 doi:10.20944/preprints202008.0699.v1

Type of the Paper (Review)

Saccharomyces Cerevisiae an Interesting Producer of Bioactive Plant Polyphenolic Metabolites

Grzegorz Chrzanowski *

Department of Biotechnology, University of Rzeszow, 1 Pigonia Street, 35-310 Rzeszow;
* Correspondence: gchrzanowski@ur.edu.pl; Tel.: +48-17-851-8753

Abstract: Secondary phenolic metabolites are defined as valuable natural products synthesized by different organisms that are not essential for growth and development. These compounds play an essential role in plant defense mechanisms, and an important role in the pharmaceutical, cosmetics, food, and agricultural industries. Despite the vast chemical diversity of natural compounds, their content in plants is very low, in consequence, it eliminates the possibility of the production of these interesting secondary metabolites from plants. Therefore, microorganisms are widely used as cell factories by industrial biotechnology to the production of different non-native compounds. Among microorganisms commonly used in biotechnological applications, yeasts are prominent host for the diverse secondary metabolite biosynthetic pathways. *Saccharomyces cerevisiae* is often regarded as the better host organism for the heterologous production of phenolics compounds, especially if the expression of different plant genes is necessary.

Keywords: heterologous production; shikimic acid pathway; phenolic acids; flavonoids; anthocyanins; stilbenes

1. Introduction

Secondary metabolites are defined as valuable natural products synthesized by different organisms that are not essential for growth and development. The plants produce over 200,000 compounds, which mostly arise from specialized metabolite pathway.

Since chemical compounds play an essential role in interspecific competition and plant defense mechanisms against biotic and abiotic stresses [1], radiation, and acting as regulatory molecules, pigments or fragrances [2]. These compounds are an integral part of our daily lives, and they play an important role in the pharmaceutical, cosmetics, food and agricultural industries. Usually, plant natural products have not shown nutritional value, but a diet rich in these substances may boost the immune system [3] or decrease level of free radicals and thus could prevent or suppress carcinogenesis [4].

Therefore, active compounds useful in medicine were intensively extracted directly from plant material. However, these methods are uneconomical or show destructive effect for the environment, in the aftermath of harvesting plants, especially protected ones. Despite the vast chemical diversity of natural compounds, their content in plants can frequently be at a low level, and it eliminates the possibility of producing these interesting secondary metabolites from plant [5]. Interesting organic compounds are also produced via chemical synthesis. However, the structural and stereochemical complexity of distinctive plant metabolites requires sophisticated methods for the synthesis. And although the chemical industry can provide varietal and useful products, it heavily relies on crude petroleum and environmentally damaging processes [6]. However, due to the limited availability and high price of natural products, their synthetic analogues have gained more and more importance in the food technology and other industries in the last decades. Following this demand, the intensive development of the chemical industry has been observed since the 1970s [7]. In Poland alone, there was in the 2018 year, the sold production of only chemicals and chemical products as well as pharmaceutical products has been estimated at over 21 billion USD [8].

However, both consumer's environmental awareness and proven toxicological effects of some synthetic compounds have nowadays caused a higher demand for natural products [9]. Microorganisms are widely used as platform cell factories by industrial biotechnology to the production of different non-native compounds, such as alcohols, terpenoids, alkaloids, phenylpropanoids, and polyketides [10]. Microbial production based on

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renewable feedstocks is relatively cheap, and the intensive growth of microorganisms provides short production times. Unlike traditional synthetic chemistry-based routes, microbial fermentations are readily scalable from the laboratory conditions to industrial-sized bioreactors [11]. Since recombinant microorganisms are usually devoid in competing pathways in relation to the heterologously expressed pathways from plants, desired natural products are typically made in the cell as chemically distinct substances [2].

Among microorganisms commonly used in biotechnological applications, yeast species are proving to be particularly suited to host diverse secondary metabolite biosynthetic pathways [12].

2. Yeast metabolism for polyphenols biosynthesis

2.1. Pyruvate and acetyl-CoA

During growth, fungi can employ two major strategies for energy production: oxidative respiration or nonoxidative fermentation. Both respiration and fermentation employ glycolysis as the central pathway. The pyruvate is an important connection between assimilatory and dissimilatory reactions, as well as it is the precursor in many metabolic pathways (Figure 1). In yeast pyruvate is oxidized into carbon dioxide and water via the tricarboxylic acid cycle (TCA). Acetyl-CoA used as the primary substrate for the TCA cycle, is generally synthesized from pyruvate during direct oxidative decarboxylation, reaction is catalyzed by the pyruvate dehydrogenase (PDH, EC 1.2.1.51) complex. Acetyl-CoA may also be produced from the pyruvate in other indirect reactions. This pyruvate dehydrogenase bypass involves three enzymes: pyruvate decarboxylase (PDC, EC 4.1.1.1), acetaldehyde dehydrogenase (ALDH, EC 1.2.1.3) and acetyl-CoA synthetase (ACS, EC 6.2.1.1) [13].

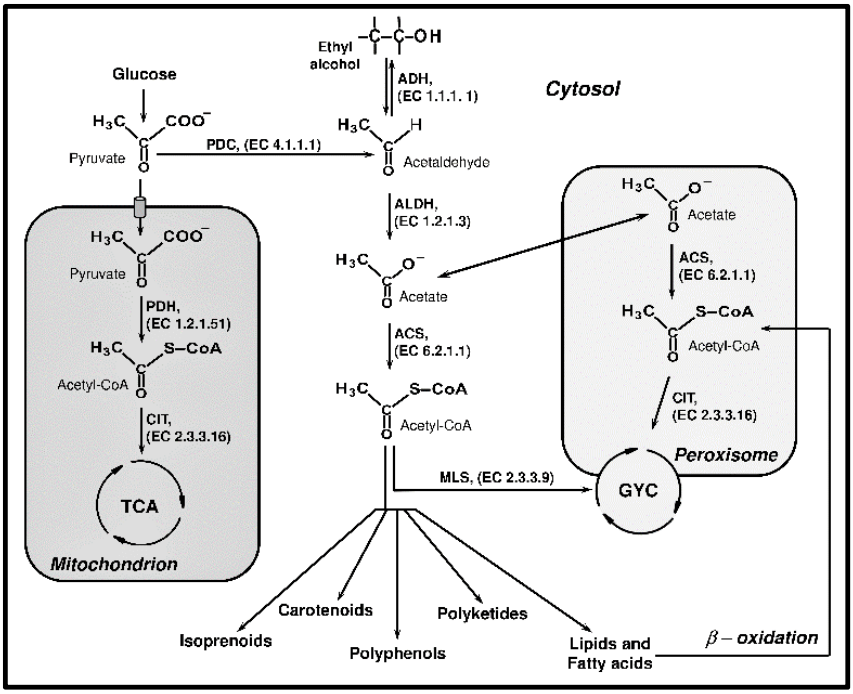

Figure 1. Metabolism of the pyruvate and acetyl-CoA in *S. cerevisiae*.

Acetyl-CoA, as well as some intermediates synthesized in the TCA cycle, are an essential biosynthetic building block in primary and secondary metabolites pathways. During fermentation, yeast produces from the pyruvate different classes of compounds, including isoprenoids, carotenoids, polyketides, polyphenols and lipids, and fatty acids.

However, these metabolites are synthesized by consuming cytosolic acetyl-CoA. Thus, it needs a transporter of it out of mitochondria or deletion of the genes encoding enzymes utilizing acetyl-CoA in mitochondria and peroxisome compartments. Moreover, some metabolic engineering manipulations have been carried out to boost the availability of acetyl-CoA in yeast [14,15].

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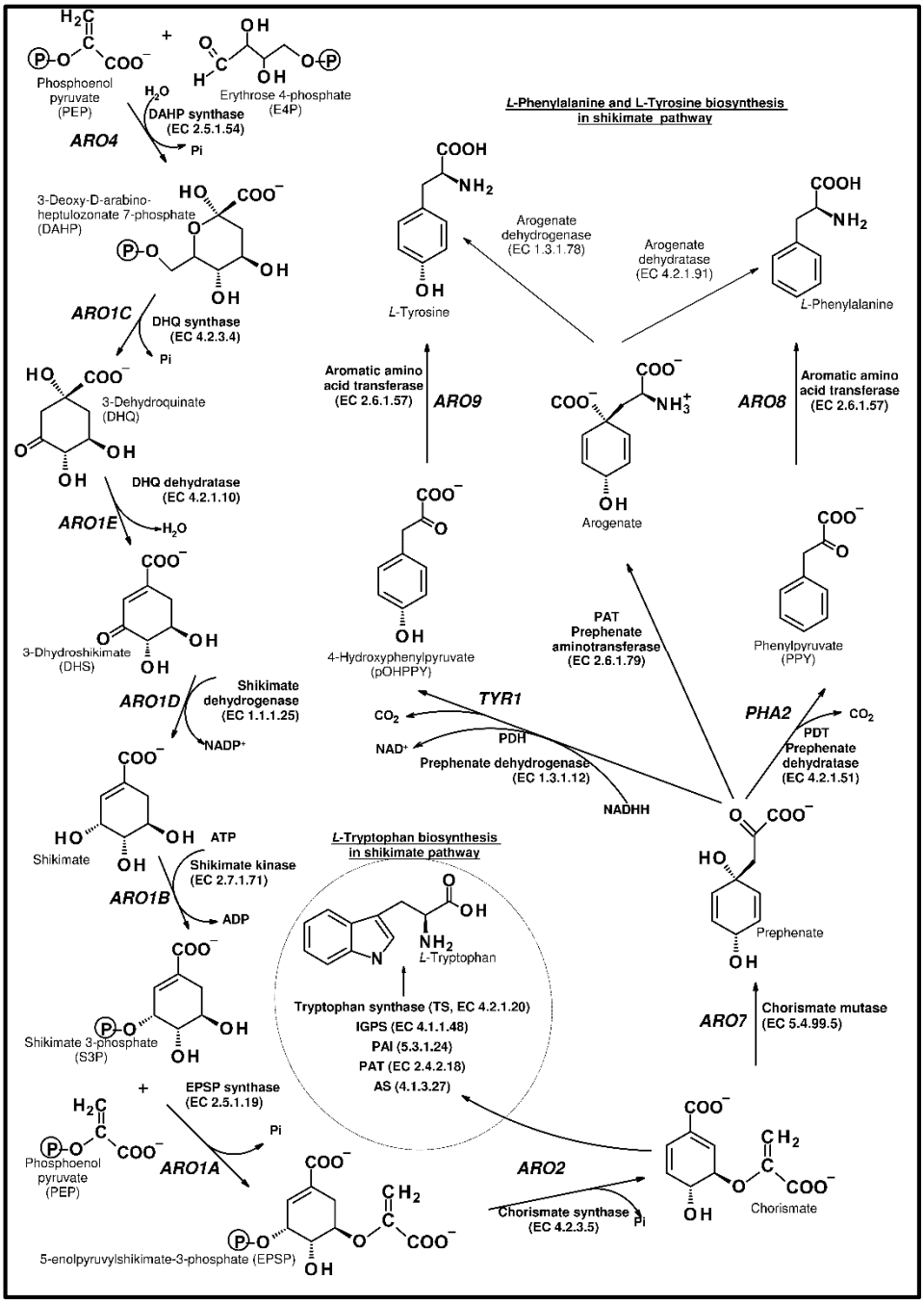

Figure 2. The aromatic amino acid pathway.

