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MINI-REVIEW

Biosynthesis of resveratrol and piceatannol in engineered microbial strains: achievements and perspectives

Anil Shrestha[1] · Ramesh Prasad Pandey[1,2] · Jae Kyung Sohng[1,2]

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Abstract

Resveratrol (3,5,4′-trihydroxystilbene) and piceatannol (3,5,3′,4′-tetrahydroxystilbene) are well-known natural products that are produced by plants. They are important ingredients in pharmaceutical industries and nutritional supplements. They display a wide spectrum of biological activity. Thus, the needs for these compounds are increasing. The natural products have been found in diverse plants, mostly such as grapes, passion fruit, white tea, berries, and many more. The extraction of these products from plants is quite impractical because of the low production in plants, downstream processing difficulties, chemical hazards, and environmental issues. Thus, alternative production in microbial hosts has been devised with combinatorial biosynthetic systems, including metabolic engineering, synthetic biology, and optimization in production process. Since the biosynthesis is not native in microbial hosts such as *Escherichia coli*, *Saccharomyces cerevisiae*, and *Corynebacterium glutamicum*, genetic engineering and manipulation have made it possible. In this review, the discussion will mainly focus on recent progress in production of resveratrol and piceatannol, including the various strategies used for their production.

Keywords Resveratrol · Piceatannol · Metabolic engineering · Pathway engineering · Combinatorial synthesis

Introduction

Resveratrol (3,5,4′-trihydroxystilbene) (Fig. 1), a well-known plant-derived polyphenol, is basically used as a nutritional supplement and medicinal component (Baur and Sinclair 2006). It is a naturally occurring secondary metabolite biosynthesized by plants commonly found in grapes, peanuts, and berry fruits. Since the discovery of resveratrol for the first time from white hellebore *Veratrum grandiflorum* (Takaoka 1939), various beneficial aspects of resveratrol have been reported. A familiar "French Paradox" phenomenon was believed to be the consequences of resveratrol, one of the vital components in red wine (Bhullar and Hubbard 2015; Borriello et al. 2014; Yang et al. 2014). On this basis, various studies, including preclinical and human trials, concluded the commodious effect on the cardiovascular system, and significant protective activities against cancers (Hubbard and Sinclair 2014; Baur and Sinclair 2006). Moreover, the clinical trial results demonstrated that the direct scavenging effects of resveratrol was significantly lower but exhibited as a gene regulator of redox genes, like inhibiting NADPH oxidase and mitochondrial superoxide production (Xia et al. 2017). The pharmacological potency in the treatment against neurodegenerative disorders, like Parkinson's, Alzheimer's, and Huntington's diseases, amyotrophic lateral sclerosis had been evaluated in vivo, as well as in vitro (Tellone et al. 2015; Rege et al. 2014; Anekonda 2006; Wang et al. 2006; Vingtdeux et al. 2008).

Bru and colleagues in 2006 reported that abiotic and biotic stresses induce the synthesis of resveratrol in grapevine culture. Potent anti-fungal activities have also been reported, indicating that the *trans*-isoform of resveratrol is more biologically active than *cis*-conformation (Fernández-Mar et al. 2012; Savoia 2012; Mikulski et al. 2010). The pharmacological activities have been apprehended due to having multiple targets in cells, cellular processes, and signaling pathways in inflammation. Likewise, it has been reported that it diminishes

Anil Shrestha and Ramesh Prasad Pandey contributed equally to this work.

✉ Jae Kyung Sohng
sohng@sunmoon.ac.kr

[1] Department of Life Science and Biochemical Engineering, Sun Moon University, 70 Sunmoon-ro 221, Tangjeong-myeon, Asan-si, Chungnam 31460, Republic of Korea

[2] Department of Pharmaceutical Engineering and Biotechnology, Sun Moon University, 70 Sunmoon-ro 221, Tangjeong-myeon, Asan-si, Chungnam 31460, Republic of Korea

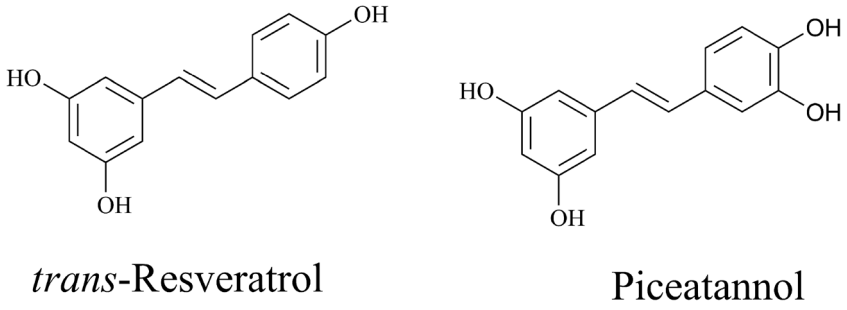

Fig. 1 Structures of *trans*-resveratrol and piceatannol

oxidative stress and plays significant roles in apoptosis, anti-aging effects, and potent anti-influenza activities (Poulsen et al. 2015; Frombaum et al. 2012; Kalra et al. 2008; Baur and Sinclair 2006; Maeurer et al. 2016). Similarly, it scavenges free radicals, inhibits lipid peroxidation, increases the efflux of cholesterol, and offers beneficial effects on the neurological and cardiovascular systems of human health (Lorenz et al. 2003; Berrougui et al. 2009; Okawara et al. 2007; Bradamante et al. 2004). Resveratrol has been found to enhance transcription factor miR-200c in lung cancer cell, showing stronger anti-tumor activity (Bai et al. 2014). Moreover, Jang and colleagues in 1997 concluded that resveratrol also acts as an anti-mutagen and anti-initiation activity, anti-promotion activity, and anti-progression activity in the major stages of carcinogenesis process. On the other hand, it has been reported that resveratrol can elongate the life span of lab-tested microorganisms like yeast, and eukaryotes, like fruit flies, nematodes, and mice, indicating it is a potential anti-aging agent (De La Lastra and Villegas 2005; Holthoff et al. 2010; Stervbo et al. 2007; Whitlock and Baek 2012; Lancon et al. 2012; Baur et al. 2006).

