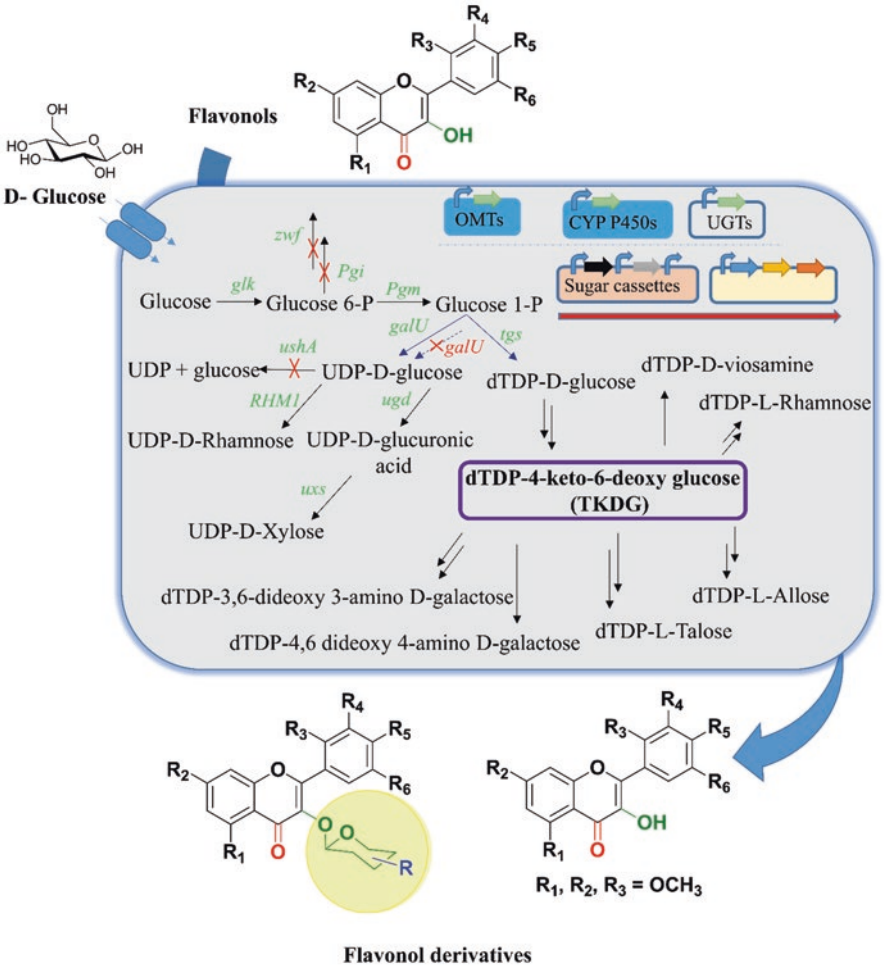

Fig. 5.5 Engineering natural and nonnatural NDP-sugar biosynthetic pathway in *E. coli* for regio-specific modification of flavonols catalyzed by specific uridine diphosphate glycosyltransferase. Red cross indicates the blocked pathway. Single black arrow is one step reaction while double black arrow indicates two steps reaction. *glk*: hexokinase, *pgm* phosphoglucomutase, *galU* UDP-glucose synthase, *ugd* dehydrogenase, *uxs* decarboxylase, *RHM1* UDP-rhamnose synthase, *tgs* TDP-D-glucose synthase

aglycon into their analogous glycosides or *O*-methoxides using post-modifying enzymes. Utilizing primary metabolites of *E. coli* including cofactors, donor substrates, or coenzymes for modification of flavonols has been cheap and simple, since they are indigenously present in bacterial cell. These natural commodities have been valued by engineering strategy to enhance the production of target compounds. A range of molecular biology tools, including metabolic engineering and synthetic biology, has been used to achieve significant bioconversion in host cells.

Engineering *E. coli* either by deletion or extra copy overexpression of glycolysis pathway genes enabled the carbon flux toward target NDP-sugar/s accumulation, where glycosyltransferase expression facilitates regiospecific modification of flavonols to their natural and nonnatural glycoside analogues (Simkhada et al. 2010; Yoon et al. 2012; Parajuli et al. 2015; Pandey et al. 2016b). However, for flavonol *O*-methoxides, few engineering approaches have been reported to increase production from microbial cell factories beside protein fusion for double modification and the simple expression of *O*-methyltransferases. Thus, expression of glycosyltransferases and *O*-methyltransferases from plant and microbial sources and rewiring native pathway via diversion of carbon flux toward primary precursor were more efficient to modify and synthesize target-based flavonol derivatives rather than anonymous microbial whole-cell biotransformation. Even through the biotransformation, modern microbial engineering approaches have helped to program and control bacterial robustness in production.

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