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Review Paper

New progress in biocatalysis and biotransformation of flavonoids

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Flavonoids have many beneficial health effects, such as antimicrobial, antioxidant, antiviral, antiplatelet, anti-ischemic, antitumor, anti-inflammatory, antiallergic, estrogenic, and radical-scavenging activity. However, their practical applications are often strongly limited due to low solubility and stability in hydrophilic media. Biocatalysis and biotransformation of flavonoids have been introduced to modify their structure and increase natural flavonoid diversity, which could alter physicochemical properties and improve the bioavailability and biological properties of maternal compounds. In this paper, biocatalytic structural modification of flavonoids by different kinds of enzymes or microorganism, the process and possible mechanism were discussed. The main achievements and key factors affecting biocatalysis of flavonoids were discussed and the research prospect was outlined.

Key words: Flavonoids, biocatalysis and biotransformation, hydrophilic media, heterologous expression, medium engineering.

INTRODUCTION

Flavonoid, a class of plant natural products that occur in a large variety of plants, fruits and vegetables, exhibit a wide range of beneficial effects on human health, including cardiovascular disease, chronic diseases and certain forms of cancer activity (Shi et al., 2001; Larsson et al., 2005; Martinez Conesa et al., 2005; Kawai et al., 2008; Bellocco et al., 2009). Various flavonoids possess antimicrobial, antioxidant, antiviral, anti-platelet, anti-ischemic, anti-tumor, anti-inflammatory, anti-allergic, estrogenic, and radical-scavenging activities (Russo et al., 2003; Ito et al., 2004; Stocker et al., 2004; Zhang et al., 2008; Tuberoso et al., 2009). Due to these numerous properties and applications, flavonoids have gained growing interest.

However, most of them have a low solubility and poor stability in both polar and nonpolar media, which strongly restricts their incorporation in many formulations (Ishihara and Nakajima, 2003; Tommasini et al., 2004; Chebil et al., 2007). Poor bioavailability of prenylated flavonoids results in their poor intrinsic permeation and transporter-mediated efflux (Chen et al. 2008). Improvement of the hydrophilic nature and stability of flavonoids can be accomplished by chemical, enzymatic or chemo-enzymatic structural modification (Haddad et al., 2006). Chemical methods are concerns over adverse environmental impact, safety and waste. In addition, when chemical method is used, many protection/de-protection steps are required to obtain selective functionalization because of the numerous reactive hydroxyl groups in flavonoid structures. On the contrary, biocatalytic methods can be used in both simple and complex transformations without the tedious blocking and deblocking steps that are common in enantio- and regioselective organic synthesis. Such high selectivity also affords efficient reactions with few by-products (Clark, 1999; Schmid et al., 2001). Moreover, the advantage of biocatalysis lies in the ability to produce enantiomer specific products and operate at near neutral pH, ambient temperatures and atmospheric pressures. Some reactions, not possible or not economically feasible by traditional chemical synthesis, could be carried out using biocatalysis (Straathof et al., 2002).

In the biocatalytic structure modification of flavonoids,

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the conversion yield and the number and the relative proportions of the synthesized products were found dependent on the nature of the enzyme, solvent and flavonoid structure, etc. This article reviews the main work done on the microbial and enzymatic transformations of flavonoids including the origin of enzyme and microorganism, the nature of reaction, operating conditions and the composition of the reaction media used in the transformation. The reconstruction of flavonoids biosynthetic pathways in heterologous microorganisms was also discussed.

ENZYMATIC TRANSFORMATIONS OF FLAVONOIDS

The use of enzymes to modify structure and improve physicochemical and biological properties of various natural products has been of a great scientific and industrial interest due to their large availability, low cost, wide substrate spectrum and no need of added cofactors (Benkovic and Hammes-Schiffer, 2003) in flavonoid biotransformation. Various types of enzymes have been tested for flavonoid biocatalysis, such as lipase, transferase, isomerase, esterase and protease. Lipases was the most frequently used enzyme in catalyze flavonoids.

Lipase

Candida Antarctica lipase B (CALB) is most frequently used in enzymatic transformation of flavonoids. The acetylation of two flavonoid glycosides, rutin and isoquercitrin, was investigated through molecular modeling using CALB catalysis. The results show that the aglycon part of both rutin and isoquercitrin was localized at the entrance of the binding pocket, stabilized by hydrogen bond and hydrophobic interactions. In particular, only the primary 6'-OH of the isoquercitrin glucose and the secondary 4'-OH of the rutin rhamnose were expected to be acetylated (De Oliveira et al., 2009) (Figure 1). While the acetylation occurred only on 3'-OH, 5'-OH, and 7-OH hydroxyls when *Pseudomonas cepacea* lipase (PSL-C) was used (Chebil et al., 2006).

