



Overview of resveratrol properties, applications, and advances in microbial precision fermentation

Carlos E. Costa, Aloia Romaní & Lucília Domingues

To cite this article: Carlos E. Costa, Aloia Romaní & Lucília Domingues (24 Nov 2024): Overview of resveratrol properties, applications, and advances in microbial precision fermentation, Critical Reviews in Biotechnology, DOI: [10.1080/07388551.2024.2424362](https://doi.org/10.1080/07388551.2024.2424362)

To link to this article: <https://doi.org/10.1080/07388551.2024.2424362>



Published online: 24 Nov 2024.



Submit your article to this journal [↗](#)



Article views: 110



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 1 View citing articles [↗](#)



REVIEW ARTICLE



Overview of resveratrol properties, applications, and advances in microbial precision fermentation

Carlos E. Costa^{a,b} , Aloia Romaní^c, and Lucília Domingues^{a,b}

^aCEB – Centre of Biological Engineering, University of Minho, Braga, Portugal; ^bLABBELS – Associate Laboratory, Braga/Guimarães, Portugal; ^cDepartment of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), Ourense, Spain

ABSTRACT

Resveratrol is an antioxidant abundant in plants like grapes and peanuts and has garnered significant attention for its potential therapeutic applications. This review explores its chemical attributes, stability, and solubility, influencing its diverse applications and bioavailability. Resveratrol's multifaceted therapeutic roles encompass: antioxidant, cardioprotective, anti-inflammatory, neuroprotective, anti-aging, and anticancer properties. While traditionally studied in preclinical settings, a surge in clinical trials underscores resveratrol's promise for human health. Over 250 recent clinical trials investigate its effects alone and in combination with other compounds. Commercially utilized in food, cosmetics, supplements, and pharmaceuticals, the resveratrol market is expanding, driven by microbial fermentation. Microbes offer advantages over plant extraction and chemical synthesis, providing cost-effective, pure, and sustainable production. Microbial biosynthesis can be attained from carbon sources, such as glucose or xylose, among others, which can be obtained from renewable resources or agro-industrial wastes. While *Saccharomyces cerevisiae* has been the most used host, non-conventional yeasts like *Yarrowia lipolytica* and bacteria like *Escherichia coli* have also demonstrated potential. Genetic modifications such as increasing acetyl-CoA/malonyl-CoA pools, boosting the shikimate pathway, or multi-copy expression of pathway genes, allied to the optimization of fermentation strategies have been promising in increasing titers. Microbial biosynthesis of resveratrol aligns with the shift toward sustainable and renewable bio-based compounds, exemplifying a circular bioeconomy. Concluding, microbial fermentation presents a promising avenue for efficient resveratrol production, driven by genetic engineering, pathway optimization, and fermentation strategies. These advances hold the key to unlocking the potential of resveratrol for diverse therapeutic applications, contributing to a greener and sustainable future.

ARTICLE HISTORY

Received 25 September 2023
Revised 3 January 2024
Accepted 13 July 2024

KEYWORDS

Resveratrol; microbial hosts; metabolic engineering; process engineering; precision fermentation; biorefinery

Introduction

Resveratrol (3,4',5-trihydroxystilbene) is a stilbenoid with strong antioxidant activity that plays a role in plant defence against environmental stresses [1]. It was first isolated in 1939 from *Veratrum grandiflorum*, a poisonous plant used at that time for therapeutic applications [2]. The generic name “resveratrol” is speculated to arise from the combination of its chemical structure and the plant source used for isolation: a derivative from resorcinol occurring in *Veratrum* species, comprising hydroxy groups that form alcohol [3]. A couple of decades later, resveratrol was found in root extracts of the Japanese knotweed, *Fallopia japonica*, also known as *Polygonum cuspidatum* [4], a Chinese medicinal plant considered an invasive species, which is currently the main source of commercialized resveratrol [5]. It was

later isolated from the grapes of *Vitis vinifera* and classified as a phytoalexin, due to its production in plants being driven by the response to fungal infection or exposure to UV light [6]. After the detection of resveratrol in red wine [7], the “French Paradox” was coined in 1992, which relied on epidemiological data from the French population and correlated the low incidence of coronary heart diseases with moderate consumption of red wine, despite the significant content of high saturated fat in their diet [8]. Even though this concept has evolved over the years, as more detailed information has arisen on the negative health impact of heavy ethanol consumption [9], and the presence of other phenolic compounds with therapeutic properties like flavonols or phenolic acids in red wine [10], it drove the focus to the understanding of the underlying

mechanisms behind the concept. After the reports of its pleiotropic effect connected to cancer prevention and other disease conditions in the late 1990s [11], resveratrol started being the subject of extensive research, with thousands of scientific reports arising in the following decades (Figure 1).

Resveratrol naturally occurs in more than 70 plant species [12] most of which are edible, such as grapes (*V. vinifera*) [13] or peanuts (*Arachis hypogea*) [14] among many others [15]. Together with the Japanese knotweed, grapes (particularly grape skin) and peanuts are the most abundant natural sources of resveratrol being the main sources used for chemical extraction processes, traditionally with organic solvents, to obtain resveratrol [16]. However, plant extraction of resveratrol is highly dependent on the availability of plant resources, relying heavily on environmental factors like weather, and has low efficiency [17], with high variations in its purity [18].