Microorganisms need to synthesize amino acids, necessary for the proteins production. Especially, aromatic amino acids are fundamental for the synthesis of these primary metabolism molecules. The pentose phosphate pathway (PPP) plays an important role in the synthesis of ribonucleotides and amino acids. Moreover, *L*-phenylalanine (*L*-Phe), *L*-Tyrosine (*L*-Tyr), and *L*-tryptophan (*L*-Trp) are produced via the shikimate pathway. These aromatic acids are not only crucial components of protein biosynthesis but also, they are precursors of the diverse phenolic secondary metabolites [12,16,17]. The shikimate pathway starts from erythrose 4-phosphate (E4P) obtained from glycolysis and phosphoenolpyruvate (PEP) derived from pentose phosphate pathway (Figure 2). And the series of reactions is invariable in different organisms, including all eukaryotic and prokaryotic cells [18]. The chorismic acid is the last intermediate of the all protein aromatic amino acids, and their derivatives such as vitamin K, ubiquinone and *p*-aminobenzoate.

A lot of important natural compounds are synthesized in mixed biosynthetic pathways. Therefore, the precise boundaries between the origin of individual classes of secondary metabolites are blurred. Shikimic and

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chorismic acids produced via the shikimic path can access the main classes of phenolic compounds, starting off simple structurally phenolic acids, such as benzoic acids, containing one benzoic ring only (C6-C1) [19]. Moreover, phenylalanine starts the phenylpropanoid path and it is the precursor of several phenylpropanoid compounds (with structure C6-C3). On the other hand, many functionalized phenylpropanoids, especially, the flavonoids are produced in the polyketide pathway involving chain elongation by malonyl-CoA. Monoterpenoid indole alkaloids arise through condensation of the tryptamine - biogenic amine produced from tryptophan and a monoterpene - secologanin. Whereas, condensation of secologanin with dopamine, giving rise to emetine and cephaeline. Several other alkaloids are formed from products of the phenylpropanoid pathway, such as substituted amphetamines through condensation of pyruvate and benzoic acid [20]. Modified amphetamines are also called phenylpropylamino alkaloids to show their origin from phenylalanine and pyruvate.

2.2. Aromatic amino acids and phenolic compounds

Aromatic amino acids are precursors of many phenolic secondary metabolites, as well as molecules such as vitamins and cofactors [21]. Many of them found applications as nutraceutical and pharmaceutical ingredients. Therefore, the shikimic acid path is attractive for the discovery of biological systems and biotechnological applications in the biosynthesis of new bioactive substances [21,22].

Enzymatic steps involved in aromatic compounds biosynthesis are similar in many, even genetically different, organisms, such as bacteria, fungi, and plants, but do not occur in animals. However, there are some fundamental differences connected with the regulation of the pathway and the function of enzymes [23].

In *S. cerevisiae*, the biosynthesis of the aromatic ring (Figure 2) starts from the reaction of the erythrose 4-phosphate (E4P) and phosphoenolpyruvate (PEP). DAHP synthase (EC 4.1.2.15) catalyses this aldol condensation, and deoxy-d-arabino-heptulosonate-7-phosphate (DAHP) is produced. In the yeast were found two synthases (ARO3 and ARO4), in bacteria *Escherichia coli* there are three isoenzymes AroF, AroG and AroH, and in *Arabidopsis thaliana* two DAHPS1 and DAHPS2. These synthases are allosterically regulated in yeast and *L*-tyrosine regulates ARO4, whereas *L*-phenylalanine controls ARO3. In other microorganisms and plants, there is no feedback regulation, but enzymes are activated by L-tryptophan [24].

In the next steps, pentafunctional ARO1 enzyme converts DAHP into 5-enolpyruvylshikimate-3-phosphate. The ARO1 is a large polypeptide that represents the fusion of five different genes. This enzyme is a mosaic of five monofunctional domains and catalyzes five different reactions. Further, ARO2 (chorismate synthase, EC 4.2.3.5) catalyzes the production of the chorismate. The flavin mononucleotide is a cofactor for the chorismate synthase. According to the capacity for regeneration of the cofactor, yeast chorismate synthase is the bifunctional enzyme with oxidoreductase activity [25]. In this point, pathway divides into two branches, one connected with phenylalanine and tyrosine biosynthesis and second towards the tryptophan production [26–28].

Analysis and characterization of the enzymatic steps leading to the synthesis of phenylalanine in bacteria have shown two alternative pathways. One is similar yeast pathway, phenylpyruvate is generated which followed transamination to Phe. The second starts with the transamination of prephenate to arogenate, which then undergoes decarboxylation/dehydration. Thus, phenylalanine may be formed from phenylpyruvate or arogenate, whereas tyrosine synthesis proceeds from either arogenate or 4-hydroxyphenylpyruvate [29]. In contrast to some bacterial species, in *S. cerevisiae* only the phenylpyruvate and 4-hydroxyphenylpyruvate paths have been suggested [16].

Overexpression of *ARO1* and *ARO2* (chorismate synthase) in *S. cerevisiae* positively influenced the production of *p*-coumaric acid. The yeast strain overexpressing *ARO1* produced 1.69 g × dm^{-3} of *p*-coumaric acid, whereas the one overexpressing *ARO2* produced 1.41 g × dm^{-3}. The simultaneous overexpression of *ARO1* and *ARO2* increased the production of *p*-coumaric acid to 1.72 g × dm^{-3}. Thus, the synthesis of p-coumaric acid by the strains with overexpression of the *ARO1* and overexpression of the both *ARO1* and *ARO2* genes was on a similar level [23].

Later, in the branch for the synthesis of *L*-phenylalanine and *L*-tyrosine, chorismic acid is converted into prephenate (PPA) with the use of chorismate mutase (EC 5.4.99.5; ARO7). Then phenylpyruvate is generated from prephenate with the use of prephenate dehydratase activity (EC 4.2.1.51, PHA2). The generation of phenylpyruvate, processes through a decarboxylation/dehydration reaction. *S. cerevisiae* has a single *PHA2* coding sequence [30], while both ADT1 and ADT2 *Arabidopsis* enzymes have demonstrated dehydratase (PDT) activity. In *Escherichia coli*, two enzymes, prephenate dehydratase and chorismate mutase (CM EC 5.4.99.5, PDT

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EC 4.2.1.51) are combined in the bifunctional P-protein (PheA), both activities are regulated by Phe-induced feedback inhibition (Zhang et al. 2000). And this catalytic protein is usually encoded by the *pheA* gene [31]. In the same branch as phenylalanine, p-hydroxyphenylpyruvate is produced from prephenate, but prephenate dehydrogenase activity (EC 1.3.1.12, TYR1) is used in this reaction instead of dehydratase during phenylpyruvate synthesis. That dehydrogenase catalyzes reactions of oxidative carboxylation and dehydration. And similarly, as dehydratase in the *E. coli*, it is bifunctional T-protein, encoded by the *tyrA* gene [31], contains discrete separable mutase, dehydrogenase and regulatory domains. Mutagenesis studies on the T-protein and kinetic experiments using substrate analogs suggest that the CM and PDH reactions occur at overlapping or perhaps proximal active sites [32,33].

The 2-oxo acids can be then transaminated to phenylalanine by aromatic amino acid transaminase (EC 2.6.1.57) – transferase I (ARO8) or II (ARO9) [29,34], using *L*-glutamate as amino group donor [35]. ARO8 is mainly effective in the generation of *L*-phenylalanine and *L*-tyrosine, whereas ARO9 involves in the catabolism of L-tryptophan. However, in strains with *ARO8* deletion, ARO9 can perform the biosynthetic function of ARO8.

Urrestarazu et al. [34] during *in vitro* research have shown, that aromatic aminotransferase I shows activity to other substrates than the aromatic amino acids. There was methionine, α-aminoadipate, and leucine also used, when phenylpyruvate was exploited as the amino group acceptor, or with their oxo-acid analogues and phenylalanine as the amino donor in the reverse reactions. Thus, it suggests, the aminotransferases may also take part in the metabolism of other than aromatic amino acids.

On the other hand, many microorganisms and in particular several yeast species, produce phenylethanol (with the characteristic rose-like aroma), directly from phenylalanine or bypassing the biosynthesis of amino acids (Figure 3), via the Ehrlich pathway [36]. In *S. cerevisiae*, decarboxylation of phenylpyruvate to phenylacetaldehyde is primarily catalyzed by the thiamine pyrophosphate - dependent 2-oxo acid decarboxylase ARO10 (EC 4.1.1.43) [37]. Earlier studies [38,39] were connected with the use of *S. cerevisiae* for the production of phenylethanol using a media supplemented with phenylalanine. However, Romagnoli et al. [40] suggest that an *ARO8Δ* mutation may be useful for *de novo* production of phenylethanol from glucose in ammonium-containing medium. They found that a combination of *ARO8* deletion with other mutations have a positive impact on the biosynthesis of aromatic alcohols. Combination of *ARO8* and *ARO3* deletions with the overexpression of feedback-insensitive DAHP synthase (*ARO4*) and chorismate mutase (*ARO7*) caused an increase in the concentration of the alcohols. Moreover, further deletion of *TYR1* gene caused an increase in phenylethanol concentration.

