Polish Journal of Microbiology
2016, Vol. 65, No 2, 137–151

MINIREVIEW

Microbial Glycosylation of Flavonoids

SANDRA SORDON*, JAROSŁAW POPŁOŃSKI and EWA HUSZCZA

Faculty of Food Science, Department of Chemistry, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Submitted 6 March 2015, revised 6 October 2015, accepted 11 February 2016

Abstract

Flavonoids constitute a large group of polyphenolic compounds naturally found in plants, which have a wide range of biological activity. Although flavonoids are beneficial to human health, their application is limited by their low bioavailability and poor water-solubility. Therefore, recently there has been a particular interest in glycosylated forms of flavonoids, which usually are better soluble, more stable, and more functional compared to their aglycones. Microbial transformation of natural flavonoids may be an attractive way of receiving their glycosylated derivatives in amounts sufficient for the research on the effect of glycoside group on compound properties and for further application of these compounds as ingredients of dietary supplements and pharmaceuticals.

Key words: biotransformation, flavonoids, glycosides, microbial glycosylation

Introduction

Many natural bioactive compounds are found in nature in the form of glycosides and the sugar part is necessary for their biological activity. Several therapeutically important antibiotics (such as erythromycin), antifungal agents (amphotericin B) or anticancer drugs (doxorubicin) contain sugars attached to the aglycone core, which facilitate transport of the drugs to their targets in cells (Salas and Méndez, 2007). Also flavonoids which are plant secondary metabolites are found mostly in the form of *O*- or *C*-glycosides. However, the sugar moiety in compounds being in the centre of interest of scientific institutions and pharmaceutical industry plays different role in plant physiology than in the processes important for their application as medications. In the case of valuable flavonoids present in nature in the form of aglycones, such as xanthohumol found in hop cones, we can easily and efficiently transform them into glycosides, using microbial methods. Such biotransformations are usually highly regioselective.

In this review we discuss the prospect of microbial transformation of flavonoids as a cost-effective and environment protective tool for food supplements and drug designing. We summarize current knowledge regarding the glycosylation of flavonoids by means of various microbes and health benefits that may result from the use of such designed compounds.

Enzymes involved in glycosylation

Glycosylation is a common biochemical transformation which proceeds with the help of glycosyltransferases. These enzymes catalyse formation of glycoside bonds *via* transfer of a saccharide (usually monosaccharide) from a donor substrate to a nucleophilic glycosyl acceptor molecule, such as proteins, lipids, steroids, flavonoids or other small molecules.

In the case of Leloir-type glycosyltransferases the sugar residue is transferred from an activated donor, such as sugar-nucleotide derivatives (Palcic, 2011). Nine such derivatives used by mammals glycosyltransferases were identified: UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, UDP-Xyl, UDP-GlcA, GDP-Man, GDP-Fuc and CMP-NeuAc. Whereas, non-Leloir glycosyltransferases use sugar mono- and diphosphates and glycosylated isoprenoid mono- and diphosphates as donors of a glycosyl residue. Acceptors of saccharide moieties in both cases may be carbohydrates, proteins, lipids, nucleic acids, natural compounds (*e.g.* antibiotics) or xenobiotics, precisely their nucleophilic oxygen atom from hydroxyl group or nucleophilic nitrogen, carbon or sulfur atoms (Lairson *at al.*, 2008). Thus, we observe formation of *O*-glycosides, *N*-glycosides, *C*-glycosides and *S*-glycosides.

The reactions catalyzed by glycosyltransferases are stereoselective and regioselective. Transfer of a glycosyl

* Corresponding author: S. Sordon, Wrocław University of Environmental and Life Sciences, Faculty of Food Science, Department of Chemistry, Wrocław, Poland; **e-mail: sandra.sordon@up.wroc.pl**

group may proceed with either retention or inversion of configuration at the anomeric carbon atom of the substrate (donor), involved in the new glycosydic bond. Therefore, glycosyltransferases may be divided into retentive and invertive ones (Palcic, 2011). Glycosyltransferases have been classified by aminoacid sequence homology, substrate specificity and glycoside bond stereochemistry into 96 families (Gloster, 2014). One of them is GT1 comprising UDP-glucosyltransferases (UGTs), which were found in plants, animals, fungi and bacteria. These enzymes use UDP-activated sugar moiety as a saccharide donor and such molecules as flavonoids, alkaloids, antibiotics and plant hormones as the acceptors (Hyung *et al.*, 2006). UDP-glucose and UDP-glucuronic acid are the most common donors used by GT1, less often UDP-rhamnose, UDP-xylose and UDP-galactose are observed (Paquette *et al.*, 2003).

Physiological role of plant flavonoids

Flavonoids constitute the largest group of plant polyphenols. They exert considerable influence on growth and development of plants, protect them from UV radiation and bacterial and fungal infections and provide colour to fruits and flowers (Forkmann and Martens, 2001). A great diversity of flavonoids arises not only from their different carbon cores, but also from different substituents, being effects of hydroxylation, and hydroxyl group methylation and acylation. One of the common modifications is also glycosylation (Gachon *et al.*, 2005; Desmet *et al.*, 2012). For example, there are known about 300 different quercetin glycosides with potentially different biological properties (Wang, 2009).

Conjugation of plant secondary metabolites to saccharides enhances their stability by protection of reactive nucleophilic groups. For instance, glycosylation of anthocyanins at C-3 OH is crucial for the stability of their heteroaromatic ring (Chemler *et al.*, 2009; Gachon *et al.*, 2005). Moreover, it protects hydroxyl groups of secondary metabolites from autooxidation, enables their transport across cell membranes to the specific cellular compartments and augments their solubility in aqueous cell environment (Kumar and Pandey, 2013; Wang, 2009; Wang *et al.*, 2010; Zhao and Dixon, 2009).

By conjugation of a flavonoid molecule to a sugar moiety it is possible to store plant secondary metabolites in specific cellular compartments. It is believed that reactive aglycones are converted into stable and unreactive forms, which can be accumulated for example in vacuoles, and their interactions with other cell ingredients become inhibited. If necessary, the opposite process is possible, in which feeding deterrents stored in vacuoles in the form of conjugates, after cell decompartmentation due to its disintegration, are released and then cleaved by β-glycosidases to the more active forms (Kim *et al.*, 2006b; Gachon *et al.*, 2005).

Recently, there has been growing interest in plant *C*-glycosides, which except for typical for phenols antioxidant activity may play different roles, for example being repellents or attractants. Stable and resistant to hydrolysis *C*-glycosides of flavones were also found in cereals, sweet corn, wheat and rice (Xiao *et al.*, 2014a).

Influence of glycosylation on human metabolism

Flavonoids present in food have beneficial effects on human health. They have antioxidant, antibacterial, antifungal, antiviral, anticancer, anti-inflammatory and antiallergenic properties (Forkmann and Martens, 2001; Hyung *et al.*, 2006; Wang *et al.*, 2010). Despite intensive studies on absorption and metabolism of flavonoids, several issues have not been fully elucidated, so far. It is not clear whether they are absorbed as aglycones, glycosides or in the both forms. It is known, however, that the type of sugar molecule in glycosides of flavonoids influences their absorption, distribution and to some degree – their metabolism (Xiao *et al.*, 2014b). It was observed that after oral administration of quercetin glucoside, the maximal concentration of quercetin in plasma was 20 fold higher than after the intake of quercetin ruthoside. Therefore, it seems that quercetin glucoside is absorbed from small intestine in unchanged form, whereas absorption of quercetin ruthoside takes place after its deglycosylation (Hollman *et al.*, 1999).

In order to be absorbed to the body, flavonoids must reach small intestine in the unchanged form. The majority of flavonoid glycosides retain their structures even after cooking processing. They are also resistant to low pH in stomach and to digestive enzymes found there. Absorption of flavonoids from food depends on their physicochemical properties, size of molecules, lipophilicity and solubility.

Aglycones of flavonoids may be transported by passive diffusion, whereas conjugation with saccharides increases hydrophilicity of the compound, which makes it better water-soluble and less capable of passive diffusion. Flavonoid glycosides may be actively transported by Na^+/glucose transporters from intestinal lumen to enterocytes, where they are subsequently cleaved to aglycones by cytosolic glucosidases. Because the absorption of flavonoid glycosides in small intestinal is weak, it is suggested that flavonoid glycosides are hydrolysed by β-glucosidase, secreted by the brush border of human small intestine epithelial cells, known as LPH (lactase phloridzin hydrolase) (Kumar and Pandey, 2013). LPH is characterized by substrate specificity and the glycosides which are not substrates

for this enzyme are transported to the large intestine, where they are hydrolysed by intestinal bacteria enzymes (*e.g.* α-arabinofuranosidase, α-fructosidase, α-rhamnosidase, β-fructosidase, β-glucosidase and β-glucuronidase). The aglycones are absorbed in colon (Kumar and Pandey, 2013; Xiao *et al.*, 2014b).

