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Engineering stilbene metabolic pathways in microbial cells

Philippe Jeandet[a], Eduardo Sobarzo-Sánchez[b], Christophe Clément[a], Seyed Fazel Nabavi[c], Solomon Habtemariam[d], Seyed Mohammad Nabavi[c], Sylvain Cordelier[a]

[a]*Research Unit, Induced Resistance and Plant Bioprotection, EA 4707, SFR Condorcet FR CNRS 3417, Faculty of Sciences University of Reims Champagne-Ardenne, PO Box 1039, 51687 Reims Cedex 2, France*

[b]*Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain, and Instituto de Investigación e Innovación en Salud, Facultad de Ciencias de la Salud, Universidad Central de Chile, Chile*

[c]*Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran 14359-16471, Iran*

[d]*Pharmacognosy Research Laboratories and Herbal Analysis Services, School of Science, University of Greenwich, Central Avenue, Chatham-Maritime, Kent ME4 4TB, UK*

Corresponding author: Philippe Jeandet, philippe.jeandet@univ-reims.fr

ABSTRACT

Numerous *in vitro* and *in vivo* studies on biological activities of phytostilbenes have brought to the fore the remarkable properties of these compounds and their derivatives, making them a top storyline in natural product research fields. However, getting stilbenes in sufficient amounts for routine biological activity studies and make them available for pharmaceutical and/or nutraceutical industry applications, is hampered by the difficulty to source them through synthetic chemistry-based pathways or extraction from the native plants. Hence, microbial cell cultures have rapidly became potent workhorse factories for stilbene production. In this review, we present the combined efforts made during the past 15 years to engineer stilbene metabolic pathways in microbial cells, mainly the *Saccharomyces cerevisiae* baker yeast, the *Escherichia coli* and the *Corynebacterium glutamicum* bacteria. Rationalized approaches to the heterologous expression of the partial or the entire stilbene biosynthetic routes are presented to allow the identification and/or bypassing of the major bottlenecks in the endogenous microbial cell metabolism as well as potential regulations of the genes involved in these metabolic pathways. The contributions of bioinformatics to synthetic biology are developed to highlight their tremendous help in predicting which target genes are likely to be up-regulated or deleted for controlling the dynamics of precursor flows in the tailored microbial cells. Further insight is given to the metabolic engineering of microbial cells with "decorating" enzymes, such as methyl and glycosyltransferases or hydroxylases, which can act sequentially on the stilbene core structure. Altogether, the cellular optimization of stilbene biosynthetic pathways integrating more and more complex constructs up to twelve genetic modifications has led to stilbene titers ranging from hundreds of milligrams to the gram-scale yields from various carbon sources. Through this review, the microbial production of stilbenes is analyzed, stressing both the engineering dynamic regulation of biosynthetic pathways and the endogenous control of stilbene precursors.

1. Introduction

Synthetic biology as part of combinatorial biosynthesis (Trantas et al., 2015) can be seen as an engineering approach to generate cells and new platform organisms or microorganisms with customized functionality (Nemhauser and Toril, 2016). This strategy has been applied to obtain complex molecules whose production through synthetic chemistry-based routes or extraction from native plant sources are unrealistic (Marienhagen and Bott, 2013; Xu et al., 2013). Through this approach, a number of compounds of pharmaceutical significance have been produced including alkaloidal antibiotics (berberine, Minami et al., 2008), antibiotics of the polyketides class (erythromycin A, Pfeiffer et al., 2001), anticancer drugs with diterpene skeleton (taxol, Ajikumar et al., 2010) and terpenoid-based antiparasitic agents (artemisinin, Ro et al., 2006). Among the naturally-occurring compounds with significant impact on human health and diseases, stilbenes and their iconic representative, resveratrol, have attracted considerable attention during the past two decades. Interestingly, resveratrol displays a broad spectrum of pharmacological activities, indicative of its promising applications for human health (Pezzuto, 2011). Previous studies have outlined the role of this compound as a phytoalexin (Langcake and Pryce, 1976), an antimicrobial molecule of low molecular weight produced by and accumulated in plants as a response to various biotic or abiotic stresses. The antifungal properties of resveratrol have been clearly demonstrated through *in vitro* bioassays and at an ultrastructural level (Adrian et al. 1997; Adrian and Jeandet, 2012). Twenty years after the characterization of resveratrol as a phytoalexin, the first report on its chemopreventive efficacy was published in 1997 (Jang et al., 1997). At that time, resveratrol was shown to exert activity against all major stages of the carcinogenesis process such as the initiation, promotion and progression by acting as an inhibitor of cyclooxygenases-1 and -2 (Jang et al., 1997). This pioneering work has encouraged a plethora of research on the discovery of natural products displaying activity both as pharmaceutical and/or nutraceutical agents. As grapes and red wine are the major sources of resveratrol in the diet, the discovery of the cancer chemopreventive activity of this compound was a timely extension of the vision of the French Paradox according to which a moderate consumption of red wine inversely correlates with risks of cardiovascular diseases in humans (Renaud and De Lorgeril, 1992). A direct experimental evidence on the involvement of resveratrol in the French Paradox rapidly gained acceptance through the demonstration of its protecting effect on low-density lipoprotein oxidation which is a crucial stage in artherosclerosis development. In this connection, resveratrol was reported as a far better antioxidant than α-tocopherol (Frankel et al., 1993). Since then, considerable number of studies have been designed to look at the various aspects of resveratrol's pharmacology leading to thousands of publications in the scientific literature.

Of the numerous mechanisms involved in the antitumor effect of resveratrol are cell cycle arrest (Estrov et al., 2003; Hsieh et al., 1999), induction of apoptosis, inhibition of angiogenesis and neovascularization (Brakenhielm et al., 2001; Igura et al., 2001), and modulation of various signaling pathways related to malignant transformation or cell survival (Fulda and Debatin, 2006). Resveratrol induces apoptosis through the regulation of multiple pathways, modulation of the tumor suppression protein p53, activation of caspases and the proapoptotic Bax proteins (Kim et al., 2003), depletion of the level of the antiapoptotic molecules Bcl-2 and Bcl-x^L (Park et al., 2001; Zhou et al., 2003), inhibition of cyclins and cyclin-dependent kinases (Ahmad et al., 2001; Hsieh et al., 1999), and interference with the nuclear transcription factors, NF-κB and AP-1 mediated cascades (Pezzuto, 2011). Besides, resveratrol was described to have therapeutic promise for Parkinson's (Karlsson et al., 2000) and Alzheimer's diseases (Anekonda, 2006), to act as an antibacterial agent in respiratory tract infections (Schriever et al., 2003), chronic gastritis (Mahady and Pendland, 2000), skin infections

(Chan, 2002) and against candidiasis as an antifungal agent (Jung et al., 2005). The role of resveratrol in health and diseases was extended to other phytostilbenes such as pinosylvin (Jeong et al., 2013; Park et al., 2012), and methylated stilbenes (Paul et al., 2009; Rimando et al., 2002). Based on evidences from *in vivo* experimental trials (Baur and Sainclair, 2006), the interest for resveratrol and its derivatives has constantly increased, though there is some skepticism regarding its bioavailability in humans (Walle et al., 2004).

Phytostilbenes are secondary plant metabolites widely represented in the plant kingdom (Rivière et al., 2012). These comprise numerous compounds (hydroxylated, methylated, isoprenylated) deriving from the stilbene skeleton (Jeandet et al., 1997, 2002, 2010 and 2014). Stilbenes are synthesized through the universal phenylpropanoid route which also leads to flavonoids and lignins formation in plants (Ververidis et al., 2007). The biosynthesis of phenylpropanoids starts from two aromatic amino acids, phenylalanine and tyrosine which are obtained from the shikimate pathway. The shikimate route begins with the condensation of erythrose-4-phosphate (pentose phosphate pathway) and phosphoenolpyruvate (glycolysis pathway) (Fig. 1). Phenylalanine gives rise to *trans* cinnamate and *trans p*-coumarate (or 4-hydroxy cinnamate) by the sequential action of phenylalanine ammonia lyase (PAL) and the cinnamate-4-hydroxylase (C4H). An alternative route is the generation of *p*-coumarate directly from tyrosine by the tyrosine ammonia lyase (TAL). *Para*-coumarate, and more generally, cinnamic acids are then converted into their respective coenzyme A-thioesters by ligation to coenzyme A (CoA) by cinnamoyl-CoA ligases (CLs), often acting as 4-cinnamoyl-CoA (or *p*-coumarate) ligases (4-CLs). The last step of the stilbene biosynthesis is catalyzed by stilbene synthase (STS) by condensation of *p*-coumaroyl-CoA with three units of malonyl-CoA, the latter being formed from acetyl-CoA or directly from malonic acid by the malonyl-CoA synthase. STS competes with chalcone synthase (CHS) responsible for the production of the C6-C3-C6 flavonoid skeleton (Austin et al., 2003). The stilbene biosynthetic pathway thus presents a large overlap with the flavonoid pathway (Pandey et al., 2016), which can be seen as a diversification of the phenylpropanoid metabolism in plants (Trantas et al., 2015). Pinosylvin, another stilbene whose metabolic pathway was also engineered in microorganisms, undergoes a very similar biosynthetic route beginning with phenylalanine to form cinnamate and then cinnamoyl-CoA. The further condensation by STS leads to the formation of pinosylvin. Finally, modified monomeric stilbenes can be generated by the subsequent action of "decorating" enzymes, such as hydroxylases, *O*-glycosyltransferases and *O*-methyltransferases (Fig. 1).

The first studies on molecular engineering of the resveratrol pathway were reported in plants in the 1990s for disease resistance and functional food (Hain et al., 1993; Delaunois et al., 2009; Jeandet et al., 2012, 2013a and 2017). Stilbene production for therapeutic applications was also developed using recombinant plant cell systems (Martinez-Marquez et al., 2018), but microbial cell cultures have rapidly became potent workhorse factories for stilbene production (Braga et al., 2018a; Donnez et al., 2009; Dudnik et al., 2018; Jeandet et al., 2013b; Lu et al., 2016; Marienhagen and Bott, 2013; Milke et al., 2018; Nandagopal et al., 2017; Pandey et al., 2016; Wang et al., 2018). In this review, we present the combined efforts made during the past 15 years to engineer stilbene pathways in microbial cells, mainly the *Saccharomyces cerevisiae* baker yeast (Table 1) and the *Escherichia coli* and *Corynebacterium glutamicum* bacteria (Table 2). Rationalized approaches based on bioinformatics applications to the heterologous expression of the partial or the entire stilbene biosynthetic pathways are described. The identification and resolution of the major bottlenecks in the endogenous microbial cell metabolism as well as the tight cellular regulations of the genes involved in these metabolic pathways are also systematically presented. The review further analyzed

the microbial production of stilbenes by stressing both the engineering dynamic regulation of biosynthetic pathways and the endogenous control of stilbene precursors.

2. General bases for the engineering of stilbene biosynthetic pathways in microorganisms

For stilbene production, molecular engineering of yeast and bacteria addresses two main strategies: at first, engineering the biosynthetic route to target the B ring of stilbenes (phenylpropanoid pathway) and secondly engineering the upstream and downstream pathways of malonyl-coenzyme A responsible for building the A ring of stilbenes (Fig. 1). Engineering the phenylpropanoid pathway can be done at two different levels depending on the precursor/carbon source used: most exclusively on the phenylpropanoid biosynthetic route in microorganisms harboring the entire pathway starting from the aromatic amino acids, or the introduction of selective genes coding downstream enzymes using cinnamic acid substrates. Additionally, engineering of the shikimate pathway to phenylalanine or tyrosine is a more and more developed strategy to achieve direct production of phytostilbenes from simple carbon sources such as D-glucose, ethanol or glycerol. Engineering *E. coli* for the biosynthesis of tyrosine from glucose or use of tyrosine over-producing bacterial strains has indeed been considered as critical for the production of flavonoids (Santos et al., 2011) or stilbenoids (see below). As it will be emphasized further, the conversion of aromatic amino acids to their corresponding cinnamic acid derivatives involving PAL or TAL appears to be a limiting factor in the stilbene biosynthetic pathway engineering. This fully justifies the search for novel synthetic pathways to optimize stilbene production by shunting the endogenous metabolism of these compounds in microbes (Kallscheuer et al., 2017a).

On the other hand, malonyl-CoA can also be seen as a major bottleneck in those metabolic pathways (Katsuyama et al., 2017a; Leonard et al. 2007; Miyahisa et al., 2005; Yang et al., 2015) since this compound is a node at the intersection of multiple biosynthetic routes (flavonoids, stilbenoids and fatty acids) and because of its limited intracellular pool, ranging from 0.01 to 0.23 nmol/mg dry weight in *E. coli* (Johnson et al., 2017). Several strategies have thus been developed to improve the availability of malonyl-CoA. Overexpressing enzymes of the anabolic pathway of malonyl-CoA as well as down-regulating its sink pathway (fatty acid biosynthesis) are good ways to improve plant polyketide production in microbial cells (Lussier et al., 2012 and references below). In the same way, redirecting the central metabolism into this compound, was reported to increase the production of the flavonoid precursor, naringenin in *E. coli* (Xu et al., 2011). We will decipher how all these strategies have been used to engineer stilbene pathways in microorganisms.

3. Building the B-ring of stilbenes: Shikimate and phenylpropanoid pathways

Appropriate engineering the phenylpropanoid pathway in microorganisms can result in the production of resveratrol or pinosylvin. Aromatic amino acids are used as starting molecules in microorganisms harboring either *PAL* or *TAL* gene as well as *C4H* and *4CL* genes for resveratrol or the combination of *PAL* and *CL*/*4CL* for pinosylvin, and final condensations being insured by the STS (Fig. 1; Tables 1 and 2). This appears to be the most applied method for stilbene engineering in microorganisms upon feeding with phenylalanine or tyrosine. However, integration of the *TAL* gene instead of *PAL* may be an interesting option for by-passing the cytochrome-P450 (CP450)-dependent hydroxylation step of cinnamate conversion to *p*-coumarate in bacteria and yeast (Becker et al., 2003; Beekwilder et al., 2006; Li et al. 2015, Zhang et al., 2006 and 2015). Indeed, C4H requires the co-expression of cytochrome P450 reductase (CPR). Moreover, it has been reported that in bacteria,

a CP450-redox partner electron transfer complex was needed to insure the full catalytic activities of P450 enzymes (Sevrioukova et al., 1999). Because of these limitations, co-expression of a *CPR* gene derived from a *Populus* hybrid was achieved in *S. cerevisiae* leading to a 4-fold increase in *p*-coumarate production (Trantas et al., 2009). These results thus indicate that efficient metabolon requires supporting the yeast endogenous CPR with additional plant CPR (Li et al., 2016; Trantas et al., 2009). For many reasons, however, heterologous expression of *TAL* genes in microorganisms is seen as apivotal step in stilbene production engineering. It has indeed been reported that *in vivo TAL* gene overexpression in yeast did not result in the formation of the respective TAL protein in the resveratrol biosynthetic route despite the high *TAL* transcripts levels (Wang et al., 2011; Zhang et al., 2006). Moreover, TAL activity is inhibited by *p*-coumaroyl-CoA, thus limiting resveratrol production (Santos et al., 2011). In order to overcome the lack of enzyme activity observed in a recombinant yeast carrying a bacterial *TAL* gene from *Rhodobacter sphaeroides*, an entire *TAL* gene was re-synthesized by replacing the bacterial codons with yeast-preferred codons leading to a successful increase in *p*-coumarate and then resveratrol production (Wang et al., 2011). In the same way, the TAL activity in a recombinant *E. coli* strain was increased by replacement of the *Rhodotorula glutinis TAL* gene by a more active *Petroselinum crispum TAL* gene (Wu et al., 2017b).

Still in *E. coli*, optimization of both *TAL* and *4CL* genes expression was also obtained by reducing the 5' region secondary structure of the open reading frame of their mRNAs (Wu et al., 2017a and b). Indeed, it has previously been reported that reducing this mRNA region of the target gene could improve protein expression (Wu et al. 2016). As TAL activities remain low in microbes, some efforts were also directed to the construction of different TAL variants (Wu et al., 2017b), testing their activity in bacteria and yeast (Jendresen et al., 2015) or the use of codon-optimized *TAL* genes (Heo et al., 2017). Some PAL enzymes, which display catalytic activity on phenylalanine but also on tyrosine, such as the PAL from *Rhodosporidium toruloides* could offer an interesting alternative for engineering the production of cinnamic acid derivatives (Jiang et al., 2005; Shin et al., 2012).