Piceatannol (3,5,3′,4′-tetrahydroxystilbene) (Fig. 1) is a naturally occurring 3′-hydroxylated product of resveratrol that is found in various plants. It was first isolated from the heartwood of *Vouacapoua americana* (King et al. 1956) and has been extracted from various plants like *Polygonum cuspidatums*, passion fruit (*Passiflora edulis*), *Arachis hypogaea*, *Vitis thunbergii*, and many more (Beňová et al. 2008; Matsui et al. 2010; Sano et al. 2011; Lin et al. 2007). But the important sources of piceatannol in edible form are grapes and wine. However, the amount of piceatannol in these natural sources is relatively low, compared to resveratrol (Cantos et al. 2003). Previous reports suggested that the biosynthesis of piceatannol was increased due to abiotic stimuli such as UV irradiation, and heavy metal contamination in soils, as well as by fungal infection (Ku et al. 2005). Piceatannol was metabolized by cytochrome P450 (CYP1B1), which was indeed overexpressed by human tumors and enabled the removal of selective cancer cells (Potter et al. 2002; McFadyen and Murray 2005). Cell culture model study showed its enhanced pharmaceutical activity against DNA damage caused by –OH radicals in certain leukemic cells due to *ortho*-dihydroxy structure (Ovesná et al. 2006). Moreover, it was reported that piceatannol was also responsible for preventing the cells from free radical associated damage induced by cumene hydroperoxide. Likewise, it has shown anti-tyrosinase activity during melanogenesis, thus inhibiting the production of melanin (Wittgen and van Kempen 2007). Reports have concluded that piceatannol had stronger anti-tyrosinase activity than resveratrol (Yokozawa and Kim 2007). Lucas et al. in 2018 demonstrated that resveratrol and piceatannol modulated the expression of programmed cell death ligand 1 (PD-L1) in breast and colorectal cancer cells. Recent finding in animal model study showed that resveratrol and piceatannol enact positive direct effects on atrial electrophysiology and stabilize atrial repolarization (Frommeyer et al. 2018). Moreover, along with the piceatannol in human mesenchymal stem cells, it endowed polyphenol with the ability to restrict the formation of lipids in these adipocyte-originated matured cells. It was also revealed that it lowered the glucose transport into adipocytes and diminished the major components of lipogenic pathway (Carpéné et al. 2018). In a different study carried by Maruki-Uchida et al. (2018), it was concluded that the oral consumption of piceatannol-enriched passion fruit seed extract improved the moisture content of the skin. Piceatannol also promulgated anti-biofilm activity against the *Streptococcus mutans* via the inhibition of the virulence factor Gtfs. On the other hand, it inhibited *S. mutans*-induced carcinogenicity in vivo (Nijampatnam et al. 2018). All these recent studies demonstrated the significance of resveratrol and piceatannol in diverse area.

This mini-review concentrates on the metabolic engineering of microbial cells for their use in the enhanced production of resveratrol and piceatannol. The review briefly discusses the biosynthetic pathway and the key genes involved in resveratrol and piceatannol biosynthesis that contemporary researchers manipulate for enhanced production.

Biosynthesis of resveratrol and piceatannol

The biosynthesis of resveratrol and its derivatives is initiated through the shikimate pathway from phenylpropanoid acids.

These phenylpropanoid acids are derived from aromatic amino acids. The starting basic amino acids, like L-phenylalanine and L-tyrosine, are converted to the phenylpropanoid acids: cinnamic acid or *p*-coumaric acid, respectively, via non-oxidative deamination by phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), which are then converted to cinnamoyl-CoA and *p*-coumaroyl-CoA by 4-coumaroyl-CoA ligase (4-CL) (MacDonald and D'Cunha 2007; Rosler et al. 1997; Hamberger and Hahlbrock 2004). Finally, malonyl-CoA is condensed with cinnamoyl-CoA or *p*-coumaroyl-CoA to produce pinosylvin or resveratrol, respectively, catalyzed by stilbene synthases (STSs) (Fig. 2). Resveratrol can be a platform for achieving several derivatives through the modifications by tailoring enzymes such as hydroxylases, *O*-methyltransferases, and glucosyltransferases, whereby diverse resveratrol analogs are generated.

Bioproduction of resveratrol and piceatannol in microorganisms

Microorganisms have been the vital sources for the production of pharmaceutical valued compounds for a long time, because of the low-cost value, as well as ease of manipulation of the genetic constituents; metabolic engineering, protein engineering, and synthetic biotechnological tools can be efficiently and economically employed thereof. Moreover, heterologous production in non-native host diminishes the growth rate to be

