Biosynthesis of resveratrol using metabolically engineered *Escherichia coli*

Jin Yeong Park†, Jeong-Hyeon Lim‡, Joong-Hoon Ahn§ and Bong-Gyu Kim*†

**Abstract**

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a phenolic compound widely used in pharmaceutics and nutraceuticals. Although resveratrol is produced by some plant species, including grapes, peanuts, and berries, the content of resveratrol and its derivatives are very low. Therefore, an alternative biosynthetic method using microorganisms, such as *Escherichia coli*, has been developed over the past two decades. In the present study, a resveratrol-over-producing *E. coli* strain was developed using three strategies. First, we increased the synthesis of *p*-coumaric acid, a precursor of resveratrol, by manipulating genes in the shikimate pathway of *E. coli*. Second, three genes involved in resveratrol biosynthesis, such as tyrosine ammonia lyase (*TAL*), 4-coumaroyl CoA ligase (*4CL*), and stilbene synthase (*STS*), were cloned from diverse sources, such as plants and microorganisms, and the best combination was selected to maximize resveratrol production in *E. coli*. Finally, culture conditions, such as cell concentration, culture temperature, and carbon sources, were established for optimal resveratrol production. Through these strategies, approximately 80.4 mg/L of resveratrol was biosynthesized after 48 h of culture using glycerol as a carbon source.

**Keywords:** *Escherichia coli*, Metabolic engineering, Phenolic compounds, Resveratrol

**Introduction**

Polyphenols are plant secondary metabolites and valuable sources for the development of cosmetic materials, functional food ingredients, and pharmaceuticals [1–3]. Polyphenols are generally derived through the phenylpropanoid pathway [4]. Depending on their carbon skeleton, they can be classified into four classes, such as phenolic acids including gallic acid and salicylic acid composed of C₆-C₁ skeleton, hydroxycinnamic acid including cinnamic acid, *p*-coumaric acid, and caffeic acid composed of C₆-C₃ skeleton, stilbene including resveratrol, piceatannol, and palidil composed of C₆-C₂-C₆ skeleton, flavonoids including naringenin, quercetin, and genistein composed of C₆-C₃-C₆ skeleton [4–6].

*Resveratrol* (3,5,4′-trihydroxy-trans-stilbene) is a polyphenolic compound containing a C₆-C₂-C₆ skeleton. It is synthesized naturally in several plants in response to pathogenic fungi and bacteria or wounds caused by insects and herbivores [7–9]. Resveratrol was first isolated from the white hellebore roots and subsequently isolated from *Polygonum cuspidatum* [10, 11]. Most of the commercial resveratrol throughout the world is extracted from *P. cuspidatum* E. et Z. In recent years, the biological effects of resveratrol on atherosclerosis and coronary heart disease, and its anticancer, antileukemic, and immune-modulating activities have been well documented through a variety of physiological and pharmacological studies [12, 13]. In plants, resveratrol is synthesized from the aromatic amino acid phenylalanine through the sequential reaction of four enzymes, phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (C₄H), 4-coumaroyl-CoA synthase (*4CL*), and stilbene synthase (*STS*) (Fig. 1) [14]. PAL is responsible for the deamination of L-phenylalanine to produce trans-cinnamic acid. Subsequently, trans-cinnamic acid is converted into *p*-coumaric acid (4-hydroxy cinnamic acid) by C₄H. After condensation to *p*-coumaroyl-CoA, stilbene synthase (STS) catalyzes the formation of resveratrol from the *p*-coumaroyl-CoA.
P-coumaric acid is converted into p-coumaroyl-CoA by 4CL with coenzyme A as a co-substrate. p-Coumaroyl-CoA is condensed with three malonyl-CoA units through the sequential reaction of STS, belonging to polyketide III (PKS III), to produce resveratrol [15]. Because the functional expression of C4H (Fig. 1), a member of the P450 genes, is generally difficult in E. coli, tyrosine ammonia lyase (TAL), which catalyzes the deamination of tyrosine to p-coumaric acid, has been recently employed for the biosynthesis of various polyphenols [16]. To synthesize resveratrol from tyrosine in E. coli, three genes, TAL, 4CL, and STS, are required (Fig. 1). However, tyrosine is a limiting factor for the synthesis of resveratrol from glucose. To increase the tyrosine content in E. coli, metabolic engineering approaches have been applied in E. coli. [17, 18].

Over many decades, natural compounds derived from plants have been the materials of great medicinal value associated with human health benefits [19, 20]. However, there are some obstacles that must be overcome, including seasonal and spatial limitations, low yields, and complicated refining processes, such as extraction...
and isolation, before the commercial usage of these biologically active molecules [21]. For these reasons, some alternative production methods have been developed, including plant cell or hairy root culture and simple biotransformation or de novo synthesis using microorganisms, such as *E. coli* and yeast, for the production of pharmaceutically useful substances of plant origin [18, 22–24]. Among them, *E. coli* is an excellent host for producing large-scale commercial commodities because their metabolic pathway is well known and various techniques of gene manipulation are well established [26]. In addition, the synthesized phenolic compounds can be easily purified using simple organic solvents such as ethyl acetate [18, 25]. Using *E. coli*, phenolic important compounds, such as hydroxycinnamic acids, coumarins, stilbenoids, flavonoids, terpenoids, and alkaloids, have been successfully synthesized [18, 27-29, 35].

Most of the previous studies related to the production of resveratrol in *E. coli* started from *p*-coumaric acid. The titer was approximately 16 mg/L resveratrol from 800 mg of *p*-coumaric acid, when *E. coli* BL21 (DE3) containing the gene 4CL from *Nicotiana tabacum* and the gene STS from *Vitis vinifera* was used [30], whereas when the *E. coli* JM109 strain transformed with the gene 4CL from *Arabidopsis thaliana* and STS from *Arachis hypogaea* was used, the amount of resveratrol was over 100 mg/L [31]. Lim et al. [32] developed a metabolically engineered *E. coli* strain capable of producing high-yield resveratrol (2.3 g/L) by feeding on 15 mM *p*-coumaric acid. However, because *p*-coumaric acid is relatively expensive compared to glucose, the synthesis of resveratrol from glucose, a renewable source, is an attractive alternative approach. Recently, resveratrol was successfully synthesized from glucose by introducing three genes, TAL, 4CL, and STS, into *E. coli* [28]. Yuan et al. [33] reported resveratrol production from glucose using modular engineering of an *E. coli*-Saccharomyces cerevisiae co-culture, and the yield of resveratrol was 28.5 mg/L. Wang et al. [28] reported the production of 114.4 mg/L of resveratrol from glucose, but 3 mM tyrosine was added to the culture medium for resveratrol biosynthesis. Although previous studies have demonstrated the de novo production of resveratrol from glucose, the potential to improve productivity remains.