In intestinal cells flavonoids usually undergo phase II metabolism, *i.e.* they are conjugated with glucuronic acid in the reaction catalysed by UDP-glucuronyltransferase, conjugated with sulphuric acid with the help of sulphotransferase, and flavonoids which contain a catechol moiety are methylated by catechol-*O*-methyltransferase. From intestines flavonoids are transported with blood to liver, where they may be deconjugated and again conjugated with either glucuronic or sulphuric acid with the help of enzymes, such as β-glucuronidase and sulphatase, or they may be metabolised to smaller phenolic compounds (Kumar and Pandey, 2013). Next the flavonoid glucuronates and sulphates formed in liver are delivered with blood to tissues of the whole organism (Desmet *et al.*, 2012). An excess of flavonoids delivered to the body is not accumulated. There are two ways of elimination of flavonoids from the organism: flavonoids absorbed in intestines are eliminated with urine, whereas the ones which were not absorbed are eliminated with faeces in the form of glycosides (Xiao *et al.*, 2014b).

Microbial glycosylation of flavonoids

Due to their valuable biological properties, recently flavonoids have been being the subject of numerous researches in the field of food technology, biotechnology, medicine and pharmacy.

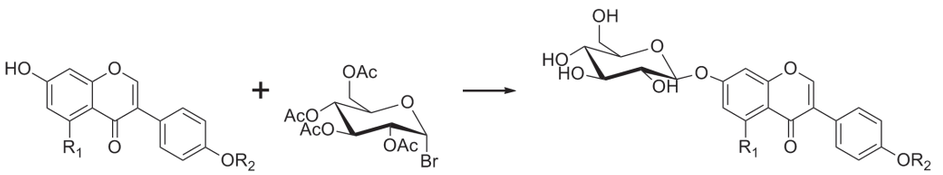
Application of flavonoids is limited by their poor water-solubility and short time spent in intestine, which result in low absorption. One of the method to overcome these drawbacks is conjugation of plant polyphenols with sugars (Hyung *et al.*, 2006; Kumar and Pandey, 2013; Tronina *et al.*, 2013). Glycosides of flavonoids are better water-soluble than their aglycones. For example, 5-*O*-α-D-glucopyranosyl-(+)-catechin is at least 40-fold better soluble in water than its aglycone – (+)-catechin (Ochiai *et al.*, 2010). Some of flavonoid glycosides demonstrate also better thermal stability than their aglycones, which is in the case of the mentioned 4'-*O*-α-D-glucopyranosyl-(+)-catechin and its aglycone (+)-catechin (Ochiai *et al.*, 2010).

Synthesis of glycosylated flavonoids, especially with the use of biological catalysts, may be an attractive method of receiving these compounds in amounts sufficient for the research concerning influence of sugar residue in flavonoid molecules on their properties and subsequent application of these compounds as dietary supplements or pharmaceuticals (Alluis and Dangles, 1999).

An example of chemical synthesis of flavonoids may be the reaction of isoflavones, such as daidzein, genistein, formononetin or biochanin A with α-tetraacetylbromoglucose in the presence of tetrabutylammonium bromide as a catalyst, leading to *O*-glucosides of the mentioned aglycones (Lewis *et al.*, 1998) (Fig. 1). The catalyst used in this reaction is harmful for the environment. Another disadvantage of chemical glycosylation is the necessity of protection of these hydroxyl groups that are not meant to conjugate with saccharide.

Biotransformation processes, as opposed to the classic chemical synthesis, are environmentally friendly, proceed under mild conditions and allow regio- and stereoselective modifications of the substrates (Cao *et al.*, 2015; Wang *et al.*, 2010). Moreover, according to the European Union Law, the products obtained by biotransformation of natural compounds are classified as natural ones (EU Directive 88/388/EEC). Because the reaction of microbial transformation of flavonoid aglycones to their glycosides is similar to the mammal metabolic processes (Xiao *et al.*, 2014c), it may serve as a model of mammalian metabolism (Miyakoshi *et al.*, 2010). Selected examples of microorganisms with identified substrate specificities that have been described in the last years are listed in Table I.

Prenylated flavonoids naturally found in common hop (*Humulus lupulus*), such as xanthohumol, isoxanthohumol and 8-prenylnaringenin, display a wide range of biological activity and naturally occur in the



1. R1=R2=H, daidzein
2. R1=OH, R2=H, genistein
3. R1=H, R2=Me, formononetin
4. R1=OH, R2=Me, biochanin A
5. R1=R2=H, daidzein 7-O-ß-D-glucopyranoside
6. R1=OH, R2=H, genistein 7-O-ß-D-glucopyranoside
7. R1=H, R2=Me, formononetin 7-O-ß-D-glucopyranoside
8. R1=OH, R2=Me, biochanin A 7-O-ß-D-glucopyranoside

Fig. 1

Table I
Microbial glycosylation of flavonoids

	Microorganism	Strain origin	Compound	Glycosylated form	Transformation yield	Comment	Reference
Fungus	*Absidia coerulea* AM93	Institute of Biology and Botany of the Medical Academy of Wrocław	xanthohumol	xanthohumol 4'-*O*-β-D-glucopyranoside	29.0%	Biotransformation at 25°C for 9 days	Tronina *et al.*, 2013
			8-prenylnaringenin	8-prenylnaringenin 7-*O*-β-D-glucopyranoside	49.3%	Biotransformation at 25°C for 5 days	Bartmańska *et al.*, 2012
	Absidia glauca AM177	Institute of Biology and Botany of the Medical Academy of Wrocław	isoxanthohumol	isoxanthohumol 7-*O*-β-D-glucopyranoside	61.6%	Biotransformation at 25°C for 7 days	Bartmańska *et al.*, 2009
	Beauveria bassiana AM446	Institute of Biology and Botany of the Medical Academy of Wrocław	xanthohumol	xanthohumol 4'-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside	23.0%	Biotransformation at 25°C for 3 days	Tronina *et al.*, 2013
	Beauveria bassiana AM278	Institute of Biology and Botany of the Medical Academy of Wrocław	isoxanthohumol	isoxanthohumol 7-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside	50.2%	Biotransformation at 25°C for 12 days	Bartmańska *et al.*, 2009
	Beauveria bassiana AM278	Institute of Biology and Botany of the Medical Academy of Wrocław	8-prenylnaringenin	8-prenylnaringenin 7-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside	32.9%	Biotransformation time: 6 days	Bartmańska *et al.*, 2012
	Beauveria bassiana ATCC 7159	American Type Culture Collection	quercetin	quercetin 7-*O*-β-D-(4'-*O*-methyl)-glucopyranoside	87.0%	ND	Zhan and Gunatilaka, 2006
	Cunninghamella echinulata AS 3.3400	China General Microbiological Culture Collection Center	kurarinone	kurarinone-7-*O*-β-glucopyranoside	2.2%	Biotransformation at 28°C for 5 days	Shi *et al.*, 2012
	Cunninghamella echinulata CBS 596.68	Centraalbureau voor Schimmelcultures (Netherlands)	kaempferol	kaempferol 3-*O*-β-D-glucopyranoside	ND	Biotransformation at 28°C for 2–5 days	Miyakoshi *et al.*, 2010
			morin	morin 3-*O*-β-D-glucopyranoside	ND		
			3-hydroxyflavone	flavone 3-*O*-β-D-glucopyranoside	ND		
	Cunninghamella elegans ATCC 9245	American Type Culture Collection	quercetin	3-O-β-D-glucopyranoside	55.7%	Biotransformation at 28°C for 4 days	Zi *et al.*, 2011
				kaempferol 3-*O*-β-D-glucopyranoside	2.9%		
				isorhamnetin 3-*O*-β-D-glucopyranoside	4.9%		
	Cunninghamella elegans var. *elegans* KCTC 6992	Korean Collection for Type Cultures	xantohumol	isoxsanthohumol 7-*O*-β-D-glucopyranoside	9.3%	Biotransformation at 25°C for 5 days	Kim and Lee, 2006
	Mortierella isabellina ATCC3 38063	American Type Culture Collection	daidzein	daidzein 4'-*O*-rhamnopyranoside	3.0%	Biotransformation time: 12 days	Maatooq and Rosazza, 2005
	Mortierella mutabilis AM404	Institute of Biology and Botany of the Medical Academy of Wrocław	xantohumol	isoxanthohumol 7-*O*-β-glucopyranoside	49.0%	Biotransformation time: 11 days	Tronina *et al.*, 2013