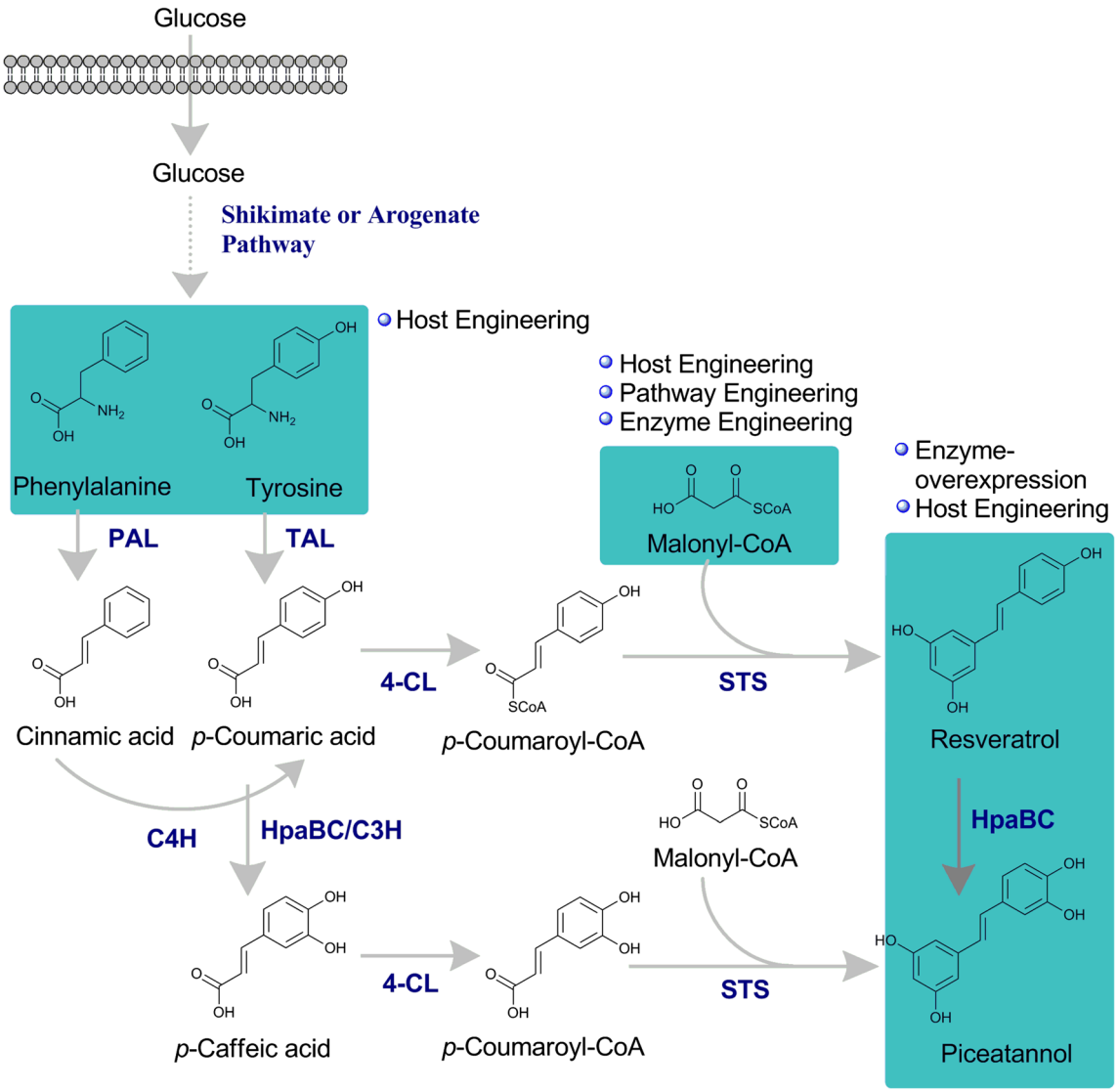

Fig. 2 Biosynthesis pathway of resveratrol and piceatannol. The heterologous biosynthesis of these two molecules is carried out in different strains. The major metabolic engineering approaches were focused on precursor biosynthesis (L-tyrosine and L-phenylalanine), biosynthesis of extender-CoA substrate (malonyl-CoA), and hydroxylation of resveratrol to piceatannol. The approaches used for the engineering are highlighted above or next to the box. TAL tyrosine ammonia lyase, PAL phenylalanine ammonia lyase, 4-CL 4-coumaroyl-CoA ligase, C4H coumarate 4-hydroxylase, C3H coumarate 3-hydroxylase, STS stilbene synthase, HpaBC monooxygenase complex

slower than that of the native host, and significant quantities of target compounds can be produced (Sun et al. 2015). Naturally, resveratrol and piceatannol are not produced by microbes. On the contrary, coadunation of the heterologous pathways from the native host such as plants to the microbes by virtue of genetic engineering has resulted in the efficient and enhanced production of these compounds (Halls and Yu 2008; Mei et al. 2015; Du et al. 2011). Various studies have reported the remarkable productions through implementation of heterologous pathway in prokaryotes, as well as eukaryotes, such as *Escherichia coli*, *Lactococcus lactis*, and *Corynebacterium glutamicum*, and in yeasts, such as *Saccharomyces cerevisiae*.

Biosynthesis of resveratrol and piceatannol in non-*E. coli* hosts

Stilbenes, particularly *trans*-resveratrol, are widely distributed in a number of plant families. The majority of them belong to the genus *Vitis* in Vitaceae family. The well-known species for this include *V. venifera*, *V. labrusca, V. riparia,* and *V. rotundifolia* (Almagro et al. 2013). Although in the course of the biosynthesis of resveratrol and its analogues, STSs play a key role, this enzyme is not ubiquitously expressed, and its number is limited in plant (Chong et al. 2009; Rivière et al. 2012). So, external stresses induce the production of these compounds in plants. Though resveratrol is biosynthesized in numerous plant species, piceatannol is only known to be produced by few species of plants. In contrast, it was isolated from *Pinus strobus* and pinosylvin synthase was suspected for biosynthesis as it showed multifunctional activities (Raiber et al. 1995). Likewise, many reports revealed the biosynthesis of piceatannol in various plant species with abiotic and biotic stimuli (Kiselev et al. 2016; Deng et al. 2017; Boue et al. 2013; Sergent et al. 2014; Lambert et al. 2013). The strong induction of UV-light enriched red wine–producing grapes with stilbenes (Cantos et al. 2003). Moreover, biotic stresses such as fungi elicited the production of resveratrol and piceatannol (Paul et al. 1998; Bavaresco et al. 2003; Vezzulli et al. 2007; Sobolev 2008). For details on biosynthesis of resveratrol and piceatannol using different stimuli, please refer to Dubrovina and Kiselev (2017).

Saccharomyces cerevisiae has been one ideal host for the production of resveratrol. It is a Generally Regarded As Safe (GRAS) microbe, used in the expression of plant-derived enzymes, and genetic manipulation as well (Krivoruchko and Nielsen 2015; Yesilirmak and Sayers 2009). Moreover, eukaryotic post translational modifications can be easily achieved (Sahdev et al. 2008; Rosano and Ceccarelli 2014). In addition, yeast cells have similar intracellular compartments like plants which facilitate the better expression of eukaryotic proteins and membrane proteins (Jiang et al. 2005; Rodrigues et al. 2015). In yeast, basically two enzymes, 4-coumarate: coenzyme A ligase (4-CL) and STS, have been implemented in heterologous biosynthetic pathway for the production of resveratrol. Milligram to almost grams level titers of resveratrol have been reported, by introducing bacterial or plants-originated 4-CL and STS. Beekwilder et al. (2006) for the first time incorporated 4-CL2 from *Nicotiana tabacum* and STS from *Vitis vinifera* to yeast and obtained the titer of 6 mg/L of resveratrol in yeast. Different reports showed various titers of resveratrol by introducing genes from different sources in engineered yeast (Sydor et al. 2010; Shin et al. 2011; Wang et al. 2011; Wang and Yu 2012; Li et al. 2015, 2016).