In this work, we describe microbial resveratrol production from glucose using *E. coli*. We engineered the tyrosine biosynthesis pathway for the stable supply of tyrosine by metabolic engineering, introduced three resveratrol biosynthetic genes, and selected the optimal combination of resveratrol biosynthetic genes. In addition, in previous studies, a mutant was used to supply more precursor (tyrosine) for resveratrol biosynthesis [16, 28], but in this study, we used not only *E. coli* mutant for tyrosine but also overexpressed genes for tyrosine biosynthesis in order to synthesize resveratrol from glucose (without supplying tyrosine). Then, we optimized the fermentation conditions such as carbon source, initial cell density, and culture temperature. Finally, the combined effect of genetic modification and culture conditions increased the final resveratrol titer to 80 mg/L. The strategy described here will be applicable as an alternative production tool of resveratrol, which can be used in various applications in food, pharmaceutical, and nutraceutical industry.

**Materials and methods**

**Plasmid constructs**

The TAL gene from *Saccharothrix espanaensis* (*SeTAL*) was previously cloned between *Eco*RI and *N*otI sites of pCACYCDuet vector [18]. Three genes of 4LC from Streptomyces coelicolor A(3) (*Sc4CL*), *Oryza sativa* (*Os4CL*), and *Lithospermum erythrorhizon* (*Le4CL*) were cloned between *Bam*HI and *N*otI of pCDFDuet, and named PC-Sc4CL, PC-Os4CL, and PC-Le4CL. The gene 4CL from *Petroselinum crispum* (*Pe4CL*) was cloned between the *Eco*RI and *N*otI sites of pCDFDuet and named PC-Pc4CL. STS from *Vitis vinifera* (*VvSTS*) was introduced into multiple cloning site 2 using *Eco*RV and *Xho*I sites of PC-Sc4CL, PC-Os4CL, PC-Pc4CL, and PC-Le4CL. The resulting plasmids, in which each gene was controlled by the T7 promoter, were named PC-Sc4CL-*VvSTS* (P), PC-Os4CL-*VvSTS* (P), and PC-Le4CL-*VvSTS* (P) (Table 1). Two STS genes from *Arachis hypogaea* (*AhSTS*) and *Picea abies* (*PaSTS*) were cloned into *Eco*RV and *Xho*I of PC-Os4CL, respectively, and named PC-Os4CL-*AhSTS* (P) and PC-Os4CL-*PaSTS* (P), which are controlled by an independent T7 promoter (Table 1). In the case of *AhSTS*, since it has a *Hind*III restriction enzyme site at the 855 position, a silencing mutation was performed using PCR. To generate a construct in which two genes were controlled by one promoter, *AhSTS*, *VvSTS*, *PaSTS* were amplified using each primer set containing *Eco*RI and *Hind*III sites and cloned into the corresponding sites of the pCDFDuet vector. Os4CL was amplified with a forward primer including a ribosomal binding site (RBS) and a *Not*I site such as 5-ATAAG CTTagaggattacaaATGATCACGGGTGGCG-3 (the underlined part indicates the *Hind*III site and the RBS is shown in lower case), and the reverse primer including a *Not*I site such as 5-ATGCCGGCGCTTACGT GCTTTGGGCGCATC-3 (the underlined part indicates the *Not*I site) (Table 1). The resulting PCR product was digested with *Hind*III I and *Not*I of the restriction enzyme after gel purification and the corresponding sites of pCDFDuet vector containing *AhSTS*, *VvSTS*, or *PaSTS*. The resulting constructs, PC-*AhSTS*-Os4CL(O),
### Table 1: Plasmids, bacterial strains, and primers used in this study