Table I
Continued

Microorganism		Strain origin	Compound	Glycosylated form	Transformation yield	Comment	Reference
Fungus	*Mucor hiemalis* CGMCC 3.14114	China General Microbiological Culture Collection Center	isoangustone A	isoangustone A 7-*O*-β-D-glucopyranoside	3.6%	Biotransformation at 30°C for 48 h	Feng *at al.*, 2015
				isoangustone A 7-*O*- β-D-glucopyranosyl-4'-O-sulfate	1.0%		
				isoangustone A 7,3'-di-*O*-β-D glucopyranoside	0.5%		
	Mucor spinosus CGMCC 3.3450	China General Microbiological Culture Collection Center	cardamonin	cardamonin 4-*O*-β-D-glucopyranoside	0.4%	Biotransformation time: 4 days	Xu *et al.*, 2011
				cardamonin 6-*O*-β-D-glucopyranoside	0.8%		
	Penicillium chrysogenum KTCC 6933	Korean Collection for Type Cultures	xanthohumol	xanthohumol 4'-*O*-β-D-glucopyranoside	5.5%	Biotransformation at 25°C for 6 days	Kim and Lee, 2006
				xanthohumol 4,4'-*O*-β-D-diglucopyranoside	1.0%		
	Rhizopus nigricans UPF701	Department of Plant Protection of Wrocław University of Environmental and Life Sciences	xanthohumol	xanthohumol 4'-*O*-β-D-glucopyranoside	14.2%	Biotransformation at 25°C for 14 days	Tronina *et al.*, 2013
	Trichoderma koningii KCTC 6042	Korean Collection for Type Cultures	silybin A	silybin A 3-*O*-β-D-glucopyranoside	18.0%	Biotransformation at 25°C for 96 h	Kim *et al.*, 2006a
				silybin A 7-*O*-β-D-glucopyranoside	7.5%		
			silybin B	silybin B 3-*O*-β-D-glucopyranoside	15.0%		
				silybin B 7-*O*-β-D-glucopyranoside	4.5%		
Bacteria	*Streptomyces* M52104	Wild strain isolated from soil	quercetin	quercetin 4'-*O*-β-D-glucuronide	50.0%	Biotransformation at 28°C for 48–192 h	Marvalin and Azerad, 2011
				quercetin 3-*O*-β-D-glucuronide	19.0%		
				quercetin 7-*O*-β-D- glucuronide	9.0%		
				quercetin 3'-*O*-β-D- glucuronide	5.0%		
			naringenin	naringenin 7-*O*-β-D-glucuronide	25.0%		
				naringenin 4'-*O*-β-D-glucuronide	5.0%		
	Streptomyces rimosus subsp. *rimosus* ATCC 10970	American Type Culture Collection	quercetin	quercetin 7-*O*-β-4'-deoxy-hex-4'-enopyranosiduronic acid	ND	Biotransformation at 28°C for 96 h	Ma *et al.*, 2013

Table I
Continued

Microorganism		Strain origin	Compound	Glycosylated form	Transformation yield	Comment	Reference
Bacteria	*Bacillus cereus*	Wild strain isolated from soil	quercetin	quercetin 3-*O*-glucopyranoside (unspecified α or β)	ND	Biotransformation at 30°C for 24–36 h	Rao and Weisner, 1981
	Lysinibacillus fusiformis CGMCC 4913	China General Microbiological Culture Collection	puerarin	puerarin 7-*O*-α-D-fructofuranoside	91.6%	Biotransformation in aqueous hydrophilic media at 30°C for 48 h	Wang *et al.*, 2014
	Microbacterium oxydans CGMCC 1788	China General Microbiological Culture Collection	puerarin	puerarin 7-*O*-α-D-glucopyranoside	40.0%	Optimal biotransformation at 30°C for 12 h	Jiang *et al.*, 2008
				puerarin 7-*O*-α-D-isomaltoside	5.0%		
	Staphylococcus saprophyticus CQ16	Organic solvent-tolerant bacteria screened from soil	naringenin	naringenin 7-*O*-β-D-glucopyranoside	86.0%	Biotransformation in aqueous hydro philic media at 30°C for 60–96 h	Chu *et al.*, 2014
			quercetin	quercetin 7-*O*-β-D-glucopyranoside	96.0%		
			daidzein	daidzein 7-*O*-β-D-glucopyranoside	90.0%		
			genistein	genistein 7-*O*-β-D-glucopyranoside	86.0%		
			formononetin	formononetin-7-*O*-β-D-glucopyranoside	82.0%		
			baicalein	baicalein-7-*O*-β-D-glucopyranoside	84.0%		
			luteolin	luteolin-7-*O*- β-D-glucopyranoside	99.0%		
			apigenin	apigenin-7-*O*-β-D-glucopyranoside	98.0%		
			isosilybin	isosilybin-7-*O*-β-D-glucopyranoside	82.0%		
	Xanthomonas campestris	Department of Chemistry of Okayama University of Science	hesperetin	hesperetin 3'-*O*-α-D-glucopyranoside	12.0%	Biotransformation product were obtained by incubation of lyophilized cells with substrate and a sugar donor	Shimoda and Hamada, 2010
				hesperetin 5-*O*-α-D- glucopyranoside	10.0%		
				hesperetin 7-*O*-α-D- glucopyranoside	15.0%		
	Escherichia coli (GMO)	A glucosyltransferase cDNA, RF5 from *Oryza sativa*	quercetin	quercetin 3-*O*-glucopyranoside	ND	Complete conversion after 7 h of incubation at 30°C	Kim *et al.*, 2006b
				(unspecified α or β)			
			kaempferol	kaempferol 3-*O*-glucopyranoside	ND	Complete conversion after 24 h of incubation at 30°C	
				(unspecified α or β)			