Besides yeast, engineered *Corynebacterium glutamicum* has also been employed for the production of resveratrol. Kallscheuer et al. (2016) depicted engineered *C. glutamicum* (Δ*phd B*, Δ *pcaF*, and Δ*pobA*) for the production of resveratrol using *p*-Coumaric acid as substrate along with cerulenin in which 158 mg/L of resveratrol was obtained. On the other hand, in the presence of 25 μl cerulenin and 5 mM caffeic acid in *C. glutamicum*, almost 55 mg/L piceatannol was isolated. This is only one result reported for production of piceatannol in *C. glutamicum* to date. No reports showed production of piceatannol from yeast. Braga et al. (2018a) also successfully produced the compound in the *C. glutamicum* DelAro4 strain from glucose and supplementing cerulenin, a fatty acid synthase inhibitor that facilitates the malonyl-CoA availability in the host organism (Lim et al. 2011; van Summeren-Wesenhagen and Marienhagen 2015).

Streptomyces venezuelae has been also engineered to produce a wide range of natural products including flavonoids and stilbenes. Park et al. (2009) for the first time reported the manipulation of *Streptomyces* sp. for the heterologous expression of phenylpropanaoid biosynthetic pathway genes. The authors used 4-coumarate: coenzyme A ligase from *S. coelicolor* (ScCCL) and codon-optimized stilbene synthase gene from *Arachis hypogaea* for the production of resveratrol. However, the production was just enough for detection (0.4 mg/L). Likewise, a tyrosinase MelC2 from melanin forming *Streptomyces avermitilis* has been reported for the ortho-hydroxylation of resveratrol, producing piceatannol (Lee et al. 2012). *Lactobacillus lactis*, *Aspergillus niger*, and *A. oryzae* have also been used for the biosynthesis of resveratrol in which the heterologous pathway genes from *Arabidopsis thaliana* (PAL, C4H, 4-CL) and *Rheum tataricum* (STS) were incorporated for the production (Katz et al. 2015). Huang et al. (2010) reported the biosynthesis in *Yarrowia lipolytica*, in which 1.46 mg/L of resveratrol was obtained.

Biosynthesis of resveratrol in *E. coli*

From a long time ago, *E. coli* has been used for the production of important pharmaceutical as well as industrial molecules. Although *E. coli* is not a natural host for the production of resveratrol and piceatannol, metabolic as well as pathway engineering have been successfully implemented on this ideal platform. In this process, the heterologous pathways from the plants, as well as other prokaryotes and eukaryotes, are translocated in this beneficial host (Marienhagen and Bott 2013). The beneficial aspects for consideration of *E. coli* as ideal host are due to ease of genetic manipulation, short generation time, and fast and high growth rate. Moreover, bioconversion of exogenous precursors is efficiently achieved, and de novo production of the target compounds from renewable carbon source has been illustrated in microorganisms (Liu et al. 2016; Kang et al. 2014; Wang et al. 2016). Besides this, basic precursor tyrosine and malonyl-CoA are easily assessable and enhanced in *E. coli* through metabolic engineering. To date, most reports show that the production of resveratrol in *E. coli* is based on the exogenous supply of precursors like tyrosine and *p*-coumaric acid. One noticeable advantage of using *E. coli* as the host for the production of resveratrol over yeast is that *E. coli* can tolerate more than 3 g/L of *p*-coumaric acid (Shin et al. 2011; Huang et al. 2013). Metabolic engineering has exhibited better options for efficient heterologous gene expression, enhancement of precursors availability, and increment of the intracellular malonyl-CoA, which helps to maintain the physical, chemical, and physiological conditions for the production of resveratrol (Wang et al. 2018).

Pathway engineering

Pathway engineering has been one pioneer tool for designing *E. coli* for the production of value-added resveratrol. Heterologous metabolic pathway is introduced from plant source, as well as other microbes. Watts et al. (2006) used 4-CL1 from *A. thaliana* and STS from *A. hypogaea* in *E. coli*. With almost 50% bioconversion rate, 105 mg/L of resveratrol was recovered when 1 mM *p*-coumaric acid was supplied as precursor. Likewise, 80.5 mg/L of resveratrol was obtained after feeding of the same amount of substrate in *E. coli* incorporated with the fusion of 4-CL1 and STS from *A. thaliana* and *A. hypogaea*, respectively, indicating lower amount production in fusion genes from the same sources (Zhang et al. 2015). Lim et al. (2011) developed the various combinations of 4-CL and STS from the same strains in two *E. coli* strains (Fig. 3). *E. coli* BW27784 strain produced resveratrol titer in 1.3 g/L. With the best strain, the authors were able to obtain 2.3 g/L after the addition of cerulenin (Lim et al. 2011) (Table 1). Similarly, tyrosine has also been used as primary precursor for the in vivo production of resveratrol. Using PAL from *Rhodotorula rubra*, 4-CL from *Lithospermum erythrorhizon*, and STS from *A. hypogaea*, 37 mg/L of resveratrol was obtained, which was higher when compared to the *E. coli* strain harboring TAL from *R. glutinis*, 4-CL from *P. crispum*, and STS from *V. vinifera* (Katsuyama et al. 2007a, b; Wu et al. 2013). Recently, Wang et al. (2015) introduced TAL from *Saccharothrix espanaensis*, 4-CL from *A. thaliana*, and STS from *A. hypogaea* in *E. coli* and was able to obtain significantly higher titer (114.5 mg/L) of resveratrol. However, *E. coli* harboring TAL, 4-CL, and STS from *S. espanaensis*, *S. ceolicolor*, and *A. hypogaea*, respectively, in *E. coli* produced 1.4 mg/L of resveratrol from tyrosine (Choi et al. 2011). Similarly, Liu et al. (2016) for the first time used a site-specific integration strategy for resveratrol biosynthesis in *E. coli*. The authors integrated genes *TAL*, *4-CL*, and *STS* into the loci of genes *tyrR* and *tyr RD* in the chromosome of *E. coli* BW25113 (DE3) and reported the production of 4.612 mg/L of resveratrol (Fig. 2; Table 1).