As enzyme immobilization has been revealed in the last times as a very powerful tool to enhance enzyme properties such as stability and regioselectiviey in nonaqueous media (Blanco et al., 2007), CALB immobilized on a macroporous acrylic resin was used to catalyze the enzymatic synthesis of acylated derivatives of phloridzin a flavonoid from the dihydrochalcone family. The acylation was shown to be total and perfectly regioselective in favor of phloridzin-6'-*O*-cinnamate (Enaud et al., 2004). Moreover, there have been more reports on the synthesis of flavonoid derivatives in non-conventional medium. The acylated derivatives of a monosaccharidic flavonoid chrysoeriol-7-*O*-β-d-(3'-*E*-*p*-coumaroyl)-glucopyranoside and a disaccharidic flavonoid chrysoeriol-7-[6'-*O*-acetyl-β-D-allosyl-(1→2)-β-D-glucopyranoside] have also been synthesized in non-toxic organic solvents using an immobilized CALB, the introduction of an acyl group into glucosylated flavonoids (P1 and P2) significantly improved their antioxidant activity towards both LDL and serum model in vitro (Mellou et al., 2005) (Figure 2). These syntheses are often influenced by the physicochemical property of medium, carbon chain length and substitution pattern of the acyl donors (Ardhaoui et al., 2004). The regioselectivity of the process was higher in [bmim] BF4 than in [bmim] PF6 or organic solvents. Reaction rates observed in ionic liquids were up to four times higher than those reported for organic media and the highest conversion yield was obtained with short chain acyl donors (Katsoura et al., 2006).

Stevenson et al. have also reported that donors has a strong effect on the reactions, when flavonoid glycosides were acylated in 2-methyl-2-propanol with immobilized CALB, conversions ranged from 25 - 95% using palmitic, cinnamic and phenylpropionic (PPA) acids or hydroxylated derivatives of PPA as acyl donors (Stevenson et al. 2006). Using palmitic methyl ester as acyl donor, the acylation rate of naringin was 10 fold higher than that of rutin. Under optimal conditions, that are a molar ratio acyl donor/naringin of 7:1 and 200 mbar, 92% naringin was acylated (Passicos et al., 2004).

Transferase

Transferases are the enzymes that transfer a chemical group from one compound (donor) to another (acceptor). NovQ, can serve as a useful biocatalyst for the synthesis of prenylated flavonoids, is the first reported prenyltransferase (PT) capable of catalyzing the transfer of a dimethylallyl group to the B-ring of flavonoids. Incubation with naringenin and genistein yielded two products with a dimethylallyl group at C-3' or O-4' in the B-ring, naringenin was most highly converted to its prenylated derivatives with 98.3% yield under suitable reaction condition (Ozaki et al., 2009). A new prenyltransferase derived from the microsomal fractions of cell cultures of *Morus nigra* was shown to be able to prenylate exclusively chalcones with a 2',4' dihydroxy substitution and the isoflavone genistein (Vitali et al., 2004). The prenylation occurred with chalcones 1, 2, 3, bearing two hydroxyl groups (C-2', C-4') on ring A, while the position of substituents in ring B appeared to be critical for the prenylation.

Enzymatic glucosylation with glycosyltransferases has also been used to enhance the water solubility of aglycone and flavonoids (Xiao et al., 2009). The flavonol quercetin is usually found glycosylated on one or more of its five hydroxyl groups to increase its solubility and stability (Haddad et al., 2006). Hesperidinase has been

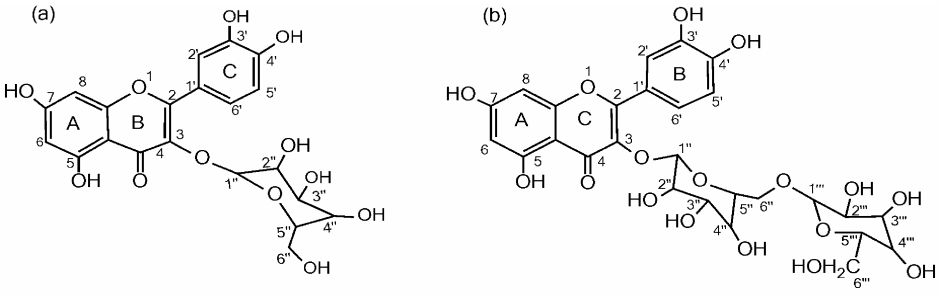

Figure 1. Chemical structure of (a) isoquercitrin and (b) rutin, with the systematic numbering of carbon atoms and nomenclature of aglycon rings.