| Plasmids/Strains | Description | Sources or reference |
|------------------|-------------|----------------------|
| **Plasmids**     |             |                      |
| pACYCDuet        | P15A ori, Cm' | Novagen              |
| pCDFDuet         | CloE13 ori, Str' | Novagen              |
| PA-SeTAL         | pACYCDuet carrying TAL from *Saccharothrix espanaensis* |          |
| PC-Pc4CL-VvSTS(P)| pCDFDuet carrying Pc4CL from *Petroselinum crispum* and VvSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Sc4CL-VvSTS(P)| pCDFDuet carrying Sc4CL from *Streptomyces coelicolor* A(3) and VvSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Os4CL-VvSTS(P)| pCDFDuet carrying Os4CL from *Oryza sativa* and VvSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Ah4CL-WvSTS(P)| pCDFDuet carrying Ah4CL from *Arabidopsis thaliana* and VvSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Le4CL-VwSTS(P)| pCDFDuet carrying Le4CL from *Lithospermum erythrorhizon* and VvSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Os4CL-AhSTS(P)| pCDFDuet carrying Os4CL from *Oryza sativa* and AhSTS from *Arachis hypogaea*. The genes are regulated by the respective T7 promoter | This study |
| PC-Pc4CL-VwSTS(P)| pCDFDuet carrying Os4CL from *Petroselinum crispum* and VwSTS from *Vitis vinifera*. The genes are regulated by the respective T7 promoter | This study |
| PC-Os4CL-PaSTS(P)| pCDFDuet carrying Os4CL from *Oryza sativa* and PaSTS from *Picea abies*. The genes are regulated by the respective T7 promoter | This study |
| PC-AhSTS-Os4CL(O)| pCDFDuet carrying Os4CL from *Oryza sativa* and AhSTS from *Arachis hypogaea*. The genes are regulated by one T7 promoter | This study |
| PC-PaSTS-Os4CL(O)| pCDFDuet carrying Os4CL from *Oryza sativa* and PaSTS from *Picea abies*. The genes are regulated by one T7 promoter | This study |
| PC-VwSTS-Os4CL(O)| pCDFDuet carrying Os4CL from *Oryza sativa* and VwSTS from *Vitis vinifera*. The genes are regulated by one T7 promoter | This study |
| PA-aroG-SeTAL-tyrA| pACYCDuet carrying TAL from *S. espanaensis*, aroG, and tyrA from *E. coli* | Kim el al. [18] |
| PA-aroG<sup>br</sup>-ppsA-tktA-SeTAL-tyrA<sup>br</sup>| pACYCDuet carrying TAL from *S. espanaensis*, aroG<sup>br</sup>, ppsA, tktA, and tyrA<sup>br</sup> from *E. coli* | Kim el al. [18] |
| **Strains**      |             |                      |
| BL21(DE3)        | F<sup>-</sup> ompT hisD5<sup>rec</sup> (r<sub>1600</sub> m<sub>1600</sub>) gal dcm lon (DE3) | Kim et al. [18] |
| B-TP             | BL21(DE3), ΔtyrR::FRT-apheA::FRT-kan<sup>5</sup>::FRT | This study |
| B-TPFI           | BL21(DE3), ΔtyrR::FRT-apheA::FRT-Δumc::FRT-AcidA::FRT-kan<sup>5</sup>::FRT | This study |
| BP-Pc4CL         | BL21(DE3) harboring PC-Pc4CL-VwSTS(P) | This study |
| BP-Os4CL         | BL21(DE3) harboring PC-Os4CL-VwSTS(P) | This study |
| BP-Ah4CL         | BL21(DE3) harboring PC-Ah4CL-VwSTS(P) | This study |
| BSa4CL           | BL21(DE3) harboring PC-Sa4CL-VwSTS(P) | This study |
| BP-Le4CL         | BL21(DE3) harboring PC-Le4CL-VwSTS(P) | This study |
| BP-AhSTS         | BL21(DE3) harboring PC-Os4CL-AhSTS(P) | This study |
| BP-PaSTS         | BL21(DE3) harboring PC-Os4CL-PaSTS(P) | This study |
| BO-VwSTS         | BL21(DE3) harboring PC-Os4CL-VwSTS(O) | This study |
| BO-AhSTS         | BL21(DE3) harboring PC-Os4CL-AhSTS(O) | This study |
| BO-PaSTS         | BL21(DE3) harboring PC-Os4CL-PaSTS(O) | This study |
| BL101            | BL21(DE3) harboring PA-SeTAL and PC-VwSTS-Os4CL(O) | This study |
| BL102            | BL21(DE3) harboring PA-aroG-SeTAL-tyrA and PC-VwSTS-Os4CL(O) | This study |
| BL103            | BL21(DE3) harboring PA-aroG<sup>br</sup>-ppsA-tktA-SeTAL-tyrA<sup>br</sup> and PC-VwSTS-Os4CL(O) | This study |
| BTP-S            | BTP harboring PA-aroG<sup>br</sup>-ppsA-tktA-SeTAL-tyrA<sup>br</sup> and PC-VwSTS-Os4CL(O) | This study |
| BTPFI-S          | BTPFI harboring PA-aroG<sup>br</sup>-ppsA-tktA-SeTAL-tyrA<sup>br</sup> and PC-VwSTS-Os4CL(O) | This study |
| **Primers**      |             |                      |
| tyrR-F-Del       | GTGCTCATATCATATATATTGTTCTTTTTTCCAGGTGAAGGTTCACCATTGtaaccctcaaaa-999<sup>fg</sup> |           |
| tyrR-Del         | TGTGACCATACGACATATCTCGGCTCTACTCTTCTCCTCTCCTCATcaatacgactcactatatagggctc |           |
| pheA-F-Del       | CTTCCCAATTCGGGGGTCTTTTTTATTGATAACAAAAAGGCAACACTATAaggataacaccctcaaaaa-999<sup>fg</sup> |           |
| Plasmids/Strains | Description | Sources or reference |
|------------------|-------------|----------------------|
| **pheA-F-Del**   | CACATCATCCGCCACCTTTTCATCAGGTTGGATCAAACAGGCACACTAGGTCGTAataacgactcactcatagaggttc | |
| **fumC-F-del**   | TTAACGCCCGGTTCTTACATGCGGACCACATCTGGTGGCGTACCCAGGTagaataccctcactataaggggcc | |
| **fumC-R-del**   | ATGGAATACGATCGACGGAAAGAATCTCGATGGGGGCGATTTAGTGCTCCCtaataacgactcactcatagaggttc | |
| **icdA-F-del**   | ATGGGAATAAAGAATGTTGGTCCGGCACAAGGCAAGAAGATACCAAACCCGCAatataacgactcactataaggggcc | |
| **icdA-R-del**   | TTATCATGTCTTGATGATCGCATCACAATTTCTGAACATTTACACAGCTAACCAatataacgactcactcatagaggttc | |
| Os4CL-BamHI      | ATggatccGATGGGTCGCTGGCCGCG | |
| Os4CL-NotI       | ATgcggccgcTTA GCT GCT TTT GGG CGC | |
| Os4CL2-HindIII-RBS | ATaagcttTCATGGCCACCTCCGAGCGATCCGCA | |
| **Os4CL2-NotI**  | ATgcggccgcCTAGCGACGGCGGAGCTTGGCAT | |
| **Sc4CL-BamHI**  | AAGgtatccGATGCCAGCCCCGAGTACGCA | |
| **Sc4CL-NotI**   | AAGgcggccgcTATCGGCGGCTCCCCGAGCTTGGCAT | |
| **Le4CL-BamHI**  | ATgtatccGATGCCAGCCCCGAGTACGCA | |
| **Le4CL-NotI**   | CAGgcggccgcTAATTTGTCACACCCATTTGG | |
| **Pc4CL-EcoRI**  | ATgaattcATGGGGGGTCGCTGGCCGCG | |
| **Pc4CL-NotI**   | ATgcggccgcTTA ATG GCT TTT GGG AAG ATC ACC GG | |
| **VvSTS-EcoRI**  | ATgaattcGATGGGTCGCTGGCCGCG | |
| **VvSTS-NotI**   | ATgcggccgcTTA ATG GCT TTT GGG AAG ATC ACC GG | |
| **VvSTS-HindIII**| GCaagcttTTAATTTGTAACCATAGGAA | |
| **VvSTS-smal**   | ATccggggGATGGGTCGCTGGCCGCG | |
| **VvSTS-Xhol**   | ATctcgagTTATTTGTAACCATAGGAA | |
| **AhSTS-EcoRV**  | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **AhSTS-Xhol**   | CATctcgagTTATTTGTAACCATAGGAA | |
| **PcSTS-EcoRV**  | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **PcSTS-Xhol**   | CATctcgagTTATTTGTAACCATAGGAA | |
| **PsSTS-EcoRI**  | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **PsSTS-SalI**   | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **PsSTS-HindIII**| ATaagcttTGGGTCGCTGGCCGCG | |
| **SeTAL-affl**   | atcttgctATATCGGCGAGCTTGGCAT | |
| **T7-SeTAL-NotI**| ATtcgagCGCTGCCCCGAGCTTGGCAT | |
| **aroG-EcoRI**   | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **aroG-SalI**    | ATgatatcGATGGGTGTATGGAAGTGAATTT | |
| **tyrA-NotI**    | ATcatactGATGGGTGTATGGAAGTGAATTT | |
| **tyrA-KpnI**    | CATtgatatcTGGGTCGCTGGGTAACCATAGGAA | |
| **ppsA-RBS-Xhol**| ATtcgagCGCTGCCCCGAGCTTGGCAT | |
| **ppsA-SalI-NotI**| ATgcgccgcgcGTTATTTGTCGCTGGGTCGCT | |
| **tktA-RBS-Xhol**| ATtcgagCGCTGCCCCGAGCTTGGCAT | |
| **tktA-NotI**    | CATtgatatcTGGGTCGCTGGGTAACCATAGGAA | |
PC-VvSTS-Os4CL(O), and PC-PaSTS-Os4CL(O) contained a single promoter but an RBS site in front of each gene.