Pathway engineering for the enhancement of precursors

Intracellular malonyl-CoA is one prime precursor in the course of the biosynthesis of resveratrol. In the resveratrol biosynthesis, three molecules of malonyl-CoA are utilized to condense one molecule of *p*-coumaroyl-CoA. Since the major concentration of malonyl-CoA is utilized in the primary activity of fatty acid biosynthesis, minimal level is used in resveratrol biosynthesis. Naturally, malonyl-CoA in *E. coli* is synthesized in low amount (Takamura and Nomura 1988). Thus, the approaches for the enhancement of this precursor in host platform have been devised and implemented for the heterologous pathway implantation in *E. coli* host. For the enhancement of cytosolic malonyl-CoA pool, two strategic steps have been carried out in microbial platform: repression of fatty acid biosynthesis to halt the utilization of malonyl-CoA and increasing the carboxylation of acetyl-CoA, which in turn enhances the intracellular malonyl-CoA. Zha et al. (2009) concluded that overexpression of acetyl-CoA carboxylase (ACC) resulted in 3-fold increase in cytosolic malonyl-CoA concentration. Moreover, along with the overexpression of acetate assimilating enzyme (*acs*), the authors also deleted the competing pathway enzymes encoding genes such as *pta* and *ackA* which are involved in acetyl-CoA degradation to form acetate and *adhE* gene which is involved in ethanol production utilizing the same acetyl-CoA, showing 15-fold higher production (Fig. 3). Different reports on the inhibition of fatty acid biosynthesis have demonstrated the efficient production of resveratrol titers. So, different approaches have been employed to inhibit the fatty acid biosynthesis to increase intracellular malonyl-CoA. One simple approach is the addition of cerulenin, a covalent inhibitor of FabB and FabF, key

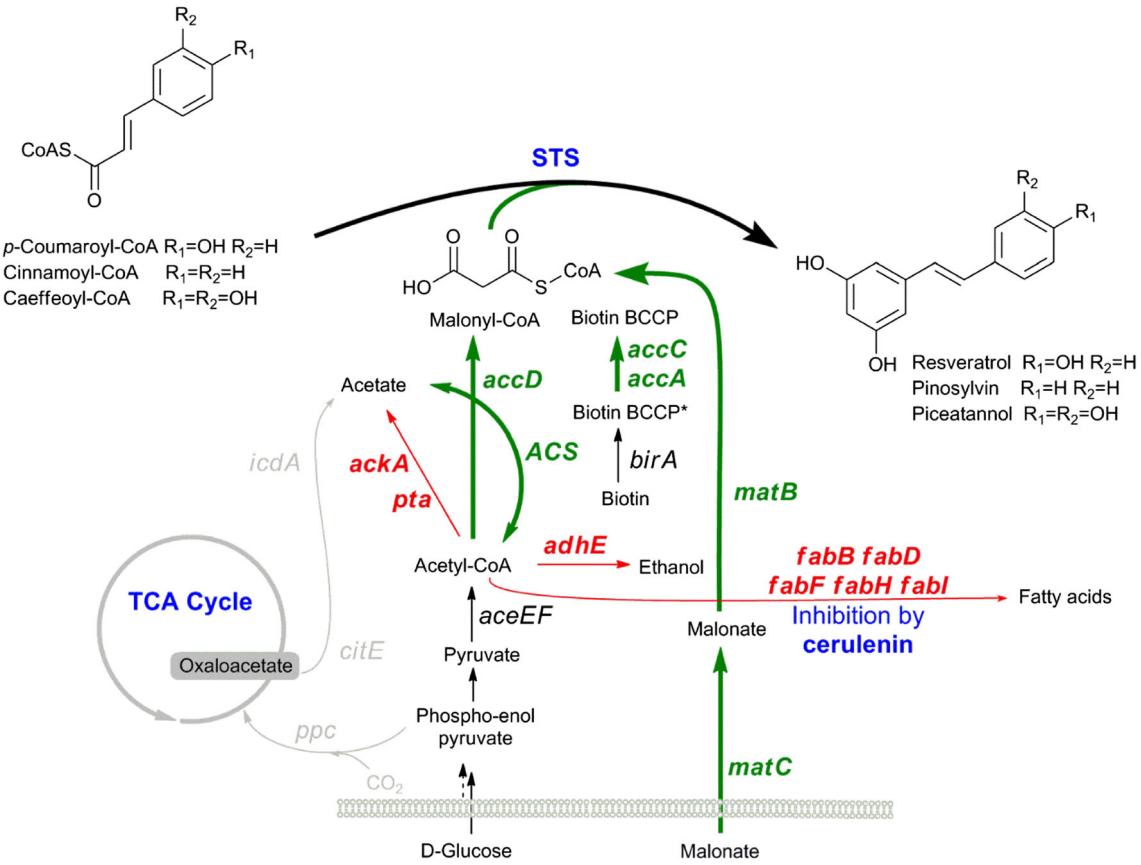

Fig. 3 Metabolic pathways of malonyl-CoA, an extender substrate of STS, engineered in *E. coli* for enhanced production of resveratrol and piceatannol. The enzymes involved in green arrows are overexpressed while enzymes shown in red arrows are either inhibited, or encoding genes are downregulated or knocked out. ACS acetyl-CoA synthase, AccACD acetyl-CoA carboxylase complex encoding genes, *matB* malonyl-CoA synthetase, *matC* dicarboxylate carrier protein, *ack* acetate kinase, *pta* phosphotransacetylase, *FabBDFHI* fatty acid biosynthesis complex encoding genes