Resveratrol can also be chemically synthesized with relatively high yields. However, it is very challenging, often requiring multiple steps and harsh conditions (high temperatures, pollutant catalysts, etc.), with contamination and control of the region selectivity and stereo-chemistry of the synthetic process being a major concern [19]. The intricacy of the chemical synthesis procedures and the availability of

several undesirable by-products, restrict the use of chemically synthesized resveratrol produced, which helps to understand why plant extraction is still the preferred choice for resveratrol [20]. In its turn, microbial production of resveratrol is a valuable alternative, having several advantages over petroleum-based processes, such as being environmentally friendly, low cost, leading to a high purity product, with high stereoselectivity [21]. In order to establish an efficient bioprocess, from the first engineering steps to the final stages encompassing product purification, it is vital to be aware of all the product's characteristics and specificities. Despite the rising interest in this molecule, information in the literature regarding the chemical properties of resveratrol, such as its stability and solubility, is often hard to find. Additionally, while there are a vast number of reports on the beneficial properties of resveratrol, many of them lack evidence and/or were not tested in a clinical context. Therefore, this review compiles resveratrol's main properties, discloses the state-of-the-art on clinically tested health benefits and provides an extensive overview of the breakthroughs accomplished in the last decades on synthetic biology approaches for resveratrol production by precision fermentation, to increase the usefulness of this high-value molecule.

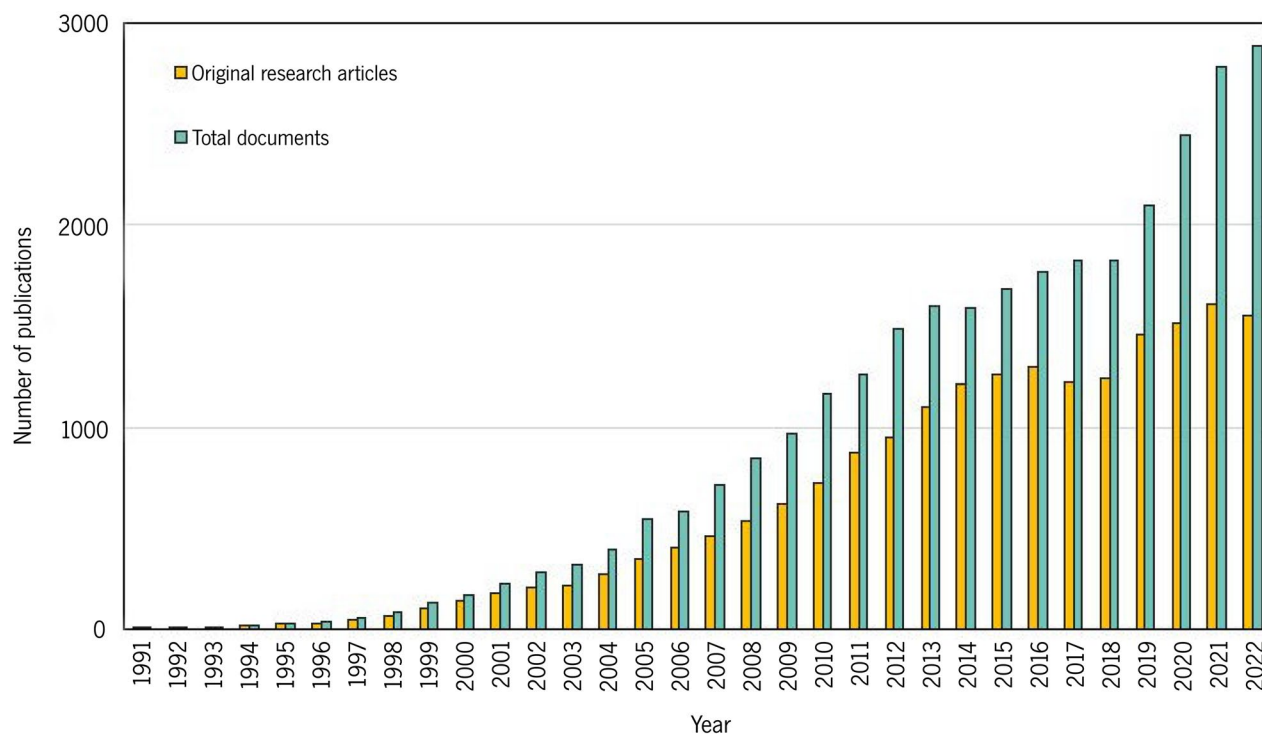


Figure 1. Total number of publications contemplating resveratrol in the last 30 years (1991–2021). Data were obtained from Scopus (<http://www.scopus.com/>) using the queries: TITLE-ABS-KEY (resveratrol) AND (LIMIT-TO (PUBYEAR, YYYY)) for the total number of documents in each year; and TITLE-ABSKEY (resveratrol) AND (LIMIT-TO (PUBYEAR, YYYY)) AND (LIMIT-TO (DOCTYPE, "ar")) for Original Research Articles in each year, where YYYY corresponds to each year from 1991 to 2022. Accessed in September 2023.