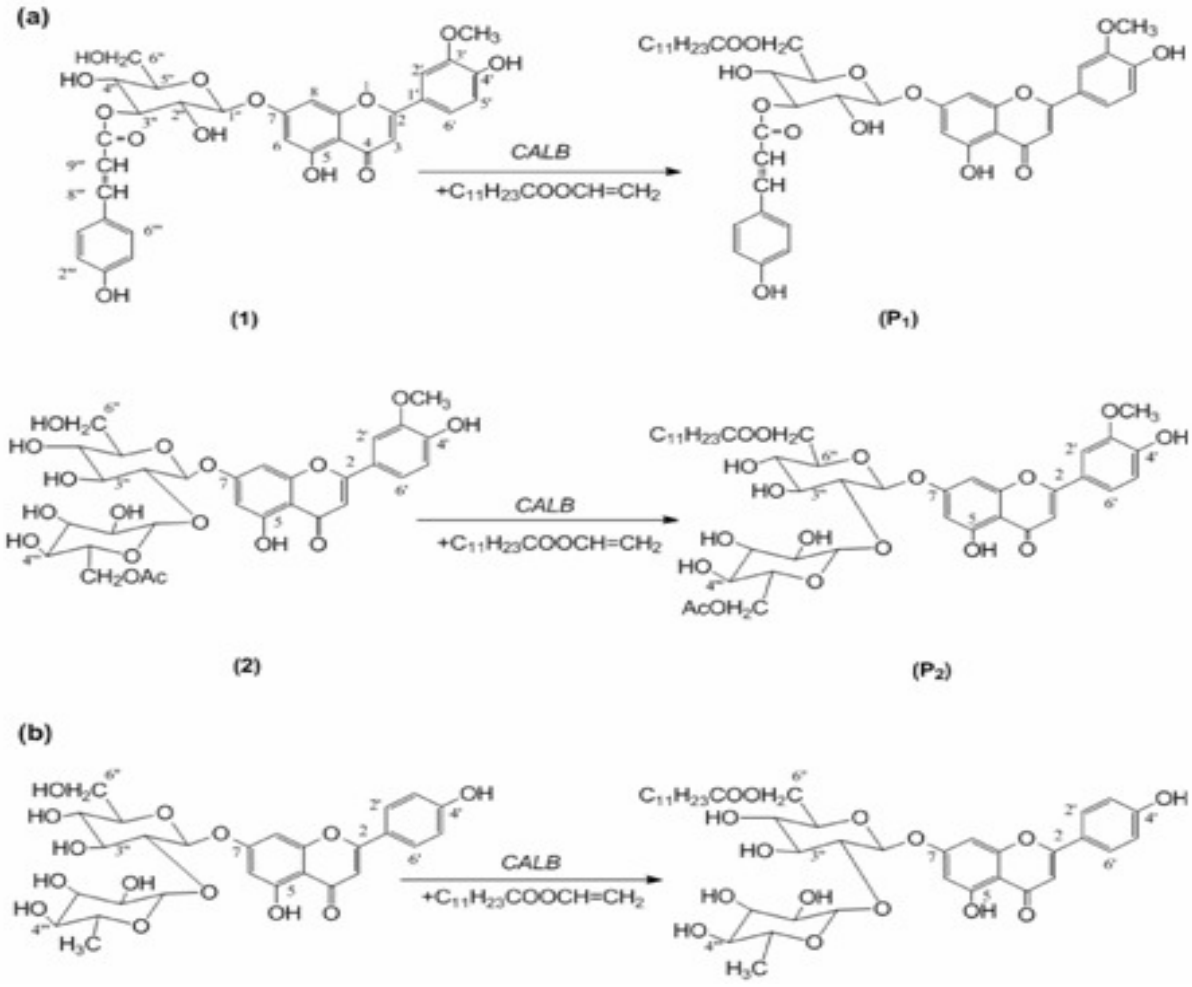

Figure 2. (a) Enzymatic esterification of chrysoeriol-7-*O*-β-D-(3''-*E*-*p*-coumaroyl)-glucopyranoside (1), and chrysoeriol-7-[6'''-*O*-acetyl-β-D-allosyl-(1→2)-β-D-glucopyranoside] (2), and (b) enzymatic esterification of naringin with vinyl laurate catalyzed by immobilized CALB.

also reported to catalyze the formation hesperetin-7-glucoside, which modulated the bioavailability of hesperidin and changed the absorption site from the colon to the small intestine (Nielsen et al., 2006). With the genomics approach to (iso) flavonoid glycosylation about *Medicago truncatula*, eight glycosyltransferase (UGT) were identified with activity against isoflavones, flavones, flavonols or anthocyanidins, and several showed high catalytic specificity for more than one class of (iso)flavonoid substrate (He et al., 2008).