The aroG, tyrA, and feedback resistance mutants of aroG (aroGbr) and tyrA (tyrAfr) were cloned in our previous study [18]. Briefly, to make the pA-aroGbr-ppsA-tktA-SeTAL-tyrAfbr construct, aroGbr and tyrAfr were introduced into the EcoRI and SalI sites and the Nde I and KpnI sites of pACYCDuet, respectively, and was named PA-aroGbr-tyrAfbr. ppsA (phosphoenolpyruvate synthase) was amplified using PCR with the forward primer containing Sal I and NotI sites, and a reverse primer including an RBS and synthase) was amplified using PCR with the forward primer containing an RBS site of PA-aroGbr-tyrAfbr and named PA-aroGbr-ppsA-tyrAfbr. The resulting PCR product was digested with the corresponding restriction enzymes and cloned into the SalI and NotI sites of pA-aroGbr-tyrAfbr and named PA-aroGbr-ppsA-tkyfr. tktA (Transketolase 1) was amplified using PCR with the forward primer containing an RBS and Xhol site and the reverse primer containing a NotI site. The resulting PCR product was digested with the corresponding restriction enzymes and cloned into the SalI and NotI sites of PA-aroGbr-tyrAfbr and named PA-aroGbr-ppsA-tkyfr. SeTAL containing the T7 promoter and RBS was amplified with two primers expanded with the NotI and aflII sites using PA-SeTAL as a template. The PCR product was digested with NotI and aflII restriction enzymes. The resulting DNA fragment was cloned into the corresponding site of PA-aroGbr-ppsA-tkyfr and named PA-aroGbr-ppsA-tktA-SeTAL-tyrAfbr (Table 1).

**Deletion of ΔtyrR, ΔpheA, ΔicdA, and ΔfumC**

The Quick and Easy Conditional Knockout Kit (Gene Bridges, Heidelberg, Germany) was used to prepare E. coli BL21 (DE3) mutants of four genes, tyrR (DNA-binding transcriptional dual regulator), pheA (chrimate mutase/prephenate dehydratase), icdA (isocitrate dehydrogenase), and fumC (fumarase C). Briefly, the tyrR gene of E. coli BL21 (DE3) was deleted using tyrR-FRT-PGK-gb2-neo-FRT-tyrR cassette generated through PCR using two primers containing the tyrR-specific sequence of 50 bp, with FRT-PGK-gb2-neo-FRT as a template (Table 1). Luria–Bertani (LB) agar plates supplemented with 50 μL/mL kanamycin were used for the selection of positive colonies. The positive clones of tyrR deletion were checked using colony PCR. The positive clone selected was named BL21ΔtyrR. To make ΔtyrR and ΔpheA double mutants, BL21ΔtyrR was used. A 708-FLPe expression plasmid encoding FLPe recombinase was used to remove the kanamycin cassette in BL21ΔtyrR. Colony PCR was used to verify the positive clone. The BL21ΔtyrR strain, a kanamycin cassette-free, was used to remove pheA using pheA-FRT-PGK-gb2-neo-FRT-pheA cassette generated through PCR. The double deletion mutant of tyrR and pheA was designated as BL21ΔtyrR-pheA. The quadruple mutant, BL21ΔtyrR-pheA-fumC-icdA, was generated by repeating the same methods as described above.

**Production of resveratrol from glucose and glycercol in E. coli**

To compare the effects of different constructs on the production of resveratrol, each construct was transformed into E. coli BL21 (DE3). Each E. coli transformant was grown overnight in LB broth containing the appropriate antibiotics at a concentration of 50 μg/mL. The overnight cultured cells were transferred into fresh LB medium containing appropriate antibiotics, and the cells were grown until the OD600 reached 1.0. The cells were collected through centrifugation and then washed briefly with the M9 medium. The cell concentration was controlled to an OD600 of 1.0, with 2 mL of M9 medium supplemented with 1% glucose or 1% glycercol, 1% yeast extract, and 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside). The resulting culture was incubated at 30 °C for 48 h with shaking at 200 rpm. Samples (200 μL) were collected and mixed with 1 mL of ethyl acetate. After vortexing and centrifugation, the upper ethyl acetate layer was transferred into a new 1.5 mL Eppendorf tube. The organic layer was evaporated to dryness, and the remaining residues were dissolved in 100 μL of dimethyl sulfoxide (DMSO). The samples were analyzed using a Varian HPLC (High performance liquid chromatography) system equipped with a photodiode array detector and an Agilent Polaris 5 C18-A column (250 × 4.6 mm) was used for the analysis of the reaction products using 20 μL injection. The mobile phases consisted of 0.1% formic acid in water or acetonitrile. The program was 20% acetonitrile at 0 min, 45% acetonitrile at 10 min, 80% acetonitrile at 20 min, 90% acetonitrile at 21.0 min, 90% acetonitrile at 25 min, 20% acetonitrile at 25.1 min, and 20% acetonitrile at 30 min. The flow rate was 1 mL/min, and UV detection for the reaction products was dually monitored at 270 and 310 nm.