As already mentioned, expressing *TAL* genes or averting the rate-limiting hydroxylation step of cinnamate to *p*-coumarate in bacteria or yeast remains an obstacle. For this reason, a lot of studies have focused on the introduction of selective genes such as *4CL* and *STS* genes using *p*-coumarate as a precursor for resveratrol synthesis (see below) (Tables 1 and 2).

When the host microbial systems are supplemented with simple carbon sources such as glucose and ethanol in yeast and bacteria (Li et al., 2015) or glycerol in *E. coli* (Camacho-Zaragoza et al., 2016), the formation of phenylalanine and tyrosine then results from the shikimate pathway (Fig. 2 and 3). However, the biosynthesis of these two amino-acids is tightly regulated at the first committed step of the shikimate pathway catalyzed by the 3-deoxy-D-arabino-heptulosonate 7-phosphate (DHAP) synthase and the chorismate branch point (chorismate mutase) (Jossek et al., 2001; Lütke-Eversloh and Stephanopoulos, 2005). A feed-back inhibition is exerted by phenylalanine and tyrosine on the shikimate pathway at these two enzymes level. Deregulation of this inhibition is then necessary to improve the phenylalanine and tyrosine production and consequently the downstream biosynthesis of stilbenes (Li et al., 2015). There are several studies related to the design of aromatic amino-acid-overproducing host strains to solve the problem of this low precursor availability in microbial cells. An interesting strategy involves the introduction of genes encoding feed-back insensitive enzymes of the shikimate pathway leading to the enhancement of the intracellular pool of aromatic amino-acids. In an engineered *S. cerevisiae* strain harboring TAL, 4CL and STS enzymes, the metabolic flow towards tyrosine was successfully increased upon overexpression of the feed-back inhibition resistant versions of the *ScARo4p*^{K229L} DAHP synthase and

the *ScARo7p*^{G1415}chorismate mutase (Fig. 2). This resulted in a 78% improvement of the resveratrol titer compared to the strain harboring the TAL-based resveratrol pathway (Li et al., 2015) (Table 1). A similar strategy was used in a recombinant *E. coli* strain by introducing a *TAL* gene together with an *aroG*^{fbr}gene encoding a feed-back-inhibition resistant version of DAHP synthase and an inactivated form of the *pheA* gene encoding the chorismate mutase/prephenate dehydratase. This redirects the biosynthesis pathway to the formation of tyrosine (Camacho-Zaragoza et al., 2016) (Fig. 3). Deregulation of the shikimate pathway allowing efficient production of resveratrol or pinosylvin from D-glucose was also explored by overexpressing in *Corynobacterium glutamicum* the *AroH*$_{EC}$ gene encoding the DHAP synthase from *E. coli* (Braga et al., 2018b; Kallscheuer et al., 2016). Similar strategy was utilized in *E. coli* by overexpressing the *aro*^{Fwt} gene and *PheA*^{fbr} gene encoding, respectively a wild type DAHP synthase and a feed-back inhibition-resistant version of the chorismate mutase/prephenate dehydratase (Wu et al. 2017a). In the same way, site-specific integration of a heterologous resveratrol pathway in *E. coli* comprising *4CL* and *STS* genes at the loci of both the *tyR* and *trpED* genes was reported (Liu et al., 2016). The initial purpose of this study was to delete the *tyR* transcriptional regulatory gene of *E. coli*, since its inactivation enhances the expression of several genes of the shikimate pathway such as *aroF*/*G*, *aroL* and *tyrA* encoding respectively the DAHP synthase, the shikimate kinase and the chorismate mutase/prephenate dehydrogenase (Fig. 3) (Pittard et al., 2005). Besides, inactivation of the *trpED* gene encoding the anthranilate synthase was carried out to inhibit the competitive route to tryptophan (Patnaik et al., 2008). However, the resveratrol yield from this system remained low (4.6 mg/L). This suggests that a complex engineering of the metabolic pathway targeting the B ring of stilbenes without managing the intracellular pool of malonyl-CoA, remains an incomplete strategy.

Interestingly, it was suggested that both the low availability of phenylalanine and tyrosine and the low activity of heterologous PAL and TAL enzymes could be by-passed by engineering a new microbial production pathway of cinnamoyl-CoA thioesters that is not found in nature, and totally independent from these two amino acids (Kallscheuer et al., 2017a). This novel synthetic pathway is based on the reversal of a β-oxidative phenylpropanoid degradation from *Azoarcus* spp. For this purpose, a *Corynebacterium glutamicum* strain, insensitive to phenylpropanoid and benzoic acid degradation, was fed with 4-hydroxybenzoic acid (HBA) as a starting precursor (Kallscheuer et al., 2016); enabling the formation of *p*-coumaroyl-CoA, independently of any supplementation with aromatic amino acids. This was achieved with a genetic construction comprising several genes encoding a HBA:CoA ligase, a β-ketothiolase catalyzing a two carbon chain elongation step, an hydroxyacyl-CoA dehydrogenase, an enoyl-CoA hydratase and two additional *4CL* and *STS* genes (Fig. 4).

The introduction of selective genes appears to be a simpler transformation strategy with only two genes, *CL* or *4CL* and *STS* to by-pass the low availability of aromatic amino-acids in yeast and bacteria (Tables 1 and 2). The CL or 4CL and STS enzymes are responsible for the biosynthesis of cinnamoyl-CoA or *p*-coumaroyl-CoA and the subsequent stilbenes, respectively under use of cinnamate or *p*-coumarate. Using *p*-coumarate may also divert the limiting hydroxylation step from cinnamate. Most of these two-gene-based genetic constructs relied on the overexpression of the *4CL* gene from *A. thaliana*, *P. crispum*, the Gram-positive bacterium *Streptomyces coelicolor* or less frequently the *4CL* gene from *Nicotiana tabacum*, *Glycine max* and poplar. The choice of 4CL from *S. coelicolor* may be dictated by its substrate specificity in favor of cinnamic acid over *p*-coumaric acid. This strategy is supposed to be more suitable for pinosylvin production in *E. coli* (Van Summeren-Wesenhagen and Marienhagen, 2015). The 4CL from *A. hypogea* offers interesting properties since it

displays affinity for a wide range of phenylpropanoid substrates compared to 4CL from other species (Lozoya et al., 1988).

4. Final condensation step with STS

The final condensation between cinnamoyl-CoA or *p*-coumaroyl-CoA and the three malonyl-CoA units involves overexpression of the *STS* gene in microbial cells. Most of the *STS* genes used to engineer bacteria and yeast for resveratrol production originate from *V. vinifera* or *A. hypogea* (Tables 1 and 2). Introducing the STS from *A. hypogea* with the purpose of producing structurally diverse stilbenes is an interesting option because of its reported broad substrate specificity (Abe et al., 2004; Morita et al., 2001; Watts et al., 2006). A recent work also described the engineering of *E. coli* for resveratrol production with a newly identified *STS* gene from mulberry (*Morus atropurpurea* Roxb.) (Wang et al., 2017). A high stilbene production is dependent on the expression efficiency of STS in microorganisms, its ability for stilbene synthesis (kinetic constants) as well as its solubility (Jeong et al., 2015; Lim et al., 2011; Van Summeren-Wesenhagen and Marienhagen, 2015). Six *STS* genes from *A. hypogea*, *V. vinifera*, *Pinus strobus*, *Pinus densiflora*, *Pinus massoniana*, *Psilotum nudum* and two genes from *Polygonum cuspidatum* were tested in different combinations in *E. coli* to optimize resveratrol production from *p*-coumarate (Lim et al., 2011). Based on the functional expression of each *STS* gene and the enzyme kinetic parameters, it appeared that STS from *V. vinifera* and *A. hypogea* were the most efficient enzymes for resveratrol production.

Engineering the pinosylvin biosynthetic pathway in microbes usually goes through the expression of *STS* genes originating from either *Pinus* spp, *V. vinifera* or *A. hypogea* (Tables 1 and 2). In order to adapt the STS from *P. strobus* to a given microbial host system, a directed protein evolution study generated two STS clones, each bearing a single amino-acid substitution and showing an increased ability for pinosylvin production (Van Summeren-Wesenhagen and Marienhagen, 2015). Namely, it was suggested that the presence of an L-alanine at position 248 of STS could simply improve the flexibility of the protein, explaining its increased activity in *E. coli*. Most interestingly, it was reported that the replacement of L-glutamine at position 361 of STS with the more hydrophilic L-arginine amino-acid, could increase the stability or the solubility of this protein resulting in increased enzymatic activity (Van Summeren-Wesenhagen and Marienhagen, 2015). Indeed, the solubility of STS in *E. coli* has been found to be a major bottleneck for engineering stilbene pathways in microorganisms (Jeong et al., 2015). Watts et al. (2006) observed that the *A. hypogea* STS expressed in *E. coli* was largely located in the soluble fraction of bacterial cell lysates. On the other hand, six different variants of STS differing at the 5' mRNA region resulted in many different pinosylvin production levels in *E. coli* (Wu et 2017a). STS expression could also be altered by the growing medium. Resveratrol titers indeed drastically decreased from > 100 mg/L to less than 4 mg/L in an engineered *E. coli* strain when the carbon source was changed from glycerol to glucose. This is likely to be the consequence of either the reduced STS production or the catabolic repression of the phenylpropanoid precursor transport in *E. coli* grown in a glucose-based medium (Watts et al., 2006). Rational engineering of STS proteins could also lead to interesting applications for the production of unnatural stilbenes (see section 8.2).

5. Building the A-ring of stilbenes: Regulating the intracellular pool of malonyl-CoA

Malonyl-CoA is a metabolic node at the intersection of both the flavonoid and the stilbenoid pathways as well as of the fatty acid biosynthesis. An increase in the internal pool of malonyl-CoA

turned out to be a key-point target to achieve overproduction of stilbenes in engineered microorganisms by redirecting the carbon flux into this compound (Van Summeren-Wesenhagen and Marienhagen, 2015; Salas-Navarrete et al., 2018). The intracellular pool of malonyl-CoA is determined by the orchestrated interaction between its upstream biosynthetic pathway and its downstream utilization towards fatty acids (Fig. 2 and 3). Herein, we present the most frequently used approaches to study the dynamic regulation of both the malonyl-CoA-origin and the malonyl-CoA-sink pathways.

5.1 Regulating the upstream malonyl-CoA pathway

Engineering the upstream biosynthetic pathway of malonyl-CoA may include gene overexpression of the bifunctional alcohol-aldehyde dehydrogenase (*adhE*) and the acetyl-CoA synthase (*ACS*) according to the fermentative pathway in yeast (Fig. 2). It can also target the overproduction of the acetyl-CoA carboxylase complex (ACC), a key enzyme in the acetate assimilation pathway responsible for the conversion of acetyl-CoA with one molecule of CO_2 to malonyl-CoA in *E. coli* (Fig. 3) (Katsuyama et al., 2007a; Lim et al., 2011) or yeast (Li et al., 2015; Shin et al., 2012). Surprisingly, overexpression of *adhE* and *ACS* genes failed to improve resveratrol production in yeast (Li et al., 2015). However, overproduction of a post-translational deregulated version of ACC insensitive to phosphorylated-targeted degradation, resulted in a 31% increase in resveratrol biosynthesis in yeast (Li et al., 2015). Co-expression of this *ACC* gene with feedback inhibition resistant versions of DAHP synthase and chorismate mutase genes enhanced resveratrol titers by 234% in yeast (Table 1) (Li et al., 2015). On the other hand, overexpression of the *ACC* gene in *E. coli* has resulted in a 15.7-fold increase of the intracellular malonyl-CoA pool for the direct biosynthesis of methylated stilbenes (Katsuyama et al., 2007a). Simultaneous expression of *ACC* and biotin ligase (*BirA*) genes also allowed a more efficient conversion of acetyl-CoA to malonyl-CoA in *E. coli* (Lim et al., 2011). Conversely, heterologous expression of the *C. glutamicum ACC* gene failed to increase the intracellular generation of malonyl-CoA from acetyl-CoA in *E. coli* (Van Summeren-Wesenhagen and Marienhagen, 2015).

Malonyl-CoA can also be formed directly from malonic acid by the action of malonyl-CoA synthase (*matB* gene) (An and Kim, 1998). For example, in the *Rhizobium trifolii* bacterium, this gene is part of the *matABC* operon, with *matA* encoding a malonyl-CoA decarboxylase (to form acetyl-CoA) and *matC* encoding a malonate transporter (Chen et al., 2011). Recombinant bacteria (*E. coli*) or yeast (*Yarrowia lipolytica*) indeed displayed an increase of their intracellular malonyl-CoA pool upon expression of the malonyl-CoA biosynthetic operon including *matB* and *matC* genes (Huang et al., 2006; Shrestha et al., 2018; Wu et al., 2013 and 2017b) (Fig. 2 and 3).

5.2 Controlling the sink malonyl-CoA pathway

This approach is based on the chemical inhibition of fatty acid biosynthesis with the cerulenin antibiotic, or by engineering dynamic regulation pathways (fatty acid synthesis, glycolysis and tricarboxylic acid cycle) to redirect the malonyl-CoA flux to stilbene production. Different approaches were used based on antisense RNA (Yang et al., 2015) or Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi) techniques (Liang et al., 2016; Wu et al., 2017a and b). These two latter strategies were able to optimize malonyl-CoA availability in *E. coli* without compromising cell growth.

Cerulenin is an antifungal antibiotic produced by *Cephalosporium caerulens* that inhibits fatty acid and steroid biosynthesis and more precisely acts as a specific inhibitor of the *fabB-fabF* (β-ketoacyl-acyl synthases I and II) gene products (Fig. 3 and Table 2). Its addition at a concentration of 50 μM in the fermentation medium was found to increase the resveratrol titer by 64% in *E. coli* (Lim et al., 2011). Interestingly, the use of cerulenin at 200 μM boosted the intracellular malonyl-CoA pool by a 50-fold factor in *E. coli* (Van Summeren-Wesenhagen and Marienhagen, 2015), leading to an 18-fold increase in pinosylvin production without any reduction in the level of cinnamoyl-CoA. This observation hints that the basal cinnamoyl-CoA pool is sufficient to insure a high pinosylvin production. It also indicates that the intracellular cinnamoyl-CoA pool was not the limiting factor for stilbene biosynthesis when cerulenin was added to the growth medium (Van Summeren-Wesenhagen and Marienhagen, 2015). Furthermore, none of the studies using cerulenin reported any effect on the biomass or the growth of the microorganisms, underlying the interest of using this compound to improve stilbene titers in engineered microorganisms. However, the high cost of cerulenin (> 20,000 USD / g) constitutes a major drawback for microbial stilbene production at an industrial scale.

Down-regulating the malonyl-CoA consumption pathway to enhance its intracellular availability through conventional gene knockout strategies could compromise fatty acid biosynthesis and in turn the physiology of the transgenic organism. Other methods such as synthetic antisense RNAs or the CRISPRi techniques have been used to repress genes acting on the fatty acid biosynthesis route. Synthetic small regulatory RNAs have already been used for metabolic engineering in *E. coli* (Na et al., 2013). A synthetic antisense RNA strategy was thus developed to down-regulate fatty acid biosynthesis with a correlated increase of the malonyl-CoA bioavailability in *E. coli* (Yang et al., 2015). When the expression of the *fabD* gene (encoding the malonyl-CoA-ACP transacylase) was interfered by its *fabD* antisense version, the malonyl-CoA pool in the bacterium was increased by 4.5-fold and the resveratrol titer by 1.7-fold (280 mg/L *vs* 160 mg/L in the control) (Table 2). Repression of the *fabB* and *fabF* genes (encoding respectively the β-ketoacyl-ACP synthases I and II), only exerted an effect on the malonyl-CoA pool in case of low interference efficiencies, resulting in an increased resveratrol titer. Higher interference efficiencies on the fatty acid pathway may indeed seriously impair cell viability (Yang et al., 2015). On the other hand, the CRIPSPRi system presents the advantage of simultaneously manipulating multiple genes in *E. coli* compared to the previously described antisense RNA strategy (Wu et al., 2017b). This system has been applied to repress various genes operating on the fatty acid biosynthesis: *fabD*, *fabB* (encoding the β-ketoacyl-ACP synthase I), *fab F* (encoding the β-ketoacyl-ACP synthase II), *fabH* (encoding the β-ketoacyl-ACP synthase III) and *fabI* (encoding the enoyl-ACP reductase) thereby increasing the intracellular malonyl-CoA pool for the biosynthesis of flavonoids or stilbenes in *E. coli* (Liang et al., 2016; Wu et al., 2015; Wu et al., 2017a and b).