2.2.1. Flavonoids

Among different plant secondary metabolites, flavonoids play an important role in accordance with their antioxidant, antibacterial and anti-inflammatory activities [41,42]. According to the low concentration of flavonoids in plant sources and some difficulties with the extraction of these compounds from plant, there is a lot of interest for their producing using cell factories. Furthermore, many valuable flavonoids and stilbenoids were only found in relatively small number of plant species [43]. That group constitute a relatively diverse family of aromatic molecules that are derived from phenylalanine (or tyrosine) and malonyl-coenzyme A [44].

In plants, biosynthesis of flavonoids (Figure 3) starts from hydroxylation of cinnamic acid to *p*-coumaric acid by trans-cinnamate 4-monooxygenase (C4H, EC 1.14.14.91), or directly from *p*-coumaric acid. These phenylpropenoid acids are produced from aromatic amino acids, via their deamination with use of ammonia lyases (PAL, EC 4.3.1.23 and TAL EC 4.3.1.24). It is suggested, that PAL could catalyse the conversion of tyrosine into *p*-coumaric acid in the absence of C4H activity. *p*-Coumaric acid is then activated to *p*-coumaroyl-CoA by 4-coumarate-CoA ligase (4CL, EC 6.2.1.12). Further, chalcone synthase (CHS, EC 2.3.1.74) catalyzes the condensation of three acetate units with p-coumaroyl-CoA, and naringenin chalcone is formed. Moreover, chalcone synthase may catalyse the condensation of cinnamoyl-CoA or caffeoyl-CoA with malonyl-CoA and trihydroxychalcone and pentahydroxychalcone are formed [45].

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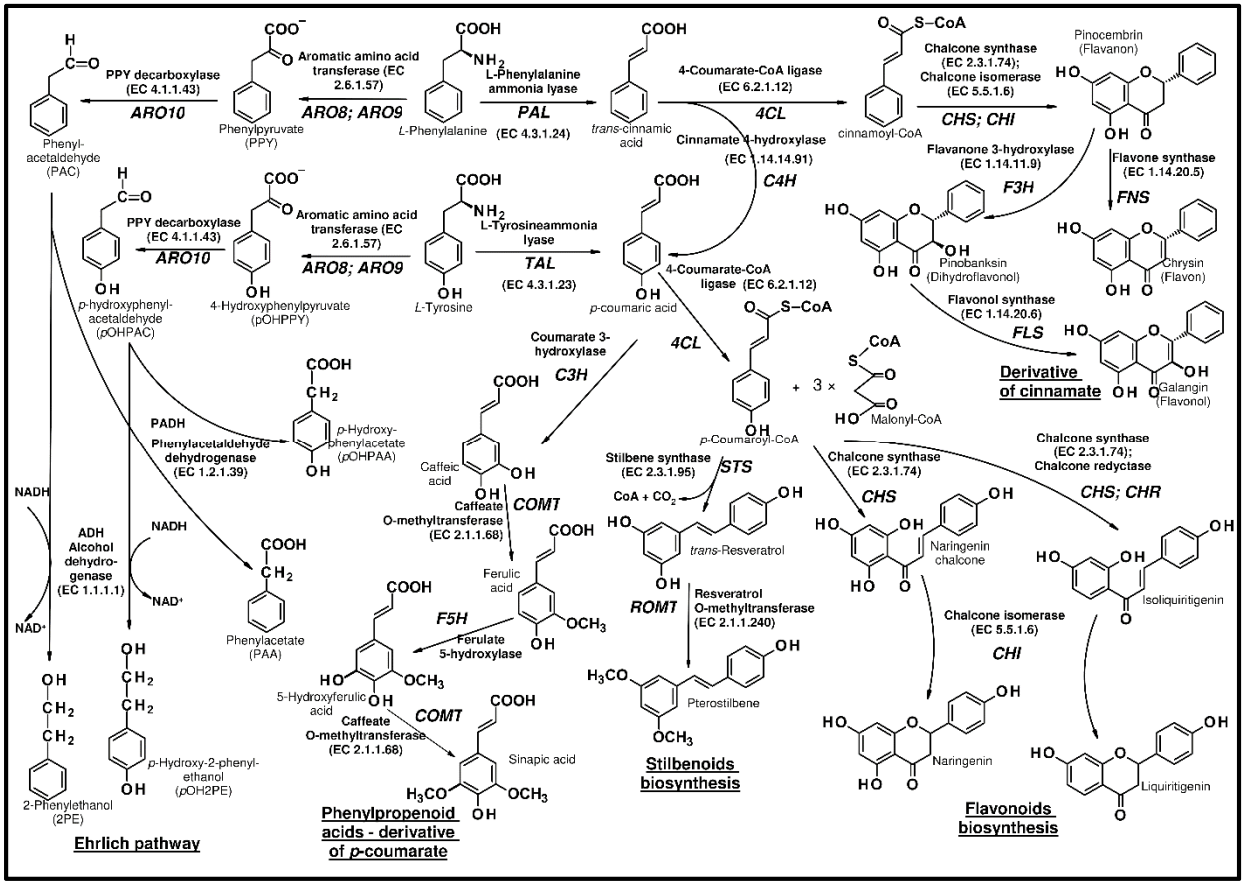

Figure 3. Metabolism of aromatic amino acids and biosynthetic pathway of polyphenols.

Following this reaction, chalcone isomerase (CHI, EC 5.5.1.6) performs stereospecific isomerization of tetrahydroxychalcone into (2S)-flavanone, which is the branch point precursor of many important downstream flavonoids. Different subclasses of flavonoids are generally classified: flavones, flavanols, flavonols, isoflavonoids, anthocyanins and proanthocyanidins [19]. On the other hand, stilbene synthase (STS, EC 2.3.1.95) catalyzes subsequent folding and cyclization of the generated tetraketide intermediate results in the production of the stilbene ring structure [45,46]. Whereas coupled catalytic action of two polyketide enzymes, chalcone synthase and chalcone reductase generate linear di-, tri-, and tetra-ketide-CoA intermediate, yielding deoxychalcone [47,48].

Subsequent transformation of naringenin (Figure 4) may produce flavanones and flavanols in reactions catalyzed by flavanone 3-hydroxylase (F3H, EC 1.14.11.9) and flavonoid 3'-hydroxylase (F3'H, EC 1.14.14.82) or flavonoid 3' 5' hydroxylase (F3'5'H, EC 1.14.14.81). F3H belongs to the 2-oxoglutarate-dependent dioxygenase (2ODD) family of enzymes. This enzyme catalyzes the hydroxylation of naringenin at 3-position and makes dihydrokaempferol (DHK), belonging to dihydroflavonols (DHF). In turn, F3'H and F3'5'H, which are P450 enzymes, catalyze the hydroxylation of the B ring derived from *p*-coumaroyl-CoA, in both flavonoids and anthocyanins [49].

As previously shown, flavonoids are biosynthesized by plants. However, yeast does not naturally produce phenylpropanoid phenolics, although its metabolism provides the necessary aromatic amino acids precursors for the further phenolic biosynthesis pathway. Thus, knowledge of microbial synthesis of these compounds is particularly attractive.

In recent years, a series of molecular biology tools have been described, and some of these techniques have already been used for transformation of the yeast for the production of the valuable secondary metabolites. Generally, these yeast strains synthesize phenolic secondary metabolites by heterologous expression of various genes from the plants and other microorganisms [50–52].

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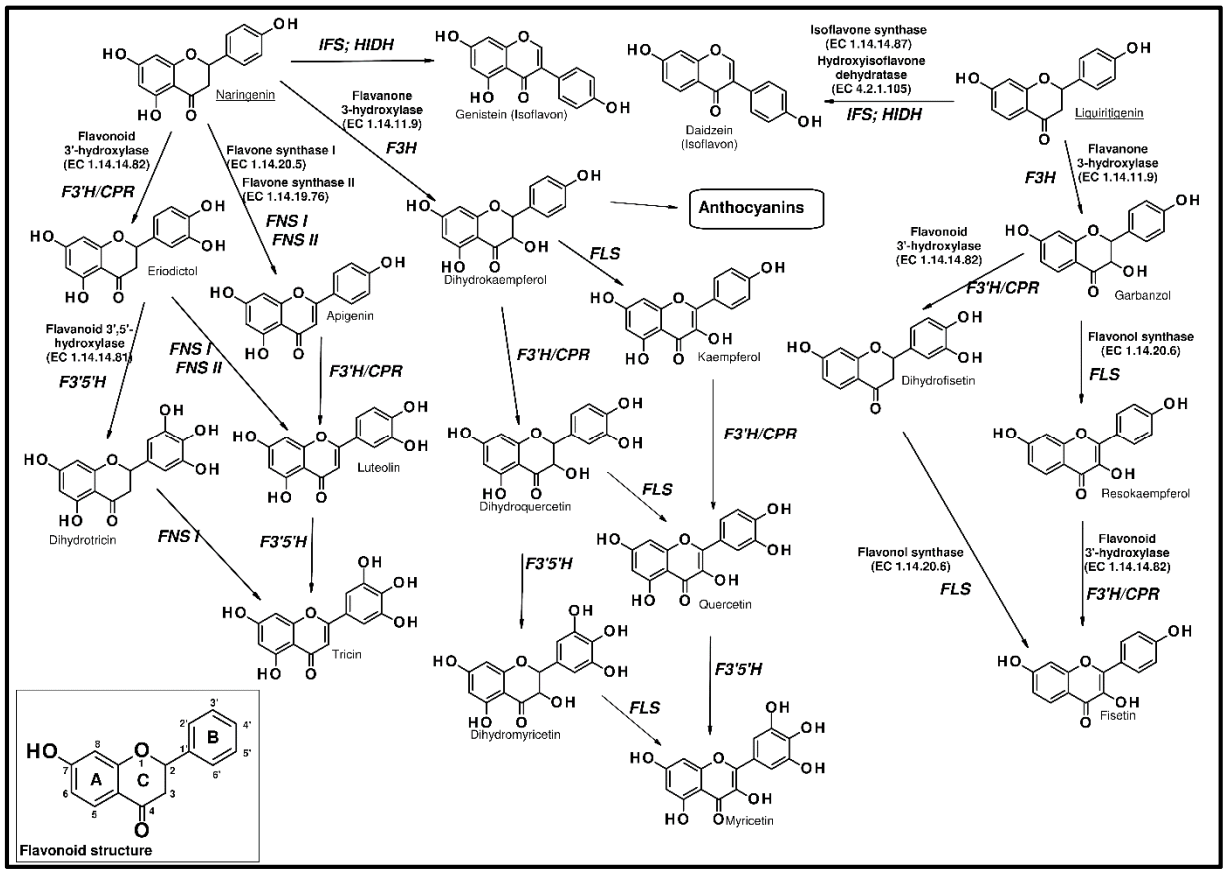

Figure 4. Flavonoid structure and biosynthetic pathways of major flavonoid compounds.