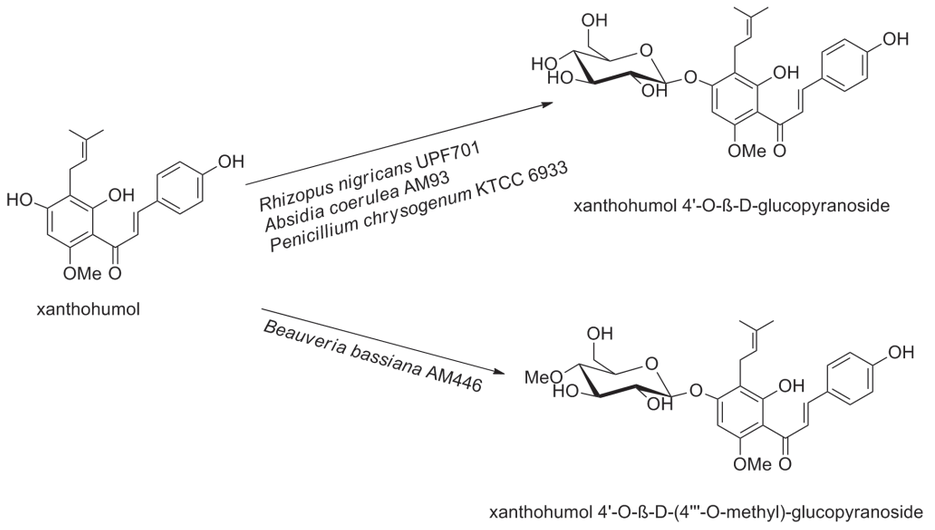


Fig. 2

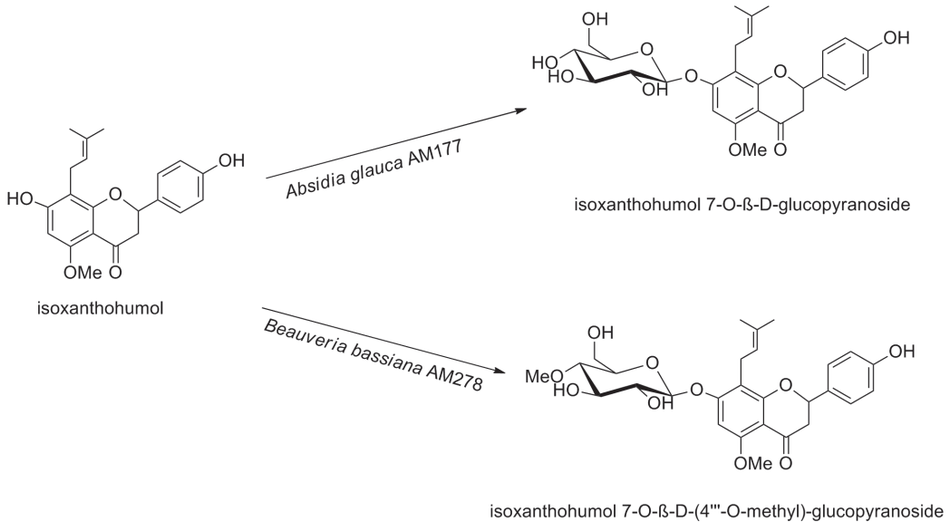


Fig. 3

form of aglycones. Biotransformation of xanthohumol in the cultures of filamentous fungi: *Absidia coerulea* AM93 and *Rhizopus nigricans* UPF701 led to xanthohumol 4'-*O*-β-D-glucopyranoside in 29.0% and 14.2% yields, respectively (Fig. 2). The identical product, but with a much less efficient conversion compared to these processes (5.5% yield) was previously observed by Kim and Lee (2006), who applied *Penicillium chrysogenum* KTCC 6933 as a biocatalyst. Whereas fungus *Beauveria bassiana* AM446 transformed xanthohumol to its 4'-*O*-β-D-(4'-*O*-methyl)-glucopyranoside in 23.0% yield (Tronina *et al*., 2013) (Fig. 2).

Very efficient biotransformations were described for isoxanthohumol. They led to isoxanthohumol 7-*O*-β-D-glucopyranoside with 61.6% yield using the culture of *Absidia glauca* AM177 and isoxanthohumol 7-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside with 50.2% yield using the fungal strain *B. bassiana* AM278 (Bartmańska *et al*., 2009) (Fig. 3). The analogous products were received when 8-prenylnaringenin was used as a substrate: in the culture of *A. coerulea* AM93 it was transformed to the 7-*O*-β-D-glucopyranoside and in the culture of *B. bassiana* AM278 to the 7-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside (Bartmańska *et al*., 2012) (Fig. 4). The yields were 49.3% and 34.0%, respectively.

Quercetin is a strong antioxidant found in many plants and it is widely used in dietary supplements. Bacteria *Bacillus cereus* transformed quercetin to isoquercetin (quercetin 3-*O*-glucopyranoside) with 20.0% yield (Rao and Weisner, 1981). Whereas, fungus *B. bassiana* ATCC 7159 transformed quercetin to quercetin 7-*O*-β-D-(4'''-*O*-methyl)-glucopyranoside with 87.0% yield

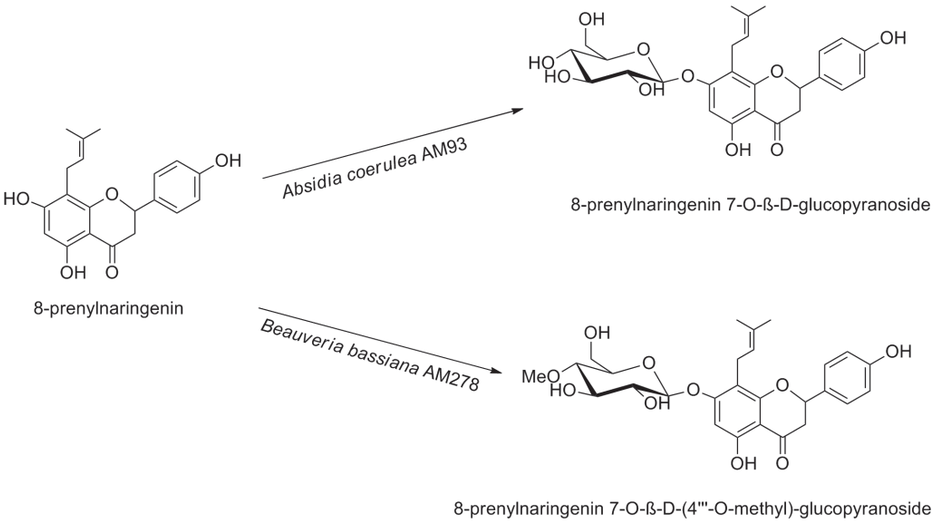


Fig. 4

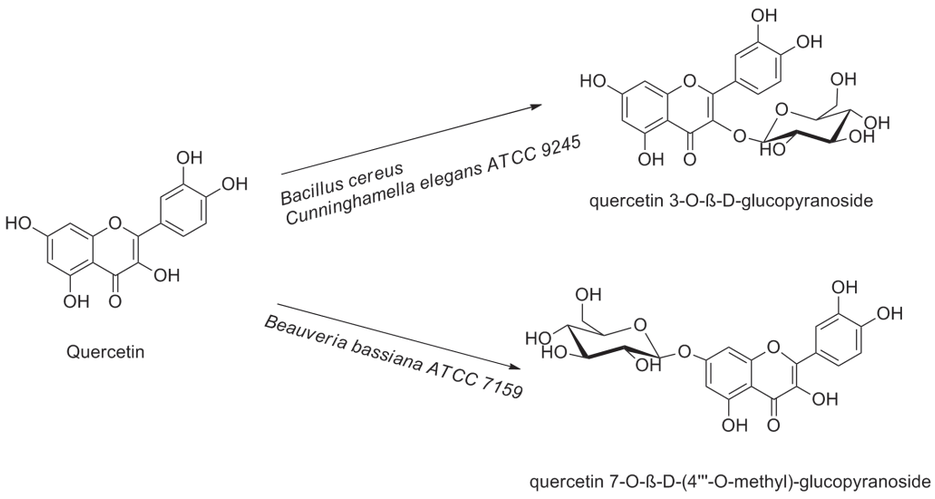


Fig. 5

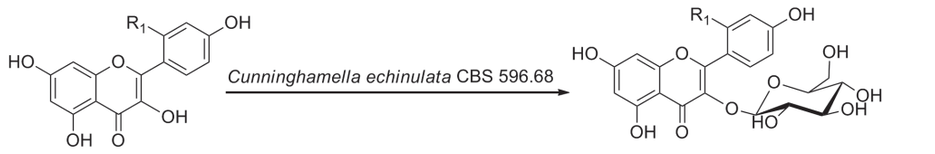


Fig. 6

(Zhan and Gunatilaka, 2006), and *Cunninghamella elegans* ATCC 9245 to quercetin 3-O-β-D-glucopyranoside with 55.7% yield (Zi *et al*., 2011) (Fig. 5).

Fungus *Cunninghamella echinulata* CBS 596.68 is also capable of glucosylation of kaempferol and morin. These transformations gave kaempferol 3-*O*-β-D-glucopyranoside in 67.0% yield and morin 3-*O*-β-D-glucopyranoside in 20.0% yield (Miyakoshi *et al*., 2010) (Fig. 6). It was proved that kaempferol 3-*O*-β-D-glucopyranoside has antibacterial properties (Mary and Merina, 2014), whereas morin 3-*O*-β-D-glucopyranoside has antifungal and anticancer activity (Hussain *et al*., 2014).