Resveratrol properties

Chemical properties

Resveratrol ($C_{14}H_{12}O_3$, IUPAC name 5-[(E)-2-(4-Hydroxyphenyl)ethen-1-yl]benzene-1,3-diol) has a molecular weight of 228.24 g/mol and has two isomers, *trans*-resveratrol (or (E)-resveratrol, according to the *E-Z* system) and *cis*-resveratrol (or (Z)-resveratrol) (Figure 2), differing in the conformation of the double hydrogen bond that ligates both phenolic rings. These are connected through an ethylene bridge, encompassing two different groups, 4-hydroxystyryl and *m*-hydroquinone, which are responsible for their biological properties, by modulation of molecular targets [22]. Due to its aromatic rings, resveratrol is known to display superior free radical scavenging properties in comparison with other antioxidants like vitamin C or E [23]. Both isoforms of resveratrol can be distinguished by distinct UV spectra, with a reported maximum absorbance between 304 nm [24] and 306 nm [25] for *trans*-resveratrol, and between 280 nm [26] and 286 nm [27] for *cis*-resveratrol. *Trans*-resveratrol is the primary biologically active isoform, being responsible for the majority of the therapeutic properties of resveratrol [28].

Stability

Resveratrol is highly reactive to light, pH, and temperature changes, mainly due to its unstable hydroxyl groups and C—C double bond [16]. The most common and stable isoform in nature is *trans*-resveratrol, having a higher bioavailability [29], though it can suffer *cis*-isomerization under exposure to sunlight [30] or natural and artificial UV radiation [27]. Nevertheless, *trans*-resveratrol can maintain its stability for several months when in the absence of light and a low-pH environment [31]. Resveratrol was found to keep its stability at low pH conditions for several days at 37°C. After 7 days of incubation, approximately 90% of resveratrol was still preserved at pH 4 or less, and more

than 70% at pH 5 or 6 [32]. Still, cultivation conditions at 35°C were previously found to cause some degradation of resveratrol [33], so this phenomenon can be also correlated with the incubation temperature and not exclusively dependent on pH. At pH 8 or above, less than 2% of resveratrol remained intact, showing its clear instability in basic conditions [32].

Solubility

Resveratrol has relatively high lipophilicity ($\log p=3.4$) [34], being practically insoluble in water [35], which hinders severely its bioavailability (Table 1). It is widely reported to be soluble in ethanol [32, 36], and slightly soluble in natural food-grade oils, such as soybean, almond, or olive oils [37], which has additional interest due to its possible incorporation into foods with constant oil phase, like butter or cooking oils [38]. Resveratrol is also very soluble in surfactants/detergents, like Tween 20 [36], and the nonionic emulsifier polyethylene glycol 400 (PEG 400), with a reported solubility of up to 374 g/L [32]. Recent studies using natural deep eutectic solvents (DES), a nontoxic and highly recyclable class of ionic liquids [39], also showed their

Table 1. Solubility of resveratrol in different solvents.

Compound	Solubility (g/L)	Temperature (°C)	Reference
Water	0.03–0.05	Room	[35,133]
Glycerine	3.83	37 °C	[36]
Soybean oil	4.004	Room	[37]
Almond oil	4.066	Room	[37]
Olive oil	5.23	Room	[37]
Tween 80	7.46–76.8	Room – 37°C	[32,37,134]
Ethanol	87.98–129.86	Room – 37°C	[32,36]
Tween 20	107.75–248.38	37°C	[36,134]
Tween 60	105.16	37°C	[36]
PEG 400	139.95–373.85	Room	[32,36]
DES 1,2-propanediol: choline chloride:water (1:1:1)	>20	40°C	[135]
DES Choline chloride:glycerol (1:2)	321	n.r.	[40]

n.r.: not reported.

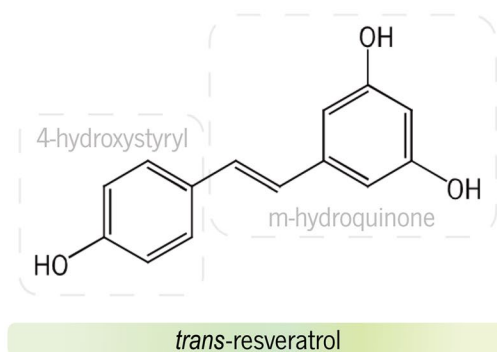
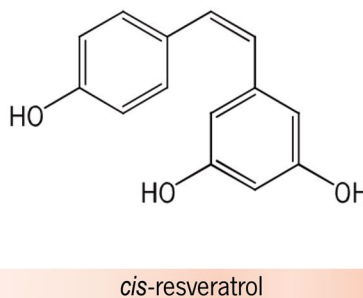


Figure 2. Chemical structures of *trans*-resveratrol, with identification of both moieties of the molecule, and *cis*-resveratrol.

potential as solvents for resveratrol, with DES like Choline chloride:glycerol, at a molar ratio of 1:2, exhibiting a solubility of 321 g/L [40].