Ro and Douglas [53] have shown, that co-expression of phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and cytochrome P450 reductase (CPR) genes from poplar, caused in the production of *p*-coumaric acid by recombinant yeast. Further modification of the strains by introducing *Rhodospidrium toruloides* tyrosine ammonia-lyase (TAL) activity (that red yeast possesses the meagre PAL/TAL ratio) showed an increase in *p*-coumaric acid synthesis. There was observed conversion of tyrosine instead of phenylalanine and bypass of C4H, during the production of the phenylpropenoid acid [54].

Moreover, yeast with overexpression of the *Flavobacterium johsoniaeu TAL* and with a double knockout of *ARO10* (phenylpyruvate decarboxylase) and *PDC5* (pyruvate decarboxylase) produced 0.55 g × dm^{-3} of *p*-coumaric acid. Whereas, yeast with a single PDC5 or ARO10 deletion produced approx. 0.3 g × dm^{-3} of that phenylpropanoid acid, in contrast to wild-type strain which produce 0.24 g of that coumaric acid. On the other hand, strains overexpressing *ARO4* (DAHP synthase) and *ARO7* (chorismate mutase) were able to synthesize *p*-coumaric acid. It was also found that overexpression of some genes from *E. coli*, including dehydroquinate synthase (*AroB*), shikimate dehydrogenase (*YdiB*) or EPSP synthase (*AroA*) leads to improved production of the *p*-coumaric acid. However, overexpression of shikimate kinase (*AroL*) generated the highest enhance of the synthesis of coumaric acid, reaching 1.9 g × dm^{-3} [23] (table 1).

Therefore, construction of strains with overproduction of flavonoid-intermediates is an important and essential route for further engineering of the yeast able to the biosynthesis of polyphenols and flavonoids.

Koopman et al. [52] have demonstrated *de novo* production of naringenin (the key intermediate during flavonoid biosynthesis) from glucose, by an engineered *S. cerevisiae* strain. Combined expression of the product pathway, codon optimization, improvement of precursor supply and reduction of byproduct formation led to concentrations of over 400 µM naringenin in aerobic, glucose-grown batch cultures. Results indicate over 4-fold increase than reported in previous Santos et al. [51] study for engineered *E. coli* strain. It suggests that, *de novo* production of naringenin in *S. cerevisiae* is an important step for the production of plant-derived flavonoids from glucose.

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245 **Table 1.** Genes modification and formation of polyphenolic compounds by recombinant *Saccharomyces cerevisiae* strains

Metabolite	Yeast strain	Productivity	Genes modification [1]	References
p-Coumaric acid	ST4067	1.71 g × dm^{-3}	*ARO10*Δ, *PDC5*Δ, Fj*TAL*,Sc*ARO4*^{fbr}, Sc*ARO7*^{fbr}, Sc*ARO1*, Sc*ARO2*	Rodriguez et al. (2015) [23]
Liquiritigenin	ST5069	5.31 mg × dm^{-3}	*ARO10*Δ, *PDC5*Δ, Fj*TAL*,Sc*ARO4*^{fbr}, Sc*ARO7*^{fbr}, Pc*4CL*, Ph*CHS*, Ms*CHI*, Amo*CHR*	Rodriguez et al. (2017) [55]
Kaempferol	ST5070	26.57 mg × dm^{-3}	*ARO10*Δ, *PDC5*Δ, Fj*TAL*,Sc*ARO4*^{fbr}, Sc*ARO7*^{fbr}, Pc*4CL*, Ph*CHS*, Ms*CHI*, Am*F3H*, At*FLS*	Rodriguez et al. (2017) [55]
Quercetin	ST5074	20.38 mg × dm^{-3}	*ARO10*Δ, *PDC5*Δ, Fj*TAL*,Sc*ARO4*^{fbr}, Sc*ARO7*^{fbr}, Pc*4CL*, Ph*CHS*, Ms*CHI*, Am*F3H*, At*FLS*, Cr*CPR*, Ph*F3'H*	Rodriguez et al. (2017) [55]
Breviscapine (scutellarin and apigenin-7-O-glucuronide)	ΔMC-FU-FC-AAA	108 mg × dm^{-3} and 185 mg × dm^{-3}	Eb*4CL*, Eb*CHS*, Eb*CHI*, Eb*FNSII*, Eb*PAL*, Eb*C4H*, Eb*F6H*, Eb*CPR*, Eb*F7GAT*, Eb*UDPGDH*, *mls1*Δ, *cit2*Δ, Se*ACS*, *ALDH6*, *ADH2*	Liu et al. (2018) [56]
Resveratrol	ST4990	272.6 mg × dm^{-3}	At*PAL2*, At*C4H*, At*4CL2*,Vv*VST1*, *ACC*, *ARO7*^{fbr}, *ARO4*^{fbr}, *ARO10*Δ, Se*ACS*, At*ATR2*, Sc*CYB5*	Li et al. (2016) [57]
Pterostilbene	ST4994	5.5 mg × dm^{-3} (mineral medium) 34.9 mg × dm^{-3} (FIT medium)	At*PAL2*, At*C4H*, At*4CL2*,Vv*VST1*, *ACC*, *ARO7*^{fbr}, *ARO4*^{fbr}, *ARO10*Δ, Se*ACS*, At*ATR2*, Sc*CYB5*, Vv*ROMT*	Li et al. (2016) [57]
Pinocembrin	Yeast, harboring plasmid Ycc4c-181	16.3 mg × dm^{-3} (from cinnamic acid)	At*C4H*, Pc*4CL2*, Ph*CHS*, Ph*CHI*	Yan et al. (2005) [58]
Cyanidin 3-Oglucoside	CANS3	2.0 mg × dm^{-3}	Ha*CHS*, Ms*CHI*, At*4CL2*, At*PAL2*, Am*C4H*, Sc*CPR1*, Ph*F3'H*, At*CPR1*, Md*ANS*, Fa*A3GT2*, Md*F3H*, Pt*DFR*	Eichenberger et al. (2018) [59]
Delphinidin 3-Oglucoside	DANS6	2.1 mg × dm^{-3}	Ha*CHS*, Ms*CHI*, At*4CL2*, At*PAL2*, Am*C4H*, Sc*CPR1*, Sl*F3'5'H*, At*CPR1*, Pco*ANS*, Fa*A3GT2*, Md*F3H*	Eichenberger et al. (2018) [59]

246 [1] 4CL, 4-coumarate-CoA ligase; 4CL2, 4-coumarate-CoA ligase 2; A3GT2, Anthocyanidin 3-O-glucosyltransferase; ACC, Acetyl-CoA carboxylase; ACS, Acetyl-CoA synthetase; ADH2,
247 Alcohol dehydrogenase; ALDH6, acetaldehyde dehydrogenase; ANS, Anthocyanidin synthase; ARO1, pentafunctional enzyme converting DAHP into 5-enolpyruvylshikimate-3-
248 phosphate; ARO2, Chorismate synthase; ARO4, Deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHP synthase); ARO7, Chorismate mutase; ARO10, 2-oxo acid
249 decarboxylase; ATR2, Cytochrome P450 reductase; C4H, Cinnamate-4-hydroxylase; CHI, Chalcone isomerase; CHR, Chalcone reductase; CHS, Chalcone synthase; CIT2, Citrate
250 synthase; CPR, Cytochrome P450 reductase; CPR1, Cytochrome P450 reductase; CYB5, Cytochrome-b5 reductase; DFR, Dihydroflavonol-4-reductase; F3'5'H, Flavonoid 3' 5'
251 hydroxylase; F3'H, Flavonoid 3'-hydroxylase; F3H, Flavanone 3-hydroxylase; F6H, Flavone-6-hydroxylase; F7GAT, Flavonoid-7-O-glucuronosyltransferase; FLS, Flavonol synthase;

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FNSII, Flavone synthase II; MLS1, Malate synthase; PAL, Phenylalanine ammonia lyase; PAL2, Phenylalanine ammonia lyase 2; PDC5, Pyruvate decarboxylase; ROMT, Resveratrol O-methyltransferases; TAL, L-tyrosine ammonia lyase; UDPGDH, UDP-glucose dehydrogenase; VST1, stilbene synthase;
Am, *Ammi majus*; Amo, *Astragalus mongholicus*; At, *Arabidopsis thaliana*; Cr, *Catharanthus roseus*; Eb, *Erigeron breviscapus*; Fa, *Fragaria x ananassa*; Fj, *Flavobacterium johnsoniae*; Ha, *Hypericum androsaemum*; Md, *Malus x domestica*; Ms, *Medicago sativa*; Pc, *Petroselinum crispum*; Pco, *Pyrus communis*; Ph, *Petunia x hybrida*; Pt, *Populus trichocarpa*; Sc, *Saccharomyces cerevisiae*; Se, *Salmonella enterica*; Sl, *Solanum lycopersicum*; Vv, *Vitis vinifera*
fbr, Feedback-inhibition resistant

To enable naringenin biosynthesis in *S. cerevisiae*, Koopman et al. [52] constructed two expression vectors which include the five genes required for flavonoid biosynthesis. Moreover, cytochrome P450 reductase (CPR) gene necessary for C4H activation was added to the construct, and finally, strain IMU011 containing all these genes was built.