**Results and discussion**

**Production of resveratrol in E. coli from p-coumaric acid**

To synthesize resveratrol from glucose in E. coli, at least three genes, namely TAL, 4CL, and STS are necessary (Fig. 1). The TAL gene is mainly found in microorganisms, and the TAL gene of Saccharothrix espanaensis, which has been widely used in previous studies, was used in this study [18, 36]. Two genes, 4CL and STS, are more important for resveratrol production. Therefore, we decided to find the best combination of 4CL and STS for resveratrol production from p-coumaric acid in E. coli BL21 (DE3). The 4CL gene is found simultaneously
in microorganisms and plants. To select most effective genes for resveratrol biosynthesis, we tested four 4CL genes, Pc4CL, Os4CL, and Le4CL from plants and Sc4CL from microorganisms, through combination with VvSTS for resveratrol production (Fig. 2). Four different combinations of 4CLs and VvSTS, which are regulated by independent T7 promoter, were tested for resveratrol production from p-coumaric acid (Fig. 2a). BP-Os4CL harboring Os4CL and VvSTS showed the highest productivity of 16.2 mg/L after 12 h of incubation, followed by BP-Pc4CL containing Pc4CL and VvSTS (8.1 mg/L). However, Strains containing Sc4CL and VvSTS (BP-Sc4CL) or Le4CL and VvSTS (BP-Le4CL) produced resveratrol of less than 4 mg/L. Based on the above results, we decided to use Os4CL in subsequent experiments. Next, we tested six different combinations of three STSs (VvSTS, AhSTS, and PaSTS) and Os4CL, which were constructed either pseudo-operon or operon regulated by the T7 promoter for resveratrol production from p-coumaric acid in E. coli (Fig. 2b). E. coli harboring Os4CL and VvSTS pseudo-operon (BP-VvSTS) regulated by each T7 promoter or Os4CL and VvSTS operon (BO-VvSTS) regulated by one T7 promoter, showed the highest productivity at 15.1 and 19.8 mg/L after 24 h of incubation, respectively. Interestingly, BO-AhSTS strain containing the AhSTS and Os4CL operon produced only small amounts of resveratrol, whereas BP-AhSTS strain containing the AhSTS and Os4CL pseudo-operon produced 13.8 mg/L of resveratrol. E. coli containing either PaSTS and Os4CL pseudo-operon (BP-PaSTS) or PaSTS and Os4CL operon (BO-PaSTS) produced only small amounts of resveratrol (less than 4 mg/L). These results indicate that the optimum combination of STS and 4CL for balanced gene expression is critical for the production of resveratrol. We chose the VvSTS-Os4CL(O) operon (E. coli strain BO-VvSTS) for further experiments.

**Production of resveratrol from glucose in E. coli**

Resveratrol biosynthesis in plants begins with cinnamic acid catalyzed by PAL with phenylalanine as the substrate (Fig. 1). Cinnamic acid is catalyzed by C4H to form p-coumaric acid [34]. Since C4H is a member of the P450 family, it is difficult to express it functionally in E. coli. For this reason, many studies related to the biosynthesis of phenolic compounds have used TAL, which converts tyrosine to p-coumaric acid [18, 35]. In previous studies, TAL cloned from S. espanaensis was used to biosynthesize various phenolic compounds in E. coli based on its excellent enzyme activity [18, 36]. pA-SeTAL was transformed into the E. coli BL 100 strain, and the resulting strain, BL 101, was examined for the production of resveratrol from glucose. After a 24 h incubation, HPLC analysis of the reaction products showed two new peaks at 8.82 and 10.29 min. Based on the HPLC retention...
time, molecular mass, and UV absorbance, the two peaks at 8.82 and 10.29 min turned out to be \( p \)-coumaric acid and resveratrol, respectively (Fig. 3a–c). To further clarify the structure of P2, MS (Mass spectrometry) and NMR (Nuclear Magnetic Resonance) analyses were performed. In the negative ESI mode, P2 showed a molecular ion \([M–H]^–\) at \( m/z \) 227, which was consistent with the molecular weight of resveratrol (Fig. 3d–f). For NMR analysis, one liter was cultured, the reaction product was extracted twice with the same amount of ethyl acetate, purified first using TLC, and then finally purified using HPLC and the same method as the analysis conditions. Finally, 5 mg of the pure reaction product was obtained and used for \(^1\)H-NMR analysis. NMR was conducted as described by Kim et al. [37]. The structure of the reactants was determined by comparison with the structure published by Amalfitano et al. [38].

The NMR data was analyzed as follows: \(^1\)H-NMR (400 MHz, Acetone-\( d_6 \); \( \delta \) 7.41 (2H, \( d \), 8.58 Hz), \( \delta \) 7.01(1H, \( d \), 16.3 Hz), \( \delta \) 6.88(1H, \( d \), 16.3 Hz), \( \delta \) 6.83(2H, \( d \), 8.6 Hz), \( \delta \) 6.5 (2H, \( d \), 2.11 Hz), and \( \delta \) 6.26(1H, \( t \), 2.11 Hz). The results were consistent with previously published results [38]. These results indicated that resveratrol was successfully synthesized from glucose in strain BL101 harboring \( SeTAL \), \( VvSTS \), and \( Os4CL \), with a yield of 13.8 mg/L (Fig. 4a). The production of BL101 (13.8 mg/L) was lower than that of \( E. coli \) strain BO-VvSTS (19.8 mg/L). This result is most likely due to insufficient supply of endogenous tyrosine in \( E. coli \) for resveratrol biosynthesis. Therefore, we decided to increase the supply of tyrosine, which is used as a precursor for \( p \)-coumaric acid biosynthesis.