Biological activities and therapeutic applications

The 21st century has been particularly prolific in an extensive study of the biological activities of resveratrol and its application as a therapeutic agent. These activities are mainly based on the ability of resveratrol to modulate multiple cell signaling molecules, such as sirtuin type 1 (SIRT1), highly associated with aging [41], insulin-like growth factor 1 (IGF-1), insulin-like growth factor-binding protein 3 (IGFB-3), Ras association domain family 1 α (RASSF-1 α), cytokines, caspases, among many others, therefore being considered a multitargeting agent [42]. As long-term use of rationally designed medications is frequently associated with many adverse effects, the shift to multitargeted therapies, that are less potent but still modify several targets to elicit multiple responses, is desired [42]. Resveratrol has been recognized as having multiple bioactive properties, such as antioxidant, cardioprotective, anti-inflammatory, anti-aging, neuroprotective, and anticancer activities, among others [12].

Resveratrol is rapidly absorbed and metabolized in the human body and has low bioavailability (roughly 1% when taken orally), being some of the main limitations to its therapeutic use [43]. This is particularly challenging when addressing organs that are far from the digestive tract [44], but clinical research has shown that resveratrol is effective on its own or in conjunction with other medications [45], and efforts are being made deal with this restraint. Micronised resveratrol (SRT501) has been reported to have 3.6-fold higher levels in plasma (1942 ng/mL) from a single dose administration when compared to standard resveratrol [46], and its concentration in hepatic tissues was equivalent to pharmacologically effective doses shown in preclinical tests [47]. Resveratrol has been taken in the quotidian diet for a long time, being therefore well-tested from a safety standpoint. It was found to be safe in doses of up to 5 g per day [48]. Certain side effects were observed in a relatively small number of patients, consisting mainly of mild and sporadic conditions like headache, dizziness, and epididymitis, by recurrent administration in short periods of time or high doses (2.5 and 5 g/day) [49].

Even though the pleiotropic activities of resveratrol have been explored for a long time [11, 50], the vast part of the discussion on these effects relied primarily on preclinical tests. Currently, there are more than 250 clinical trials, either complete or ongoing, focusing on

the resveratrol effects on human health, as the sole therapeutic agent or combined with other pharmacological compounds, with more than 95% being developed in the last decade. Some of the outcomes of clinical trials completed to date are discussed below.

Aging

Resveratrol, as an anti-aging agent, is one of its most prominent properties since the first studies on its ability to mimic calorie restriction effects and expand the lifespan of several (micro)organisms by activating the sirtuin pathways, were reported [41, 51]. While it indirectly activates human SIRT1, direct evidence for increased human lifespan remains inconclusive. Commercially, resveratrol finds application in facial skin products as it displays a superior antioxidant capacity compared to idebenone [52]. Additionally, distinct nanocarriers for topical administration and micro-emulsions (e.g., containing a large amount of natural deep eutectic solvents) [53] can also: improve resveratrol solubility, provide photoprotection, enhance skin penetration, and inhibit *trans*-to-*cis* isomerization (de Vries et al. 2021). Resveratrol topical use includes wrinkle reduction and enhanced skin vitality [55], anti-scarring [56], acne reduction [57], and wound healing properties [58].

Cancer

Micronised resveratrol SRT501 enhanced cleaved caspase-3 expression in malignant hepatic tissue of colorectal cancer patients, potentially benefiting disease outcomes [46]. In colon cancer patients, resveratrol downregulated CD133 and LGR5, associated with colon cancer cell growth inhibition [59]. However, resveratrol administration in multiple myeloma patients had adverse effects [60]. Resveratrol was found to diminish IGF-1 and IGFBP-3 levels in healthy volunteers [48], which are associated with different types of cancer, showing potential as a cancer chemopreventive agent, though more clinical trials are needed to corroborate preclinical findings.

Diabetes

Resveratrol demonstrates efficacy against metabolic disorders, such as diabetes mellitus and obesity. Noteworthy outcomes include reductions in fasting blood sugar, hemoglobin A1c (HbA1c), insulin levels, and insulin resistance in type-2 diabetes patients [61]. Notably, the significant reduction in HbA1c levels observed in this study was comparable with the reduction in type-2 diabetes patients treated with metformin, a first-line commercial treatment for diabetes

[62]. Other trials with patients with type-2 diabetes revealed a reduction in oxidative stress and blood sugar levels and enhanced insulin solubility [63]. Effects on quicker healing and size reduction of foot ulcers [64] and enhanced vasodilator function were also reported [65]. Regarding other metabolic diseases, such as obesity, resveratrol administration led to significant loss of weight in obese patients after 6 months of treatment [66]. The use of resveratrol alone or as an adjuvant has been established in various clinical studies, with very encouraging results in the field of diabetes treatment and other metabolic diseases. Still, there is a lot to learn about the best resveratrol dosages for auxiliary treatment.

Cardiovascular diseases

The French Paradox sparked interest in studying resveratrol's impact on cardiovascular function, showing promise as a treatment for cardiovascular disease (CVD), a leading global cause of mortality [67]. Limited clinical trials support these claims, often combining resveratrol with other agents, obscuring its individual effects. Some trials noted reduced cholesterol, apolipoprotein-B, and improved lipid profiles in primary CVD [68], as well as improved diastolic function and reduced LDL cholesterol post-myocardial infarction [69] and enhanced diastolic function of the left ventricle and endothelial function was observed in post-myocardial infarction patients, as well as a reduction in low-density lipoprotein (LDL cholesterol) [70]. Effects of factors like ethnicity, age, and gender on resveratrol's CVD efficacy warrant comprehensive exploration in future trials [42].