Bifunctional enzymes generally contain two large structural domains whose association facilitate metabolic pathway control and/or allow more efficient substrate conversion (Schuster et al., 1999). An in-frame fusion of the flavonoid O-glucosyltransferase (OsUGT-3) and sucrose synthase (AtSUS) genes was made (Son et al., 2009). The resulting fusion protein is useful for the enzymatic production of flavonoid O-glucosides. When sucrose and UDP were supplied, the obtained fusion protein was able to convert quercetin into quercetin O-glucoside without the addition of UDP-glucose. It is the advantage of metabolic channelliing in biocatalysis and biotransformation that protein fusion and metabolic channelliing technology have been applied in the structure modification of flavonoid (Hossain et al., 2004; Jorgensen et al., 2005).

Though some natural-product glycosyltransferases (GTs) are sufficiently promiscuous for use in altering these glycosylation patterns, the stringent specificity of others remains a limiting factor in natural-product diversification (Williams et al., 2007). On the other hand, the positions, number, and length of the sugar moieties are predicted to be significant factors (Sato et al., 2000; Karakaya, 2004; Moon et al., 2006; Modolo et al., 2007).

Isomerase

Chalcone isomerase (CHI) catalyzes the cyclization of chalcone to form flavanone and plays a central role in flavonoid biosynthetic pathways, catalyzing the transformation of chalcone and 6'-deoxychalcone into (2*S*)-naringerin and (2*S*)-5-deoxyflavanone. The structure and mutational analysis of this enzyme suggest a mechanism in which shape complementarity of the binding cleft locks the substrate into a constrained conformation that allows the reaction to proceed with a second-order rate constant and a diffusion controlled limit (Jez et al., 2000; Tian and Dixon, 2006; Ruiz-Pernia et al., 2007). It's also found that some of the newly identified soybean CHIs do not require the 4'-hydroxy moiety on the substrate for high enzyme activity (Ralston et al., 2005).

Laccase

Using M*yceliophthora* laccase, a flavonoid polymer and high molecular fraction of extracted flavonoids have been prepared with rutin as substrate in the mixture of methanol and buffer (Kurisawa et al., 2003). The obtained polymer exhibited enhanced physiological properties, compared with native rutin. In addition, under selected conditions, the resulting polymer showed greatly improved superoxide scavenging activity and inhibition effects on human low-density lipoprotein (LDL) oxidation initiated by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Laccase has also been used to catalyze oxidation of catechin in the presence of gelatin and synthesize the gelatin-catechin conjugate (Chung et al., 2003). The conjugates had a good scavenging activity against superoxide anion radicals. Moreover, these conjugates also showed an amplified inhibition effect on human low density lipoprotein oxidation, same as the former report.

Peroxidase

Horseradish peroxidase (HRP) was used to catalyze the conjugation of green-tea catechin with amine substituted octahedral silsesquioxane (Ihara et al., 2005). Compared to intact catechin, the conjugate against superoxide anion was greatly improved. Moreover, the conjugate strongly inhibited xanthine oxidase activity and would allow amplification of the beneficial physiological properties of flavonoids

Cellulase

The pectinolytic and cellulolytic cellulase were characterized for main-chain and side-chain polysaccharide hydrolyzing activities and also against pure samples of various flavonoids previously identified in bergamot peel to determine various glycosidase activities (Mandalari et al., 2006). Commercial cellulase preparations from *Trichoderma viride* was also found to show transglucosylation activity toward (+)-catechin and (-)-epigallocatechin gallate (EGCG) using dextrin as a glucosyl donor (Noguchi et al., 2008). Some EGCG derives were prepared using this enyzme as biocatalyst. One of them, EGCG 5-*O*-α-D-glucopyranoside, showed higher heat stability and solubility, lower astringency and astringent stimulation than its aglycon, which suggested that EGCG glucosides were functionally superior to EGCG as food additives.

Protease

It has been reported that flavonoid glycosides, rutin and flavonoid disaccharide monoglycosides were acylated by the catalytic action of the protease subtilisin in anhydrous pyridine. The acylation occurred with high yield with rutin giving a single monoester on its glucose moiety. Moreover, using the subtilisin, modification reaction showed excellent selectivity. But it occurred with low yield on the glucose moiety of the two flavonoid triglycosides (Danieli et al., 1995). In the flavonoid acylation, proper enzyme often plays a multiple roles and significantly influences the regioselectivity of the reaction. Alkaline protease from *Bacillus subtilis* provided 3''-O-substituted vinyl rutin esters in pyridine, and Novozym 435 gave 4''-

O-substituted vinyl rutin esters in tert-butanol. Using this enzyme, it is also effective to control the acylation position of rutin by transesterification with divinyl carboxylates in non-aqueous media (Xiao et al., 2005).