Despite the above-mentioned progresses, overproducing enzymes involved in the biosynthesis of malonyl-CoA or deleting those implicated in its consumption do not avail a sufficient improvement in malonyl-CoA levels (Lim et al., 2011). Hence, the entire metabolic pathway involving malonyl-CoA and its related precursors should be taken into consideration. Firstly, the effects of redirecting the malonyl-CoA flux towards the biosynthesis of resveratrol in *E. coli* had to be evaluated. For this, the Optforce algorithm, which is designed to consider the genome-scale metabolic flux measurements that are available, was used to prioritize the implementation of genetic manipulation of metabolic pathways (Bhan et al., 2013). In that sense, multiple genetic alterations of the central carbon metabolism in *E. coli* were shown to increase resveratrol titers by 60% (1.6 g/L) *vs* control (Table 2). These genetic modifications targeted the tricarboxylic acid cycle and the glycolysis

pathway, which were used to redirect the carbon flux into malonyl-CoA. These included the gene overexpression of the pyruvate dehydrogenase multi enzyme complex, a phosphoglycerate kinase, a glyceraldehyde-3-phosphate dehydrogenase and deletion of the fumarase C encoding gene (Bhan et al., 2013) (Fig. 3).

One of the latest strategies described in the literature takes the control of entire metabolic pathways into consideration. This consists of implementing a CRISPRi system in *E. coli* to redirect the endogenous metabolism to malonyl-CoA on one side, and to engineer both the shikimate and phenylpropanoid routes to pinosylvin, on the other side (Wu et al., 2017a) (Fig. 3). To increase the production of phenylalanine from D-glucose, three genes were overexpressed encoding respectively the DAHP synthase ($aroF^{wt}$), a feed-back inhibition resistant chorismate mutase/prephenate dehydratase ($pheA^{fbr}$) and the *PAL* gene. A two-gene downstream pathway to pinosylvin including overexpression of *4CL* and *STS* was also introduced. A CRISPRi-based redirection of the central metabolism to malonyl-CoA included gene repressions respectively involved in a) the fatty acid biosynthetic pathway (*fabB/fabF*), b) the tricarboxylic acid cycle (succinate CoA ligase, *SucC*, and fumarate hydratase, *fumC*), and c) glycolysis (enolase, *eno*) and the fermentative pathway through ethanol formation (aldehyde-alcohol dehydrogenase, *adhE*) (Wu et al., 2017a) (Fig. 3). The positive performance of the system was obtained by a) a low repressing efficacy towards the *eno*, *adhE* and *fabB* genes, b) by a medium repressing efficacy towards genes involved in the citric acid cycle (*SucC* and *FumC*) and c) by a high repressing efficacy towards *fabF*. These last observations suggest that repressing the *fabF* gene of the fatty acid biosynthetic pathway is a determinant point in redirecting the carbon flux into malonyl-CoA without altering cell growth. Repressing the *SucC* and *FumC* genes from the tricarboxylic acid cycle also appears to be crucial for the redirection of the central metabolism to malonyl-CoA. This confirms the previous works of Bhan et al. (2013) showing that down-regulation of the *FumC* gene has a positive effect on resveratrol production in *E. coli*. Conversely, repressing the *eno*, *adhE* and *fabB* genes seems to be less important for redirecting the carbon flow into malonyl-CoA. These transformations resulted in the highest pinosylvin titer (281 mg/L) reported to date by using a simple starting precursor easily usable in industrial process.

6. Fused and scaffolded proteins

The exploration of various genetic constructions leading to efficient platforms composed of exogenous genes, that encode enzymes displaying a desired activity, continues to be a challenging task (Lim et al., 2011; Lu et al., 2016). Co-localizing stilbene biosynthetic pathway enzymes such as 4CL and STS and reducing the number of constructs in a heterologous expression system by the use of fused proteins or scaffolded proteins could represent a valuable strategy. The advantages of this strategy relate to the simplification of the reconstitution of metabolic pathways, the reduction of the loss of pathway intermediates and the increase of turnover rates, all designed to enhance enzyme efficiency and stilbene production yields (Lu et al., 2016). For gene fusion technology, both *4CL* and *STS* genes are linked by a few amino-acids flexible linker. For example, a genetic construct was designed to encode a translational fusion protein system of the 4CL from *A. thaliana* and the STS from *V. vinifera*, linked together by a three amino-acids linker, but leading to a low resveratrol production in yeast (5.25 mg/L) (Zhang et al., 2006). This protein fusion strategy was also applied to unexpected cellular systems such as a co-transfected mammalian kidney cell line where *TAL* and a fused *4CL::STS* gene construct were expressed (Zhang et al., 2006). Another approach based on a synthetic protein scaffolds strategy was used to improve resveratrol production in yeast cells (Wang and Yu, 2012). Here, the scaffold bearing the 4CL and STS proteins resulted in a 5-fold improvement

in resveratrol biosynthesis in yeast (14.4 mg/L) over the non-scaffolded control and a 2.7-fold increase compared to the previous short-linker fusion protein construct (Zhang et al., 2006) (Table 1). This was likely related to a more flexible linkage of the two enzymes by scaffolding as compared to the more rigid protein fusion constructs (Wang and Yu, 2012; Zhang et al., 2006). Recently, insertion of a flexible peptide of 15 neutral amino-acids was successfully used between the *4CL* and the resveratrol synthase (*RS*) genes to ensure the correct folding of the two corresponding proteins in the fusion gene construct (Zhang et al., 2015) (Table 1). The latter was the best performing system as yet reported in an *E. coli* strain harboring the *A. thaliana 4CL* and *A. hypogea RS* fusion gene, leading to a resveratrol production higher than 80 mg/L along with a 35% *p*-coumarate conversion yield.

7. Rational modular design approaches

Significant insights in metabolic engineering and synthetic biology arose in 2010 by the use of a new approach termed "multivariate-modular pathway engineering" and characterized by the partitioning of entire pathways into small modules whose expression can be regulated. This approach was first carried on to engineer the isoprenoid pathway for the production of taxadiene, a precursor of the anticancer drug taxol in *E. coli* (Ajikumar et al., 2010).

In fact, the main difficulty encountered when engineering stilbene pathways in microorganisms was that *de novo* synthesis of these compounds required the manipulation of multi-gene pathways which were subjected to tight cellular regulations, such as the feedback inhibition effect exerted by aromatic amino acids on their biosynthesis (Wu et al., 2017a). Rational modular design approaches were thus developed to overcome the necessary metabolic flux imbalances observed when implementing a heterologous synthetic pathway (Wu et al., 2013 and 2017a). This type of approach was useful for the fine-tuning of synthetic pathways as well as for identifying and averting potential pathway bottlenecks such as the build-up of cinnamate or *p*-coumaroyl-CoA. The complete pathways are thus redefined as a collection of distinct modules including the source pathways (formation of cinnamate or *p*-coumaroyl-CoA, malonyl-CoA and final stilbenes) and the malonyl-CoA sink pathway.

Through this approach, the resveratrol heterologous biosynthetic pathway was partitioned into two modules separated at the *p*-coumaroyl-CoA level using tyrosine as a starting precursor in *E. coli* (Wu et al., 2013). The first module comprises the genes encoding TAL and 4CL while the second module contains the gene encoding STS. A third module was added to increase the intracellular malonyl-CoA pool and was composed of genes encoding a malonate transporter (*matC*) and a malonyl-CoA synthase (*matB*) (Wu et al., 2013). Even so, the reported resveratrol titers remained modest (around 35 mg/L). A pinosylvin pathway including a total of 11 genes was partitioned into three modules and introduced in *E. coli* utilizing D-glucose as the carbon source (Wu et al., 2017a). The first module was consisted of a three-gene synthetic construct directing metabolic flow from D-glucose to cinnamate. The second module was composed of *4CL* and *STS* genes to produce pinosylvin while a third module was added to increase the malonyl-CoA pool by redirecting the central metabolism. Using this successful strategy, the pinosylvin titer reached 281 mg/L (Table 2). Very recently, a similar approach was applied for the production of resveratrol and piceatannol in *E. coli* (Shrestha et al., 2018). The employed strategy targeted the intracellular pool of acetyl-CoA and malonyl-CoA stressing on the malonate assimilation and anabolic pathways by overexpression of *matB* and *matC* genes or the *ACC* gene. Doping with either sodium acetate or disodium malonate aimed at increasing the levels of acetyl-CoA and malonyl-CoA. Module 1 comprises *4CL* and *STS* genes for resveratrol production. Module 2 targets the malonyl-CoA intracellular pool with module 2a

harboring *ACC* genes from *E. coli*, module 2b, *ACC* genes from *Nocardia farcinica* or module 2c, bearing the *matB* and *matC* genes from *S. coelicolor*. A third module was added for piceatannol production consisting of two genes encoding regiospecific 3'-hydroxylating enzymes, *HpaB* and *HpaC* from *E. coli* (Table 2). The combination of modules 1 and 2c upon supplementation with disodium malonate led to a resveratrol titer of 151 mg/L. High piceatannol yield (124 mg/L) was obtained from resveratrol after the addition of module 3 to the latter construct (Table 2) (Shrestha et al., 2018).

8. Engineering microbial cells with decorating enzymes

8.1 Natural stilbenes

Other stilbene derivatives including hydroxylated, glycosylated and methylated stilbenes can also be obtained by inserting decorating enzymes such as hydroxylases, glycosyl- or methyl-transferases in bacteria (Choi et al., 2014; Heo et al., 2017; Jeong et al., 2014 and 2015; Kallscheuer et al., 2017b; Kang et al., 2014; Katsuyama et al., 2007a; Li et al., 2016; Ozaki et al., 2012; Wang et al., 2014 and 2015) and yeast (Li et al., 2016; Wang et al., 2014) (Tables 1 and 2). Piceatannol, **1**, (3,3',4',5-tetrahydroxystilbene) is a natural resveratrol analog that presents an additional hydroxyphenyl group at the C'-3 position (Fig. 5). Surprisingly, this compound was obtained by expression of the *p*-coumarate 3-hydroxylase (*C3H*)-encoding gene from the bacterium *Saccharothrix espanaensis* NRRL 15764 in a recombinant *E. coli* strain that additionally bear the *TAL*, *4CL* and *STS* genes (Wang et al., 2015). This indicates that C3H enzyme, aside from its conventional activity on *p*-coumarate, may act directly on resveratrol to form piceatannol. Albeit resveratrol is not known as the usual substrate for C3H, piceatannol was recovered with a titer of 21 mg/L (Wang et al., 2015). This compound was also obtained at a detectable level in *E. coli* (13 mg/L) and *C. glutamicum* (56 mg/L) (Kallscheuer et al., 2016) harboring *4CL* and *STS* genes following feeding with caffeic acid, and probing the transformation of other phenylpropanoic acids by STS (Watts et al., 2006). Conversely, ferulic acid failed to be converted to the corresponding stilbene, isorhapontignenin (Watts et al., 2006). A high piceatannol titer (124 mg/L) was also recovered by overexpression of two monooxygenases with hydroxylating activity, HpaB and HpaC, in *E. coli* (see section 7) (Shrestha et al., 2018).

Glycosylation of the stilbene backbone can be seen as a way to increase the solubility of phytostilbenes that are reputed not to be readily soluble in their aglycone forms in biological matrices. In plant cells, free stilbenes are produced and glycosylated by endogenous glycosyltransferases. A bi-functional glucosyltransferase has been characterized in grapevine and shown to transfer a glycosyl moiety both to stilbenes and hydroxycinnamic acids (Hall and De Luca, 2007). However, the functional expression of glucosyltransferases from other sources was chosen to form glycosylated stilbenes in engineered *E. coli* strains, such as the *Pa*GT3 from *Phytolacca americana* (Ozaki et al., 2012; Thuan et al., 2018) or the UDP-glycosyltransferase gene *yjiC* from *Bacillus* spp. (Choi et al., 2014) (Table 2). This resulted in the production of piceid, **2** (the resveratrol-3-*O*-β-D-glucoside), and the resveratrol-4'-*O*-β-D-glucoside, **3**, indicative of a moderate regioselectivity of the glycosylation process (Fig. 5). Recently, a two-population *E. coli* co-culture system comprising an upstream module for resveratrol biosynthesis (*4CL* from *N. tabacum*, *V. vinifera STS*) and a module dedicated to resveratrol glycosylation via the UDPG/ *Pa*GT3 from *P. americana*, was used to produce piceid (Thuan et al., 2018). Channeling the carbon flux towards UDP-glucose was obtained by knock-out of the bacterial genes *pgi* and *zwf* encoding respectively the glucose-6-phosphate isomerase and the glucose-6-phosphate dehydrogenase, and overexpression of

the gene *hasC* encoding the glucose-1-phosphate uridylyltransferase. Upon feeding with *p*-coumarate, the resultant production of 15 mg/L of piceid was reported. Interestingly, the piceid titer followed a 6-fold increase upon scale-up fermentation from 50 mL flasks to a 3-L fed-batch bioreactor.

Engineering of methylated stilbene production in *E. coli* and, to a lesser extend in yeast, has attracted wide interest as methylation of the stilbene scaffold leads to compounds with enhanced bioactivity (Heo et al., 2017; Jeong et al., 2014 and 2015; Kang et al., 2014; Katsuyama et al, 2007a; Wang et al., 2014 and 2015) (Tables 1 and 2). The production of methylated stilbene derivatives requires the action of *O*-methyltransferases (OMT) able to catalyze the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to the hydroxyphenyl groups of stilbenes (Tables 1 and 2). Few numbers of characterized OMTs function as methyltransferases for stilbenes. Some of them catalyze ring-specific substitutions as, for example, the *Sorghum bicolor O*-methyltransferase 3 (SbOMT3) or the resveratrol-*O*-methyltransferase (SbROMT3), which presents an A-ring specific 3,5-bis-*O*-methylating activity on resveratrol, whereas the SbOMT1/SbROMT1 predominantly catalyzes the B-ring 4'-*O*-methylation (Kang et al., 2014; Rimando et al., 2012). Surprisingly, expression of the *SbOMT3* gene in recombinant *E. coli* using resveratrol as a substrate led to high titers (34 mg/L) of pinostilbene, **4**, (4',5-dihydroxy-3-methoxystilbene) but to an unexpectedly very low production of pterostilbene, **5**, (< mg/L), suggesting incomplete 3,5-bis-*O*-methylation of resveratrol (Jeong et al., 2014) (Fig. 5). In contrast, the ROMT activity from a multifunctional caffeic acid *O*-methyltransferase (COMT) from *A. thaliana* has successfully been used for pterostilbene production in *E. coli*, indicating that a non-specific OMT can insure the transfer of methyl groups to stilbenes (Heo et al., 2017). In the latter case, a complete construct including *TAL*, *4CL* and *STS* genes co-expressed with the *COMT* gene in presence of methionine as a SAM precursor, led to a high pterostilbene titer of *ca* 34 mg/L in an engineered L-tyrosine-overproducing *E. coli* strain.

Besides, SbROMT1 and SbROMT3 can lead to unusual natural stilbenes such as 3,5-dihydroxy-4'-methoxystilbene, **6**, 3,4'-dimethoy-5-hydroxystilbene, **7**, and 3,5,4'-trimethoxystilbene, **8**, in the order of milligrams per liter in engineered *E. coli* (Kang et al., 2014) (Fig. 6). Two other OMTs have been over-expressed in *E. coli* for methylated stilbene production such as the ROMT from *Vitis riparia* (VrROMT) exhibiting 98% homology at the amino-acid level with the ROMT from *V. vinifera* (VvROMT) (Schmidlin et al., 2008) and the pinosylvin *O*-methyl transferase from *Oryza sativa* (OsPMT) (Katsuyama et al., 2007a; Jeong et al., 2014 and 2015). The *O*-methylating activity of the OsPMT was demonstrated via the precursor-directed biosynthesis of stilbene methyl ethers in *E. coli* (Katsuyama et al., 2007a). When phenylalanine or tyrosine were used as precursors, a construct composed of *PAL*, *4CL*, *STS* and *OsPMT* led to ten milligrams quantities of pinosylvin, pinosylvin monomethyl- and dimethyl ethers on one hand, and to resveratrol, pinostilbene and pterostilbene on the other (Table 2). Expression of the *VrROMT* gene results in no methylating activity in *E. coli* compared to the expression of the *SbROMT* gene (Jeong et al., 2014 and 2015). In contrast, by co-expressing in *E. coli* and the *S. cerevisiae* yeast the *VvROMT* gene together with the *4CL* and *STS* gene fusion from the resveratrol pathway, pterostilbene was obtained as the major methylated derivative (50 mg/L and 2 mg/L, respectively) (Wang et al., 2014). Pinostilbene (5.5 mg/L) and pterostilbene (34.9 mg/L) were also produced in *S. cerevisiae* yeast harboring the *VvROMT* and *SbROMT* genes associated with a dozen of genetic modifications related to the resveratrol biosynthetic pathway (Li et al., 2016) (Table 1). A recent work has also shown that the subsequent methylation of stilbenes might be limited by the intracellular pool of the methyl donor SAM and the low OMTs solubility (Kallscheuer et al., 2017b). To alleviate the hindrance to the action of OMTs in engineered microbial cells, a *C. glutamicum* strain harboring *4CL* and *STS* was modified with the *E. coli metK* gene encoding

a SAM synthase and a gene fusion of *VvOMT* with a maltose-binding protein *MalE* known to increase the solubility of the targeted proteins (Carter and Hausinger, 2010; Chen et al., 2011) (Fig. 4). A high pterostilbene level was thus reached (42 mg/L). Subsequent stilbene modifications with decorating enzymes can be obtained by optimizing their transport into the cell. Expression of a non-specific arabinose-H^+ transporter (*araE*) in yeast was indeed suspected to favor the resveratrol transport in yeast (Wang et al., 2011).