**Engineering of \( E. coli \) to increase the production of resveratrol**

Although resveratrol was biosynthesized from glucose using the wild type \( E. coli \) strain containing three genes, the yield was not high. This was due to the limited supply of tyrosine. Therefore, we decided to increase the amino acid and tyrosine pool, which is the TAL substrate for \( p \)-coumaric acid biosynthesis, which was used as an entry point for resveratrol biosynthesis. To increase tyrosine levels, we overexpressed \( aroG \) and \( tyrA \), which encodes the rate-limiting enzymes in the shikimic acid biosynthetic pathway [17, 18]. The \( aroG \) is converted into 3-deoxy-d-arabinoheptulosonate-7-phosphate using both phosphoenolpyruvate and erythrose 4-phosphate as substrates. tyrA is responsible for a two-step reaction from chorismate via prephenate to 4-hydroxy-phenylpyruvate. Additionally, two genes, \( aroG^{fr} \) and \( tyrA^{fr} \),
were overexpressed because aroG and tyrA are feedback inhibited by tyrosine. aroG<sub>fr</sub>, a mutant form of aroG, is not inhibited by the end product, tyrosine, unlike aroG (Fig. 1). In addition, tyrA was changed to tyrA<sub>fr</sub>, which is not inhibited by the end product, tyrosine (Fig. 1) [17, 18]. Two genes, ppsA and tktA, which were overexpressed are involved in the biosynthesis of phosphoenolpyruvate and erythrose-4-phosphate, which are used as entrance compounds for the shikimic acid biosynthetic pathway.

*E. coli* BL21(DE3) was used as the host strain for resveratrol production using three different vector sets, pA-SeTAL (BL101), pA-aroG-SeTAL (BL102), and pA-aroG<sub>fr</sub>-ppsA-tktA-SeTAL-tyrA<sub>fr</sub> (BL103), along with the PC-VvSTS-Os4CL operon. The resulting three *E. coli* strains were compared for resveratrol production. BL103 containing pA-aroG<sub>fr</sub>-ppsA-tktA-SeTAL-tyrA<sub>fr</sub> produced a much greater amount (42.9 mg/L) of resveratrol, followed by strain BL102 (23.6 mg/L) (Fig. 4a). These results indicate that the increased supply of tyrosine in *E. coli* had a great effect on the biosynthesis of resveratrol. Thus, we decided to increase the tyrosine supply in *E. coli* by the deletion of two genes, tyrR and pheA, which have been reported to increase tyrosine levels in several studies. tyrR encodes a transcriptional regulatory protein for tyrosine biosynthesis. Its transcription is regulated by the feedback inhibition of the end product, tyrosine. In previous studies, the deletion of tyrR in *E. coli* increased tyrosine production [18]. Because both tyrA and pheA compete to use prephenate as a substrate, pheA was deleted to induce tyrosine biosynthesis. Next, the removal of the icdA and fumC genes encoding isocitrate dehydrogenase and fumarate hydratase, respectively, has been reported to increase the amount of CoA in *E. coli* (Fig. 1) [18]. Therefore, the two genes were selected for engineering. This was expected to increase the biosynthesis of p-coumaroyl-CoA, which is used as an intermediate metabolite of resveratrol biosynthesis. As a result, three strains, BL21 DE3 (wild type), B-TP (tyrA/pheA double mutant), and B-TPFI (tyrA/pheA/icdA/fumC quadruple mutant) were used to create three different strain sets along with two different vector sets, pA-aroG<sub>fr</sub>-ppsA-tktA-SeTAL-tyrA<sub>fr</sub> and PC-VvSTS-Os4CL operon. The three strains were named BL103, BTP-S, and BTPFI-S, and resveratrol production was compared for each strain. BTP-S and BTPFI-S produced 41.3 mg/L and 38.9 mg/L, respectively, which was less than the yield of BL103 (50.3 mg/L) (Fig. 4b). In both the BTP-S and BTPFI-S strains, more than 150 mg/L of tyrosine was detected, whereas in the wild type, 21.7 mg/L of tyrosine was detected (Fig. 4b). In addition, BTP-S (54.3 mg/L) and BTPFI-S (50.1 mg/L) accumulated more p-coumaric acid than BL103 (32.1 mg/L). These results show that both tyrosine and p-coumaric acid increased in the mutant strains, but resveratrol did not. It seemed that the extra tyrosine or p-coumaric acid...
might inhibit the next two steps, \( p \)-coumaroyl-CoA synthesis or resveratrol synthesis. This resulted in a lower final yield of resveratrol in the mutant strains than in the wild type. Therefore, when resveratrol is synthesized from simple carbon sources, such as glucose and glycerol, balancing between the precursor and the product is critical for increasing the final yield. Recently, various methods, such as the co-culture methods, introduction of promoters of different strengths, and gene integration into the genome, have been attempted to resolve the metabolic imbalances. Among them, a co-culture method that divides the metabolic pathway necessary for the biosynthesis of phytochemicals into two or three strains has been frequently used as a method to overcome metabolic imbalance [35, 39]. To increase resveratrol biosynthesis, subsequent research such as a co-culture method should be conducted.

We explored the optimum culture method for resveratrol production using BL 103. First, we optimized the culture temperature. The cells were cultured at 25, 30, and 37 °C. The production of resveratrol was the highest with 44.3 mg/L at 30 °C, followed by 23.1 mg/L at 25 °C and 3.2 mg/L (Fig. 5a). Although the production of resveratrol was the lowest at 37 °C, the production of

![Fig. 5](image-url)
$p$-coumaric acid was the highest at 118.9 mg/L among the temperatures tested (Fig. 5a). These results suggest that the production of high amounts of proteins such as 4CL and STS may form inclusion bodies. The cells grown at 25 °C produced a lower amount of $p$-coumaric acid and resveratrol than those at 30 °C. These results suggest that the cells cultivated at 25 °C did not produce enough proteins for resveratrol production owing to the low culture temperature. We also optimized cell culture density. The cell density was adjusted to 0.5, 1, 1.5, and 2 at OD$_{600}$ The optimal cell density was 0.5 at OD$_{600}$ at which approximately 58.7 mg/L resveratrol was produced (Fig. 5b). However, as cell density increased, the production of resveratrol decreased. Next, we determined the optimal carbon sources and supply concentrations. The cell densities were adjusted to 0.5 at OD$_{600}$ and glucose and glycerol were supplied at a rate of 1, 2, 4, 6, 8, and 10%. Overall, the productivity of resveratrol was higher when glycerol was used as a carbon source than glucose. When glycerol was used as a carbon source, the productivity of resveratrol increased as the supply concentration increased. The highest productivity of resveratrol was observed at a concentration of 8%, at which approximately 78.9 mg/L of resveratrol was produced (Fig. 5c). In the case of glucose, the productivity was highest at a concentration of 2%, at which approximately 45.4 mg/L of resveratrol was produced. However, as the concentration of glucose increased, the productivity of resveratrol gradually decreased.