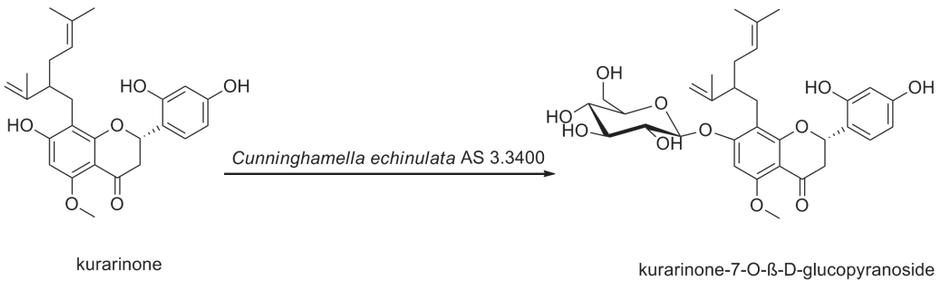


Fig. 7

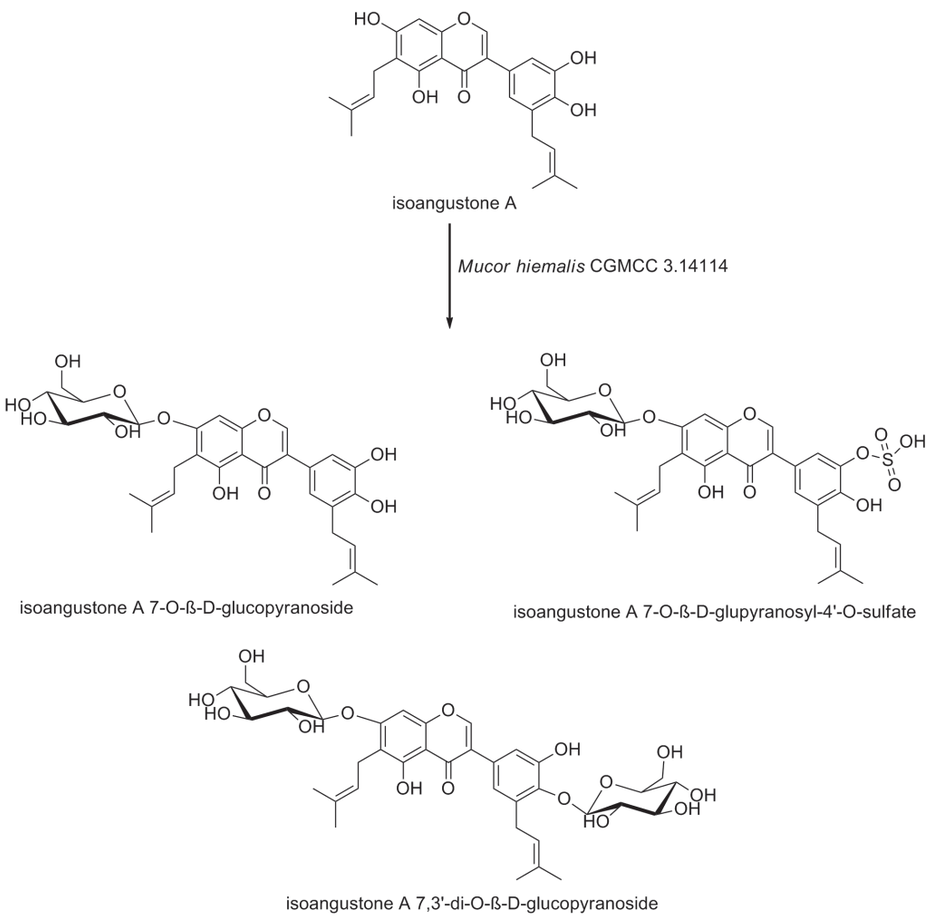


Fig. 8

Strain *C. echinulata* AS 3.3400 is also capable to perform glucosylation of kurarinone to kurarinone 7-*O*-β-D-glucopyranoside (Fig. 7). In recent years, pharmacological research of kurarinone indicate that it had the significant cytotoxicity, tyrosinase inhibitors and glycosidase inhibitor. Microbial transformation of this lavandulyl flavonoid can improving the bioactivities or water solubility and therefore enhances the chances of this bioactive compound for their potential application in medicinal use (Shi *et al*., 2012).

Isoangustone A is a flavonoid isolated from licorice and exhibit various pharmacological properties, such as antimicrobial, antioxidative, anti-inflammatory and antitumor. Biotransformation of isoangustone A in the culture of *Mucor hiemalis* CGMCC 3.14114 at 30°C for 48 h affords three new derivatives: isoangustone A 7-*O*-β-D-glucopyranoside (3.6%), isoangustone A 7-*O*-β-D-glupyranosyl-4'-*O*-sulfate (1.0%) and isoangustone A 7,3'-di-*O*-β-D-glucopyranoside (0.5%) (Feng *et al*., 2015) (Fig. 8).

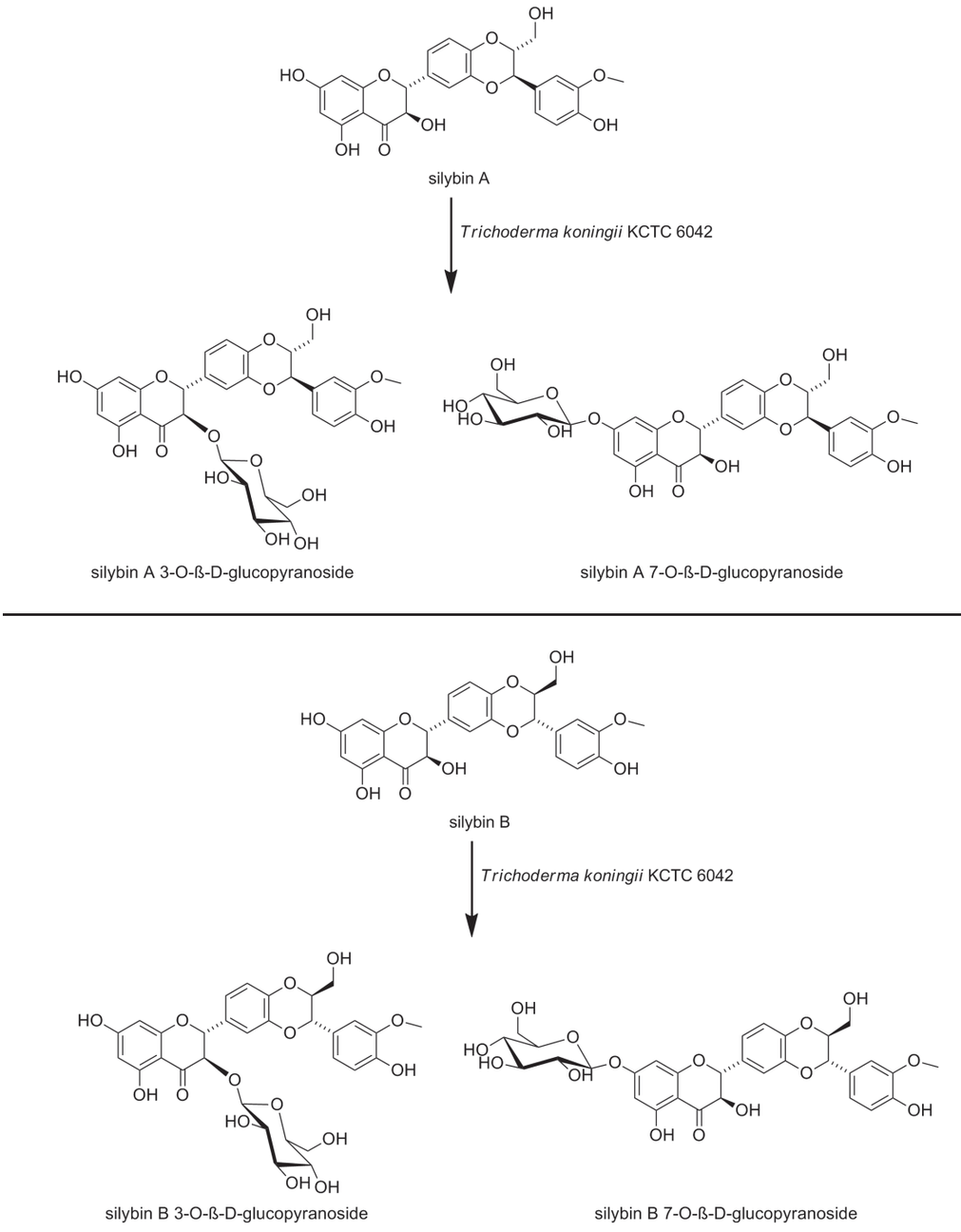


Fig. 9

Fungus *Trichoderma koningii* KCTC 6042 transform two silybin diastereomers: silybin A and silybin B and gave two pairs of glucosylated derivatives, silybin 3-*O*-β-D-glucopyranosides and silybin 7-*O*-β-D-glucopyranosides (Fig. 9). Biotransformation of silybin by microbes can be useful methods to achieve selective conversions of compounds to derivatives which are difficult to produce synthetically. Silybin is a major active constituent of silymarin, which have hepatoprotective and antioxidant activity (Kim *et al*., 2006a).

Microbial transformations may also lead to unusual products, which we observed in the case of *Mortierella isabellina* ATCC 38063, which was able to metabolize daidzein, one of the main isoflavones found in soybean, to the untypical metabolite daidzein 4'-*O*-rhamnopyranoside (Maatooq and Rosazza, 2005) (Fig. 10).