Microbial cells could thus represent valuable platforms for the synthesis of stilbene derivatives, mainly methylated and glycosylated stilbenes. However, methylation of the stilbene hydroxyphenyl groups by methyltransferases is largely unpredictable because of the randomized introduction of the methyl groups on the stilbene backbone. The same difficulties are encountered during the glycosylation of stilbenes since the transfer of the glycosyl moiety on the stilbene core is rarely regioselective. Such a situation also prevails upon the introduction of *O*-methyltransferases or glycosyltransferases in plants, leading to unexpected substitutions (Delaunois et al., 2009; Jeandet et al., 2013a; Rimando et al., 2012). No production of resveratrol oligomers has been reported to date in engineered microbial cells. It is well known that stilbene oligomers present some interesting anticancer activities (Nivelle et al., 2017a and b; Tisserant et al., 2016) and are produced upon a polymerization process starting from stilbene radicals (Bavaresco et al., 2012). Final condensation of these oligomers is achieved by peroxidases but whose heterologous expression in microorganisms has not yet been described, albeit stilbene dehydrodimers such as ε-viniferin (Fig. 1) and pallidol have already been produced artificially by peroxidases of horseradish (Langcake and Pryce, 1977) and soybean (Mora-Pale et al., 2015).

8.2 Unnatural stilbenes

Stilbenes display a great deal of structural diversity depending on the nature of the starting substrates, the number of condensation steps and the mode of ring closure of the polyketide chains formed under the control of CHS or STS, respectively (Horinouchi, 2008; Katsuyama at al., 2007b). Moreover, it has been reported that naturally-occurring stilbenes have a weaker activity than synthetic chemistry-based compounds. For example, effective doses of stilbene phytoalexins usually fall within one order of magnitude (10^{-5} to 10^{-4}M), which is lower than the efficiency of chemical fungicides (Smith, 1982). Thus, in the search of more active compounds, efforts have been made to produce unnatural stilbenes by combinatorial biosynthesis (Horinouchi, 2008; Katsuyama at al., 2007b) or expanding the chemical space of stilbenes through structure-guided mutagenesis of the *V. vinifera* STS (Bhan et al., 2015a and b).

A recombinant *E. coli* strain carrying a three-gene construct composed of *4CL* from *L. erythrorhizon*, *STS* from *A. hypogea* and *ACC* from *C. glutamicum*, and supplied with various carboxylic acids, led to the production of 15 unnatural stilbenes with yields of 50 to 150 mg/L (Horinouchi, 2008; Katsuyama at al., 2007b). A variety of unnatural carboxylic acids such as 3 or 4-fluorocinnamic acids, 4-methylcinnamic acid and a series of furyl-, thienyl- and 3-(3-pyridilic) cinnamic acids, form the corresponding stilbenes, **9-13** (Fig. 6). Interestingly, the 4'-fluoro-3,4-dihydroxystilbene, **9**, and the two stilbenes containing a furan ring, **12a** (*trans*-5-(2-furan-2-yl)vinyl)benzene-1,3-diol), or a thiophen ring, **12b** (*trans*-5-(2-thiophen-2-yl)vinyl)benzene-1,3-diol), showed a significant inhibitory activity of CYP1B1, an enzyme catalyzing hydroxylation of 17-β-estradiol and implied in the metabolism of procarcinogens (Chun and Kim, 2003; Katsuyama et al 2007b). Moreover, the presence on the stilbene core of electron-attracting groups such as halogens

(chlorine or fluorine; **9**,**10**) was thought to increase the biological activity of the corresponding stilbenes (Pont and Pezet, 1990).

Rational engineering of STS enzymes, particularly the *V. vinifera* STS, was shown to be an interesting option for the production of unnatural polyketides by controlling the number of malonyl-CoA condensations, varying the CoA starter substrates and managing the cyclization mechanisms of the resultant polyketide chain (Bhan et al., 2015a and b). A resorcinol-structure-based stilbene **14**, was obtained by a structure-guided *V. vinifera* STS mutant including a single point threonine to glycine mutation at amino acid 197 (Bhan et al., 2015b). This mutation allowed the attachment of an extra-acetyl unit leading to compound **14** (*trans*-1-(3,5-dihydroxyphenyl)-4-(4-hydroxyphenyl)but-3-en-2-one), with a 4-carbon chain between the two rings (instead of 2 carbons in natural stilbenes). In another study, the wild type STS of *V. vinifera* was evaluated in the presence of various non-natural CoA starter substrates such as propionyl-CoA, myristoyl-CoA or methylmalonyl-CoA (Bhan et al., 2015a). An unnatural stilbene-like compound **15** (2-ethyl-6-(4-hydroxyphenethyl)-3,4-dihydro-2*H*-pyran-2,4-diol), was obtained for example, when the wild type STS of *V. vinifera* was supplied with propionyl-CoA (Fig. 6). Non-natural polyketides were produced, as well, using this series of CoA starter substrates with various structure-based mutants of *V. vinifera* STS (Bhan et al., 2015a). Overall, these studies also showed that enzymes such as CoA ligases and STS, which operate on the stilbene pathway, are able to accept numerous precursor molecules which are structurally very different from their natural substrates (Bhan et al., 2015a and b; Katsuyama et al., 2007b).

9. Best producing microbial systems

A distinction should be made between systems using simple carbon sources such as D-glucose and ethanol, or source precursors such as cinnamate, for the biosynthesis of pinosylvin on one hand, or *p*-coumarate, for resveratrol biosynthesis on the other (Tables 1 and 2). The high cost and toxicity of *p*-coumarate towards cell growth constitute a major drawback for the large-scale production of stilbenes by microorganisms (Shin et al., 2012). Supplementing a microbial system with expensive sources may be realistic for small-scale experiments but remains unsuitable for industrial applications. Lim et al. (2011) showed that feeding engineered microorganisms with simple phenylpropanoid compounds was efficient enough for the biosynthesis of pynosylvin or resveratrol through the expression of only two *CL*/*4CL* and *STS* genes. An impressive titer of 2.3 g/L resveratrol was indeed reported in a recombinant *E. coli* by feeding it with an increased *p*-coumarate supply (15 mM, *i.e.* 2.46 g/L) and addition of cerulenin in the growth broth (Lim et al., 2011). In the same study, resveratrol production was optimized by testing various promoter sequences. The constitutive promoter of the glyceraldehyde-3-phosphate dehydrogenase (*gap)* gene gave better results than the commonly used IPTG-inducible promoter (pT7). The former was constitutively activated in the presence of D-glucose, allowing the continuous transcription of the *4CL* and *STS* genes. In turn, when both the *A. thaliana 4CL* and *V. vinifera STS* genes were expressed under the control of separate *gap* promoters or expressed together in an operon, the resveratrol titer increased by 4-fold (Lim et al., 2011). This underlines the significance of the promoter to obtain a high-yield resveratrol production. A high resveratrol titer of 1.6 g/L was also reported in *E. coli* without the addition of cerulenin but in presence of a high concentration of *p*-coumarate (15 mM *i.e.* 2.46 g/L) as a substrate and by redirecting the carbon central metabolism to the synthesis of malonyl-CoA (Bhan et al., 2013). Regulating malonyl-CoA metabolism via a synthetic antisense RNA strategy led to a resveratrol titer of 280 mg/L in *E. coli* by addition of *p*-coumarate (Yang et al., 2015) (Table 2). Finally, a high resveratrol production (391 mg/L) was obtained with an industrial Brazilian sugar cane yeast by

adding 5 mM (0.82 g/L) *p*-coumarate in a rich medium, underlying the role played by the culture medium to reach high stilbene production titers (Sydor et al., 2010) (Table 1).

In other similar studies, the *de novo* pinosylvin and resveratrol biosynthesis have been reported in yeast and *E. coli*, in the scale of hundreds milligrams per liter of culture utilizing cheap carbon sources such as glucose or ethanol (Li et al., 2015; Wu et al., 2017a and b). Using glucose as the source for stilbene production implies that generation of the aromatic amino acids needed to supply the phenylpropanoid pathway are efficiently synthesized through the shikimate pathway. Such a strategy requires engineered strains exhibiting enhanced capacity for phenylalanine or tyrosine biosynthesis (Li et al., 2015; Wu et al., 2017a and b), coupled with an engineered stilbene pathway and a CRISPRi-based redirection of the central carbon metabolism to malonyl-CoA. These systems led respectively to a resveratrol production of 305 mg/L (Wu et al., 2017b) and a pinosylvin titer of 281 mg/L (Wu et al., 2017a).

In a yeast strain over-expressing feedback-insensitive alleles of DAHP synthase and chorismate mutase genes, along with the *TAL*, *4CL* and *STS* genes together with the *ACC* gene, a following pulse feeding strategy using D-glucose or ethanol as primary carbon sources, led to high resveratrol titers of 415 and 531 mg/L respectively (Li et al., 2015). A complex genetic construct of 12 genetic modifications was also recently introduced in *S. cerevisiae* yeast leading to the highest value (800 mg/L) ever reported for resveratrol production in an engineered yeast (Li et al., 2016). This construct was designed to i) engineer the shikimate pathway with resistant versions to feedback inhibition of DAHP synthase and chorismate mutase combined to the overexpression of a shikimate kinase gene (*aroL*), ii) increase the intracellular malonyl-CoA pool with a deregulated variant of ACC and a non-regulated version of ACS, iii) introduce the whole pathway of resveratrol biosynthesis (*PAL*, *4CH*, *4CL*, *STS* genes) including overexpression of a cytochrome P450 reductase gene. Additionally, the engineered yeast strain harbored a gene deletion of the ARO10 phenylpyruvate decarboxylase, which could divert phenylalanine towards the production of phenyl-2-ethanol, a higher alcohol produced through the Ehrlich pathway in yeast (Fig. 2) (Li et al., 2016).

Overall, the highest performances ever reported in terms of resveratrol production, respectively, 2.3 g/L (Lim et al., 2011) and 1.6 g/L (Bhan et al., 2013) in recombinant microorganisms, were obtained in the presence of an elevated concentration of *p*-coumarate with or without addition of cerulenin, that is, two costly and toxic compounds to cell growth. All other high resveratrol or pinosylvin titers (in the order of hundreds of milligrams), were recovered using glucose or ethanol, cheap carbon sources without any addition of cerulenin (Li et al., 2015 and 2016; Wu et al., 2017a and b). However, it should be noted that most of these studies were performed in flasks with very small culture volumes ranging from 10 to 50 mL (Bhan et al., 2013; Lim et al., 2011; Sydor et al., 2010; Wu et al., 2017a and b). This raises the question of up-scaling from small flasks to industrial applications in higher volume-bioreactors. Two studies have reported high resveratrol production using fed-batch fermentation systems with larger volume (400 mL) to grow recombinant yeast strains, reaching titers respectively of 415 and 800 mg/L (Li et al., 2015 and 2016). It is thus difficult to clearly define which systems are more suitable for obtaining high stilbene titers in recombinant microorganisms. Feeding tailored yeast or bacteria with *p*-coumarate as a substrate led to the best results in terms of resveratrol production (> g/L) (Bhan et al., 2013; Lim et al., 2011). Conversely, lower resveratrol or pinosylvin titers (hundreds of milligrams per liter) were obtained in yeast or bacteria from cheap carbon sources, needing the introduction of complex genetic constructs.

10. Conclusions and perspectives

The strategies of engineering stilbene biosynthetic pathways in microorganisms have considerably evolved. The applicable genetic constructs are becoming more and more complex. The simplest systems initially employed harbored the two ending genes of the phenylpropanoid pathway, *4CL* and *STS* genes, using precursors located downstream of the pathway such as *p*-coumarate or cinnamate. In another side, strategies based on the use of the aromatic amino acids phenylalanine and tyrosine, were facing the low activity of the heterologous PAL and TAL ammonia lyases in the transgenic organisms as well as the feed-back inhibition exerted by these two amino acids on the shikimate pathway. It quickly became necessary to engineer feed-back resistant versions of the DAHP synthase and the chorismate mutase (Camacho-Zaragoza et al., 2016; Li et al., 2015; Wu et al., 2017a; Kallscheuer et al., 2016). Moreover, to alleviate the low expression of the *TAL* gene in recombinant microorganisms, some efforts have been directed on attempts for resynthesizing the entire *TAL* genes, for instance by replacing bacterial codons with yeast ones (Wang et al., 2011) or by modifying the 5' region of the *TAL* mRNAs (Wu et al., 2017a and b). Protein engineering will thus be useful in the future to improve the performance of these enzymes in microbial hosts (Milke et al., 2018). Structure-guided mutagenesis of the STS with resultant modification of the substrate specificity of this enzyme would also be of interest to expand the chemical spaces of stilbenes (Bhan et al., 2015a and b). Interesting strategies are also under development to by-pass the use of phenylalanine or tyrosine. These consist of engineering novel synthetic pathways, that are not found in nature, and totally independent from the endogenous metabolism of these two amino acids. For example, the reversal of the β-oxidative phenylpropanoid degradation has been proposed to form *p*-coumaroyl-CoA upon *C. glutamicum* feeding with 4-hydroxybenzoic acid (Kallscheuer et al., 2017a).

The current strategies developed for engineering stilbene pathways in microbial cells are based on synthetic biotechnolology techniques. They implement advanced gene constructs by integrating both the anabolic and metabolic routes of malonyl-CoA. In fact, the metabolic utilization of malonyl-CoA through fatty acid synthesis has been taken as a crucial approach to improve stilbene titers in tailored microorganisms. The contributions of bioinformatics to synthetic biology have further allowed to predict which target genes are likely to be up-regulated or deleted for controlling the intracellular malonyl-CoA pool. This showed that a redirection of the central carbon metabolism by regulating the fatty acid synthesis, but also other enzymes operating on glycolysis, the tricarboxylic acid cycle or the fermentation pathway resulted in the concomitant increases of malonyl-CoA and resveratrol titers in *E. coli* (Bhan et al., 2013). The development of biosensor-based dynamic regulation techniques can also be considered for the control of both the malonyl-CoA anabolic pathway and its sink pathway (Johnson et al., 2017. Schallmey et al., 2014; Siedler et al., 2017; Trantas et al., 2015; Xu et al., 2014). The design of two malonyl-CoA sensors displaying opposite transcriptional activities and controlled by a single regulatory protein (FapR) could be attempted to increase the stilbene titers in engineered microorganisms as previously reported in the production and yield optimization of the fatty acids in *E. coli* (Xu et al., 2014). In the case of stilbene production in bacteria, a similar malonyl-CoA switch could be indeed developed, comprising on one hand, the malonyl-CoA source pathway enzyme gene (*ACC* gene) driven by a promoter with an "active" box and, on the other hand, the malonyl-CoA sink pathway (genes operating on the fatty acid synthesis) driven by a promoter with a "repressive" box. The two promoters could be controlled by the same transcription factor simultaneously targeting active or repressive boxes and minimizing any damage to the cell (Trantas et al., 2015). Such approaches thus afford new paradigms in

synthetic biology offering the possibility to optimize metabolic pathways for the high-yield production of malonyl-CoA–derived stilbenes.