Subsequently, resveratrol production from glycerol using the optimized conditions was monitored for 48 h. $p$-Coumaric acid was observed after 8 h of incubation and it slightly increased with incubation time. The cell density gradually increased with incubation time. The highest density was observed after 24 h of incubation, at which time the cell density reached approximately 7.5 at OD$_{600}$. After 24 h incubation, the cell density showed a tendency to gradually decrease. Resveratrol production was initiated after 8 h of incubation and it rapidly increased until 24 h of incubation. At this time, approximately 68.9 mg/L resveratrol was produced. After 24 h of incubation, resveratrol production slowly increased. After 40 h of incubation, resveratrol production was highest at 80.4 mg/L and then it slightly decreased (Fig. 6). Wang et al. [28] reported the biosynthesis of 114.4 mg/L of resveratrol from glucose, which was 1.4 times higher than that of this study. However, in the study of Wang et al. [28], resveratrol was biosynthesized by adding 3 mM tyrosine to the culture medium, but we synthesized resveratrol from glucose without supplying tyrosine to the medium. For commercial application of resveratrol, a yield of at least 1.0 g/L must be reached. However, the yield for this study...

**Fig. 6** Production of resveratrol by *E. coli* strain BL103. The concentration of the pre-cultured cells was adjusted to OD$_{600}$ = 0.5, with 25 mL of M9 medium supplemented with 8% glycerol, 1% yeast extract, and 1 mM IPTG at the final concentration. The flask was incubated at 30 °C for 49 h with shaking at 200 rpm, and the sample was periodically collected. The reaction products were extracted with two volumes of ethyl acetate and analyzed via HPLC. The error bars indicate mean values ± from three independent experiments.
is 80.4 mg/L. Of course, it is difficult to apply commercial with the current production amount, but if the culture conditions are optimized using a fermentor and the pathway for resveratrol biosynthesis is slightly improved, it will be possible to produce a sufficient amount required for commercial production.

**Conclusion**

Resveratrol is attracting much attention due to its various health benefits such as anticancer, antileukemic, and immune-modulating activities [12, 13]. Since resveratrol is supplied by extraction from plants [10, 11], researchers recently attempted to biosynthesize resveratrol by introducing the resveratrol biosynthesis gene into microorganisms as an alternative production method for resveratrol [30–33]. There are several things to consider in order to biosynthesize resveratrol from a simple carbon source using microorganisms. First, resveratrol biosynthetic genes such as 4CL and STS with high turnover rates should be selected. Second, a stable supply of tyrosine, the starting material for resveratrol biosynthesis, must be established. For the stable supply of tyrosine, the metabolic engineering of the tyrosine biosynthetic pathway of microorganisms must be achieved. Third, it is important to balance the metabolic pathways for resveratrol production without the metabolic load of intermediates such as tyrosine, p-coumaric acid and p-coumaroyl CoA. In this study, we attempted to produce resveratrol from simple carbon source, considering the problems presented above and optimized the culture system such as cell concentration, culture temperature, and carbon sources. Under optimized conditions, approximately 80.4 mg/L of resveratrol was produced after 48 h of culture using glycerol as a carbon source. Although we have successfully biosynthesized resveratrol from a simple carbon source, we need to further improve the biosynthesis of resveratrol in microorganisms. For this purpose, various methods, such as the co-culture methods, introduction of promoters of different strengths, and gene integration into the genome, must be attempted.

**Abbreviations**

PAL: Phenyalanine ammonia lyase; C4H: Cinnamic acid 4-hydroxylase; TAL: Tyrosine ammonia lyase; 4CL: 4-Coumarate-CoA ligase; STS: Stilbene synthase; HFLC: High-performance liquid chromatography; IPTG: Isopropyl-β-D-thiogalactoside; LB: Luria broth; ppsA: Phosphoenolpyruvate synthetase; ktnA: Transketolase; tyrP: Phenyalanine DNA-binding transcription repressor; aroC: Deoxyphosphoheptonate aldolase; aroC: Chorismate synthase; tyrA: Phenylalanine dehydrogenase; pheA: Prephenate dehydratase; tyrB: Phenylalanine aminotransferase.

**Acknowledgements**

This study was supported by a grant from the Korea Forest Service’s Forest Convergence Specialist Training Project (Support for Forest Industry Characterization Research, FTIS Assignment No. 2020186A00-2022-AA02) and by a grant from the Basic Science Research Program (NRF-2016R1D1A1B03933610).

**Authors’ contributions**

BGK and JHA designed the experiments. JYP, JKL, and BGK performed the experiments and analyzed the data. JYP, JKL, JHA, and BGK wrote the manuscript. All authors read and approved the final manuscript.

**Funding**

Funding was received from the Korea Forest Service’s Forest Convergence Specialist Training Project (Support for Forest Industry Characterization Research, FTIS Assignment No. 2020186A00-2022-AA02) and from the Basic Science Research Program (NRF-2016R1D1A1B03933610).

**Availability of data and materials**

All data generated or analyzed during the present study are included in this published article.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1 Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea. 2 Department of Forest Resources, Gyeongnam National University of Science and Technology, 33 Dongjin-ro, Jinju 52725, Gyeongsangnam-do, Korea.