Neurological diseases

Resveratrol plays a vital role in neuroprotection by mitigating oxidative damage, mitochondrial dysfunction, and chronic inflammation in the brain and nervous system [71]. Studies indicate that resveratrol enhances memory by fortifying hippocampal functional connectivity and improving glucose metabolism in healthy older adults [72]. Although resveratrol shows therapeutic potential for Alzheimer's disease, a prevalent neurodegenerative disorder linked to oxidative stress and mitochondrial damage [73], clinical trials have yet to report significant treatment effects [74].

Resveratrol biosynthesis

The resveratrol market size reached \$118.6 million in 2022, with a projected 8% compound annual growth rate (CAGR) from 2023 to 2033 [75]. Europe currently

dominates the worldwide resveratrol market, and while plant extraction is still the main source of commercial resveratrol, chemical synthesis (DSM, Netherlands) and microbial fermentation (Evolva, Switzerland) are gaining ground in the market [5, 76].

Resveratrol microbial production by precision fermentation can side many of the aforementioned drawbacks associated with the alternative routes to obtain this polyphenol. It has several advantages over traditional processes, such as reduced costs, high product purity, proneness to produce *trans*-resveratrol, straightforward purification processes [77] and it is independent of season changes [5], being sustainable and environmentally friendly.

The yeast *Saccharomyces cerevisiae* as resveratrol producer

The first reports on microbial resveratrol production relied on *p*-coumaric acid as a substrate (Table 2). The first study goes back to 2003 when Becker et al. [78] co-expressed the coenzyme-A ligase gene from a hybrid poplar and the grapevine resveratrol synthase gene in a laboratory *S. cerevisiae* strain. This led to a titer of 1.45 µg/L of resveratrol in the form of piceid, a resveratrol glucoside, by feeding 10 mg/L of *p*-coumaric acid to the yeast culture. Soon after, the integration of a 4-coumarate-CoA ligase from *Nicotiana tabacum*, and stilbene synthase (*STS*) from *V. vinifera*, led to a production of 5.8 mg/L of non-glycosylated resveratrol from 820.8 mg/L of *p*-coumaric acid [77]. All these studies used laboratory *S. cerevisiae* strains. In its place, Sydor et al. [79] expressed *4CL1* from *A. thaliana* and *STS* from *V. vinifera* into an industrial yeast, isolated from a sugar cane plantation in Brazil. This resulted in the production of 391 mg/L of resveratrol from 2.46 g/L *p*-coumaric acid in YPD medium.

Although the claim for the first report on *de novo* resveratrol production came only a few years later, the first steps toward it came with the attempt to use the amino acid precursors of the phenylpropanoids pathway (Table 2). After a failed first attempt at resveratrol production from tyrosine, due to complications with the expression of tyrosine ammonia lyase (*TAL*) from *Rhodobacter sphaeroides* in *S. cerevisiae* [80], resveratrol production from this amino acid was accomplished by using a codon-optimised *TAL* gene. Together with the expression of a fused 4-coumaroyl-CoA ligase::stilbene synthase with a Gly-Ser-Gly linker, previously reported to enhance resveratrol yield [80], and an arabinose-proton symporter from *E. coli*, the strain achieved a titer of 1.90 mg/L from 12 mg/L tyrosine on induction media with SD dropout and 2% (w/v) of

Table 2. Studies reporting microbial production of resveratrol using *S. cerevisiae* strains.