Since biosynthesis of aromatic amino acids is feedback inhibited by phenylalanine and tyrosine, as well as phenylethanol produced via the Ehrlich pathway, causes a reduction of flavonoids biosynthesis, further necessary modifications were made. In the received IMX198 strain, deletions of genes (*ARO3*, *ARO10*, *PDC5*, *PDC6*), and introduction feedback resistant DAHP synthase allele $ARO4^{G226S}$, *A. thaliana* genes (*AtPAL1*, *coC4H*, *coCPR1*, *AtCHI1*, *AtCHS3*, *coCHS3*, *At4CL3*) as well as *Rhodobacter capsulatus coTAL1* gene, were applied.

Further utilisation of naringenin by engineered yeast strains may cause biosynthesis of genistein, kaempferol and quercetin [60]. Addition of naringenin as the flavonoid precursor, yeast has produced kaempferol at the level of 4.6 mg × dm^{-3} after 70 h of growth, while genistein producing strain, generated 7.7 mg × dm^{-3} of that isoflavonoid after 180 h. The lowest concentration of flavonoids, during naringenin feeding, created quercetin producing yeast strain, and its level reached only 0.38 mg × dm^{-3} after 70 h. Jiang et al. [61] found that *S. cerevisiae* strains are able to production of the naringenin and pinocembrin. However, it required an introduction of the phenylpropanoid pathway within yeast cells. It was accomplished by expression of phenylalanine ammonia-lyase (*PAL*) from *Rhodosporidium toruloides*, 4-coumarate-CoA ligase (*4CL*) from *A. thaliana*, and chalcone synthase (*CHS*) from *Hypericum androsaemum*.

Rodriguez et al. [55] constructed platform strains for the production of different classes of flavonoids by modification parental yeast strains produced *p*-coumaric acid. They have shown that naringenin was produced in strain with overexpression of three genes (*4CL*, *CHS* and *CHI*). Whereas for liquiritigenin synthesis, chalcone reductase gene (*CHR*) was additionally overexpressed. The naringenin- and liquiritigenin-producing strains were further engineered for a generation of kaempferol and resokaempferol, respectively. There were overexpressing the genes encoding flavanone 3-hydroxylase (*F3H*) and flavonol synthase (*FLS*). In another experiment, they constructed strains for biosynthesis of quercetin and fisetin. However, the overexpression of cytochrome P450 flavonoid monooxygenase (*FMO*) and cytochrome P450 reductase (*CPR*) was necessary.

2.2.2. Anthocyanins

Anthocyanins are one of the most important plant pigments, and they are responsible for most of the red, blue, and purple colours of leaves, fruits, and flowers. Anthocyanins are considered as the flavonoids due to their C6-C3-C6 chemical structure, although they have a positive charge at the oxygen atom of the C-ring of basic flavonoid structure (Figure 5) [62].

Based on cell-line studies, animal models, and human clinical trials, it has been suggested that anthocyanins exhibit anti-inflammatory, anti-carcinogenic activity, and prevent cardiovascular diseases. They effectively diminish the level of free radicals and terminate the chain reaction that is responsible for the oxidative damage [63].

Anthocyanins are produced in a specific branch of the flavonoid pathway. From naringenin, they are biosynthesized by flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR, EC 1.1.1.219), and anthocyanidin synthase (ANS, EC 1.14.20.4). Especially, anthocyanidin synthase has been characterized as a multifunctional protein catalyzing several reactions with different flavonoid substrate intermediates. The final reaction, converted anthocyanidins into anthocyanins, is catalyzed by an anthocyanidin 3-*O*-glucosyltransferase (3GT, EC 2.4.1.115) [64,65].

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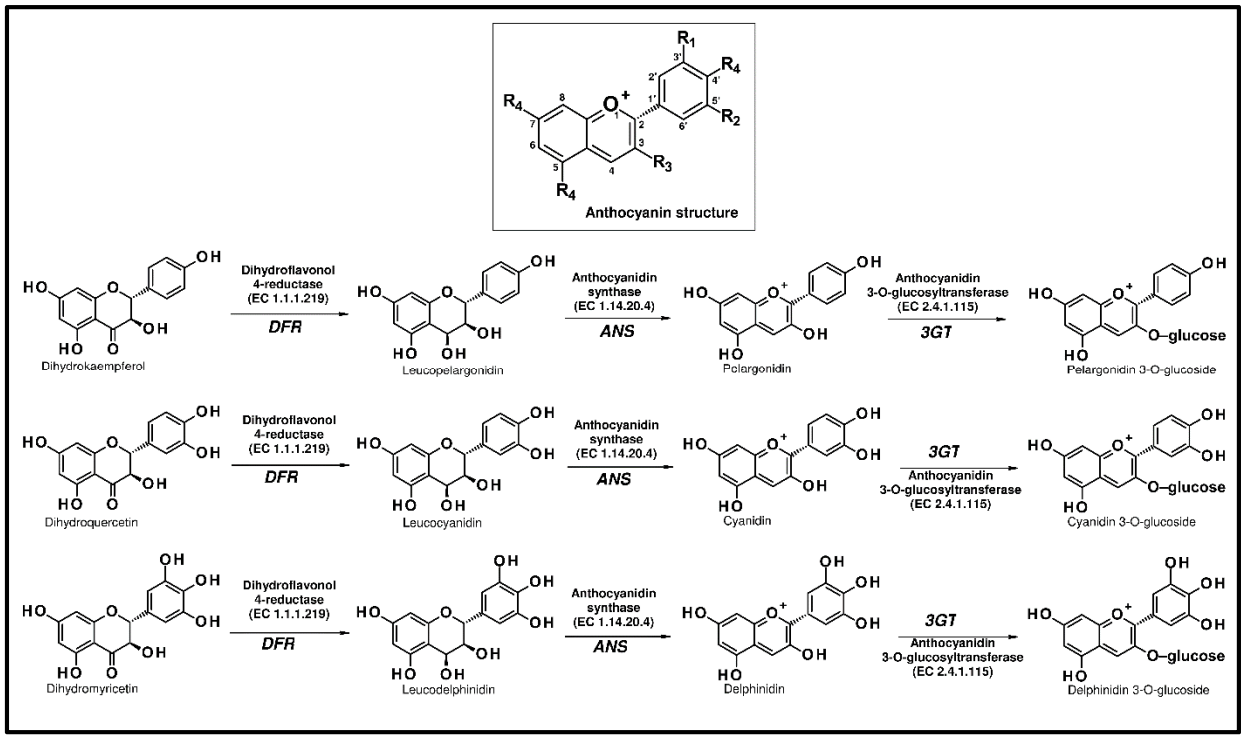

Figure 5. Anthocyanin structure and biosynthetic pathway (ANS, anthocyanidin synthase; DFR, dihydroflavonol 4-reductase; 3GT: flavonoid 3-*O*-glucosyltransferase).

Plant cells generally produce complex mixtures of polyphenolic compounds, and they are difficult to stabilize and engineer. Levisson et al. [65], for the biosynthesis of pelargonidin 3-*O*-glucoside, have used the earlier constructed by Koopman et al. (2012) naringenin-producing IMX106 strain. They showed the production of anthocyanins from naringenin after subsequent insertion of the genes (*F3H*, *ANS*, and *3GT*). Moreover, further modification of the strain IMK393 showed *de novo* production of kaempferol, kaempferol 3-*O*-glycoside, and pelargonidin. There were introduced genes for naringenin biosynthesis and the elimination of the Ehrlich pathway. In the next step, the genes of anthocyanin synthesis were incorporated, deleted genes encoding glucosidases, as well as the pathway of phloretic acid synthesis was abolished. The total sum of extracellular flavonoids at the end of the glucose consumption phase was 70.4 µM, consisting mostly of dihydrokaempferol with 59.9 µM. Whereas, the concentration of flavonoids reached 202.3 µM at the end of the fermentation.

Initially, Wellman et al. [66] have proposed that anthocyanidins derive from naringenin via dihydroflavonols and leucocyanidins. And these compounds may eventually be oxidized by anthocyanidin synthase (ANS). However, their investigations were carried out on *E. coli*. On the other hand, they have been put into question the role of ANS, because the recombinant enzyme from *Arabidopsis* exhibited primarily flavonol synthase (FLS) activity, with negligible ANS activity. They were shown that ANS and FLS, might select dihydroflavonoid substrates for the catalyzed reaction. Recombinant ANS from *Gerbera hybrida* converted (+)-catechin into two primaries and one vestige products, whereas (−)-catechin, (−)-epicatechin, (+)-epicatechin and (−)-gallocatechin were not accepted. The Km value for (+)-catechin was determined at 175 µM, and LC-MS and NMR analysis showed a presence of the 4,4-dimer of oxidized (+)-catechin (93%), cyanidin (7%) and a traces of quercetin.

The full-length pathways to the most ACN structures require the action of multiple plant CYP enzymes, and they are usually difficult to express in bacterial hosts (Kim et al. 2011). Thus, *S. cerevisiae* is often regarded as the better host organism for the heterologous production of anthocyanins.

Eichenberger et al. [59] successfully have reconstituted the full pathway to the biosynthesis of pelargonidin-3-*O*-glucoside (P3G), cyanidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside within *S. cerevisiae*. It was also reported, that yeast strain, used in the experiment has not been specifically optimized for providing the relevant precursors,as well as the optimization of the conditions of growth were not performed. Moreover, they suggest the potential for efficient ACN production in yeast. In particular, an efficient hydroxylation of naringenin by F3'H and F3'5'H demonstrates the ability of S. cerevisiae to functionally express plant CYPs. The concentration of eriodictyol obtained from glucose is within the same order of magnitude as the highest titers reported in *E.*

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coli by feeding with phenylalanine [67] or caffeic acid [68]. They have also suggested, heterologous production of 5,7,3',4',5'-pentahydroxy-flavanone (PHF) has not been reported in microorganisms.