**Received:** 5 October 2020 **Accepted:** 25 January 2021 **Published online:** 05 February 2021

**References**

1. Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, Ishiqia A, Husain S, Suleria HAS (2017) Natural polyphenols: an overview. Int J Food Prop 20:1689–1699
2. Newman DJ, Cragg GM (2020) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83:770–803
3. Del Bubba M, Di Serio C, Renai L, Scordo CVA, Checchini L, Ungar A, Tarantino F, Bartoletti R (2020) Vactinium myrtillus L. extract and its native polyphenol-recombined mixture have anti-proliferative and pro-apoptotic effects on human prostate cancer cell lines. Phytother Res. https://doi.org/10.1002/ptr.6879
4. Vogt T (2010) Phenylpropanoid biosynthesis. Mol Plant 3:2–20
5. Lattanzio V, Lattanzio V, Cardinali A, Imperato F (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F (ed) Phytochemistry: advances in research. Research Signpost, Trivandrum, Kerala, pp 23–67
6. Vattem DA, Ghaedarian R, Shetty K (2005) Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. Asia Pac J Clin Nutr 14:120–130
7. Pezet R, Perret C, Jean-Denis JS, Tabacchi R, Girdro K, Viret O (2003) Deltaviniferin, a resveratrol dehydrodimer: one of the major stilbenes synthesized by stressed grapevine leaves. J Agric Food Chem 51:5488–5492
8. Borie B, Jeander P, Parize A, Bessis R, Adrian M (2004) Resveratrol and stilbene synthase mRNA production in grapevine leaves treated with biotic and abiotic phytoalexin elicitors. Am J Enol Vitic 55:60–64
9. Jeandet P, Clement C, Cordelier S (2019) Regulation of resveratrol biosynthesis in grapevine: new approaches for disease resistance? J Exp Bot 70:375–378
10. Kimura Y, Kozawa M, Baba K, Hata K (1983) New constituents of roots of Polygonum cuspidatum. Planta Med 48:164–169
11. Das S, Das DK (2007) Resveratrol: a therapeutic promise for cardiovascular diseases. Recent Patents Cardiovasc Drug Discov 2:133–138
12. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5:493–506
13. Singh AP, Singh R, Verma SS, Rai V, Kaschula CH, Maiti P, Gupta SC (2019) Health benefits of resveratrol: evidence from clinical studies. Med Res Rev 39:1851–1891
14. Giovannazzo G, Ingrosso I, Paradiso A, Gara LD, Santino A (2012) Resveratrol biosynthesis: plant metabolic engineering for nutritional improvement of food. Plant Foods for Hum Nutr 67:191–199
15. Wang J, Yang Y, Yan Y (2018) Bioproduction of resveratrol. In: Schwab W, Lange B, Wüst M (eds) Biotechnology of natural products. Springer, Cham, pp 61–79

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

17. Juninaga D, Baidoo EEK, Redding-Johanson AM, Batth TS, Burd H, Mukhopadhyay A, Petzold CJ, Keasling JD, (2012) Modular engineering of L-tyrosine production in Escherichia coli. Appl Environ Microbiol 78:89-98

18. Kim MJ, Kim BG, Ahn JH (2013) Biosynthesis of bioactive O-methylated flavonoids in Escherichia coli. Appl Microbiol Biotechnol 97:7195–7204

19. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA (2019) The effects of polyphenols and other bioactives on human health. Food Funct 10:514–528

20. Durazzo A, Lucarini M, Souto EB, Carla C, Caiazzo E, Izzo AA, Novellino E, Santini A (2019) Polyphenols: A concise overview on the chemistry, occurrence, and human health. Phytother Res 33:2221–2243

21. Adams RP (1987) Yields and seasonal variation of phytochemicals from Juniperus species of the United States. Biomass 12:129–139

22. 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

23. Chung IM, Rekha K, Rajakumar G, Thiruvengadam M (2018) Secondary metabolism and gene expression alterations in hairy root cultures of chinese cabbage elicited by copper oxide nanoparticles. Plant Cell, Tissue Organ Cult 134:95–106

24. Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, Frukh A (2018) Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield. Plant Cell, Tissue Organ Cult 132:239–265

25. Kim BG (2019) Optimization of bioactive isorhamnetin 3-O-glucoside production in Escherichia coli. J Appl Biol Chem 62:361–366

26. Yang D, Park SY, Park YS, Eun H, Lee SY (2020) Metabolic Engineering of Escherichia coli for Natural Product Biosynthesis. Trends Biotechnol 38:745–765

27. Yang SM, Shim GY, Kim BG, Ahn JH (2015) Biological synthesis of coumarins in Escherichia coli. Microb Cell Fact 14:65. https://doi.org/10.1186/s12934-015-0248-y

28. Wang S, Zhang S, Xiao A, Rasmussen M, Skidmore M, Zhang JN (2015) Metabolic engineering of Escherichia coli for the biosynthesis of various phenylpropanoid derivatives. Metab Eng 29:153–159

29. Bian G, Deng Z, Liu T (2017) Strategies for terpenoid overproduction and new terpenoid discovery. Curr Opin Biotechnol 48:234–241

30. Beekwilder J, Wolswinkel R, Monner H, Hall R, de Voos C, Verstuyft Y, Boey A (2006) Production of resveratrol in recombinant microorganisms. Appl Environ Microbiol 72:5670–5672

31. Watts KT, Lee PC, Schmidt-Dannert C (2006) Biosynthesis of plant specific stilbene polyketides in metabolically engineered Escherichia coli. BMC Biotechnol 6:22

32. Lim CG, Fowler ZL, Hueller T, Schaffer S, Koffas MAG (2011) High-yield resveratrol production in engineered Escherichia coli. Appl Environ Microbiol 77:3451–3460

33. Yuan SE, Yi X, Johnston TG, Alper HS (2020) De novo resveratrol production through modular engineering of an Escherichia coli–Saccharomyces cerevisiae co-culture. Microb Cell Fact 19:143

34. Gomes E, Coutos-Thevenot P (2009) Molecular aspects of grapevine-pathogenic fungi interactions. In: Roubelakis-Angelakis KA (ed) Grapevine molecular physiology & biotechnology. Springer, Dordrecht, Netherlands, pp 407–428

35. Wang X, Shao A, Li Z, Policarpio L, Zhang H (2020) Constructing E. coli co-cultures for de novo biosynthesis of natural product acacetin. Biotech Method 15:2000131

36. Berner M, Knud B, Bihlmayer C, Vente A, Muller R, Bechthold A (2006) Genes and enzymes involved in caffeic acid biosynthesis in the actinomycete Saccharothrix espanaensis. J Bacteriol 188:2666–2673

37. Kim BG, Jung BR, Lee Y, Hur HK, Lim Y, Ahn JH (2006) Regiospecific flavonoid 7-O-methylation with Streptomyces avermitilis O-methyltransferase expressed in Escherichia coli. J Agric Food Chem 54:823–828

38. Amalitano C, Evidente A, Surico G, Iegi S, Bertelli E, Mugnai L (2000) Phenols and stilbene polyphenols in the wood of esca-diseased grapevines. Phytopathol 39:178–183

39. Jones JA, Vernacchio VR, Collins SM, Shirke A, Xiu Y, Englaender JA, Cress B, McCutcheon CC, Linhardt R, Gross RA, Koffas MAG (2017) Complete biosynthesis of anthocyanins using E. coli polycultures. eBio 8.00621–17

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.