Enzymatic transformations of flavonoids were affected by the type, origin and concentration of enzymes, nature of flavonoids, donor and optimal conditions (temperature, substrates and solvent). For the advantages of immobilized enzyme such as its reuse and simple isolation, enzymes are often used in immobilized forms in the biocatalysis of flavonoids. In addition, enzyme immobilization has been revealed in the last times as a very powerful tool to enhance enzyme stability and regioselectiviey in non-aqueous media, which the cost of enzymatic structure modification of flavonoids would be reduced.

MICROBIAL TRANSFORMATIONS OF FLAVONOIDS

Microbial transformation is an effective tool besides enzyme for the structural modification of bioactive natural and synthetic compounds including flavonoids. Microbial factories pose advantages such as rapid growth, ease of cultivation, convenient genetic manipulations, and high-level production on natural products biotransformation. Moreover, microbial production increases product selectivity and reduces the usage of toxic chemicals while conserving energy usage (Chebil et al., 2007). Biotransformations of flavonoid by many microorganisms including species of *Aspergillus* and *Bacillus* have been examined and investigated.

Aspergillus

Previous work had shown that commercial lipases and esterases from *Aspergillus* sp, are effective in the biocatalytic of flavonoids. In addition, using an *A. niger* strain, regioselective *O*-demethylation of the flavones tangeretin (1) and 3-hydroxytangeretin (6) into their 4'-*O*-demethylated metabolites was performed. The products obtained by incubation of 1, 6, and 3-methoxytangeretin (7) were found more polar than the three substrates. However, contrary to 1 and 6, 7 was slowly and only partially metabolized by both strains of *A. niger* (Buisson et al., 2007)(Figure 3).

Biotransformation of flavanone (1) by means of *A. niger MB* culture led to formation of three products: 6-hydroxyflavanone (2), 2', 5'-dihydroxydihydrochalcone (3) and 2'-hydroxydihydrochalcone (4). Their yields were 18%, 6% and 7.5% after 9 days, respectively (Kostrzewa-Sus ow et al., 2008) (Figure 4).

Flavanone (1) and 6-hydroxyflavanone (2) were subjected to transformation by one wild strain of *A. niger KB* and three UV mutants of *A. niger* (13/5, *IBR* 6/2, *SBP*). For both substrates, the biotransformation resulted in the reduction of carbonyl group (5 and 7) and dehydrogenation at C-2 and C-3 (3 and 8). Additionally, for flavanone (1), reduction of C-4 together with hydroxylation at C-7 (6) , dehydrogenation at C-2, C-3 and hydroxylation at C-3 (4) were observed (Kostrzewa-Susow et al., 2006) (Figure 5).

In the high molecular weight fraction of the culture extract of *Aspergillus oryzae*, genistein was transformed into shoyuflavone B in the presence of (±)-trans-epoxysuccinic acid (Kinoshita et al., 2000). Epigallocatechin gallate (EGCG) has been reported to convert into epigallocatechin (EGC) by a hydrolase from *Aspergillus oryzae* and the yield of EGC could reach at least 70%. However, without EGCG induction, the cultures did not show any EGCG hydrolysis activity (Zhong et al., 2008). Molds belonging to *A. niger* species are effective biocatalysts for transformations of flavonoids, which is proved by the diversity of the transformation products and high yield.

Bacillus

Bacillus was chosen to be used in transformations of flavonoids because of its safety, grow rapidly and easy to be scale-up for mass cultures. *Bacillus subtilis* natto NTU-18 in black soymilk could effectively hydrolyzed the glycosides from isoflavone and the fermented black soymilk has the potential to be applied to selective estrogen receptor modulator products (Kuo et al., 2006). However, hydrolysis of glycosides to aglycones does not enhance the bioavailability of isoflavones in humans (Richelle et al., 2002). When neohesperidin dihydrochalcone (NHDC) was modified to a series of its oligosaccharides by transglycosylation activity of *Bacillus stearothermophilus* maltogenic amylase (BSMA), major transglycosylation product was determined to be maltosyl-R-(1, 6)-neohesperidin dihydrochalcone. Additionlally, the obtained maltosyl-NHDC was 700 times more soluble in water and 7 times less sweet than NHDC (Cho et al., 2000).

The solubility can also be enhanced for puerarin when it was transglycosylated by using *Bacillus stearothermophilus maltogenic* amylase (BSMA), 14 and 168 times higher than that of puerarin (Kulikov et al., 2009). Intestinal bacteria are indispensable for the hydrolysis of flavonoid diglycosides, three anaerobic *Lactobacillus*-like strains designated as MF-01, MF-02 and MF-03 from the cecum of chicken were found to be active in the conversion of rutin and hesperidin into their aglyconic forms, while no metabolites were detected after the fermentation tests with naringin (Iqbal and Zhu, 2009).