Interesting computations have indicated that the resveratrol production yield obtained in an engineered yeast strain fed with D-glucose was 7 mmol/mol for a maximum theoretical yield of 280 mmol/mol glucose (Li et al., 2016). Such discrepancies underline that even when using a recombinant yeast strain harboring multiple genetic modifications, stilbene production yields remain low. There is thus still a lot of room left for improvement of strategies used for engineering stilbene production in microbial cells. It also becomes more and more evident that the efficient microbial production of natural compounds of pharmaceutical significance depends on the molecular engineering of multiple pathways including the engineering of the endogenous cell metabolism (Nielsen and Keasling, 2016). Thus, the regulation of various pathways relies on complex constructs harboring multiple, occasionally more than ten genetic modifications (Kallscheuer et al., 2017a; Li et al., 2016; Wu et al. 2017a and b). The genetic constructs could also target interfering pathways such as the phenyl-2-ethanol production by deleting the phenyl acrylic acid decarboxylase *PAD 1* gene in yeast (Shin et al., 2011), the phenylpropanoid compounds degradation by deleting the *phdBCDE* operon in *C. glutamicum* (Kallscheuer et al., 2016) or the 4-vinylphenols production from *p*-coumarate by deleting the phenylpyruvate decarboxylase *ARO10* gene in yeast (Li et al., 2016).

Recent molecular techniques could also be applied to develop rapid assembly of synthetic operons in various pathways such as the Operon-PLICing (phosphorothiolate-based ligase-independent gene cloning) method (Blanusa et al., 2010; Van-Summeren et al., 2015). The development of an operon composed of several target genes under the same promoter can optimize the expression of an entire metabolic pathway. The Operon-PLICing method allows the combinatorial assembly of several genes involved in a biosynthetic pathway by optimizing the Shine-Delgarno sequence-start codon space. All possible combinations can be simply and rapidly generated producing a large number of recombinant microbial strains. A screening of the variant strains easily identifies the operons with the most efficient assembly. This technique also offers the possibility of fine-tuning (balancing) heterologous gene expression of all pathway genes and could be applicable to stilbene production in engineered microbial cells, as was successfully applied in *E. coli* for the biosynthesis of *p*-coumaroyl alcohol (Van Summeren-Wesenhagen et al., 2015).

On the other hand, approaches such as combinatorial biosynthesis will constitute exciting options for the production of unnatural stilbenes with expected increased biological activities (Bhan et al., 2015a and b; Katsuyama et al., 2007b).

Finally, increasing resveratrol and related stilbene titers in recombinant microorganisms will require optimization of various growth parameters such as operating modes (batch or fed batch), substrate concentration and oxygen supply. Raising the glucose concentration from 40 to 80 g/L in the growth medium of an engineered *C. glutamicum* strain, led to a 3-fold increase of resveratrol production (Braga et al., 2018b). This confirms previous observations regarding the increase of resveratrol production in recombinant yeast by using a rich medium (Sydor et al., 2010). In the same experimental system, high oxygen concentrations negatively affected the resveratrol production, likely due to its action on resveratrol oxidation. Optimization of culture conditions for engineered microbial cell factories will also raise the important question of the up-scaling from flask experiments to higher volumes in bioreactors for application to the industry.

References

Abe, I.; Watanabe, T.; Noguchi, H., 2004. Enzymatic formation of long-chain polyketide pyrones by plant type III polyketide synthases. Phytochemistry 65, 2447-2453.

Adrian, M.; Jeandet, P., 2012. Effects of resveratrol on the ultrastructure of *Botrytis cinerea* conidia and biological significance in plant/pathogen interactions. Fitoterapia 83, 1345-1350.

Adrian, M.; Jeandet, P.; Veneau, J.; Weston, L.A.; Bessis, R., 1997. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. J. Chem. Ecol. 23, 1689-1702.

Ahmad, N.; Adhami, V.M.; Afaq, F.; Feyes, D.K.; Mukhtar, H., 2001. Resveratrol causes WAF-1/p21-mediated G(1) phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin. Cancer Res. 7, 1466-1473.

Ajikumar, P.K.; Xiao, W.H.; Tyo, K.E.J.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T.H.; Pfeifer, B.; Stephanopoulos, G., 2010. Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli*. Science 330, 70-74.

An, J.H.; Kim, Y.S., 1998. A gene cluster encoding malonyl-CoA decarboxylase (MatA), malonyl-CoA synthetase (MatB) and a putative dicarboxylate carrier protein (MatC) in *Rhizobium trifolii*-cloning, sequencing, and expression of the enzymes in *Escherichia coli*. Eur. J. Biochem. 257, 395-402.

Anekonda, T.S., 2006. Resveratrol - A boon for treating Alzheimer's disease? Brain Res. Rev. 52, 316-326.

Austin, M.B.; Noel, J.P., 2003. The chalcone synthase superfamily of type III polyketide synthases. Nat. Prod. Rep. 20, 79-110.

Baur, J.A.; Sindair, D.A., 2006. Therapeutic potential of resveratrol: the *in vivo* evidence. Nat. Rev. Drug. Discov. 5, 493-506.

Bhan, N.; Cress, B.F.; Linhardt, R.J.; Koffas, M.A.G., 2015a. Expanding the chemical space of polyketides through structure-guided mutagenesis of *Vitis vinifera* stilbene synthase. Biochimie 115, 136-143.

Bhan, N.; Li, L.; Cai, C.; Xu, P.; Linhardt, R.J.; Koffas, M.A.G., 2015b. Enzymatic formation of a resorcylic acid by creating a structure-guided single-point mutation in stilbene synthase. Prot. Sci. 24, 167-173.

Bhan, N.; Xu, P.; Khalidi, O.; Koffas, M.A.G., 2013. Redirecting carbon flux into malonyl-CoA to improve resveratrol titers: Proof of concept for genetic interventions predicted by optForce computational framework. Chem. Eng. Sci. 103, 109-114.

Bavaresco, L.; Mattivi, F.; De Rosso, M.; Flamini, R., 2012. Effects of elicitors, viticultural factors, and enological practices on resveratrol and stilbenes in grapevine and wine. Mini-Rev. Med. Chem. 12, 1366–1381.

Becker, J.V.W.; Armstrong, G.O.; van der Merwe, M.J.; Lambrechts, M.G.; Vivier, M.A.; Pretorius, I.S., 2003. Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine-related antioxidant resveratrol. FEMS Yeast Res. 4, 79-85.

Beekwilder, J.; Wolswinkel, R.; Jonker, H.; Hall, R.; de Vos, C.H.; Bovy, A., 2006. Production of resveratrol in recombinant microorganisms. Appl. Environ. Microbiol. 72, 5670-5672.

Blanusa, M.; Schenk, A.; Sadeghi, H.; Marienhagen, J.; Schwaneberg, U., 2010. Phosphorothiolate-based ligase-independent gene cloning (PLICing): an enzyme-free and sequence-independent cloning method. Anal. Biochem. 406, 141-146.

Braga, A.; Ferreira, P.; Oliveira, J.; Rocha, I.; Faria, N., 2018a. Heterologous production of resveratrol in bacterial hosts: current status and perspectives. World J. Microbiol. Biotechnol. 34:122.

Braga, A.; Oliveira, J.; Silva, R.; Ferreira, P.; Rocha, I.; Kallscheuer, N.; Marienhagen, J.; Faria, N., 2018b. Impact of the cultivation strategy on resveratrol production from glucose in engineered *Corynebacterium glutamicum*. J. Biotechnol. 265, 70-75.

Brakenhielm, E.; Cao, R.; Cao, Y., 2001. Suppression of angiogenesis, tumor growth and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB J. 15, 1798-1800.

Camacho-Zaragoza, J.M.; Hernandez-Chavez, G.; Moreno-Avita, F.; Ramirez-Iniguez, R.; Martinez, A.; Bolivar, F.; Gosset, G., 2016. Engineering of a microbial coculture of *Escherichia coli* strains for the biosynthesis of resveratrol. Microb. Cell Fact. 15: 163.

Carter, E.L.; Hausinger, R.P., 2010. Characterization of the *Klebsiella aerogenes* urease accessory protein UreD in fusion with the maltose binding protein. J. Bacteriol. 192, 2294-2304.

Chan, M.M., 2002. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem. Pharmacol. 63, 99-104.

Chen, H.; Kim, H.U.; Weng, H.; Browse, J., 2011. Malonyl-CoA synthetase, encoded by *acyl activating enzyme13*, is essential for growth and development of *Arabidopsis*. Plant Cell 23, 2247-2262.

Choi, O.; Lee, J.K.; Kang, S.Y.; Pandey, R.P.; Sohng, J.K.; Ahn, J.S.; Hong, YS., 2014. Construction of artificial biosynthetic pathways for resveratrol glucoside derivatives. J. Microbiol. Biotechnol. 24, 614-618.

Chun, Y.J.; Kim, S., 2003. Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. Med. Res. Rev. 23, 657-668.

Delaunois, B.; Cordelier, S.; Conreux, A.; Clément, C.; Jeandet, P., 2009. Molecular engineering of resveratrol in plants. Plant Biotechnol. J. 7, 2-12.

Donnez, D.; Jeandet, P.; Clément, C.; Courot, E., 2009. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol. 27, 706-713.

Dudnik, A.; Gaspar, P.; Neves, A.R.; Forster, J., 2018. Engineering of microbial cell factories for the production of plant polyphenols with health-beneficial properties. Curr. Pharm. Des. 24, 2208-2225.

Estrov, Z.; Shishodia, S.; Faderl, S.; Harris, D.; Van, Q.; Kantarjian, H.M.; Talpaz, M.; Aggarwal, B.B., 2003. Resveratrol blocks interleukin-1 β-induced activation of the nuclear transcription factor NFκB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells. Blood 102, 987-995.

Frankel, E.N.; Waterhouse, A.L.; Kinsella, J.E., 1993. Inhibition of human LDL oxidation by resveratrol. Lancet 341, 1103-1104.

Fulda, S.; Debatin, K.M., 2006. Resveratrol modulation of signal transduction in apoptosis and cell survival: a mini-review. Cancer Detect. Prev. 30, 217-223.

Hain, R.; Reif, H.J.; Krause, E.; Langebartels, R.; Kindl, H.; Vornam, B.; Wiese, W.; Schmelzer, E.; Schreier, P.; Stöcker, R.; Stenzel, K., 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361, 153-156.

Hall, D.; De Luca, V., 2007. Mesocarp localization of a bi-functional resveratrol/hydroxycinnamic acid glucosyltransferase of Concord grape (*Vitis labrusca*). Plant J. 49, 579-591.

Heo, K.Y.; Kang, S.Y.; Hong, Y.S., 2017. De novo biosynthesis of pterostilbene in an *Escherichia coli* strain using a new resveratrol *O*-methyltransferase from *Arabidopsis*. Microb. Cell Fact. 16: 30.

Horinouchi S., 2008. Combinatorial biosynthesis of non-bacterial and unnatural flavonoids, stilbenoids and curcuminoids by microorganisms. J. Antibiot. 61, 709-728.

Hsieh, T.C.; Juan, G.; Darzynkiewicz, Z.; Wu, J.M., 1999. Resveratrol increases nitric oxide synthase, induces accumulation of p53 and $p21^{WAF-1/Cip-1}$ and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. Cancer Res. 59, 2596-2601.

Huang, L.L.; Xue, Z.; Zhu, Q.Q., 2009. Method for the production of resveratrol in a recombinant oleaginous microorganism. US patent 2009/0082286 A1.

Igura, K.; Ohta, T.; Kuroda, Y.; Kaji, K., 2001. Resveratrol and quercetin inhibit angiogenesis *in vitro*. Cancer Lett. 171, 11-16.

Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.; Fong, H.H.; Farnsworth, N.R.; Kinghorn, A.D.; Metha, R.G.; Moon, R.C.; Pezzuto, J.M., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275, 218-220.

Jeandet, P.; Breuil, A.C.; Adrian, M.; Weston, L.A.; Debord, S.; Meunier, P.; Maume, G.; Bessis, R., 1997. HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. Anal. Chem. 69, 5172-5177.

Jeandet, P.; Clément, C.; Courot, E.; Cordelier, S., 2013a. Modulation of phytoalexin biosynthesis in engineered plants for disease resistance. Int. J. Mol. Sci. 14, 14136-14170.

Jeandet, P.; Courot, E.; Clément, C.; Ricord, S.; Crouzet, J.; Aziz A.; Cordelier, S. 2017. Molecular engineering of phytoalexins in plants: Benefits and limitations for food and agriculture. J. Agric. Food Chem. 65, 2643-2644.

Jeandet P.; Delaunois, B.; Aziz, A.; Donnez, D.; Vasserot, Y.; Cordelier, S.; Courot, E., 2012. Metabolic engineering of plants and yeast for the production of the biologically active hydroxystilbene, resveratrol. J. Biomed. Biotechnol. 2012: 579089.

Jeandet, P.; Delaunois, B.; Conreux, A.; Donnez, D.; Nuzzo, V.; Cordelier, S.; Clément, C.; Courot, E., 2010. Biosynthesis, metabolism, molecular engineering and biological functions of stilbene phytoalexins in plants. BioFactors 36, 331-341.

Jeandet, P.; Douillet, A.C.; Weston, L.A.; Debord, S.; Sbaghi, M.; Bessis, R.; Adrian, M., 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J. Agric. Food Chem. 50, 2731-2741.

Jeandet, P.; Hébrard, C.; Deville, M.A.; Cordelier, S.; Dorey, S.; Aziz, A.; Crouzet, J., 2014. Deciphering the role of phytoalexins in plant-microorganism interactions and human health. Molecules 19, 18033-18056.

Jeandet, P.; Vasserot, Y.; Chastang, T.; Courot, E., 2013b. Engineering microbial cells for the biosynthesis of natural compounds of pharmaceutical significance. BioMed Res. Int. 2013: 780145.

Jendressen, C.B.; Stahlhut, S.G.; Li, M.; Gaspar, P.; Siedler, S.; Förster, J.; Maury, J.; Borodina, I.; Nielsen, A.T., 2015. Novel highly active and specific tyrosine ammonia lyases from diverse origins enable enhanced production of aromatic compounds in bacteria and yeast. Appl. Environ. Microbiol. 81, 4458-4476.

Jeong, Y.J.; An, C.H.; Woo, S.G.; Jeong, H.J.; Kim, Y.M.; Park, S.J.; Yoon, B.D.; Kim, C.Y., 2014. Production of pinostilbene compounds by the expression of resveratrol *O*-methyltransferase genes in *Escherichia coli*. Enzyme Microb. Technol. 54, 8-14.

Jeong, E.; Lee, H.R.; Park, H., 2013. Pinosylvin induces cell survival, migration and anti-adhesiveness of endothelial cells via nitric oxide production. Phytother. Res. 27, 610-617.

Jeong Y.J.; Woo, S.G.; An, C.H.; Jeong, H.J.; Hong, Y.S.; Kim, Y.M.; Ryu, Y.B.; Rho, M.C.; Lee, W.S.; Kim, C.Y., 2015. Metabolic engineering for resveratrol derivative biosynthesis in *Escherichia coli*. Mol. Cells 38, 318-326.

Jiang, H.; Wood, K.V.; Morgan, J.A., 2005. Metabolic engineering of the phenylpropanoid pathway in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 71, 2962-2969.

Johnson, O.; Gonzalez-Villanueva, M.; Wong, L.; Steinbüchel, A.; Tee, K.L; Xu, P.; Wong, T.S., 2017. Design and application of genetically-encoded malonyl-CoA biosensors for metabolic engineering of microbial cell factories. Metab. Eng. 44, 253-264.

Jossek, R.; Bongaerts, J.; Sprenger, G.A., 2001. Characterization of a new feedback-resistant 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase AroF of *Escherichia coli*. FEMS Microbiol. Lett. 202, 145-148.

Jung, H.J.; Hwang, I.A.; Sung, W.S.; Kang, H.; Kang, B.S.; Seu, Y.B.; Lee, D.G., 2005. Fungicidal effect of resveratrol on human infectious fungi. Arch. Pharmac. Res. 28, 557-560.

Kallscheuer, N.; Vogt, M.; Marienhagen, J. 2017a. A novel synthetic pathway enables microbial production of polyphenols independent from the endogenous aromatic amino acid metabolism. ACS Synth. Biol. 6, 410-415.

Kallscheuer, N.; Vogt, M.; Bot, M.J.; Marienhagen, J. 2017b. Functional expression of plant-derived *O*-methyltransferase, flavanone 3-hydroxylase, and flavonol synthase in *Corynebacterium glutamicum* for production of pterostilbene, kaempferol, and quercetin. J. Biotechnol. 258, 190-196.