enzymes in the fatty acid biosynthesis. This approach has revealed the significant titer of malonyl-CoA in the host (Kallscheuer et al. 2016; Lim et al. 2011; Finzel et al. 2015; Lu et al. 2016). In contrast, the use of cerulenin is costly; as well, it diminishes the malonyl-CoA production even lower than the basal metabolic rate, which halts the cellular growth rate of the host itself (Santos et al. 2011; de Fouchécour et al. 2018; Subrahmanyam and Cronan 1998). Likewise, downregulation of *fab* operon has been successfully brought about for the amassment of malonyl-CoA, and finally, redirection to resveratrol. On this basis, Yang et al. (2015) repressed *fabD* gene of the operon, using anti-sense RNA in *E. coli*. This resulted in 4.5-fold enhancement in cytosolic malonyl-CoA and almost 1.5-fold increase in resveratrol (268 mg/L) titer. Lately, biosynthetic tool CRISPRi system has been used to downregulate the multiple genes of the fatty acid biosynthesis pathway. Wu et al. (2017) downregulated five genes of *fab* operon (*fabD*, *fabH*, *fabB*, *fabF*, *fabI*) and reported the increased level of resveratrol from 80.0 to 216.5% in each, individually (Fig. 3). Moreover, the authors introduced the malonate assimilation pathway genes (*matB* and *matC*) from *Rhizobium trifolii* in this system to increase the malonyl–CoA pool. This combined system increased the titer of resveratrol to 188.1 mg/L. Additionally, the synthetic pathway was further improvised by the expression level of TAL. The final strain produced 304.5 mg/L of resveratrol. Even though the production is at milligram level, the recent synthetic tool paved the efficient production of resveratrol in *E. coli* platform.

As discussed earlier, resveratrol, a major class of polyphenol, is derived from the ubiquitous aromatic amino acids: L-phenylalanine or L-tyrosine. Both amino acids are converted to phenylpropanoids by non-oxidative deamination. So, significant approaches have been devised to optimize the production of aromatic amino acids produced from shikimate pathway or their derived phenylpropanoic acids in *E. coli* (Rodriguez et al. 2015; Juminaga et al. 2012; Zhang and Stephanopoulos 2013). For this, various steps are implemented, such that carbon flux is directed towards chorismate, a branch point to phenylalanine, and tyrosine. Increasing erythrose-4-phosphate (E4P) supply and phosphoenolpyruvate (PEP) availability are the major steps for the increment of chorismate production (Fig. 4). In addition, other steps, such as the overexpression of transketolases and native PEP

Table 1 Biosynthesis of resveratrol and piceatannol in engineered microorganisms

Microbial host	Incorporated genes	Host engineered	Substrate	Titer (mg/L)	References
Escherichia coli (resveratrol)					
E. coli BW27784	4-CL (*A. thaliana*) STS (*A. hypogaea*)		*p*-Coumaric acid	0.16	Afonso et al. (2014)
E. coli BL21(DE3)	4-CL (*Nicotiana tabacum*) STS (*V. vinifera*)		*p*-Coumaric acid	16	Beekwilder et al. (2006)
E. coli BL21(DE3)	Pal (*Rhodotorula rubra*) 4-CL (*Lithospermum erythrorhizon*) STS (*A. hypogaea*)		Tyrosine	37	Katsuyama et al. (2007a)
E. coli BRB	4-CL (*L. erythrorhizon*) STS (*A. hypogaea*) ACC (*C. glutamicum*) *F3H* and *FLS* (Citrus)		Cinnamic acid p-Coumaric acid	155 171	Katsuyama et al. (2007b)
E. coli BW27784	4-CL (*A. thaliana*) STS (*A. hypogaea*)		*p*-Coumaric acid	105	Watts et al. (2006)
E. coli BW27784	4-CL (*A. thaliana*) STS (*A. hypogaea*)		*p*-Coumaric acid	404	Lim et al. (2011)
	4-CL (*A. thaliana*) STS (*V. vinifera*)			1380	
	4-CL (*P. crispum*) STS (*A. hypogaea*)			142	
	4-CL (*P. crispum*) STS (*V. vinifera*)			610	
	4-CL (*A. thaliana*) STS (*V. vinifera*)		*p*-Coumaric acid and cerulenin	2340	
E. coli C41 (DE3)	TAL (*Saccharothrix espanaensis*) 4-CL (*Streptomyces coelicolor*) STS (*A. hypogaea*)		*p*-Coumaric acid	104	Choi et al. (2011)
E. coli BW25113 (DE3	TAL (*R. glutinis*) 4-CL (*P. crispum*) STS (*V. vinifera*)	Inactivation of *tyr*R and deletion of *trp*ED by chromosomal integration	Glucose	4.6	Liu et al. (2016)
E. coli BW27784 (DE3)	4-CL (*A. thaliana*) STS (*V. vinifera*)		*p*-Coumaric acid	1600	Bhan et al. (2013)
E. coli BL21 (DE3)	TAL (*R. glutinis*) 4-CL (*P. crispum*) STS (*V. vinifera*) *matB* and *matC* (*R. trifolii*)		L-Tyrosine	35.02	Wu et al. (2013)
E. coli BL21 (DE3)	4-CL::STS, 4-CL (*A. thaliana*)- STS (*A. hypogaea*) fusion enzyme		*p*-Coumaric acid	80.5	Zhang et al. (2015)
E. coli BL21 (DE3)	TAL (*Trichosporon cutaneum*) 4-CL (*P. crispum*) STS (*V. vinifera*) *matB* and *matC* (*R. trifolii*) tyrA^{fbr} and aroG^{fbr} (*E. coli* K12)	Downregulation of *fabD*, *fabH*, *fabB*, *fabF*, *fabI*	Glucose	304.5	Wu et al. (2017)
E. coli BW25113	4-CL2 (*P. crispum*) STS (*V. vinifera*)		*p*-Coumaric acid	268.2	Yang et al. (2015)
E. coli C41 (DE3)	TAL (*S. espanaensis*) 4-CL (*S. coelicolor*) STS (*A. hypogaea*)		Glucose	5.2	Kang et al. (2014)
E. coli BL21(DE3)	TAL (*S. espanaensis*) 4-CL (*A. thaliana*) STS (*A. hypogaea*)		Tyrosine	114.2	Wang et al. (2015)
E. coli W (pheA^{-}) Rg *E. coli* W-Vv	TAL (*R. glutinis*) tktA^{fbr} and aroG ^{fbr} (*E. coli*) 4-CL (*S. coelicolor*) STS (*V. vinifera*)	Deletion of *phe*A	Glycerol	22.58	Camacho-Zaragoza et al. (2016)
E. coli (piceatannol)					
E. coli	4-CL (*A. thaliana*) STS (*A. hypogaea*)		Caffeic acid	13.3	Watts et al. (2006)
E. coli C41 (DE3)	Tal (*S. espanaensis*) CCL (*S. coelicolor*) STS (*A. hypogaea*)	Tyrosine over expression (Phenol acid decarboxylase; *pad*)	L-Tyrosine	31.5	Heo et al. (2017)