Reference	Relevant genotype	Engineering strategy	Substrate/ precursor fed	Titer (mg/L)
[78]	4CL (<i>Populus trichocarpa</i> × <i>Populus deltoides</i>) STS (<i>V. vinifera</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	1.45 · 10 ⁻³
[77]	4CL (<i>N. tabacum</i>) STS (<i>V. vinifera</i>)	Genomic integration (single copy)	<i>p</i> -Coumaric acid	6
[80]	4CL::STS (<i>A. thaliana</i> , <i>V. vinifera</i>) Fusion enzyme	Episomal plasmid	<i>p</i> -Coumaric acid	5.25
[84]	PAL, CPR (<i>P. trichocarpa</i> × <i>P. deltoides</i>) C4H, 4CL (<i>Glycine max</i>) RS (<i>V. vinifera</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	0.31
[79]	4CL (<i>A. thaliana</i>) STS (<i>V. vinifera</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	391
[81]	TAL (<i>R. sphaeroides</i>) 4CL::STS (<i>A. thaliana</i> , <i>V. vinifera</i>) <i>araE</i> (<i>E. coli</i>)	Genomic integration (single copy)	<i>p</i> -Coumaric acid	3.1
[90]	4CL1 (<i>A. thaliana</i>) STS (<i>A. hypogaea</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	3.1
[83]	PAL (<i>R. toruloides</i>) 4CL (<i>A. thaliana</i>) STS (<i>A. hypogaea</i>) ACC1 (<i>S. cerevisiae</i>)	Episomal plasmid	Galactose	3.4
[91]	4CL1 (<i>A. thaliana</i>) STS (<i>V. vinifera</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	5.8
[136]	4CL (<i>A. thaliana</i>) STS (<i>V. vinifera</i>)	Episomal plasmid	<i>p</i> -Coumaric acid	14.4
[21]	TAL (<i>H. aurantiacus</i>) 4CL (<i>A. thaliana</i>) VST (<i>V. vinifera</i>) ARO4 ^{K229L} , ARO7 ^{G141S} , ACC1 ^{S659A} , S1157A (<i>S. cerevisiae</i>)	Cre-LoxP recombination (single/multi copy)	Glucose and ethanol	8.249
[87]	PAL, C4H, 4CL, ATR2 (<i>A. thaliana</i>) VST (<i>V. vinifera</i>) ACS (<i>S. enterica</i>) ARO4 ^{trb} , ARO7 ^{trb} , CYB5, ACC1 ^{S659A} , S1157A (<i>S. cerevisiae</i>) ΔARO10	Cre-LoxP recombination, CRISPR/ Cas9-mediated gene integration (single/ multi copy)	Glucose	531.41
[89]	PAL2, 4CL1, ATR2, C4H (<i>A. thaliana</i>) TAL (<i>F. johnsoniae</i>) STS (<i>V. vinifera</i>) CYB5, ACC1 ^{S659A} , S1157A (<i>S. cerevisiae</i>) ^a	CRISPR/Cas9-mediated gene integration (single copy)	Glucose	812
[137]	4CL1 (<i>P. appendiculatum</i>) STS (<i>P. cuspidatum</i>) + 5 other STS	Episomal plasmid	<i>p</i> -Coumaric acid	263.4
[92]	STS (<i>V. vinifera</i>) TAL (<i>T. cutaneum</i>) - in <i>E. coli</i> 4CL (<i>A. thaliana</i>) - in <i>S. cerevisiae</i> ^a Co-culture	Bacterial expression vectors	Glucose	39
[94]	PAL2, 4CL2, C4H (<i>A. thaliana</i>) VST1 (<i>V. vinifera</i>)	Genomic integration (single copy)	<i>p</i> -Coumaric acid (secreted from <i>E. coli</i>)	36
[95]	PAL2, 4CL2, C4H (<i>A. thaliana</i>) VST1 (<i>V. vinifera</i>) ^a	CRISPR/Cas9-mediated gene integration (single copy)	Glucose, Ethanol	187
[96]	PAL2, 4CL2, C4H, ATR2 (<i>A. thaliana</i>) VST1 (<i>V. vinifera</i>), CYB5 (<i>S. cerevisiae</i>) ^a	CRISPR/Cas9-mediated gene integration (single copy)	Lactose	210
[88]	PAL/TAL (<i>R. toruloides</i>) STS (<i>V. vinifera</i>) ARO4 ^{K229L} , ARO7 ^{G141S} (<i>S. cerevisiae</i>) ^a	CRISPR/Cas9-mediated gene integration (single/multi-copy)	Xylose, Glucose	388
			Glucose	4100

^aMultiple modifications, refer to the original research article.

galactose [81]. Interestingly, without the addition of tyrosine, 1.06 mg/L resveratrol was accumulated in the fermentation media. An alternative to TAL, phenylalanine ammonia lyase (PAL) (Figure 3) from *Rhodospiridium*

toruloides was found to have catalytic activity on both tyrosine and phenylalanine [82], and its introduction in *S. cerevisiae* alongside *AtC4H* (encoding for cinnamic acid 4-hydroxylase) and *AhSTS* led to a resveratrol

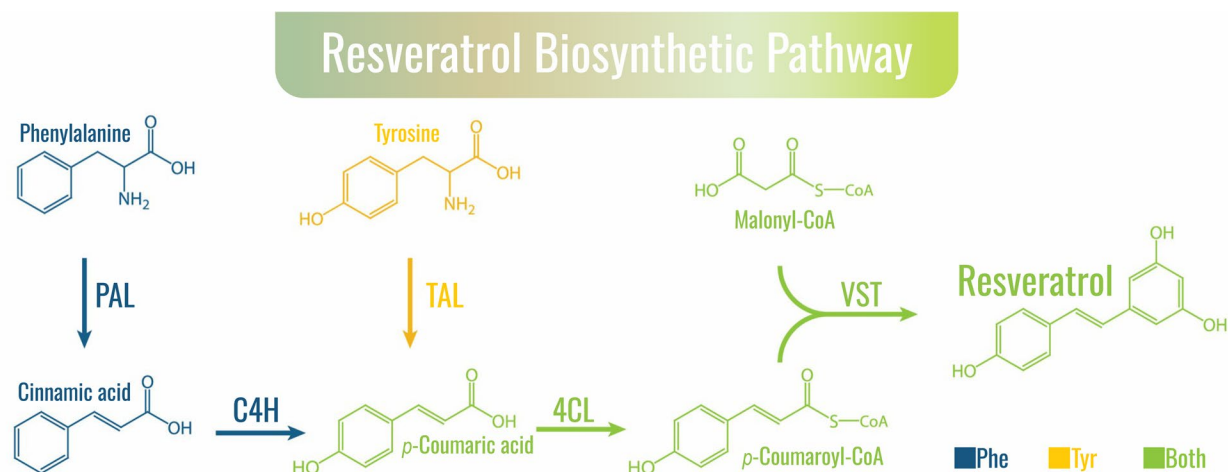


Figure 3. Resveratrol biosynthetic pathway via phenylalanine or tyrosine. PAL, Phenylalanine ammonia lyase; TAL, Tyrosine ammonia lyase; C4H, Cinnamate-4-hydroxylase; 4CL, 4-coumarate lyase; VST, Resveratrol synthase.