Eichenberger et al. (2018) have also tested the activity of DFR, by branching off the pathway towards flavan-3-ols (F3Os) by including a leukoanthocyanidin reductase (LAR) enzyme. And some DFRs converted dihydroflavonols into F3Os via the instable LCD intermediate almost entirely, demonstrating a high catalytic activity of both proteins. Therefore, following Yan et al. [69] and Huang et al. [70], it has been suggested that DFR might represent a rate-limiting step in the ACN pathway.

On the other hand, these results provide new information, which could be used in the study of proanthocyanidins, for which F3Os are the precursors.

2.2.3. Stilbenoids

Another group of compounds produced via shikimic acid and polyketide pathways are non-flavonoid polyphenolic secondary metabolites - stilbenoids. Natural stilbenes are a group of polyphenols characterized by the presence of a 1,2-diphenylethylene nucleus [71]. They are generally plant-produced substances, and similarly to flavonoids play defensive role against environmental stresses, such as UV radiation or fungal infection. Moreover, according to their anticancer and antiinflammatory activities, may be used as drug [72]. Stilbenoids are well-known chemicals produced by bacteria, however, investigations including modification of the growth media clearly show that they are synthesis by yeast [73].

Becker et al. [74], as the first, have reported resveratrol biosynthesis ability of *S. cerevisiae*. In *p*-coumaric acid - fed strains, they introduced 4-coumarate-CoA ligase gene (*4CL*) from hybrid poplar (*Populus trichocarpa* × *Populus deltoides*) and other gene trihydroxystilbene synthase (*STS*) from wine grape (*Vitis vinifera*). Three years later, Zhang et al. [75] obtained resveratrol in the same yeast strain by expression of *4CL* from *A. thaliana*, and *STS* from *V. vinifera*. Despite the apparent increase in the concentration of resveratrol in modified strains of yeast, in *E. coli* resveratrol was produced at a significantly higher level [76]. However, the possibility of heterologous gene expression in the yeast, as well as the modification of the media content for their growth, create the opportunity to acquire sufficient content of synthesized components. Sydor et al. [73] found, that *S. cerevisiae* expressing the *A. thaliana* 4-coumaroyl-coenzyme A ligase (*4CL1*) and the *Vitis vinifera* stilbene synthase (*STS*) and the use of rich medium considerably improved resveratrol production, up to 391 mg/L.

In another study, Li et al. [77] introduced multiple copies of the genes Ha*TAL*, At*4CL1*, and Vv*VST1* into a strain, over-expressing Sc*ARO4*^{K229L}, Sc*ARO7*^{G141S}, and Sc*ACC1*$^{S659A, S1157A}$. After fed-batch cultivations, the best producer named ST4152 finally synthesized approx. 415.65 mg per L of resveratrol in the glucose feeding phase, whereas with the feeding of ethanol, the highest titer reached 531.41 mg per L of resveratrol.

3. Conclusion

This review is focused on the important biosynthesis pathways for aromatic amino acids and further phenolic compounds, including crossing with the polyketides path. This branched pathway serves as a model system for understanding a yeast-based production of natural phenolic secondary metabolites. In particular, these compounds, according to their antioxidant, antibacterial, and anti-inflammatory activities, make an increasing interest in developing polyphenols-rich functional foods. During the last years, the different genes involved in the biosynthesis of polyphenols have been identified and characterized. However, a better understanding of their expression in yeast cell platforms is crucial to achieving the desired success.

In this work, it has been described the heterologous expression of the plant genes into yeast to make them able to the production of major groups of plant-specific polyphenols. Besides, it was showed an effect of the overexpression, deletions, as well as feedback regulation of mutually existing genes on the generation of the expected product.

The synthesis of natural compounds from a cheap carbon source by microbial fermentation is attractive due to short process time, feedstock uniformity, and high purity of the product. However, after establishing the appropriate pathway in the yeast and demonstrating the production of the desired metabolite, further optimization to obtain desirable *product yield* is necessary.

The author of this review hopes that this paper highlights the importance and advantages of phenylpropanoids, flavonoids, anthocyanins, and stilbenes production by yeast in order to promote further research in this field.

 doi:10.20944/preprints202008.0699.v1

Funding: This work was supported by the scientific program of the Institute of Biology and Biotechnology, University of Rzeszow.

Acknowledgments: The author wishes to thank Justyna Ruchala for her criticism and helpful suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

4CL	4-coumarate-CoA ligase
4CL2	4-coumarate-CoA ligase 2
A3GT2	Anthocyanidin 3-O-glucosyltransferase
ACC	Acetyl-CoA carboxylase
ACS	Acetyl-CoA synthetase
ADH2	Alcohol dehydrogenase
ALDH6	Acetaldehyde dehydrogenase
ANS	Anthocyanidin synthase
ARO1	Pentafunctional enzyme converting DAHP into 5-enolpyruvylshikimate-3-phosphate
ARO2	Chorismate synthase
ARO4	Deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHP synthase)
ARO7	Chorismate mutase
ARO8	Aromatic amino acid transferase
ARO9	Aromatic amino acid transferase
ARO10	2-oxo acid decarboxylase
ATR2	Cytochrome P450 reductase
C4H	Cinnamate-4-hydroxylase
CHI	Chalcone isomerase
CHR	Chalcone reductase
CHS	Chalcone synthase
CIT2	Citrate synthase
CPR	Cytochrome P450 reductase
CPR1	Cytochrome P450 reductase
CYB5	Cytochrome-b5 reductase
DFR	Dihydroflavonol-4-reductase
F3′5′H	Flavonoid 3' 5' hydroxylase
F3′H	Flavonoid 3'-hydroxylase
F3H	Flavanone 3-hydroxylase
F6H	Flavone-6-hydroxylase
F7GAT	Flavonoid-7-O-glucuronosyltransferase
FLS	Flavonol synthase
FNSI	Flavone synthase I
FNSII	Flavone synthase II
MLS1	Malate synthase
PAL	Phenylalanine ammonia lyase
PAL2	Phenylalanine ammonia lyase 2
PDC5	Pyruvate decarboxylase
ROMT	Resveratrol O-methyltransferases
TAL	*L*-tyrosine ammonia lyase
UDPGDH	UDP-glucose dehydrogenase
VST1	stilbene synthase
Am	*Ammi majus*
Amo	*Astragalus mongholicus*
At	*Arabidopsis thaliana*
Cr	*Catharanthus roseus*
Eb	*Erigeron breviscapus*
Fa	*Fragaria x ananassa*
Fj	*Flavobacterium johnsoniae*

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Ha	*Hypericum androsaemum*
Md	*Malus x domestica*
Ms	*Medicago sativa*
Pc	*Petroselinum crispum*
Pco	*Pyrus communis*
Ph	*Petunia x hybrida*
Pt	*Populus trichocarpa*
Sc	*Saccharomyces cerevisiae*
Se	*Salmonella enterica*
Sl	*Solanum lycopersicum*
Vv	*Vitis vinifera*
fbr	Feedback-inhibition resistant
co	Codon optimized

References

1. Czerniewicz, P.; Sytykiewicz, H.; Durak, R.; Borowiak-Sobkowiak, B.; Chrzanowski, G. Role of phenolic compounds during antioxidative responses of winter triticale to aphid and beetle attack. *Plant Physiology and Biochemistry* **2017**, *118*, 529–540, doi:10.1016/j.plaphy.2017.07.024.
2. Marienhagen, J.; Bott, M. Metabolic engineering of microorganisms for the synthesis of plant natural products. *Journal of Biotechnology* **2013**, *163*, 166–178, doi:10.1016/j.jbiotec.2012.06.001.
3. Licciardi, P.V.; Underwood, J.R. Plant-derived medicines: A novel class of immunological adjuvants. *International Immunopharmacology* **2011**, *11*, 390–398, doi:10.1016/j.intimp.2010.10.014.
4. Tan, A.C.; Konczak, I.; Sze, D.M.-Y.; Ramzan, I. Molecular Pathways for Cancer Chemoprevention by Dietary Phytochemicals. *Nutrition and Cancer* **2011**, *63*, 495–505, doi:10.1080/01635581.2011.538953.
5. Atanasov, A.G.; Waltenberger, B.; Pferschy-Wenzig, E.-M.; Linder, T.; Wawrosch, C.; Uhrin, P.; Temml, V.; Wang, L.; Schwaiger, S.; Heiss, E.H.; et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances* **2015**, *33*, 1582–1614, doi:10.1016/j.biotechadv.2015.08.001.
6. Dhamankar, H.; Prather, K.L. Microbial chemical factories: recent advances in pathway engineering for synthesis of value added chemicals. *Current Opinion in Structural Biology* **2011**, *21*, 488–494, doi:10.1016/j.sbi.2011.05.001.
7. Chen, Y.; Nielsen, J. Advances in metabolic pathway and strain engineering paving the way for sustainable production of chemical building blocks. *Current Opinion in Biotechnology* **2013**, *24*, 965–972, doi:10.1016/j.copbio.2013.03.008.
8. GUS Production of industrial products in 2018 Available online: https://stat.gov.pl/en/topics/industry-construction-fixed-assets/industry/production-of-industrial-products-in-2018,9,2.html (accessed on Apr 14, 2020).
9. Sowbhagya, H.B.; Chitra, V.N. Enzyme-Assisted Extraction of Flavorings and Colorants from Plant Materials. *Critical Reviews in Food Science and Nutrition* **2010**, *50*, 146–161, doi:10.1080/10408390802248775.
10. Nielsen, J. Synthetic Biology for Engineering Acetyl Coenzyme A Metabolism in Yeast. *mBio* **2014**, *5*, e02153-14, doi:10.1128/mBio.02153-14.