Table 1 (continued)

Microbial host	Incorporated genes	Host engineered	Substrate	Titer (mg/L)	References
E. coli BL21 (DE3)	C3H (*S. espanaensis*) TAL (*S. espanaensis*) 4-CL (*A. thaliana*) C3H (*S. espanaensis*) STS (*A. hypogaea*)		Resveratrol L-Tyrosine	65.4 21.5	Wang et al. (2015)
E. coli BL21 (DE3)	HpaBC (*E. coli*)		Resveratrol	1200	Lin and Yan (2014)
E. coli BL21 (DE3)	HpaBC (*Pseudomonas aeruginosa*)		Resveratrol + Tween 80	5200	Furuya and Kino (2014)
E. coli BL21 (DE3)	HpaBC (*P. aeruginosa*)		Resveratrol + β-cyclodextrin	1200	Furuya et al. (2018)
E. coli BL21 (DE3)	4-CL (*Petroselinum crispum*) STS (*V. vinifera*) HpaBC (*E. coli*)		*p*-Coumaric acid	124	Shrestha et al. (2018)
Non-*E. coli* (resveratrol)					
C. glutamicum DelAro3	STS (*A. hypogaea*) 4-CL (*P. crispum*)	Deletion of *phdB*, *pcaF* and *pobA*	*p*-Coumaric acid *p*-Coumaric acid + cerulenin	12 158	Kallscheuer et al. (2016)
C. glutamicum DelAro3	TAL (*F. johnsoniae*) 4-CL (*P. crispum*) STS (*A. hypogaea*) aroH (*E. coli*)	Deletion of *phdB*, *pcaF*, *qsuB* and *pobA*	Glucose Glucose + cerulenin Glucose (40 g/L) Glucose (80 g/L) Glucose (Fed-batch)	12 59 4 12 7	Braga et al. (2018b)
S. cerevisiae W303-1A	4-CL (*A. thaliana*) STS (*A. hypogaea*)		*p*-Coumaric acid	3.1	Shin et al. (2011)
S. cerevisiae WAT11	4-CL (*A. thaliana*)::STS (*V. vinifera*)		*p*-Coumaric acid	5.25	Zhang et al. (2006)
S. cerevisiae WAT11	TAL (*Rhodobacter sphaeroides*) 4-CL::STS, 4-CL1 *A. thaliana*)-STS *V. vinifera*) fusion enzyme		Tyrosine	1.9	Wang et al. (2011)
S. cerevisiae CEN. PK102-5B	TAL (*Herpetosiphon aurantiacus*) 4-CL (*A. thaliana*) VST (*V. vinifera*)	Overexpression of *aro4*, *aro7*, and *acc1*	Glucose (Fed-batch) Ethanol (Fed-batch)	415.65 531.41	Li et al. (2015)
S. cerevisiae ST4990	PAL (*A. thaliana*) C4H (*A. thaliana*) 4-CL (*A. thaliana*) VST (*V. vinifera*) ACS (*Salmonella enterica*) Overexpression of *atr2* (*A. thaliana*)	Overexpression of *aro4*, *aro7*, and *acc1* and deletion of *aro10*	Glucose (Fed-batch)	812	Li et al. (2016)

synthase, can also be endorsed for the enhancement of aromatic amino acids (Lütke-Eversloh and Stephanopoulos 2007; Bulter et al. 2003; Na et al. 2013; Pandey et al. 2016).

Protein engineering

Occasionally, enzymes with low turnover or poor expression property do not give sufficient levels of target products. So, the improvement of such enzymes through evolutionary or rational engineering methods may prove the solution in key enzymes to increase the target products (Pickens et al. 2011). For the efficient and enhanced production of resveratrol, protein engineering and mutagenesis of 4-CL and STS have been framed out and ratified in *E. coli*. Assuming that co-localization of the two enzymes active site might improve the efficiency, the unnatural fusion of 4-CL and STS (4-CL::STS) from *A. thaliana* and *A. hypogaea*, respectively, was constructed and introduced in *E. coli*. The result showed the production of resveratrol to 80.5 mg/L after feeding 1 mM *p*-coumaric acid (Zhang et al. 2015).

Piceatannol biosynthesis in *E. coli*

Piceatannol has been proven to be one of the potentially important pharmaceutical compounds. Various reports have been published with significant production of piceatannol in *E. coli* by heterologous pathway incorporation. Watts and colleagues in 2006 reported the piceatannol production in *E. coli* for the first time by using caffeic acid as the primary substrate. The authors used 4-CL from *A. thaliana* and STS from *A. hypogaea* and reported the production of 13.3 mg/L of piceatannol. After