production of 3.4 mg/L from 2% galactose, accumulating also 2.6 mg/L of *p*-coumaric acid [83]. After switching the *ACC1* promoter for a stronger galactose-inducible *GAL1* promoter (leading to a 2-fold increase in *ACC1* transcription levels) in an attempt to increase the pool of malonyl-CoA, and supplementing the media with 12 mM of tyrosine, a titer of 5.8 mg/L of resveratrol was obtained [83]. The introduction of *PAL* was also attempted to produce resveratrol using phenylalanine as a precursor. By the expression of *PAL* from a *Populus* hybrid, *C4H* and *4CL* from *Glycine max* and *VvSTS*, an accumulation of 0.29 mg/L of resveratrol was observed when the medium was supplemented with 10 mM phenylalanine, and a nearly identical titer of 0.31 mg/L when supplemented with 1 mM *p*-coumaric acid [84]. Vos et al. [85] provided valuable insight into the influence of specific growth rates on the physiology of resveratrol-producing previously engineered yeast strains [86]. Resveratrol production processes require high amounts of energy translated into an estimation of 13 moles of ATP per mole of resveratrol. Given this and the participation of critical precursors that are growth rate-dependent, a stoichiometric study indicated that the *de novo* generation of resveratrol was closely associated with yeast cell growth. The introduction of the resveratrol pathway into *S. cerevisiae* led to clear transcriptional changes in precursor biosynthesis genes, namely *TKL1*, *ARO7*, and *ARO9*, altogether underlining the need to decouple growth and synthesis of resveratrol for industrial use [85].

Up to this point, academic studies using *S. cerevisiae* strains were mostly based on proof-of-concept approaches and still exhibited low resveratrol titers (below 10 mg/L). Li et al. [21] reported, for the first time, efficient *de novo* production of resveratrol from

glucose or ethanol, in a minimal medium, *via* tyrosine. The authors introduced multiple copies of the genes *TAL* from *Herpetosiphon aurantiacus*, encoding for tyrosine ammonia lyase, *At4CL1* from *A. thaliana* and *VST1* from *V. vinifera*, into a strain overexpressing the feedback-inhibition resistant versions of DAHP synthase (*ARO4^{K229L}*) and chorismate mutase (*ARO4^{K229L}*), and the inactivation-resistant version of acetyl-CoA carboxylase (*ACC1^{S659A, S1157A}*). In fed-batch fermentation, the final recombinant haploid laboratory strain (a CEN.PK102-5B derivative), achieved resveratrol titers of 415.65 and 531.41 mg/L from glucose and ethanol feeds, respectively, after an initial batch phase on 40 g/L of glucose in both fermentations. Later on, the same authors reported resveratrol production from glucose *via* the phenylalanine pathway by applying a pull-push-block engineering strategy. This comprised multicopy overexpression of the resveratrol biosynthetic pathway (*AtPAL2*, *AtC4H*, *At4CL2*, and *VvVST1*), optimization of the electron transfer to the cytochrome P450 monooxygenase, increase of the precursor supply, and the block of competing pathways to avoid degradation of pathway intermediates [87]. The resultant laboratory recombinant strain produced 812 mg/L of resveratrol in fed-batch fermentation, with a feed of 88 g/L of glucose and a batch phase of 40 g/L of glucose [87].

In another study, the combination of both phenylalanine and tyrosine pathways was evaluated by expressing a bi-functional phenylalanine/tyrosine ammonia lyase from *R. toruloides*. This strategy allied with the integration of multi-copy biosynthetic genes to improve the metabolic flux to malonyl-CoA and aromatic amino acids ultimately led to a resveratrol titer of 4.1 g/L in a glucose fed-batch fermentation in minimal media, which is the highest titer reported to date using *S.*

cerevisiae [88]. Nevertheless, the reported yields so far, a parameter often overlooked and rarely reported in the vast majority of studies, are still far from the theoretical maximum of 0.28 mol/mol glucose (354.7 mg/g) [85].

The importance of rewiring the carbon flux toward aromatic amino acid (AAA) biosynthesis for high-level production of its derivatives was also shown by extensive engineering of a laboratory *S. cerevisiae* where the authors introduced a phosphoketolase-based pathway to deflect glycolytic flux toward erythrose 4-phosphate formation [89]. Using the resveratrol biosynthetic pathway *via* phenylalanine, alongside several other genetic modifications to optimize carbon distribution amid glycolysis and the AAA biosynthesis, the authors reported a resveratrol production of 263.4 mg/L, while still accumulating nearly 2 g/L of *p*-coumaric acid [89]. These studies coincide with the upsurge of revolutionary genome-editing technologies like CRISPR/Cas9. Until then, the vast majority of studies focusing on resveratrol production in yeast used expression vectors relying on the presence of auxotrophic markers and galactose induction [80, 81, 84, 90, 91]. Modular engineering and co-culture fermentation to divide the metabolic labor between two microorganisms were also assessed for resveratrol bioprocesses. Constitutive overexpression of *TcTAL*, a feedback-inhibition-resistant DAHP synthase (*aroGfbr*) and chorismate mutase/prephenate dehydrogenase (*tyrAfbr*) in a strain lacking *tyrR*, a transcriptional regulator that represses tyrosine synthesis, the upstream *E. coli* module can produce *p*-coumaric acid into the culture medium. The excreted *p*-coumaric acid was subsequently utilized by an *S. cerevisiae* strain, expressing *At4CL*, *VvSTS* and *ACC^{15659A,51157A}*, producing 36 mg/L of resveratrol after optimization of the inoculation ratio between the two populations [92].