Kallscheuer, N.; Vogt, M.; Stenzel, A.; Gätgens, J.; Bott, M.; Marienhagen, J. 2016. Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2*S*)-flavanones. Metab. Eng. 38, 47-55.

Kang, S.Y.; Lee, J.K.; Choi, O.; Kim, C.Y.; Jang, J.H.; Hwang, B.Y.; Hong, Y.S., 2014. Biosynthesis of methylated resveratrol analogs through the construction of an artificial biosynthetic pathway in *E. coli*. BMC Biotechnol. 14: 67.

Karlsson, J.; Emgard, M.; Brundin, P.; Burkitt, M.J., 2000. Trans-resveratrol protects embryonic mesencephalic cells from ter-butyl hydroperoxide: electron paramagnetic resonance spin trapping evidence for a radical scavenging mechanism. J. Neurochem. 75, 141-150.

Katsuyama, Y.; Funa, N.; Horinouchi, S., 2007a. Precursor-directed biosynthesis of stilbene methyl ethers in *Escherichia coli*. Biotechnol. J. 2, 1286-1293.

Katsuyama, Y.; Funa, N.; Miyahisa, I.; Horinouchi, S., 2007b. Synthesis of unnatural flavonoids and stilbenes by exploiting the plant biosynthetic pathway in *Escherichia coli*. Chem. Biol. 14, 613-621.

Kim, Y.A.; Lee, W.H.; Choi, T.H.; Rhee, S.H.; Park, K.Y.; Choi, Y.H., 2003. Involvement of $p21^{WAF-1/Cip-1}$, pRB, Bax and NFκB in induction of growth arrest and apoptosis by resveratrol in human lung carcinoma A549 cells. Int. J. Oncol. 23, 1143-1149.

Langcake, P.; Pryce, R.J., 1976. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. Physiol. Plant Pathol. 9, 77-86.

Langcake, P.; Pryce, R.J., 1977. Oxidative dimerisation of 4-hydroxystilbenes *in vitro*: production of a grapevine phytoalexin mimic. J. Chem. Soc., Chem. Commun. 7, 208-210.

Leonard, E.; Lim, K.H.; Saw, P.N.; Koffas M.A.G., 2007. Engineering central metabolic pathways for high-level flavonoid production in *Escherichia coli*. Appl. Environ. Microbiol. 73, 3877-3886.

Li, M.; Kildegaard, K.R.; Chen, Y.; Rodriguez, A.; Borodina, I.; Nielsen, J., 2015. *De novo* production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. Metab. Eng. 32, 1-11.

Li, M.; Schneider, K.; Kristensen, M.; Borodina, I.; Nielsen J., 2016. Engineering yeast for high-level production of stilbenoid antioxidants. Sci. Rep. 6:36827.

Liang, J.; Guo, L.; Lin, J.; He, Z.; Cai, F.; Chen, J., 2016. A novel process for obtaining pinosylvin using combinatorial bioengineering in *Escherichia coli*. World J. Microbiol. Biotechnol. 32:102.

Lim, C.G.; Fowler, Z.L.; Hueller, T.; Schaffer, S.; Koffas, M.A.G., 2011. High-yield resveratrol production in engineered *Escherichia coli*. Appl. Environ. Microbiol. 77, 3451-3460.

Liu, X.; Lin, J.; Hu, H.; Zhou, B.; Zhu, B., 2016. *De novo* biosynthesis of resveratrol by site-specific integration of heterologous genes in *Escherichia coli*. FEMS Microbiol. Lett. 363, 8.

Lozoya, E.; Hoffmann, H.; Douglas, C.; Schulz, W.; Scheel, D.; Hahlbrock, K., 1988. Primary structures and catalytic properties of isoenzymes encoded by the two 4-coumarate: CoA ligase genes in parsley. Eur. J. Biochem. 176, 661-667.

Lu, Y.; Shao, D.; Shi, J.; Huang, Q.; Yang, H.; Jin, M., 2016. Strategies for enhancing resveratrol production and the expression of pathway enzymes. Appl. Microbiol. Biotechnol. 17, 7407-7421.

Lussier, F.X.; Colatriano, D.; Wiltshire, Z.; Page, J.E.; Martin, V.J., 2012. Engineering microbes for plant polyketide biosynthesis. Comput. Struct. Biotechnol. J. 3, e20120020.

Lütke-Eversloh, T.; Stephanopoulos, G., 2005. Feedback inhibition of chorismate mutase/prephenate dehydrogenase (TyrA) of *Escherichia coli*: Generation and characterization of tyrosine-insensitive mutants. Appl. Environ. Microbiol. 71, 7224-7228.

Mahady, G.B.; Pendland, S.L., 2000. Resveratrol inhibits the growth of *Helicobacter pylori in vitro*. Am. J. Gastroenterol. 95, 1849.

Marienhagen, J.; Bott, M., 2013. Metabolic engineering of microorganisms for the synthesis of plant natural products. J. Biotechnol. 163, 166-178.

Martinez-Marques, A.; Morante-Carriel, J.A.; Palazon, J.; Bru-Martinez, R., 2018. *Rosa hybrida* orcinol-*O*-methyl transferase-mediated production of pterostilbene in metabolically engineered grapevine cell cultures. New Biotechnol. 42, 62-70.

Milke, L.; Aschenbrenner, J.; Marienhagen, J.; Kallscheuer, N., 2018. Production of plant-derived polyphenols in microorganisms: current state and perspectives. Appl. Microbiol. Biotechnol. 102, 1575-1585.

Minami, H.; Kim, J.S.; Ikezawa, N.; Takemura, T.; Katayama, T.; Kumagai, H.; Sato, F., 2008. Microbial production of plant benzylisoquinoline alkaloids. Proc. Natl. Acad. Sci. USA 105, 7393-7398.

Miyahisa, I.; Kaneko, M.; Funa, N.; Kawasaki, H.; Kojima, H.; Ohnishi, Y.; Horinouchi, S., 2005. Efficient production of (2*S*)-flavanones by *Escherichia coli* containing an artificial biosynthetic gene cluster. Appl. Microbiol. Biotechnol. 68, 498-504.

Mora-Pale, M.; Bhan, N.; Masuko, S.; James, P.; Wood, J.; McCallum, S.; Linhardt, R.J.; Dordick, J.S.; Koffas, M.A.G., 2015. Antimicrobial mechanism of resveratrol-*trans*-dehydrodimer produced from peroxidase-catalyzed oxidation of resveratrol. Biotech. Bioeng., 112, 2417-2428.

Morita, H.; Noguchi, H.; Schröder, J.; Abe, I., 2001. Novel polyketides synthesized with a higher plant stilbene synthase. Eur. J. Biochem. 268, 3759-3766.

Na, D.; Yoo, S.M.; Chung, H.; Park, H.; Park, J.H.; Lee, S.Y., 2013. Metabolic engineering of *Escherichia coli* using small synthetic regulatory RNAs. Nat. Biotechnol. 31, 170-174.

Nandagopal, K.; Halder, M.; Dash, B.; Nayak, S.; Jha S., 2017. Biotechnological approaches for production of anti-cancerous compounds, resveratrol, podophyllotoxin and zerumbone. Curr. Med. Chem. 25.

Nemhauser, J.L.; Toril, K.U., 2016. Plant synthetic biology for molecular engineering of signalling and development. Nat. Plants 2:16010.

Nielsen, J.; Keasling, J.D., 2016. Engineering cellular metabolism. Cell 164, 1185-1197.

Nivelle, L.; Hubert, J.; Courot, E.; Borie, N.; Renault, J.H.; Nuzillard, J.M.; Harakat, D.; Clément, C.; Martiny, L.; Delmas, D.; Jeandet, P.; Tarpin M., 2017a. Cytotoxicity of labruscol, a new resveratrol dimer produced by grapevine cell suspensions, on human skin melanoma cancer cell Line HT-144. Molecules 22:1940.

Nivelle, L.; Hubert, J.; Courot, E.; Jeandet, P.; Aziz, A.; Nuzillard, J.M.; Renault, J.H.; Clément, C.; Martiny, L.; Delmas, D.; Tarpin, M., 2017b. Anti-cancer activity of resveratrol and derivatives produced by grapevine cell suspensions in a 14 L stirred bioreactor. Molecules 22:474.

Ozaki, S.; Imai, H.; Iwakiri, T.; Sato, T.; Shimoda, K.; Nakayama, T.; Hamada, H., 2012. Regioselective glucosidation of *trans*-resveratrol in *Escherichia coli* expressing glucosyltransferase from *Phytolacca americana*. Biotechnol. Lett. 34, 475-481.

Pandley, R.P.; Parajuli, P.; Koffas, M.A.G.; Sohng, J.K., 2016. Microbial production of natural and nonnatural flavonoids: Pathway engineering, directed evolution and systems/synthetic biology. Biotech. Adv. 34, 634-662.

Park, J.W.; Choi, Y.J.; Suh, S.I.; Baek, W.K.; Suh, M.H.; Jin, I.N.; Min, D.S.; Woo, J.H.; Chang, J.S.; Passaniti, A.; Lee, Y.H.; Kwon, T.K., 2001. Bcl-2 overexpression attenuates resveratrol-induced apoptosis in U937 cells by inhibition of caspase-3 activity. Carcinogenesis 22, 1633-1639.

Park, E.J.; Park, H.J.; Chung, H.J.; Shin, Y.; Min, H.Y.; Hong, J.Y.; Kang, Y.J.; Ahn, Y.H.; Pyee, J.H.; Lee S.K., 2012. Antimetastatic activity of pinosylvin, a natural stilbenoid, is associated with the suppression of matrix metalloproteinases. J. Nutr. Biochem. 23, 946-952.

Patnaik, R.; Zolandz, R.R.; Green, D.A.; Kraynie, D.F., 2008. L-tyrosine production by recombinant *Escherichia coli*: fermentation optimization and recovery. Biotechnol. Bioeng. 99, 741-752.

Paul, S.; Rimando, A.M.; Lee, H.J.; Ji, Y.; Reddy, B.S.; Suh, N., 2009. Anti-inflammatory action of pterostilbene is mediated through the p38 mitogen-activated protein kinase pathway in colon cancer cells. Cancer Prev. Res. 2, 650-657.

Pezzuto, J.M., 2011. The phenomenon of resveratrol: redefining the virtues of promiscuity. Ann. New York Acad. Sci. 1215, 123-130.

Pfeifer, B.A.; Admiraal, S.J.; Gramajo, H.; Cane, D.E.; Khosla, C., 2001. Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*. Science 291, 1790-1792.

Pittard, J.; Camakaris, H.; Yang, J., 2005. The Tyr regulon. Mol. Microbiol. 55, 16-26.

Pont, V.; Pezet, R., 1990. Relation between the chemical structure and the biological activity of hydroxystilbenes against *Botrytis cinerea*. J. Phytopathol. 130, 1-8.

Renaud, S.; De Lorgeril, M., 1992. Wine, alcohol, platelets and the French paradox for coronary heart disease. Lancet 339, 1523-1526.

Rimando, A.M.; Cuendet, M.; Desmarchelier, C.; Metha, R.G.; Pezzuto, J.M.; Duke, S.O., 2002. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. J. Agric. Food Chem. 50, 3453-3457.

Rimando, A.M.; Pan, Z.; Polashock, J.J.; Dayan, F.E.; Mizuno, C.S.; Snook, M.E.; Liu, C.J.; Baerson, S.R., 2012. *In planta* production of the highly potent resveratrol analogue pterostilbene via stilbene synthase and *O*-methyl transferase co-expression. Plant Biotechnol. J. 10, 269-283.

Rivière, C.; Pawlus, A.D.; Mérillon, J.M., 2012. Natural stilbenoids: Distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. Nat. Prod. Rep. 29, 1317-1333.

Ro, D.K.; Paradise, E.M.; Ouellet, M.; Fisher, K.J.; Newman, K.L.; Ndungu, J.M.; Ho, K.A.; Eachus, R.A.; Ham, T.S.; Kirby, J.; Chang, M.C.Y.; Withers, S.T; Shiba, Y.; Sarpong, R.; Keasling, J.D., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440, 940-943.

Salas-Navarrete, C.; Hernandez-Chavez, G.; Flores, N.; Martinez, L.M.; Martinez, A.; Bolivar, F.; Barona-Gomez, F.; Gosset, G., 2018. Increasing pinosylvin production in *Escherichia coli* by reducing the expression level of the gene *fabI*-encoded enoyl-acid carrier protein reductase. Electron. J. Biotechnol. 33, 11-16.

Santos, C.N.S.; Koffas, M.A.G.; Stephanopoulos, G., 2011. Optimization of a heterologous pathway for the production of flavonoids from glucose. Metab. Eng. 13, 392-400.

Schallmey, M.; Frunzke, J.; Eggeling, L.; Marienhagen, J., 2014. Looking for the pick of the bunch: high-throughput screening of producing microorganisms with biosensors. Curr. Opin. Biotechnol. 26, 148-154.

Schmidlin, L.; Poutaraud, A.; Claudel, P.; Mestre, P.; Prado, E.; Santos-Rosa, M.; Wiedemann-Merdinoglu, S.; Karst, F.; Merdinoglu, D.; Hugueney, P., 2008. A stress-inducible resveratrol *O*-methyltransferase involved in the biosynthesis of pterostilbene in grapevine. Plant Physiol. 148, 1630-1639.

Schriever, C.; Pendland, S.L.; Mahady, G.B., 2003. Red wine, resveratrol, *Chlamydia pneumoniae* and the French connection. Atherosclerosis 171, 379-380.

Sevrioukova, I.F.; Li, H.; Zhang, H.; Peterson, J.A.; Poulos, T.L., 1999. Structure of a cytochrome P450-redox partner electron-transfer complex. Proc. Natl. Acad. Sci. USA 96, 1863-1868.

Shin, S.Y.; Han, N.S.; Park, Y.C.; Kim, M.D.; Seo, J.H., 2011. Production of resveratrol from *p*-coumaric acid in recombinant *Saccharomyces cerevisiae* expressing 4-coumarate:coenzyme A ligase and stilbene synthase genes. Enzyme Microb. Technol. 48, 48-53.

Shin, S.Y.; Jung, S.M.; Kim, M.D.; Han, N.S.; Seo, J.H., 2012. Production of resveratrol from tyrosine in metabolically-engineered *Saccharomyces cerevisiae*. Enzyme Microb. Technol. 51, 211-216.

Shrestha, A.; Pandey, R.P.; Pokhrel, A.R.; Dhakal, D.; Chu, L.L.; Sohng, J.K., 2018. Modular pathway engineering for resveratrol and piceatannol production in engineered *Escherichia coli*. Appl. Microbiol. Biotechnol. 102, 1.

Siedler, S.; Khatri, N.K.; Zsohar, A.; Kjaerbolling, I.; Vogt, M.; Hammar, P.; Nielsen, C.F.; Marienhagen, J.; Sommer, M.O.A.; Joensson, H.N., 2017. Development of a bacterial biosensor for rapid screening of yeast *p*-coumaric acid production. ACS Synth. Biol. 20, 1860-1869.

Smith, D.A., 1982. Toxicity of phytoalexins. In: Phytoalexins. Bailey, J.A.; Mansfield, J.W. (eds). Blackie, Glasgow and London, U.K. pp 218-252.

Sydor, T.; Schaffer, S.; Boles, E. 2010. Considerable increase in resveratrol production by recombinant industrial yeast strains with use of rich medium. Appl. Environ. Microbiol. 76, 3361-3363.

Thuan, N.H.; Trung, N.T.; Cuong, N.X.; Cuong, D.V.; Quyen, D.V.; Malla, S., 2018. *Escherichia coli* modular coculture system for resveratrol glucosides production. World J. Microbiol. Biotechnol. 34:75.

Tisserant, L.P.; Hubert, J.; Lequart, M.; Borie, N.; Maurin, N.; Pilard, S.; Jeandet, P.; Aziz, A.; Renault, J.H.; Nuzilllard, J.M.; Clément, C.; Boitel-Conti, M.; Courot, E., 2016. ^{13}C NMR and LC-MS Chemical profiling of major stilbenes of pharmaceutical significance produced by elicited *Vitis vinifera* cv. Pinot noir hairy root cultures. J. Nat. Prod. 79, 2846-2855.

Trantas, E.; Koffas, M.A.G.; Xu, P.; Ververidis, F., 2015. When plants produce not enough or at all: metabolic engineering of flavonoids in microbial hosts. Front. Plant Sci. 6: 7.