Bacteria strains are also capable to glycosylation flavonoids compounds. For example bacteria *Microbacterium oxydans* CGMCC 1788 converted puerarin into two novel compounds, puerarin-7-*O*-α-D-

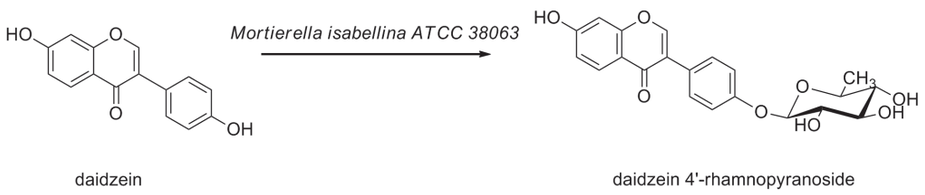


Fig. 10

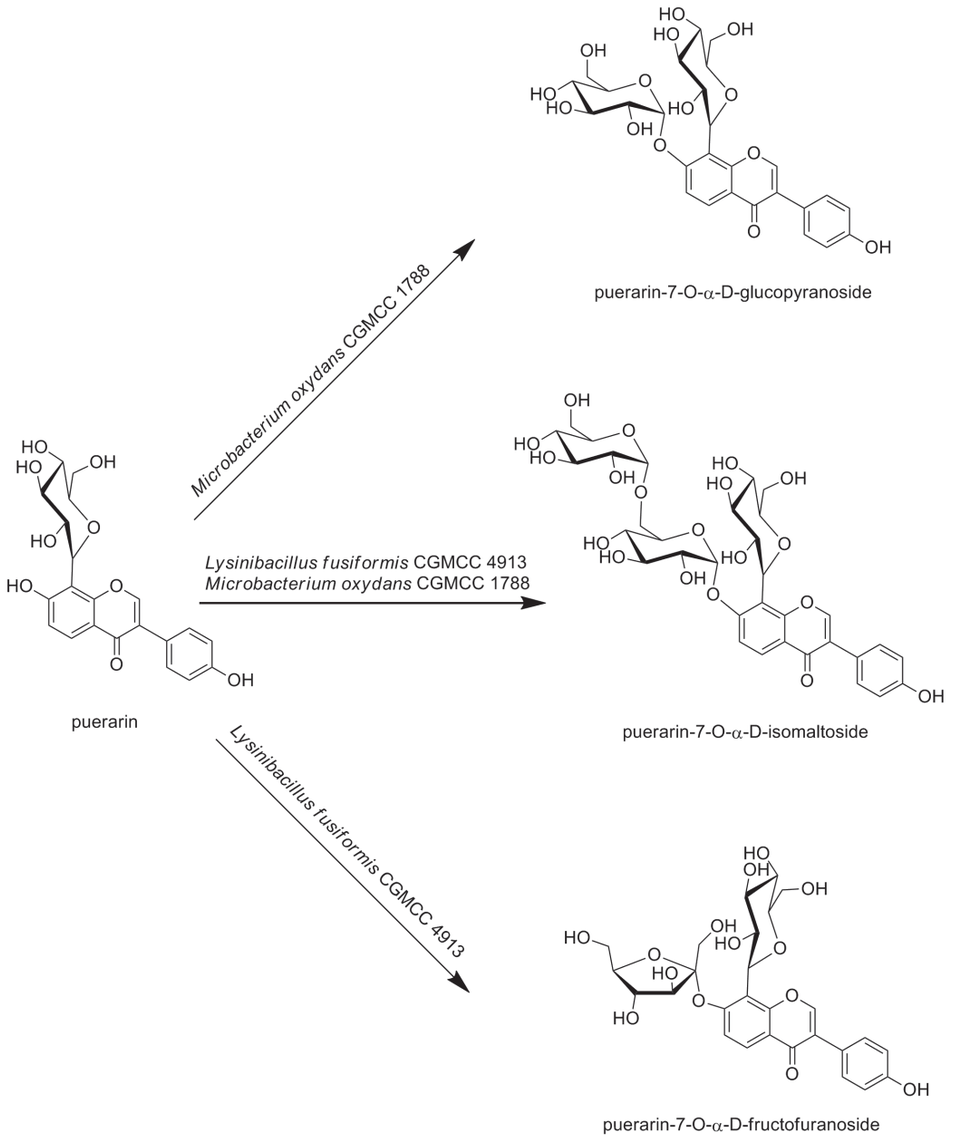


Fig. 11

glucopyranoside and puerarin-7-*O*-α-D-isomaltoside in 40.0% and 5.0% yields, respectively (Fig. 11). Puerarin is one of several known isoflavones, contains a unique *C*-glucoside moiety and is found in a number of plants and herbs, such as the root of *Pueraria* (*Radix puerariae*) and have anticancer and antioxidant activity (Jiang *et al.*, 2008). Wang *et al.* (2014) reported the efficient glucosylation of puerarin by an organic solvent-tolerant strain of *Lysinibacillus fusiformis* CGMCC 4913 in aqueous hydrophilic media at 30°C for 48 h. Incubation of this strain with puerarin led to efficient production (91.6% conversion rate) of

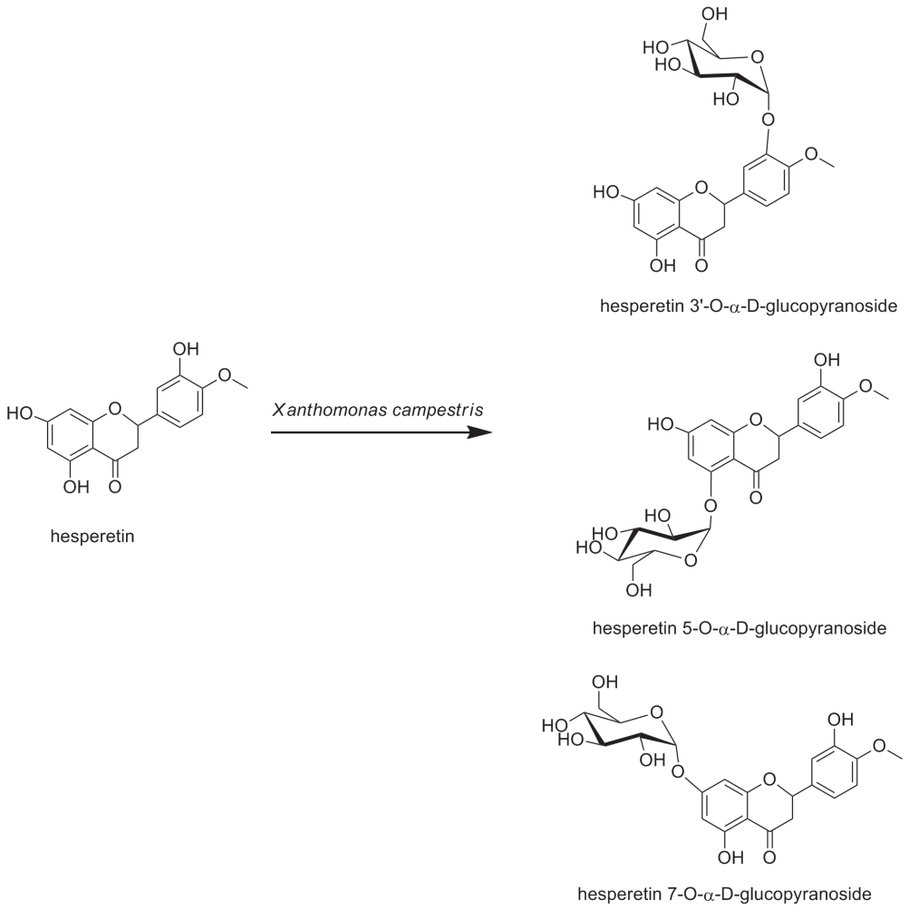


Fig. 12

puerarin-7-*O*-α-D-fructofuranoside and puerarin-7-*O*-α-D-isomaltoside (Fig. 11) with a conversion rate of less than 1% after 48 h reaction.

Chu *et al.* (2014) reported the example of highly regioselective glucosylation of various flavonoids catalysed by organic solvent-tolerant *Staphylococcus saprophyticus* CQ16. The efficient glucosylation of flavonoids was achieved in aqueous hydrophilic media. The addition of the polar solvent 15% DMSO significantly improved the glucosylation of flavonoids substrates (Table I).

Shimoda and Hamada (2010) investigated the production of hesperetin glycosides using glycosylation with bacteria *Xanthomonas campestris.* They obtained hesperetin 3'-*O*-α-D-glucopyranoside (12.0%), hesperetin 5-*O*-α-D-glucopyranoside (10.0%) and hesperetin 7-*O*-α-D-glucopyranoside (15.0%) (Fig. 12). Biotransformation products were obtained by incubation of lyophilized cells with substrate and a sugar donor.