11. Walker, R.S.K.; Pretorius, I.S. Applications of Yeast Synthetic Biology Geared towards the Production of Biopharmaceuticals. *Genes* **2018**, *9*, 340, doi:10.3390/genes9070340.
12. Pyne, M.E.; Narcross, L.; Martin, V.J.J. Engineering Plant Secondary Metabolism in Microbial Systems. *Plant Physiol.* **2019**, *179*, 844, doi:10.1104/pp.18.01291.
13. Pronk, J.T.; Steensma, H.Y.; Van Dijken, J.P. Pyruvate Metabolism in Saccharomyces cerevisiae. *Yeast* **1996**, *12*, 1607–1633, doi:10.1002/(SICI)1097-0061(199612)12:16<1607::AID-YEA70>3.0.CO;2-4.
14. Strijbis, K.; Distel, B. Intracellular Acetyl Unit Transport in Fungal Carbon Metabolism. *Eukaryot Cell* **2010**, *9*, 1809–1815, doi:10.1128/EC.00172-10.
15. Chen, Y.; Daviet, L.; Schalk, M.; Siewers, V.; Nielsen, J. Establishing a platform cell factory through engineering of yeast acetyl-CoA metabolism. *Metabolic Engineering* **2013**, *15*, 48–54, doi:10.1016/j.ymben.2012.11.002.
16. Braus, G.H. Aromatic amino acid biosynthesis in the yeast Saccharomyces cerevisiae: a model system for the regulation of a eukaryotic biosynthetic pathway. *Microbiol Rev* **1991**, *55*, 349–370.
17. Kobayashi, Y.; Sahara, T.; Ohgiya, S.; Kamagata, Y.; Fujimori, K.E. Systematic optimization of gene expression of pentose phosphate pathway enhances ethanol production from a glucose/xylose mixed medium in a recombinant Saccharomyces cerevisiae. *AMB Express* **2018**, *8*, doi:10.1186/s13568-018-0670-8.
18. Carlquist, M.; Gibson, B.; Yuceer, Y.K.; Paraskevopoulou, A.; Sandell, M.; Angelov, A.I.; Gotcheva, V.; Angelov, A.D.; Etschmann, M.; Billerbeck, G.M. de; et al. Process engineering for bioflavour production with metabolically active yeasts – a mini-review. *Yeast* **2015**, *32*, 123–143, doi:10.1002/yea.3058.
19. Ferrer, J.-L.; Austin, M.B.; Stewart, C.; Noel, J.P. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiology and Biochemistry* **2008**, *46*, 356–370, doi:10.1016/j.plaphy.2007.12.009.
20. Hagel, J.M.; Krizevski, R.; Marsolais, F.; Lewinsohn, E.; Facchini, P.J. Biosynthesis of amphetamine analogs in plants. *Trends Plant Sci.* **2012**, *17*, 404–412, doi:10.1016/j.tplants.2012.03.004.
21. Lamichhane, G.; Freundlich, J.S.; Ekins, S.; Wickramaratne, N.; Nolan, S.T.; Bishai, W.R. Essential Metabolites of Mycobacterium tuberculosis and Their Mimics. *mBio* **2011**, *2*, doi:10.1128/mBio.00301-10.
22. Scotti, L.; Bezerra Mendonça Junior, F.J.; Magalhaes Moreira, D.R.; da Silva, M.S.; Pitta, I.R.; Scotti, M.T. SAR, QSAR and docking of anticancer flavonoids and variants: a review. *Curr Top Med Chem* **2012**, *12*, 2785–2809, doi:10.2174/1568026611212240007.
23. Rodriguez, A.; Kildegaard, K.R.; Li, M.; Borodina, I.; Nielsen, J. Establishment of a yeast platform strain for production of p-coumaric acid through metabolic engineering of aromatic amino acid biosynthesis. *Metabolic Engineering* **2015**, *31*, 181–188, doi:10.1016/j.ymben.2015.08.003.
24. Maeda, H.; Dudareva, N. The Shikimate Pathway and Aromatic Amino Acid Biosynthesis in Plants. *Annual Review of Plant Biology* **2012**, *63*, 73–105, doi:10.1146/annurev-arplant-042811-105439.

25. Quevillon-Cheruel, S.; Leulliot, N.; Meyer, P.; Graille, M.; Bremang, M.; Blondeau, K.; Sorel, I.; Poupon, A.; Janin, J.; Tilbeurgh, H. van Crystal Structure of the Bifunctional Chorismate Synthase from Saccharomyces cerevisiae. *J. Biol. Chem.* **2004**, *279*, 619–625, doi:10.1074/jbc.M310380200.
26. Duncan, K.; Edwards, R.M.; Coggins, J.R. The Saccharomyces cerevisiae ARO1 gene An example of the co-ordinate regulation of five enzymes on a single biosynthetic pathway. *FEBS Letters* **1988**, *241*, 83–88, doi:10.1016/0014-5793(88)81036-6.
27. Jones, D.G.L.; Reusser, U.; Braus, G.H. Molecular cloning, characterization and analysis of the regulation of the AR02 gene, encoding chorismate synthase, of Saccharomyces cerevisiae. *Molecular Microbiology* **1991**, *5*, 2143–2153, doi:10.1111/j.1365-2958.1991.tb02144.x.
28. Helmstaedt, K.; Strittmatter, A.; Lipscomb, W.N.; Braus, G.H. Evolution of 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase-encoding genes in the yeast Saccharomyces cerevisiae. *PNAS* **2005**, *102*, 9784–9789, doi:10.1073/pnas.0504238102.
29. Bross, C.D.; Corea, O.R.A.; Kaldis, A.; Menassa, R.; Bernards, M.A.; Kohalmi, S.E. Complementation of the pha2 yeast mutant suggests functional differences for arogenate dehydratases from Arabidopsis thaliana. *Plant Physiology and Biochemistry* **2011**, *49*, 882–890, doi:10.1016/j.plaphy.2011.02.010.
30. Maftahi, M.; Nicaud, J.-M.; Levesque, H.; Gaillardin, C. Sequencing analysis of a 24·7 kb fragment of yeast chromosome XIV identifies six known genes, a new member of the hexose transporter family and ten new open reading frames. *Yeast* **1995**, *11*, 1077–1085, doi:10.1002/yea.320111109.
31. Parthasarathy, A.; Cross, P.J.; Dobson, R.C.J.; Adams, L.E.; Savka, M.A.; Hudson, A.O. A Three-Ring Circus: Metabolism of the Three Proteogenic Aromatic Amino Acids and Their Role in the Health of Plants and Animals. *Front. Mol. Biosci.* **2018**, *5*, doi:10.3389/fmolb.2018.00029.
32. Vincent, S.; Chen, S.; Wilson, D.B.; Ganem, B. Probing the overlap of chorismate mutase and prephenate dehydrogenase sites in the escherichia coli T-protein: a dehydrogenase-selective inhibitor. *Bioorganic & Medicinal Chemistry Letters* **2002**, *12*, 929–931, doi:10.1016/S0960-894X(02)00053-7.
33. Hassing, E.-J.; de Groot, P.A.; Marquenie, V.R.; Pronk, J.T.; Daran, J.-M.G. Connecting central carbon and aromatic amino acid metabolisms to improve de novo 2-phenylethanol production in Saccharomyces cerevisiae. *Metabolic Engineering* **2019**, *56*, 165–180, doi:10.1016/j.ymben.2019.09.011.
34. Urrestarazu, A.; Vissers, S.; Iraqui, I.; Grenson, M. Phenylalanine- and tyrosine-auxotrophic mutants of Saccharomyces cerevisiae impaired in transamination. *Mol Gen Genet* **1998**, *257*, 230–237, doi:10.1007/s004380050643.
35. Enzyme Database - BRENDA Available online: https://www.brenda-enzymes.org/ (accessed on Jul 31, 2020).
36. Hazelwood, L.A.; Daran, J.-M.; van Maris, A.J.A.; Pronk, J.T.; Dickinson, J.R. The Ehrlich pathway for fusel alcohol production: a century of research on Saccharomyces cerevisiae metabolism. *Appl. Environ. Microbiol.* **2008**, *74*, 2259–2266, doi:10.1128/AEM.02625-07.

37. Vuralhan, Z.; Luttik, M.A.H.; Tai, S.L.; Boer, V.M.; Morais, M.A.; Schipper, D.; Almering, M.J.H.; Kötter, P.; Dickinson, J.R.; Daran, J.-M.; et al. Physiological characterization of the ARO10-dependent, broad-substrate-specificity 2-oxo acid decarboxylase activity of Saccharomyces cerevisiae. *Appl. Environ. Microbiol.* **2005**, *71*, 3276–3284, doi:10.1128/AEM.71.6.3276-3284.2005.
38. Cui, Z.; Yang, X.; Shen, Q.; Wang, K.; Zhu, T. Optimisation of biotransformation conditions for production of 2-phenylethanol by a Saccharomyces cerevisiae CWY132 mutant. *Natural Product Research* **2011**, *25*, 754–759, doi:10.1080/14786419.2010.529441.
39. Kim, B.; Cho, B.-R.; Hahn, J.-S. Metabolic engineering of Saccharomyces cerevisiae for the production of 2-phenylethanol via Ehrlich pathway. *Biotechnology and Bioengineering* **2014**, *111*, 115–124, doi:10.1002/bit.24993.
40. Romagnoli, G.; Knijnenburg, T.A.; Liti, G.; Louis, E.J.; Pronk, J.T.; Daran, J.-M. Deletion of the Saccharomyces cerevisiae ARO8 gene, encoding an aromatic amino acid transaminase, enhances phenylethanol production from glucose. *Yeast* **2015**, *32*, 29–45, doi:10.1002/yea.3015.
41. Xu, Y.-B.; Chen, G.-L.; Guo, M.-Q. Antioxidant and Anti-Inflammatory Activities of the Crude Extracts of Moringa oleifera from Kenya and Their Correlations with Flavonoids. *Antioxidants (Basel)* **2019**, *8*, doi:10.3390/antiox8080296.
42. Adamczak, A.; Ożarowski, M.; Karpiński, T.M. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. *Journal of Clinical Medicine* **2020**, *9*, 109, doi:10.3390/jcm9010109.
43. Ververidis, F.; Trantas, E.; Douglas, C.; Vollmer, G.; Kretzschmar, G.; Panopoulos, N. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnology Journal* **2007**, *2*, 1214–1234, doi:10.1002/biot.200700084.
44. Winkel-Shirley, B. Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. *Plant Physiology* **2001**, *126*, 485–493, doi:10.1104/pp.126.2.485.
45. Petrussa, E.; Braidot, E.; Zancani, M.; Peresson, C.; Bertolini, A.; Patui, S.; Vianello, A. Plant Flavonoids—Biosynthesis, Transport and Involvement in Stress Responses. *International Journal of Molecular Sciences* **2013**, *14*, 14950–14973, doi:10.3390/ijms140714950.
46. Watts, K.T.; Lee, P.C.; Schmidt-Dannert, C. Biosynthesis of plant-specific stilbene polyketides in metabolically engineered Escherichia coli. *BMC Biotechnol* **2006**, *6*, 22, doi:10.1186/1472-6750-6-22.
47. Bomati, E.K.; Austin, M.B.; Bowman, M.E.; Dixon, R.A.; Noel, J.P. Structural Elucidation of Chalcone Reductase and Implications for Deoxychalcone Biosynthesis. *J. Biol. Chem.* **2005**, *280*, 30496–30503, doi:10.1074/jbc.M502239200.
48. Mameda, R.; Waki, T.; Kawai, Y.; Takahashi, S.; Nakayama, T. Involvement of chalcone reductase in the soybean isoflavone metabolon: identification of GmCHR5, which interacts with 2-hydroxyisoflavanone synthase. *The Plant Journal* **2018**, *96*, 56–74, doi:10.1111/tpj.14014.