Trantas, E.; Panopoulos, N.; Ververidis, F., 2009. Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. Metab. Eng. 11, 355-366.

Van Summeren-Wesenhagen, P.V.; Marienhagen, J., 2015. Metabolic engineering of *Escherichia coli* for the synthesis of the plant polyphenol pinosylvin. Appl. Environ. Microbiol. 81, 840-849.

Van Summeren-Wesenhagen, P.V.; Voges, R.; Dennig, A.; Sokolowsky, S.; Noack, S.; Schwaneberg, U.; Marienhagen, J., 2015. Combinatorial optimization of synthetic operons for the microbial production of *p*-coumaroyl alcohol with *Escherichia coli*. Microb. Cell Fact. 14:79.

Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E.; Walle, U.K., 2004. High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab. Dispos. 32, 1377-1382.

Ververidis, F.; Trantas, E.; Douglas, C.; Vollmer, G.; Kretzschmar, G.; Panopoulos, N., 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. Biotechnol. J. 2, 1214-1234.

Wang, Y.; Bhuiya, M.W.; Zhou, R.; Yu, O., 2014. Pterostilbene production by microorganisms expressing resveratrol *O*-methyltransferase. Ann. Microbiol. 65, 817-826.

Wang, Y.; Halls, C.; Zhang, J.; Matsuno, M.; Zhang, Y.; Yu, O., 2011. Stepwise increase of resveratrol biosynthesis in yeast *Saccharomyces cerevisiae* by metabolic engineering. Metab. Eng. 13, 455-463.

Wang, J.; Yang, Y.; Yan, Y., 2018. Bioproduction of resveratrol. In: Biotechnology of Natural Products. Schwab, W.; Lange, B.M.; Wüst, M. (eds), Springer Link, pp. 61-79.

Wang, Y.; Yu, O., 2012. Synthetic scaffolds increased resveratrol biosynthesis in engineered yeast cells. J. Biotechnol. 157, 258-260.

Wang, S.; Zhang, S.; Xiao, A.; Rasmussen, M.; Skidmore, C.; Zhan, J., 2015. Metabolic engineering of *Escherichia coli* for the biosynthesis of various phenylpropanoid derivatives. Metab. Eng. 29, 153-159.

Wang, C.; Zhi, S.; Liu, C.; Xu, F.; Zhao, A.; Wang X.; Ren, Y.; Li, Z.; Yu, M., 2017. Characterization of stilbene synthase genes in mulberry (*Morus atropurpurea*) and metabolic engineering for the production of resveratrol in *Escherichia coli*. J. Agric. Food Chem. 65, 1659-1668.

Watts, K.T.; Lee, P.C.; Schmidt-Dannert, C., 2006. Biosynthesis of plant-specific stilbene polyketides in metabolically engineered *Escherichia coli*. BMC Biotechnol. 6: 22.

Wu, J.; Du, G.C.; Chen, J.; Zhou, J.W., 2015. Enhancing flavonoid production by systematically tuning the central metabolic pathways based on a CRISPR interference system in *Escherichia coli*. Sci. Rep. 5:13477.

Wu, J.; Liu, P.; Fan, Y.; Bao, H.; Du, G.; Zhou, J., 2013. Multivariate modular metabolic engineering of *Escherichia coli* to produce resveratrol from L-tyrosine. J. Biotechnol. 167, 404-411.

Wu J.; Zhang, X.; Dong, M., Zhou, J., 2016. Stepwise modular pathway engineering of *Escherichia coli* for efficient one-step production of (2*S*)-pinocembrin. J. Biotechnol. 231, 183-192.

Wu, J.; Zhang, X.; Zhu, Y.; Tan, Q.; He, J.; Dong, M., 2017a. Rational modular design of metabolic network for efficient production of plant polyphenol pinosylvin. Sci. Rep. 7:1459.

Wu J.; Zhou, P.; Zhang, X.; Dong, M., 2017b. Efficient *de novo* synthesis of resveratrol by metabolically engineered *Escherichia coli*. J. Ind. Microbiol. Biotechnol. 44, 1083-1095.

Xu, P.; Bhan, N.; Koffas, M.A.G., 2013. Engineering plant metabolism into microbes: from systems biology to synthetic biology. Curr. Opin. Biotechnol. 24, 291-299.

Xu, P.; Li, L.; Zhang, F.; Stephanopoulos, G.; Koffas, M.A.G., 2014. Improving fatty acids production by engineering dynamic pathway regulation and metabolic control. Proc. Natl. Acad. Sci. USA 111, 11299-11304.

Xu, P.; Ranganathan, S.; Fowler, Z.L.; Maranas, C.D.; Koffas, M.A.G., 2011. Genome-scale metabolic network modeling results in minimal interventions that cooperatively force carbon flux towards malonyl-CoA. Metab. Eng. 13, 578-587.

Yang, Y.; Lin, Y.; Li, L.; Linhardt, R.J.; Yan, Y., 2015. Regulating malonyl-CoA metabolism via synthetic antisense RNAs for enhanced biosynthesis of natural products. Metab. Eng. 29, 217-226.

Zhang, E.; Guo, X.; Meng, Z.; Wang, J.; Sun, J.; Yao, X.; Xun, H., 2015. Construction, expression and characterization of *Arabidopsis thaliana 4CL* and *Arachis hypogea RS* fusion gene *4CL::RS* in *Escherichia coli*. World J. Microbiol. Biotechnol. 31, 1379-1385.

Zhang, Y.; Li, S.Z.; Li, J.; Pan, X.; Cahoon, R.E.; Jaworski, J.G.; Wang, X.; Jez, J.M.; Chen, F.; Yu, O., 2006. Using unnatural protein fusions to engineer resveratrol biosynthesis in yeast and mammalian cells. J. Am. Chem. Soc. 128, 13030-13031.

Zhou, H.B.; Yan, Y.; Sun, Y.N.; Zhu, J.R., 2003. Resveratrol induces apoptosis in human esophageal carcinoma cells. World J. Gastroenterol. 9, 408-411.

Legends of the figures

Fig. 1. General scheme of the stilbene biosynthetic pathways and subsequent metabolism. **Abbreviations:** PAL and TAL, respectively phenylalanine and tyrosine ammonia lyases; C4H, cinnamate-4-hydroxylase; CL, 4CL, cinnamoyl/4-cinammoyl-CoA ligases; STS, stilbene synthase; C3H, *p*-coumarate 3-hydroxylase; ROMT, resveratrol *O*-methyltransferase; POMT, pinosylvin *O*-methyltransferase; PER, peroxidases; GT, glycosyltransferases; ACC, acetyl-CoA carboxylase complex; SAM, *S*-adenosyl-methionine. Precursors or carbon sources for stilbene production are framed and in dark brown.

Fig. 2. Metabolic engineering of yeast for stilbene production. **Shikimate pathway**: *aroL*, shikimate kinase; *ARO4*^{K229L} and *ARO7*^{G141S} genes encoding feedback-inhibition resistant versions of DAHP synthase and chorismate mutase, respectively; deletion of the *ARO10* gene encoding the phenylpyruvate decarboxylase, **Phenylpropanoid pathway:** *PAL* and *TAL* genes encoding respectively phenylalanine and tyrosine ammonia lyases; *C4H*, cinnamate-4-hydroxylase; *CL*, *4CL*, cinnamoyl/4-cinammoyl-CoA ligases; *STS*, stilbene synthase; deletion of the *PAD1* gene encoding the phenyl acrylic acid decarboxylase; *ATR2* and *CYB5*, cytochrome P450 reductases. **Malonyl-CoA network:** *ACC1*^{S659A} and *ACC*^{S1157A}, acetyl-CoA carboxylase complex; *SeACS1* and *SeACS*^{L641P}, acetyl-CoA synthase; *ScAld6p*, aldehyde dehydrogenase. **Decorating enzymes:** *ROMT*, resveratrol *O*-methyltransferase. **Other abbreviations:** SAM, *S*-adenosylmethionine; *araE*, arabinose-H^+ transporter; ADH, alcohol dehydrogenase; PDC, pyruvate decarboxylase. In dark red are the introduced genes. Precursors or carbon sources for stilbene production are in dark brown.

Fig. 3. Metabolic engineering of *Eschericha coli* for stilbene production. **Shikimate pathway**: *aro*^{Fwt} gene encoding a wild type DAHP synthase; *aroG*^{fbr}gene encoding a feedback-inhibition resistant version of DAHP synthase; *PheA*^{fbr} feedback inhibition-resistant chorismate mutase/prephenate dehydratase; *TyrA*^{fbr} feedback inhibition-resistant chorismate mutase/prephenate dehydrogenase; inactivation of the *pheA* gene encoding the chorismate mutase/prephenate dehydratase; *trpED* anthranilate synthase. **Phenylpropanoid pathway:** *PAL* and TAL genes encoding respectively phenylalanine and tyrosine ammonia lyases; *C4H*, cinnamate-4-hydroxylase; *CL*, *4CL*, cinnamoyl/4-cinammoyl-CoA ligases; *STS* stilbene synthase. **Malonyl-CoA network:** *ACC*, acetyl-CoA carboxylase complex; *adhE*, bifunctional alcohol-aldehyde dehydrogenase; *eno*, enolase; *SucC*, succinate CoA-ligase; *fumC*, fumarate hydratase; *fabD*, *fabB* encoding the β-ketoacyl-ACP synthase I, *fab F* encoding the β-ketoacyl-ACP synthase II, *fabH* encoding the β-ketoacyl-ACP synthase III and *fabI* encoding the enoyl-ACP reductase. Inhibition of *fabB* and *fabF* genes (encoding respectively the β-ketoacyl-ACP synthases I and II) by cerulenin. **Decorating enzymes:** *ROMT* and *POMT*, respectively resveratrol and pinosylvin *O*-methyltransferases; *COMT*, caffeic *O*-methyltransferase; glycosyltransferases, *yjiC* and *Pa*GT3 genes. **Other abbreviations:** SAM, *S*-adenosylmethionine; UDPG, uridine diphosphoglucose. In dark red are the introduced genes. Precursors or carbon sources for stilbene production are in dark brown.

Fig. 4. Metabolic engineering of *Corynebacterium glutamicum* for stilbene production. **Shikimate pathway**: *AroH*$_{EC}$ gene encoding a feed-back inhibition resistant version of DAHP synthase.

Phenylpropanoid pathway: *TAL* gene encoding the tyrosine ammonia lyase; *C4H*, cinnamate-4-hydroxylase; *4CL*, 4-cinammoyl-CoA ligase; *STS*, stilbene synthase; deletion of the *phdBCDE* operon encoding the *p*-coumaroyl-CoA degradation. Amino-acid independent pathway: HbcL1, 4-HBA-CoA ligase; *EbA5319*, β-ketothiolase; *EbA5320*, 3-Hydroxyacyl-CoA dehydrogenase; *EbA5318*, Enoyl-CoA hydratase. **Malonyl-CoA network:** inhibition of *fabB* and *fabF* genes (encoding respectively the β-ketoacyl-ACP synthases I and II) by cerulenin. **Decorating enzymes:** *metK* gene encoding a SAM synthase; *VvOMT::MalE*, gene fusion of *Vitis vinifera O*-Methyltransferase and *MalE* encoding a maltose-binding protein. **Other abbreviations:** SAM, *S*-adenosylmethionine; ACC, acetyl-CoA carboxylase. In dark red are the introduced genes. Precursors or carbon sources for stilbene production are in dark brown.

Fig. 5. Structures of some natural stilbenes. **1**, piceatannol (3,3',4',5-tetrahydroxystilbene). **2**, piceid (resveratrol-3-*O*-β-D-glucoside). **3**, resveratrol-4'-*O*-β-D-glucoside. **4**, pinostilbene (4',5-dihydroxy-3-methoxystilbene). **5**, pterostilbene (3,5-dimethoxy-4'-hydroxystilbene).

Fig. 6. Structures of some non-usual natural stilbenes (**6**-**8**) and non-natural stilbenes (**9**-**15**). **6,** 3,5-dihydroxy-4'-methoxystilbene. **7**, 3,4'-dimethoy-5-hydroxystilbene. **8**, 3,5,4'-trimethoxystilbene. **9**, 4'-fluoro-3,5-dihydroxystilbene. **10**, 3'-fluoro-3,5-dihydroxystilbene. **11**, 4'-methyl-3,5-dihydroxystilbene. Stilbenes containing a furan ring, **12a** (*trans*-5-(2-furan-2-yl)vinyl)benzene-1,3-diol), or a thiophen ring, **12b** (*trans*-5-(2-thiophen-2-yl)vinyl)benzene-1,3-diol)**. 13,** stilbene containing a pyridine ring (*trans*-5-(2-(pyridin)-3-yl)vinyl)benzene-1,3-diol). **14**, resorcinol-structure-based stilbene (*trans*-1-(3,5-dihydroxyphenyl)-4-(4-hydroxyphenyl)but-3-en-2-one). **15**, unnatural stilbene resulting from the condensation with propionyl-CoA (2-ethyl-6-(4-hydroxyphenethyl)-3,4-dihydro-2*H*-pyran-2,4-diol).

Table 1: Metabolic Engineering of Yeast for Resveratrol Production

Abbreviations: Res, resveratrol; Ptero, pterostilbene. For gene abbreviations, refer to the legends of Fig. 2-4

Microorganism/Species/ Strain	Introduced gene(s)	Origin of genes	*Precursor or source*/ Resveratrol or stilbene quantity	References
Yeast *S. cerevisiae* FY23	*4CL*, *STS*	*Populus trichocarpa* X *Populus deltoides* (*4CL*), *Vitis vinifera* (*STS*)	+ *p-coumarate* **1.45 mg/L Res**	Becker et al. (2003)
Yeast *S. cerevisiae* CEN.PK113-3b	*4CL*, *STS*	*Nicotiana tabacum* (*4CL*), *V. vinifera* (*STS*)	+ *p-coumarate* **5.8 mg/L Res**	Beekwilder et al. (2006)
Yeast *S. cerevisiae* WAT11	*TAL*, 4CL::STS fusion protein	*Rhodobacter sphaeroides* (*TAL*), *Arabidopsis thaliana* (*4CL*), *Vitis vinifera* (*STS*)	+ *p-coumarate* **5.25 mg/L Res**	Zhang et al. (2006)
Yeast *Yarrowia lipolytica*	*PAL/TAL, C4H*, *4CL*, *STS, matB, matC*	*Rhodotorula glutinis* (*PAL/TAL*), *Streptomyces coelicolor* (*4CL*), *Vitis* sp. (*STS*), *Rhizobium trifolii* (*matB/matC*)	**1.46 mg/L Res**	Huang et al. (2009)
Yeast *S. cerevisiae* YPH499	*PAL*, Cytochrome P450 Reductase (*CPR*), *C4H, 4CL*, *STS*	*P. trichocarpia* x *P. deltoides* (*PAL, CPR*) *Glycine max* (*C4H, 4CL*), *V. vinifera* (*STS*)	+ *phenylalanine* **0.29 mg/L Res** + *p-coumarate* **0.31 mg/L Res**	Trantas et al. (2009)
Yeast *S. cerevisiae* CEN.PK2-1	*4CL1*, *STS*	*A. thaliana* (*4CL*), *V. vinifera* (*STS*)	+ *p-coumarate* **262-391 mg/L Res**	Sydor et al. (2010)
Yeast *S. cerevisiae* W303-1A	*4CL1*, *STS* Deletion of *phenyl acryl acid decarboxylase, gene PAD1*	*A. thaliana* (*4CL1*), *A. hypogea* (*STS*)	+ *p-coumarate* **3.1 mg/L Res**	Shin et al. (2011)
Yeast *S. cerevisiae* WAT11	*TAL*, 4CL::STS fusion protein, H^+ arabinose transporter *ara*E	*R. sphaeroides* (*TAL*), *E. coli* (*araE*), *A. thaliana* (*4CL*), *V. vinifera* (*STS*)	+ *tyrosine* **3.1 mg/L Res** **1.27 mg/L Res** (without *ara*E)	Wang et al. (2011)
Yeast *S. cerevisiae* WAT11	4CL::STS fusion protein	*A. thaliana* (*4CL*), *V. vinifera* (*STS*)	+ *p-coumarate* **14.4mg/L Res**	Wang and Yu (2012)
Yeast *S. cerevisiae* W303-1A Yeast *S. cerevisiae* W303-1A/ACC1	*PAL, C4H, 4CL1, STS* + overexpression of acetyl-CoA carboxylase *(ACC1)*	*Rhodosporidium toruloides* (*PAL*), *A. thaliana* (*C4H*, *4CL1*), *A. hypogea* (*STS*)	+ *phenylalanine* **3.3 mg/L Res** + *phenylalanine* **4.3 mg/L Res** *tyrosine* **5.8 mg/L Res**	Shin et al. (2012)
Yeast *S. cerevisiae* WAT11	*4CL*, *STS*, *ROMT*	*A. thaliana (4CL), V. vinifera (STS, ROMT)*	+ *resveratrol* **150 mg/L Ptero** + *p-coumarate* **2 mg/L Ptero**	Wang et al. (2014)