Microbial transformations can be also employed to receive mammalian flavonoid metabolites, such as the most common glucuronides. Glucuronides of quercetin and naringenin can be obtained with the help of bacteria *Streptomyces* M52104 (Marvalin and Azerad, 2011). Incubation of quercetin in the culture of these bacteria at 28°C for 65 h affords four derivatives: quercetin 4'-*O*-β-D-glucuronide (50.0%), quercetin 3-*O*-β-D-glucuronide (19.0%), quercetin 7-*O*-β-D-glucuronide (9.0%) and quercetin 3'-*O*-β-D-glucuronide (5.0%) (Fig. 13).

This strain is also capable to perform glucuronidation of naringenin to give naringenin 7-*O*-β-D-glucuronide in 25% yield and naringenin 4'-*O*-β-D-glucuronide in 5.0% yield (Marvalin and Azerad, 2011) (Fig. 14).

Another way of economic production of glycosylated flavonoids may be employment of genetically modified microorganisms (Simkhada *et al.*, 2010). Biotransformation of kaempferol and quercetin in the culture of *Escherichia coli*, expressing glucosyltransferase gene cloned from rice (*Oryza sativa*), afforded 3-*O*-glycosides of both flavonoid substrates. Complete conversion of quercetin to its 3-*O*-glucopyranoside took place after seven hours of the incubation, whereas complete conversion of kaempferol to the 3-*O*-glucopyranoside after 24 h of the incubation (Kim *et al.*, 2006b).

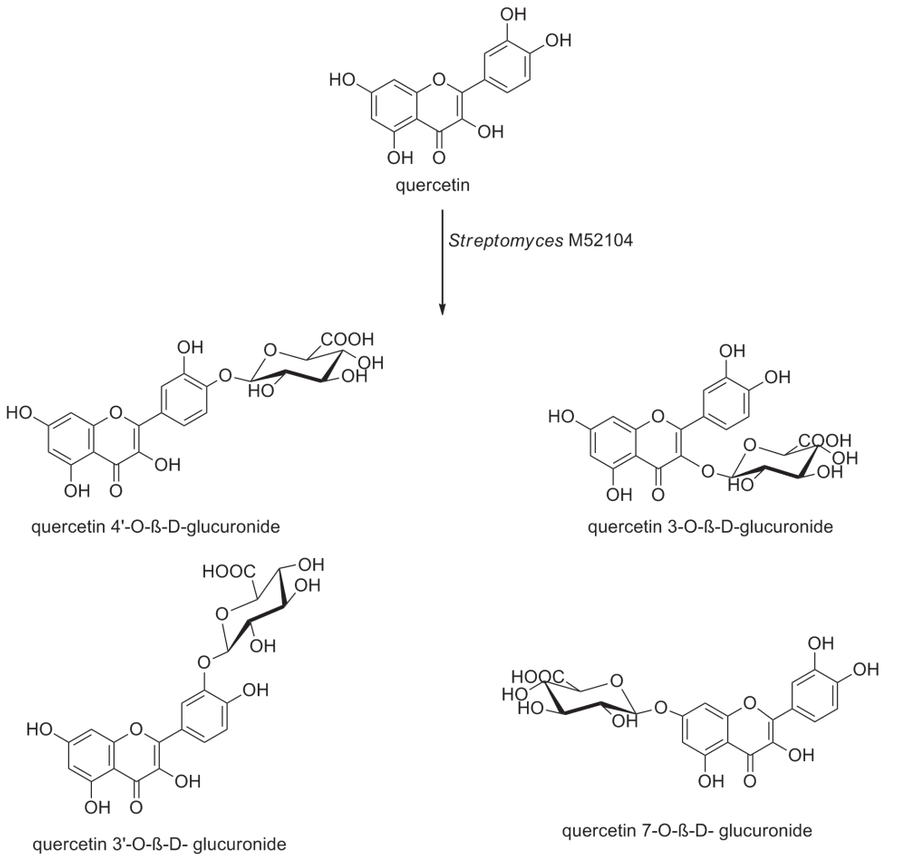


Fig. 13

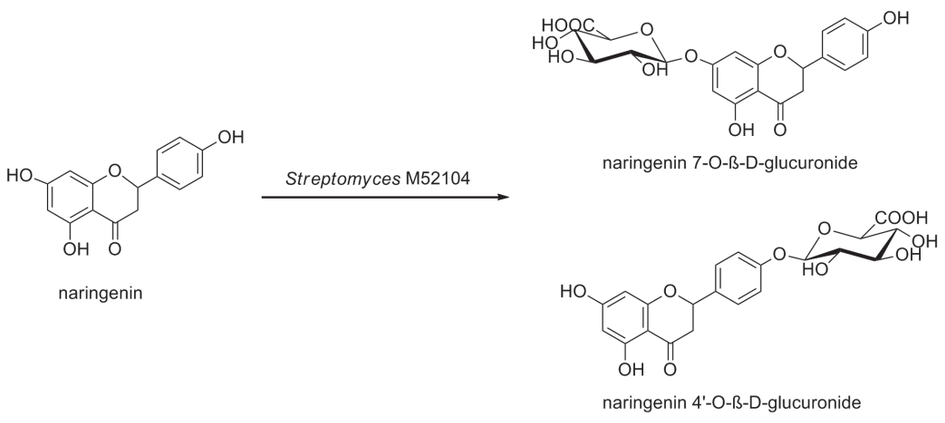


Fig. 14

Summary

Flavonoid glycosides are widespread in the plant kingdom and thus common in human diet. Pro-healthy properties of flavonoids, including their sugar derivatives, make them promising ingredients of dietary supplements and biomedical preparations. Employment of microbial transformations to obtain glycosides of the flavonoids found in nature in the form of aglycones or to obtain new derivatives of natural glycosides has many advantages. Such processes have potential industrial application, due to their relatively low costs and mild reaction conditions. Noteworthy is that glycosides obtained by microbial transformation of flavonoids

are classified as natural compounds, which facilitates their potential application as food supplements or ingredients of cosmetics and pharmaceuticals.

The achievements of researchers described in this paper indicate that an microbial transformation is a powerful approach to modify the structures of bioactive natural flavonoids to glycosylated derivatives. The use of the mentioned methods offers the possibility to receive new or known glycosides with the high regioselectivity (Chu *et al*., 2014) and yield (Zhan and Gunatilaka, 2006; Wang *et al*., 2014; Chu *et al*., 2014). In most cases microorganisms convert flavanones into the corresponding 7-*O*-glycosides, chalcones into 4'-*O*-glycosides and flavonols to 3-*O*-glycosides (Table I). In these reactions, aglycones are predominantly conjugated with the glucose moiety.

Microbial glycosylation of flavonoids leads to improvement of their water-solubility and therefore enhances the chances of bioactive compounds for their potential application in large scale (Jiang *et al*., 2008; Chu *et al*., 2014). Better solubility of flavonoids may result in their better absorption by the human body (Jiang *et al*., 2008).

The results described in this work clearly demonstrate the biotechnological potential of microbial glycosylation as a method for the preparation of the high-value flavonoids with medical applications. However, there is still too little *in vivo* data on the biological benefits of most flavonoids glycosides. Further research is also needed to develop biotransformation technologies that can be competitive alternative to the current methods that involve plant extraction or chemical synthesis.

Acknowledgements

The Project is supported by Wroclaw Centre of Biotechnology, The Leading National Research Centre Programme (KNOW) for years 2014–2018.

Literature

Alluis B. and O. Dangles. 1999. Acylated flavone glucosides: synthesis, conformational investigation, and complexation properties. *Helv. Chim. Acta* 82: 2201–2212.

Bartmańska A., E. Huszcza and T. Tronina. 2009. Transformation of isoxanthohumol by fungi. *J. Mol. Catal. B. Enzym*. 61: 221–224.

Bartmańska A., T. Tronina and E. Huszcza. 2012. Transformation of 8-prenylnaringenin by *Absidia coerulea* and *Beauveria bassiana. Bioorg. Med. Chem. Lett.* 22: 6451–6453.

Cao H., X. Chen, A.R. Jassbi and J. Xiao. 2015. Microbial biotransformation of bioactive flavonoids. *Biotechnol. Adv*. 33: 214–223.

Chemler J.A., E. Leonard and M.A.G. Koffas. 2009. Flavonoid biotransformations in microorganisms, pp. 191–255. In: Gould K., K. Davides and C. Winefield. *Anthocyanins: Biosynthesis, Functions and Applications.* Springer Science+Business Media LLC. New York.