Microorganisms grow rapidly, have ability to release products and easy to be scale-up. Metabolic engineering of microorganisms provided an alternative method of supplying valuable natural products that occur at low

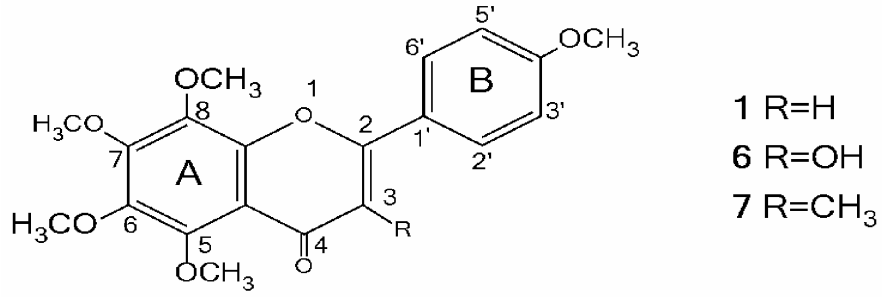

Figure 3. Tangeretin (1), 3-hydroxytangeretin (6) and 3-methoxytangeretin (7).

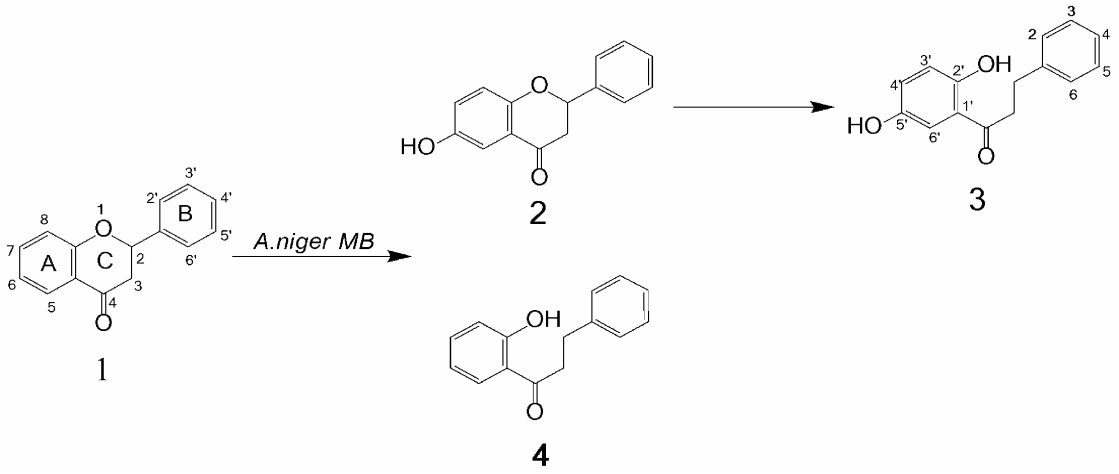

Figure 4. Biocatalysis pathway for the conversion of 1 to flavanone and altered chalcones by *A. alliaceus UI 315.*

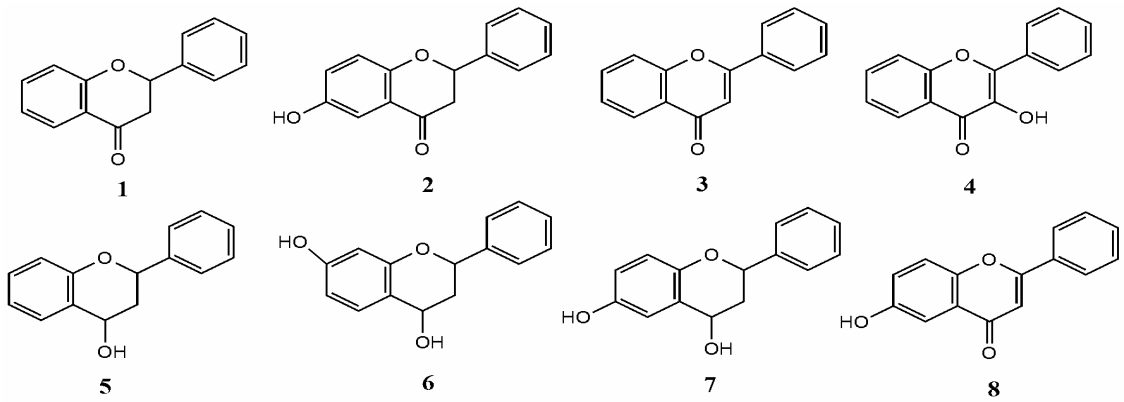

Figure 5. Chemical structure of flavanone (1) and 6-hydroxyflavanone (2) and the obtains 3, 4, 5, 6, 7, 8.

levels in nature (Cheng et al., 2009), instead of chemical synthesis. Moreover, the novel fermentation technology would help to increase the production of these natural products and render the biocatalytic process economically viable.