Table 1: Metabolic Engineering of Yeast for Resveratrol Production (continued)

			+ *glucose* **2.73 mg/L Res**	
Yeast *S. cerevisiae* ST4135	*TAL, 4CL1,* *STS* + feedback insentive alleles of DHAP synthase (*ScARO4*^{K229L}) and chorismate mutase (*ScArO7p*^{G141S}) + inactivation resistant version of acetyl-CoA carboxylase (*Acc1p*)	*Herpetosiphon aurianticus (TAL), A. thaliana* (*4CL1*), *V. vinifera* (*STS*)	+ *glucose* **4.85 mg/L Res** + *glucose* **6.39 mg/L Res**	Li et al. (2015)
Yeast *S. cerevisiae* ST4140	+ integration of multiple copies of the pathway +fed-batch fermentations		+ *glucose* **235 mg/L Res** + *glucose* **415 mg/L Res** + *ethanol* **531 mg/L Res**	
Yeast *S. cerevisiae* ST4990	Resveratrol pathway including *PAL*, *C4H*, *4CL2*, *STS* + feedback insensitive alleles of DHAP synthase (*ScARO4*^{K229L}) and chorismate mutase (*ScArO7*^{G141S}) + shikimate kinase *aroL* + deregulated variant of *ACC1* + non-regulated version of acetyl-CoA synthase (*ACS*) + deletion of phenylpyruvate decarboxylase *ARO10* + cytochrome P450 reductases *ATR2* and *CYB5*	*A. thaliana (PAL, C4H*, *4CL2*), *V. vinifera* (*STS*), *Salmonella enterica* (*ACS*), *E. coli* (shikimate kinase), *A. thaliana (ATR2), S. cerevisiae (CYB5)*	+ *glucose* **800 mg/L Res**	Li et al. (2016)
Yeast *S. cerevisiae* ST4993 and ST4994	precedent strain ST4990 + resveratrol *O*-methyltransferases (ROMT)	*Sorghum bicolor (SbROMT)* *V. vinifera (VvROMT)*	+ *glucose* **5.5 mg/L Ptero** **35 mg/L Ptero**	

Table 2: Metabolic Engineering of Stilbene Pathways in Bacteria

Abbreviations: Res, resveratrol; PS, pinosylvin; Ptero, pterostilbene. For gene abbreviations refer to the legends of Fig. 2-4

Microorganism/Species/ Strain	Introduced gene(s)	Origin of genes	*Precursor or source*/ Resveratrol or stilbene quantity	References
Escherichia coli BW27784	*4CL1*, *STS*	*Arabidopsis thaliana* (*4CL1*), *Arachis hypogea* (*STS*)	+ *p-coumarate* **106 mg/L Res** + *caffeic acid* **13 mg/L piceatannol**	Watts et al. (2006)
E. coli BL21	*4CL*, *STS*	*Nicotiana tabacum* (*4CL*), *Vitis vinifera* (*STS*)	+ *p-coumarate* **12-20 mg/L Res**	Beekwilder et al. (2006)
E. coli BLR (DE3)	*PAL*, *4CL*, *STS*, *ACC*	*Rhodotorula rubra* (*PAL*), *Lithospermum erythrorhizon* (*4CL*), *A. hypogea* (*STS*), *Corynebacterium glutamicum* (*ACC*)	+ *tyrosine* **37 mg/L Res** + *phenylalanine* **20 mg/L PS** + *tyrosine* **37 mg/L Res** **18 mg/L pinostilbene** **5.8 mg/L Ptero**	Katsuyama et al. (2007a)
	same construct + OsPMT	*Oryza sativa* (*OsPMT*)	+ *phenylalanine* **20 mg/L PS** **27 mg/L PS mono-methylether**	
	same construct + OsPMT	*O. sativa (OsPMT)*	**27 mg/L PS dimethylether**	
E. coli BL21 Star	*4CL*, *STS, ACC, BirA* two T7-promoter constructs for *4CL* and *STS*		+ *p-coumarate* **46 mg/L Res**	
E. coli BL21 Star	*4CL*, *STS* expressed under the control of separate *gap* promoters **+ cerulenin**	*Petroselinum crispum* (*4CL*), *A. hypogea* (*STS*), *Photorhabdus luminescens* (*ACC*), *P. luminescens* (*BirA*)	+ *p-coumarate* **65 mg/L Res**	Lim et al. (2011)
E. coli BW27784	*4CL*, *STS* expressed in an operon **+ cerulenin**		+ *p-coumarate* **2,340 mg/L Res**	
E. coli BW27784 (DE3)	*4CL*, *STS* overexpression of *PGK,G3PDH, pyruvate DH complex,* deletion of *fumC*	*A. thaliana* (*4CL*), *V. vinifera* (*STS*)	+ *p-coumarate* **1,600 mg/L Res**	Bhan et al. (2013)
E. coli BL21 (DE3)	**module: 1** *TAL, 4CL*; **module 2:** *STS;* **module 3:** *matB, matC*	*Rhodotorula glutinis* (*TAL*), *P. crispum* (*4CL*), *V. vinifera* (*STS*), *Rhizobium trifolii* (*matBlmatC*)	+ *tyrosine* **35 mg/L Res**	Wu et al. (2013)

Table 2: Metabolic Engineering of Stilbene Pathways in Bacteria (Continued)

E. coli C41 (DE3)	*TAL, 4CL, STS, Glycosyltransferase yjiC*	*S. Espanaensis* (*TAL*), *S. coelicolor* (*4CL*), *A. hypogea* (*STS*), *Bacillus* spp. (*yjiC*)	*+ glucose* **2.5 mg/L 3-*O*-resveratrol β-D-glucoside (piceid)** **7.5 mg/L 4'-*O*-resveratrol β-D-glucoside**	Choi et al. (2014)
E. coli BL21-CodonPlus (DE3)-RIPL	*VvROMT*	*V. riparia* (*VrROMT*)	*+ resveratrol* **0.16 mg/L pinostilbene 0.04 mg/L Ptero**	Jeong et al. (2014)
	SbROMT	*S. bicolor* (*SbROMT*)	*+ resveratrol* **34 mg/L pinostilbene 0.16 mg/L Ptero**	
E. coli C41 (DE3)	*TAL*, *CL*, *STS*	*S. espanaensis* (*TAL*), *S. coelicolor* (*4CL*), *A. hypogea* (*STS*)	*+ glucose* **5.2 mg/L Res**	Kang et al. (2014)
	TAL, *SbOMT3*, *CL*, *SbOMT1*,*STS*	*S. bicolor* (*SbOMT1* and *SbOMT3*)	*+ glucose* **2.5 mg/L 3,5-diOH-4'-methoxy-stilbene 0.2 mg/L 3,4'-dimethoxy-5-OH-stilbene**	
E. coli M15	Glycosyltransferase (*GT*) No addition of UDP-glucose needed	*Phytolacca americana* (*GT*)	*+ resveratrol* **piceid and 4'-*O*-resveratrol β-D-glucoside**	Ozaki et al. (2014)
E. coli BW27784	*4CL*, *STS*, *ROMT*	*A. thaliana* (*4CL*), *V. vinifera* (*STS*, *ROMT*)	*+ resveratrol* **170 mg/L Ptero** *+ p-coumarate* **50 mg/L Ptero**	Wang et al. (2014)
E. coli BL21-CodonPlus (DE3)-RIPL	*4CL*, *STS*, Vr*ROMT*	*S. coelicolor* (*4CL*), *A. hypogea* (*STS*), *V. riparia* (*VrROMT*) or *Sorghum bicolor* (*SbROMT*)	*+ p-coumarate* **1.9 mg/L Res no methylated derivatives**	Jeong et al. (2015)
	4CL, *STS*, *SbROMT*		*+ p-coumarate* **1.2 mg/L Res 2.4 mg/L pinostilbene**	

Table 2: Metabolic Engineering of Stilbene Pathways in Bacteria (Continued)

E. coli BL21 (DE3)	*PAL, 4CL, STS* **+ cerulenin**	*P. crispum* (*PAL*), *S. coelicolor* (*4CL*), *Pinus strobus* (two mutated *STS* T248A; Q361R)	*+ glucose* **70 mg/L PS** *+ glucose/ phenylalanine* **91 mg/L PS**	Van Summeren and Marien-hagen (2015)
E. coli BL21 (DE3)	*TAL, 4CL, STS* *TAL, 4CL, C3H, STS* *PAL, 4CL, STS*	*Saccharothrix espanaensis* (*TAL*), *A. thaliana* (*4CL*), *A. hypogea* (*STS*) *+ S. espanaensis* (*C3H*) *Trifolium pretense* (*PAL*)	*+ glucose + tyrosine* **114 mg/L Res** *+ tyrosine* **21 mg/L piceatannol** *+ phenylalanine* **13 mg/L PS**	Wang et al. (2015)
E. coli BW25113	*4CL2*, *STS* *+ fabD antisense RNA*	*P. crispum (4CL2)*, *V. vinifera* (*STS*)	*+ p-coumarate* **160 mg/L Res** *+ p-coumarate* **280 mg/L Res**	Yang et al. (2015)
E. coli BL21 (DE3)	*4CL::RS* fusion gene	*A. thaliana* (*4CL*), *A. hypogea* (*RS*)	*+ p-coumarate* **80 mg/L Res**	Zhang et al. (2015)
E. coli Dh5α	*Combination of two E. coli strains:* *-strain 1 : PAL overexpressing strain* *-strain 2: 4CL, STS* SgRNA *antifabD* SgRNA *antifabB* SgRNA *antifabF*	*A. hypogea* (*4CL*), *V. vinifera* (*STS*)	*+ glycerol and cinnamate* **25 mg/L PS** **47 mg/L PS** **37 mg/L PS** **25 mg/L PS**	Liang et al. (2016)
Corynebacterium glutamicum DelAro^4	*TAL*, *4CL*, *STS* *aroH$_{EC}$* feed-back inhibition resistant version of DAHP Deletion of the *phdBCDE* synthase operon **+ cerulenin**	*Flavobacterium johnsoniae* (*TAL*), *P. crispum* (*4CL*), *A. hypogea* (*STS*)	*+ glucose* **59 mg/L Res** *+ glucose* **158 mg/L Res**	Kallschauer et al. (2016)
E. coli BW25113 (DE3)	*TAL*, *4CL*, *STS* Inactivation of *TyR* and *trpED* genes	*Rhodotorula glutinis* (*TAL*), *Petroselinum crispum* (*4CL*), *Vitis vinifera* (*STS*)	*+ glucose* **4.6 mg/L Res**	Liu et al. (2016)

Table 2: Metabolic Engineering of Stilbene Pathways in Bacteria (Continued)

C. glutamicum DelAro[4]	*4CL*, *STS* Reversal of the β-oxidative pathway for phenylpropanoids: Hydroxybenzoate:CoA ligase (*HbcL1*) β-ketothiolase (*EbA5319*) 3-hydroxyacyl-CoA dehydrogenase (*Eb5320*) Enoyl-CoA hydratase (*Eb5318*) **+ cerulenin**	*P. crispum* (*4CL*), *A. hypogea* (*STS*), *Azoarus* sp. (*HbcL1*, *EbA5319*, *Eb5320*, *Eb5318*)	*+ p-coumarate* **5 mg/L Res**	Kallschauer et al., 2017a
C. glutamicum DelAro[4]	*4CL*, *STS* *OMT::MalE* *metK*	*P. crispum* (*4CL*), *A. hypogea* (*STS*), *V. vinifera* (*OMT*), *E. coli* (*MalE*, *metK*)	*+ p-coumarate* **42 mg/L Ptero**	Kallschauer et al., 2017b
Co-culture *E. coli* W (pheA⁻) + *E.coli* W3110 W-Ah	*TAL/aroG*^{fbr}, feedback resistant version of DAHP synthase, *tktA* transketolase Inactivation of the *pheA* gene encoding for a chorismate mutase/prephenate dehydratase; *4 CL*, *STS* (strain W3110 W-Ah)	*R. glutinis* (*TAL*), *S. coelicolor* A2 (*4CL*), *A. hypogea* (*STS*)	*+ glycerol* **22.6 mg/L Res**	Camacho-Zaragoza et al., 2016
Co-culture *E. coli* W (pheA⁻) + *E.coli* W3110	*4CL/STS* (strain W3110)	*S. coelicolor* A2 (*4CL*), *V. vinifera* (*STS*)	*+ p-coumarate* **78.1 mg/L Res**	
E. coli B21 (DE3)	*TAL*, *4CL*, *STS*, *COMT*	*S. espanaensis* (*TAL*), *N. tabacum* (*4CL*), *V. vinifera* (*STS*), *A. thaliana* (*COMT*)	*+ tyrosine and methionine* **33.6 mg/L Ptero**	Heo et al., 2017
E. coli BL21 (DE3)	**module 1**: *aroF*^{wt} encoding DAHP synthase, *pheA*^{fbr} feed-back resistant version of chorismate mutase/prephenate dehydratase, *PAL*, **module 2:** *4CL*, *STS*, **module 3:** CRISPRi targeted genes: anti-*eno*, anti-*adhE*, anti-*fabB* sg RNA; *anti-sucC*, anti-*fumC* sg RNA and anti-*fabF* sg RNA	*T. cutaneum* (*TAL*), *P. crispum* (*4CL*), *V. vinifera* (*STS*),	*+ glucose* **281 mg/L PS**	Wu et al., 2017a
E. coli BL21 (DE3)	*aroG*^{fbr} feed-back resistant version of DAHP synthase, *tyrA*^{fbr} feed-back resistant version of chorismate mutase/prephenate dehydrogenase, *TAL*, *4CL*, *STS*, *matB*, *matC*, CRISPRi targeted genes *fabD*, *fabH*, *fabB*, *fabF*, *fabI*	*Trichosporon cutaneum* (*TAL*), *P. crispum* (*4CL*), *V. vinifera* (*STS*), *Rhizobium trifolii* (*matB*, *matC*)	*+ glucose* **305 mg/L Res**	Wu et al. (2017b)

Table 2: Metabolic Engineering of Stilbene Pathways in Bacteria (Continued)

E. coli BL21	*4CL*, STS	*Morus atropurpurea Roxb.* (*4CL*) *M. atropurpurea* (*STS*)	+ *p-coumarate* **ca 0.2 mg/L Res**	Wang et al. (2017)
E. coli W3110	*4 CL*, *STS* *4 CL*, *STS* + reduced expression level of *fab I* gene	*S. coelicolor* A3 (*4CL*), *V. vinífera* (*STS*)	+ *cinnamate* **34.89 mg/L PS** + *cinnamate* **52.67 mg/L PS**	Salas-Navarrete et al. (2018)
E. coli BL21 (DE3) + *E. coli* BL21 (DE3)	*4 CL*, *STS* Knock out of *pgi* and *zwf* genes Overexpression of the *hasC* gene	*N. tabacum* (*4CL*), *V. vinifera* (*STS*)	+ *p-coumarate* **15 mg/L piceid** in flasks **92 mg/L piceid** in bioreactor	Thuan et al. (2018)
E. coli BL21 (DE3)	**module 1:** *4CL2*, *STS*, **module 2a:** *ACC* from *E. coli*, **module 2b:** *ACC* from *Nocardia farcinica*, **module 2c:** *matB, matC*, **module 3:** *HpaB/HpaC* (encoding 3'-hydroxylating enzymes)	*P. crispum* (*4CL*), *V. vinifera* (*STS*) *E. coli* (*ACC*), *Nocardia farcinica* (*ACC*), *matB*, *matC* (*S. coelicolor*), *HpaB*, *HpaC* (*E. coli*)	**module 1** + *p-coumarate* **8.6 mg/L Res** **module 1** + *p-coumarate* + **module 2b** **60 mg/L Res** + sodium acetate **64 mg/L Res** **module 1** + *p-coumarate* + **module 2c** + disodium malonate **151 mg/L Res** **module 1** + *p-coumarate* + **module 2c** + disodium malonate + **module 3** **124 mg/L Piceatannol**	Shrestha et al. (2018)