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

Desmet T., W. Soetaert, P. Bojarovā, V. Kr'en, L. Dijkhuizen, V. Eastwick-Field and A. Schiller. 2012. Enzymatic glycosylation of small molecules: challenging substrates require tailored catalysts. *Chem. Eur. J*. 18: 10786–10801.

Feng J., W. Liang, S. Ji, X. Qiao, Y. Zhang, S. Yu and M. Ye. 2015. Microbial transformation of isoangustone A by *Mucor hiemalis* CGMCC 3.14114J. *Chin. Pharm. Sci*. 2015, 24: 285–291.

Forkmann G. and S. Martens. 2001. Metabolic engineering and applications of flavonoids. *Curr. Opin. Biotechnol*. 12: 155–160.

Gachon C.M.M., M. Langlois-Meurinne and P. Saindrenan. 2005. Plant secondary metabolism glycosyltransferases: the emerging functional analysis. *Trends Plant Sci.* 10: 542–549.

Gloster T.M. 2014. Advances in understanding glycosyltransferases from a structural perspective. *Curr. Opin. Struct. Biol*. 28: 131–141.

Hollman P.C., M.N. Bijsman, Y. van Gameren, E.P. Cnossen, J.H. de Vries and M.B. Katan. 1999. The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. *Free Radic. Res.* 31: 569–573.

Hussain J., L. Ali, A.L. Khan, N.U. Rehman, F. Jabeen, J.S. Kim and A. Al-Harrasi. 2014. Isolation and bioactivities of the flavonoids morin and morin-3-*O*-β-D-glucopyranoside holl from *Acridocarpus orientalis* – a wild arabian medicinal plant. *Molecules* 19: 17763–17772.

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

Jiang J.R., S. Yuan, J.F. Ding, S.C. Zhu, H.D. Xu, T. Chen, X.D. Cong, W.P. Xu, H. Ye and Y.J. Dai. 2008. Conversion of puerarin into its 7-O-glycoside derivatives by *Microbacterium oxydans* (CGMCC 1788) to improve its water solubility and pharmacokinetic properties. *Appl. Microbiol. Biotechnol*. 81: 647–657.

Kim H.J. and I.S. Lee. 2006. Microbial metabolism of the prenylated chalcone xanthohumol. *J. Nat. Prod*. 69: 1522–1524.

Kim H.J., H.S. Park and I.S. Lee. 2006a. Microbial transformation of silybin by *Trichoderma koningii. Bioorg. Med. Chem. Lett*. 16: 790–793.

Kim J.H., K.H. Shin, J.H. Ko and JH. Ahn. 2006b. Glucosylation of flavonols by *Escherichia coli* expressing glucosyltransferase from rice (*Oryza sativa*). *J. Biosci. Bioeng.* 102: 135–137.

Kumar S. and A.K. Pandey. 2013. Chemistry and biological activities of flavonoids: an overview. *Sci. World J*. 2013:162750.

Lairson L.L., B. Henrissat, G.J. Davies and S.G. Withers. 2008. Glycosyltransferases: structures, functions, and mechanisms. *Annu. Rev. Biochem*. 77: 25.1–25.35.

Lewis P. S. Kaltia and K. Wähälä. 1998. The phase transfer catalysed synthesis of isoflavone-*O*-glucosides. *J. Chem. Soc. Perkin Trans.* 1: 2481–2484.

Ma B., J. Zeng, L. Shao and J. Zhan. 2013. Efficient bioconversion of quercetin into a novel glycoside by *Streptomyces rimosus* subsp. *rimosus* ATCC 10970. *J. Biosci. Bioeng.* 115: 24–26.

Maatooq G.T. and J.P. Rosazza. 2005. Metabolism of daidzein by *Nocardia species* NRRL 5646 and *Mortierella isabellina* ATCC 38063. *Phytochemistry* 66: 1007–1011.

Marvalin C. and R. Azerad. 2011. Microbial glucuronidation of polyphenols. *J. Mol. Catal., B Enzym.* 73: 43–52.

Mary S.J. and A.J. Merina. 2014. Antibacterial activity of kaempferol-3-*O*-Glucoside. *Int. J. Sci. Res.* (*Ahmedabad*) 3: 46–47.

Miyakoshi S., S. Azami and T. Kuzuyama. 2010. Microbial glucosylation of flavonols by *Cunninghamella echinulata. J. Biosci. Bioeng.* 110: 320–321.

Ochiai M., H. Fukami, M. Nakao and A. Noguchi. 2010. Method of glycosylation of flavonoid compounds. *United States Patent Application Publication* Pub. No.: US 2010/0256345 A1.

Paquette S., B.L. Møller and S. Bak. 2003. On the origin of family 1 plant glycosyltransferases. *Phytochemistry* 62: 399–413.

Palcic M.M. 2011. Glycosyltransferases as biocatalysts. *Curr. Opin. Chem. Biol.* 15: 226–233.

Rao K.V. and N.T. Weisner. 1981. Microbial transformation of quercetin by *Bacillus cereus. Appl. Environ. Microbiol.* 42: 450–452.

Salas J.A. and C. Méndez. 2007. Engineering the glycosylation of natural products in actinomycetes. *Trends Microbiol.* 15: 219–232.

Shi Y.Q., X.L. Xin, Q.P. Yuan, C.Y. Wang, B.J. Zhang, J. Hou, Y. Tian, S. Deng, S.S. Huang and X.C. Ma. 2012. Microbial biotransformation of kurarinone by *Cunninghamella echinulata* AS 3.3400. *J. Asian Nat. Prod. Res*. 14: 1002–1007.

Shimoda K. and H. Hamada. 2010. Production of hesperetin glycosides by *Xanthomonas campestris. Nutrients.* 2: 171–180.

Simkhada D., N.P. Kurumbang, H.C. Lee and J.K. Sohng. 2010. Exploration of glycosylated flavonoids from metabolically engineered *E. coli*. *Biotechnol. Bioprocess Eng.* 15: 754–760.

Tronina T., A. Bartmańska, M. Milczarek, J. Wietrzyk, J. Popłoński, E. Rój and E. Huszcza. 2013. Antioxidant and antiproliferative activity of glycosides obtained by biotransformation of xanthohumol. *Bioorg. Med. Chem. Lett.* 23: 1957–1960.

Wang A., F. Zhang, L. Huang, X. Yin, H. Li, Q. Wang, Z. Zeng and T. Xie. 2010. New progress in biocatalysis and biotransformation of flavonoids. *J. Med. Plant Res.* 4: 847–856.

Wang S., G. Liu, W. Zhang, N. Cai, C. Cheng, Y. Ji, L. Sun, J. Zhan and S. Yuan. 2014. Efficient glycosylation of puerarin by an organic solvent-tolerant strain of *Lysinibacillus fusiformis. Enzyme Microb. Technol.* 57: 42–7.

Wang X. 2009. Structure, mechanism and engineering of plant natural product glycosyltransferases. *FEBS Lett.* 585: 3303–3309.

Xiao J., E. Capanoglu, A.R. Jassbi and A. Miron. 2014a. The paradox of natural flavonoid *C*-glycosides and health benefits: When more occurrence is less research. *Biotechnol. Adv.* pii: S0734–9750 (14) 00177–3.

Xiao J., T. Chen and H. Cao. 2014b. Flavonoid glycosylation and biological benefits. *Biotechnol. Adv.* pii: S0734–9750(14)00092–5.

Xiao J., T.S. Muzashvili and M.I. Georgiev. 2014c. Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnol. Adv.* 32: 1145–1156.

Xu J., L. Yang, S.J. Zhao, G.X. Chou and Z.T. Wang. 2011. Microbial glycosylation of cardamonin by *Mucor spinosus. Acta Pharmaceutica Sinica.* 46: 733–737.

Zhao J. and R.A. Dixon. 2009. The 'ins' and 'outs' of flavonoid transport. *Trends Plant Sci.* 15:72–80.

Zhan J. and A.A.L. Gunatilaka. 2006. Selective 4'-O-methylglycosylation of the pentahydroxy-flavonoid quercetin by *Beauveria bassiana* ATCC 7159. *Biocatal Biotransform*. 24: 396–399.

Zi J., J. Valiente, J. Zeng and J. Zhan. 2011. Metabolism of quercetin by *Cunninghamella elegans* ATCC 9245. *J. Biosci. Bioeng*. 112: 360–362.