HETEROLOGOUS EXPRESSION OF FLAVONOIDS AND FLAVONOIDS DERIVES

Isolation and synthesis of flavonoids has become a frequent endeavor, due to their biological activities.

However, the low flavonoid concentration in planta, abundant natural resources are required for large-scale production for nutraceutical supplements. Bioreactor-based systems for mass production of flavonoids from plant cell cultures have been described for a few species, but to date, economic feasibility has not been established, partly because of engineering challenges in large-scale cultivation (Fowler and Koffas, 2009). In contrust, reconstruction of biosynthetic pathways in heterologous microorganisms offers significant promise for a scalable means to provide suitable biosynthesis pathway or sufficient quantities enzymes to product desired natural products (NPs) using inexpensive renewable resources (Sato et al., 2001). Recent efforts in flavonoid and its derivative production have focused on heterologous synthesis using well-characterized microbial hosts, such as yeast *Saccharomyces cerevisiae* and gram-negative bacterium *Escherichia coli*. The advances in the techniques of combinatorial biosynthesis have provided us with strategies to heterologously produce flavonoid compounds in bacteria.

Saccharomyces

Ro and Douglas have reported firstly to connect the two initial enzymes involved in phenylpropanoid pathway, namely phenylalanine ammonia lyase (PAL) and cinnamate 4-hydroxylase (C4H) in *Saccharomyces cerevisiae* together with a cytochrome P450 reductase (Ro and Douglas, 2004). Jiang et al. (2005) have also demonstrated the biosynthesis of flavanones, the common precursors of the vast majority of flavonoids, in *S. cerevisiae* (Jiang et al., 2005). The production of monohydroxylated naringenin and unhydroxylated pinocembrin can be carried out at levels of 7 and 0.8 mg/l, respectively. Flavanones can also be biosynthesized in *S. cerevisiae* by constructing a gene cluster that included C4H from *A. thaliana*, 4cL-2 from parsley, CHI-A (encoding for chalcone isomerase) and CHS from petunia. The recombinant *S. cerevisiae* strain was fed with phenylpropanoid acids and produced naringenin (28.3 mg/L), pinocembrin (16.3 mg/l) and the trihydroxylated flavanone eriodictyol (6.5 mg/l) (Yan et al., 2005). Isoflavonoid biosynthesis pathway can be reconstructed partially in *S. cerevisiae* by using different types of CHI and an isoflavone synthase (IFS) from soybean (Glycine max) (Ralston et al. 2005). A naringenin 8-dimethylallyltransferase cDNA (SfN8DT-1), was subcloned into a yeast expression vector (pDR196), encoding a membrane-bound enzyme which was isolated from *sophora flavescens*. The obtained enzyme is responsible for the prenylation of naringenin at the 8-position (Sasaki et al., 2009).

A bifunctional enzyme, isoflavone synthase/chalcone isomerase (IFS/CHI), was constructed by in-frame gene fusion and expressed in yeast and tobacco, petals and young leaves of IFS/CHI transgenic tobacco plants. These reconstructed hosts could produce higher levels of the isoflavone genistein and genistein glycosides as a ratio of total flavonoids produced than that plants transformed with IFS alone (Tian and Dixon, 2006). As a eukaryotic organism, *S. cerevisiae* has transcriptional and translational mechanisms similar in basic respects to the plants. This would make yeast a suitable single-celled organism for the production of secondary metabolites through the heterologous expression of plant genes. However, this expectation has never been confirmed, as in many cases the production of flavonoids in bacteria is more efficient than in eukaryotic organisms.

Escherichia coli

Escherichia coli have also been utilized as drug factory for the production of a diverse array of important pharmaceuticals. In most cases, the productivity is greatly limited by the low availability of intracellular precursors. However, engineered *E. coli* strains, which express the plant flavonoid biosynthetic pathways, allow high-yield flavonoid and its derivatives production through traditional metabolic engineering techniques (Leonard et al., 2008). Recombinant *E. coli* cells, containing four genes for a phenylalanine ammonialyase, cinnamate/ coumarate: CoA ligase, chalcone synthase, and chalcone isomerase, in addition to the acetyl-CoA carboxylase, have been established for the efficient production of (2S)-naringenin from tyrosine and (2S)-pinocembrin from phenylalanine. The engineered *E. coli* cells using the flavanone 3β-hydroxylase and flavonol synthase genes from the plant Citrus species can be applied to produce kaempferol (15.1 mg/l) from tyrosine and galangin (1.1 mg/l) from phenylalanine (Miyahisa et al., 2006).