49. Tanaka, Y.; Sasaki, N.; Ohmiya, A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Plant J.* **2008**, *54*, 733–749, doi:10.1111/j.1365-313X.2008.03447.x.
50. Leonard, E.; Lim, K.-H.; Saw, P.-N.; Koffas, M.A.G. Engineering Central Metabolic Pathways for High-Level Flavonoid Production in Escherichia coli. *Appl Environ Microbiol* **2007**, *73*, 3877–3886, doi:10.1128/AEM.00200-07.
51. Santos, C.N.S.; Koffas, M.; Stephanopoulos, G. Optimization of a heterologous pathway for the production of flavonoids from glucose. *Metabolic Engineering* **2011**, *13*, 392–400, doi:10.1016/j.ymben.2011.02.002.
52. Koopman, F.; Beekwilder, J.; Crimi, B.; van Houwelingen, A.; Hall, R.D.; Bosch, D.; van Maris, A.J.; Pronk, J.T.; Daran, J.-M. De novo production of the flavonoid naringenin in engineered Saccharomyces cerevisiae. *Microbial Cell Factories* **2012**, *11*, 155, doi:10.1186/1475-2859-11-155.
53. Ro, D.-K.; Douglas, C.J. Reconstitution of the Entry Point of Plant Phenylpropanoid Metabolism in Yeast (Saccharomyces cerevisiae) IMPLICATIONS FOR CONTROL OF METABOLIC FLUX INTO THE PHENYLPROPANOID PATHWAY. *J. Biol. Chem.* **2004**, *279*, 2600–2607, doi:10.1074/jbc.M309951200.
54. Vannelli, T.; Wei Qi, W.; Sweigard, J.; Gatenby, A.A.; Sariaslani, F.S. Production of p-hydroxycinnamic acid from glucose in Saccharomyces cerevisiae and Escherichia coli by expression of heterologous genes from plants and fungi. *Metabolic Engineering* **2007**, *9*, 142–151, doi:10.1016/j.ymben.2006.11.001.
55. Rodriguez, A.; Strucko, T.; Stahlhut, S.G.; Kristensen, M.; Svenssen, D.K.; Forster, J.; Nielsen, J.; Borodina, I. Metabolic engineering of yeast for fermentative production of flavonoids. *Bioresource Technology* **2017**, *245*, 1645–1654, doi:10.1016/j.biortech.2017.06.043.
56. Liu, X.; Cheng, J.; Zhang, G.; Ding, W.; Duan, L.; Yang, J.; Kui, L.; Cheng, X.; Ruan, J.; Fan, W.; et al. Engineering yeast for the production of breviscapine by genomic analysis and synthetic biology approaches. *Nat Commun* **2018**, *9*, doi:10.1038/s41467-018-02883-z.
57. Li, M.; Schneider, K.; Kristensen, M.; Borodina, I.; Nielsen, J. Engineering yeast for high-level production of stilbenoid antioxidants. *Scientific Reports* **2016**, *6*, 36827, doi:10.1038/srep36827.
58. Yan, Y.; Kohli, A.; Koffas, M.A.G. Biosynthesis of Natural Flavanones in Saccharomyces cerevisiae. *Appl Environ Microbiol* **2005**, *71*, 5610–5613, doi:10.1128/AEM.71.9.5610-5613.2005.
59. Eichenberger, M.; Hansson, A.; Fischer, D.; Dürr, L.; Naesby, M. De novo biosynthesis of anthocyanins in Saccharomyces cerevisiae. *FEMS Yeast Res* **2018**, *18*, doi:10.1093/femsyr/foy046.
60. Trantas, E.; Panopoulos, N.; Ververidis, F. Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in Saccharomyces cerevisiae. *Metabolic Engineering* **2009**, *11*, 355–366, doi:10.1016/j.ymben.2009.07.004.

61. Jiang, H.; Wood, K.V.; Morgan, J.A. Metabolic Engineering of the Phenylpropanoid Pathway in Saccharomyces cerevisiae. *Appl Environ Microbiol* **2005**, *71*, 2962–2969, doi:10.1128/AEM.71.6.2962-2969.2005.
62. Kong, J.-M.; Chia, L.-S.; Goh, N.-K.; Chia, T.-F.; Brouillard, R. Analysis and biological activities of anthocyanins. *Phytochemistry* **2003**, *64*, 923–933, doi:10.1016/S0031-9422(03)00438-2.
63. He, J.; Giusti, M.M. Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annu. Rev. Food Sci. Technol.* **2010**, *1*, 163–187, doi:10.1146/annurev.food.080708.100754.
64. Petroni, K.; Tonelli, C. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Science* **2011**, *181*, 219–229, doi:10.1016/j.plantsci.2011.05.009.
65. Levisson, M.; Patinios, C.; Hein, S.; de Groot, P.A.; Daran, J.-M.; Hall, R.D.; Martens, S.; Beekwilder, J. Engineering de novo anthocyanin production in Saccharomyces cerevisiae. *Microbial Cell Factories* **2018**, *17*, 103, doi:10.1186/s12934-018-0951-6.
66. Wellmann, F.; Griesser, M.; Schwab, W.; Martens, S.; Eisenreich, W.; Matern, U.; Lukačin, R. Anthocyanidin synthase from Gerbera hybrida catalyzes the conversion of (+)-catechin to cyanidin and a novel procyanidin. *FEBS Letters* **2006**, *580*, 1642–1648, doi:10.1016/j.febslet.2006.02.004.
67. Zhu, S.; Wu, J.; Du, G.; Zhou, J.; Chen, J. Efficient Synthesis of Eriodictyol from l-Tyrosine in Escherichia coli. *Appl Environ Microbiol* **2014**, *80*, 3072–3080, doi:10.1128/AEM.03986-13.
68. Fowler, Z.L.; Gikandi, W.W.; Koffas, M.A.G. Increased Malonyl Coenzyme A Biosynthesis by Tuning the Escherichia coli Metabolic Network and Its Application to Flavanone Production. *Appl Environ Microbiol* **2009**, *75*, 5831–5839, doi:10.1128/AEM.00270-09.
69. Yan, Y.; Li, Z.; Koffas, M.A.G. High-yield anthocyanin biosynthesis in engineered Escherichia coli. *Biotechnology and Bioengineering* **2008**, *100*, 126–140, doi:10.1002/bit.21721.
70. Huang, Y.; Gou, J.; Jia, Z.; Yang, L.; Sun, Y.; Xiao, X.; Song, F.; Luo, K. Molecular Cloning and Characterization of Two Genes Encoding Dihydroflavonol-4-Reductase from Populus trichocarpa. *PLoS One* **2012**, *7*, doi:10.1371/journal.pone.0030364.
71. Rivière, C.; Pawlus, A.D.; Mérillon, J.-M. Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Nat. Prod. Rep.* **2012**, *29*, 1317–1333, doi:10.1039/C2NP20049J.
72. Sirerol, J.A.; Rodríguez, M.L.; Mena, S.; Asensi, M.A.; Estrela, J.M.; Ortega, A.L. Role of Natural Stilbenes in the Prevention of Cancer. *Oxid Med Cell Longev* **2016**, *2016*, doi:10.1155/2016/3128951.
73. Sydor, T.; Schaffer, S.; Boles, E. Considerable Increase in Resveratrol Production by Recombinant Industrial Yeast Strains with Use of Rich Medium. *Appl Environ Microbiol* **2010**, *76*, 3361–3363, doi:10.1128/AEM.02796-09.
74. Becker, J.V.W.; Armstrong, G.O.; Merwe, M.J. van der; Lambrechts, M.G.; Vivier, M.A.; Pretorius, I.S. Metabolic engineering of Saccharomyces cerevisiae for the

 doi:10.20944/preprints202008.0699.v1

synthesis of the wine-related antioxidant resveratrol. *FEMS Yeast Research* **2003**, *4*, 79–85, doi:10.1016/S1567-1356(03)00157-0.

75. Zhang, Y.; Li, S.-Z.; Li, J.; Pan, X.; Cahoon, R.E.; Jaworski, J.G.; Wang, X.; Jez, J.M.; Chen, F.; Yu, O. Using Unnatural Protein Fusions to Engineer Resveratrol Biosynthesis in Yeast and Mammalian Cells. *J. Am. Chem. Soc.* **2006**, *128*, 13030–13031, doi:10.1021/ja0622094.
76. Beekwilder, J.; Wolswinkel, R.; Jonker, H.; Hall, R.; de Vos, C.H.R.; Bovy, A. Production of Resveratrol in Recombinant Microorganisms. *Appl Environ Microbiol* **2006**, *72*, 5670–5672, doi:10.1128/AEM.00609-06.
77. Li, M.; Kildegaard, K.R.; Chen, Y.; Rodriguez, A.; Borodina, I.; Nielsen, J. De novo production of resveratrol from glucose or ethanol by engineered Saccharomyces cerevisiae. *Metabolic Engineering* **2015**, *32*, 1–11, doi:10.1016/j.ymben.2015.08.007.