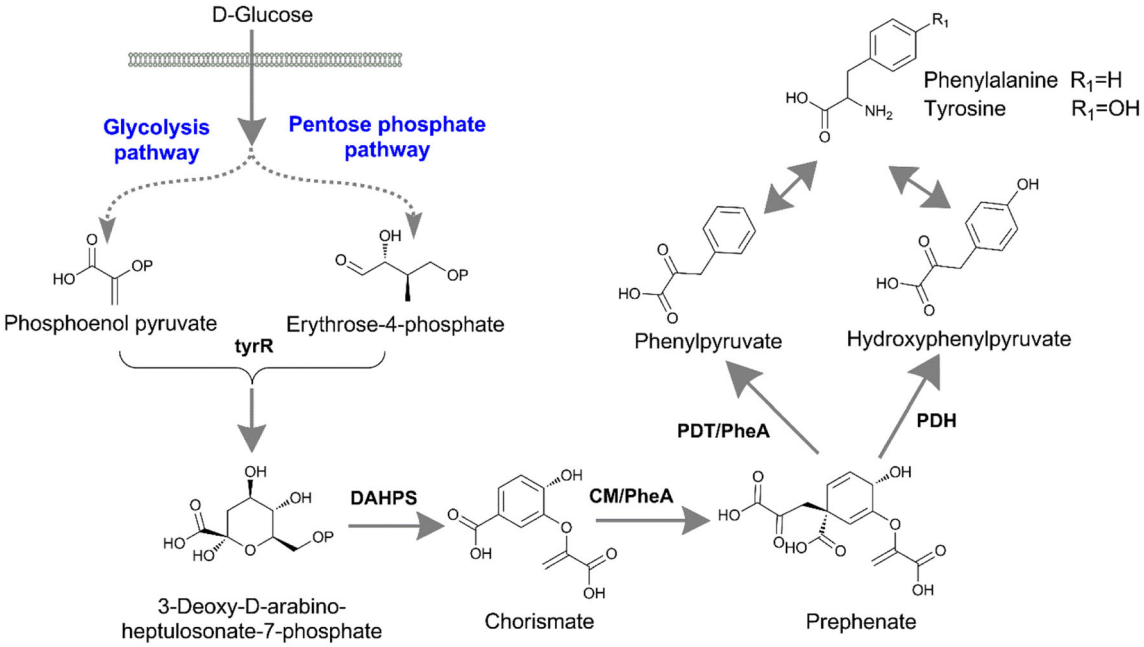

Fig. 4 Biosynthesis pathway of two aromatic amino acids L-phenylalanine and L-tyrosine. The biosynthesis of two initial precursors (phosphoenolpyruvate, an intermediate of glycolysis pathway and erythrose-4-phosphate, an intermediate of pentose phosphate pathway) are engineered to enhance biosynthesis of L-phenylalanine and L-tyrosine which are in turn converted to phenylpropanoyl-CoA, an starter–CoA substrate of STS. TyrR phenylalanine DNA-binding transcription repressor, DAHPS 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase, CM/PheA chorismate mutase/prephenate dehydratase, PDT PDH pyruvate dehydrogenase complex

almost a decade, gram scale (1.2 g/L) of the piceatannol was produced from resveratrol using non-P450 hydroxylase *hpaBC* from *E. coli* by Lin and Yan (2014). By whole cell catalysis with *hpaBC* monooxygenase, Furuya and Kino (2014) reported 5.2 g/L of piceatannol in presence of Tween 80. Similarly, 1.2 g/L of piceatannol was recently reported after the addition of β-cyclodextrin (Furuya et al. 2018). Wang et al. (2015) obtained 21.5 mg/L of piceatannol by total biosynthetic pathway in *E. coli*, in which the authors co-expressed TAL, 4-CL, C3H, and STS. On the other hand, when they used resveratrol directly as substrate, 65.4 mg/L of piceatannol was produced. In one report, Heo et al. (2017) claimed the production of 31.5 mg/L piceatannol. The authors also stated that Sam5 enzyme from *Saccharothrix espanaensis* exhibited 5.7-fold higher conversion rate of resveratrol to piceatannol, compared to coumarate 3-hydroxylase. Similarly, Shrestha et al. (2018) devised the modular pathway engineering in *E. coli* for the production of piceatannol. The biosynthetic pathway genes *4-CL* from Parsley, *STS* from *V. vinifera*, *hpaBC* from *E. coli*, *matB*, and *matC* from *Streptomyces coelicolor* were assembled in different fashion in modular approach and used *p*-coumaric acid as basic substrate. The production of 124 mg/L of piceatannol was obtained after the supplement of disodium malonate which was 2-fold higher than the non-supplied strain (Fig. 5). Heterologous expression of recombinant tyrosinase from *S. avermitilis* MA4680 (MelC2) in *E. coli* enhanced the biotransformation of *trans*-resveratrol in which they found 15.4% conversion rate from 500 μM resveratrol (77.4 μM piceatannol). Furthermore, the piceatannol conversion was increased (58.0%, 290.2 μM piceatannol), after using mutant strain along with NADH regeneration system, resulting in an 8-fold increase in product (Lee et al. 2015). Recently, piceatannol was also produced using cytochrome P450 enzymes from microbial sources. For example, a CYP129A2 from *Streptomyces peucetius* showed high flexibility of microbial CYP450 enzyme towards plant polyphenol (Rimal et al. 2018), whereas *Bacillus megaterium* CYP450 BM3 was reported to hydroxylate wide array of substrates, including resveratrol to produce piceatannol (Kim et al. 2009; Chu et al. 2016) (Fig. 5).

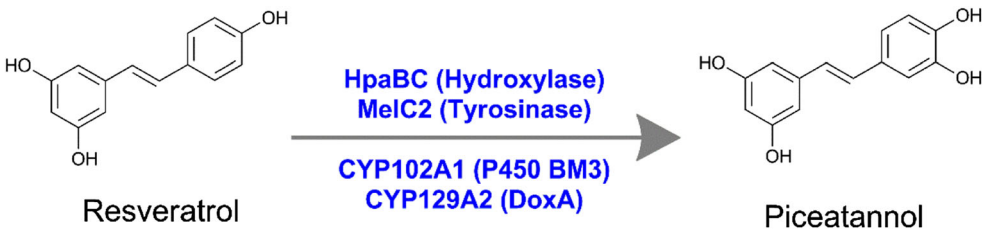

Fig. 5 Enzymes involved in conversion of resveratrol to piceatannol

Conclusion and future perspective

Plant-derived natural products including resveratrol and its derivative piceatannol are of special importance, due to their pharmaceutical and nutritional value. In recent years, the low-cost, eco-friendly, and minimal time range microbial production of these compounds has been efficiently assessed. Metabolic engineering and synthetic biology approaches have allowed the microbial platform that helps the large-scale production of these compounds. Heterologous pathway engineering in microorganisms produces relatively pure compounds and does not need extensive processing. Even though the recent techniques are endorsed for production, the overall production is not satisfactory for industrial scale production. Every gene involved in the biosynthetic pathways is well characterized; however, the heterologous production is still remained below few grams per liter. The reason could be due to the low enzyme activity in *trans*-located hosts or low precursors supply within the cell. So, each step in the biosynthetic pathway should be optimized, such that the every metabolite, as well as precursor, is directed towards the final products such as resveratrol and piceatannol. Moreover, to enhance the production from microbial platform, comprehensive knowledge of the intracellular organization should be utilized, such that its entire genome, transcriptome, proteome, and metabolome could be directed towards the production of the desired compounds.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests The authors declare that they have no competing interests.

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