Watts et al. (2004) have reported that phenylalanine ammonia lyase, cinnamate-4-hydroxylase, 4-coumarate: CoA ligase, and chalcone synthase from the model plant *Arabidopsis thaliana* could be cloned and co-expressed in *E. coli*. With the reconstructed strains, the high-level production of flavanone naringenin could be performed. Recombinant *E. coli* strains with the capabilities of biphenyl-2, 3-dioxygenase (BphA) and biphenyl-2, 3-dihydrodiol 2, 3-dehydrogenase (BphB) of *Burkholderia* sp. strain LB400, was also proved to biotransform 14 isoflavonoids synthesized in the laboratory (Seeger et al., 2003). The full-length cDNA clones for 18 glucosyltransferase (GT) genes were isolated from petal tissue of carnation. The 18 GTs encoded in the cDNAs can also be expressed in an *E. coli* and enzymatically characterized using chalcone, flavanone, flavone, flavonol and anthocyanidin as substrates. Three of the 18 were characterized as 3-GT possessing different substrate specificities, one for flavonoids and anthocyanidin and another two GTs catalyzed the transfer of glucose to the 2-hydroxyl group of chalcone

(Ogata et al., 2004). Other systems including bacteria, insect cells and plant cells, have also been used for the *in vitro* expression of the genes leading to fully active favonoid enzyme proteins in high yield. Heterologous expression has not only allowed extensive biochemical characterization of the respective enzymes, but also ensured the stereospecific synthesis of flavonoid substrates in profuse amounts (Martens et al., 2002). Synthetic biotechnology, an emerging field that aims of re-design and fabrication of existing biological systems, would be able to manipulate the information in living organisms more and more feasibly and realize the production of flavonoids and its derives as the pathways that occur in plants.

MEDIUM ENGINEERING

The solubility of flavonoid was strongly affected by the nature of both solvent and flavonoid structure. Recently a great impetus has been given to enzyme biocatalysis in nonaqueous medium because enzymatic catalysis in organic solvents exhibits additional advantages such as increased substrate solubility, elimination of unwanted reactions, enhanced enzyme thermo-stability (Hari Krishna, 2002).

When enzymatic acylation of a flavonoid (naringin) by the immobilized *Candida antarctica* lipase in organic media, the yield is greatly influenced by the water content, because the organic media modifies the thermodynamic equilibrium of the reaction and then directs the reaction towards hydrolysis or synthesis. Most of the water available for hydrolysis was provided by the solvent and the acyl acceptor in nonaqueous media. When flavonoid (naringin) was acylated in 2-methyl 2-butanol using the immobilized *C. antarctica* lipase, this atypic substrate for a lipase was esterified very selectively. Moreover, the drying of naringin and 2-methyl 2-butanol significantly affected the enzymatic synthesis. In both cases, the conversion yield rose by 45% compared with the standard reaction. The specific activity of lipase was increased by 22 and 28% when naringin and 2-methyl 2-butanol were dried (Gayot et al., 2003).

In ionic liquids, the solubility of flavonoids is strongly anion-dependent. The solubility changes of flavonoids were quantitatively associated with solvation interactions and structural characteristics of ionic liquids (Guo et al., 2007), reaction rates observed in ionic liquids were up to four times higher than the organic media. It is noteworthy that the reaction rate, the regioselectivity and the amount of flavonoids that can be enzymatically transformed in one-step process, are significantly higher in ionic liquids than in conventional organic media used for the enzymatic modification of flavonoids (Katsoura et al., 2006). The enzymatic esterification of esculin catalyzed by CALB (Novozym 435) was carried out in ionic liquid (IL)-acetone solvent mixed systems, conversion in most IL-acetone mixtures significantly decreased with the increase in the IL ratios. The [TOMA] 3 [Tf2N]-acetone mixture have the highest conversion in the different volume percentages. As the IL-organic solvent mixtures used in esterification, both esculin solubility and enzyme activity can be improved at the same time (Hu et al., 2009).

Of course, both organic solvents and ILs possess advantages and disadvantages. In organic solvents reactions low solubility of flavonoids was the limiting factors and bottleneck of the reaction in ionic liquids was the low enzyme activity. IL-organic solvent made it possible to improve the solubility of flavonoids while the effects of ILs on enzyme activity were minimized.

Conclusion

Biocatalysis and biotransformation of various flavonoids nature products to modify their physicochemical and biological properties have been of a great scientific and industrial interest. Enzymatic or microbial transformation of flavonoids was achieved by various enzymes and microbial. Reactions were carried out in free- or added-solvent systems and it took between a few hours and several days. When optimal conditions (temperature, enzyme, substrates and solvent) were applied, the conversion yield was often more than 95% and the properties are increased. Biotransformation in engineering microorganisms and their ability to release products provide an alternative method of supplying valuable natural products that occur in nature at low levels. Merging the potential of microbial genetics with biological and chemical diversity would offer a brighter future for natural products drug discovery.

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