Noteworthy, only a few studies have reported the use of industrial yeast strains for resveratrol production [79, 93–96]. Due to their increased robustness, the use of industrial strains paves the way for the valorization of several agro-industrial wastes for resveratrol production.

Renewable resources as substrate

A biorefinery consists, at its core, of the processing of biomass into commercially viable products and/or energy. This explains the utilization of different technologies to convert renewable resources into building blocks to produce biofuels or other value-added products [97]. It is imperative to demonstrate the viability of exploiting low-cost carbon sources, such as lignocellulosic materials, to manufacture high-value chemicals other than bioethanol in the search for a bio-based

economy [97]. The most plentiful and easily accessible renewable resource is lignocellulosic biomass, which contains 40–50% of cellulose, the most prevalent polymer on Earth. Numerous D-glucose molecules are joined in a linear chain by $\beta(1,4)$ -glycosidic linkages to form the structure of cellulose. To improve the enzymatic saccharification of cellulose into glucose, a pretreatment is necessary to disassemble the resistant lignocellulosic structure. Depending on the pretreatment and conditions, as well as on the lignocellulosic biomass type, different compounds may be generated during pretreatment that inhibit the subsequent steps of saccharification and fermentation. These compounds comprise furans (mainly furfural and HMF), weak carboxylic acids, and phenolic compounds and are commonly designated as inhibitors [98]. Furthermore, due to its phenolic nature, resveratrol can act synergistically with the above-mentioned inhibitory compounds [99], which can represent an additional burden to fermentation, therefore highlighting the necessity of selecting a suitable chassis for a biomass-to-resveratrol process. Yeast tolerance to these inhibitors is strain dependent with industrial isolates presenting increased robustness [100] and often beneficial intrinsic characteristics for the target metabolic engineering purpose [101–103].

In comparison to separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) processes have several advantages for process development, such as avoiding end-product inhibition or requiring fewer vessels. SSF processes are widely used to produce ethanol [104]. However, since a temperature of 45–50°C is ideal for cellulase activity and 30°C for yeast growth, the main downside of the SSF process is the requirement for a thermotolerant yeast to carry out both processes (saccharification and fermentation) simultaneously. The incorporation of resveratrol production into biorefinery schemes, traditionally focused exclusively on bioethanol production, has the potential to augment its market penetration along value chains [105].

Taking into account the constraints for lignocellulose valorization processes, Costa et al. [94] expressed a resveratrol biosynthetic pathway *via* phenylalanine in three different industrial strains, known for their tolerance to fermentation inhibitors and/or high temperatures [101, 102, 106]. The best thermotolerant resveratrol-producing strain was able to produce 2.12 g of resveratrol per kg of eucalyptus wood at 39°C using the cellulosic fraction of a pretreated *Eucalyptus globulus* raw material (5% solids). Subsequently, the authors expressed a heterologous β -galactosidase and lactose permease, both from *Kluyveromyces lactis*, in the previously engineered resveratrol-producing strain in order

to extend the range of sources of renewable substrates. The strain was able to produce 210 mg/L of resveratrol in a synthetic media with lactose as the sole carbon source [95]. Furthermore, this study reported the use of cheese whey, a highly pollutant by-product of the dairy industry [107] as a source of lactose, reaching 111 mg/L of resveratrol. A techno-economic analysis showed the potential of whey-to-resveratrol bioprocesses by obtaining a minimum resveratrol sale price, to ensure the economic viability of the process, below the market average [108]. Costa et al. [96] have also shown the positive impact of the co-utilization of glucose and xylose, both present in lignocellulosic biomasses. By expressing this resveratrol biosynthetic pathway in a xylose-consuming strain, the authors reported a 1.31-fold increase in resveratrol production when fermenting glucose and xylose simultaneously in comparison with glucose alone, achieving a titer of 388 mg/L. With this strain, the authors used several winery by-products to produce resveratrol from a wide range of substrates, namely wine lees (ethanol as C-source), the hemicellulosic hydrolysate of a hydrothermally pretreated vine pruning residue (glucose and xylose as C-source), and grape must (glucose and fructose as C-source), with reported yields of 0.52, 1.40 and 2.25 grams of resveratrol per kg of raw material [96]. Economic and environmental assessments of this resveratrol production process using Life Cycle Assessment (LCA) methodology showed that the use of wine wastes is both sustainable and economically attractive with grape must residues being the most promising alternative [109].

Non-conventional yeasts as resveratrol producers

The yeast *Scheffersomyces stipitis* due to its ability to naturally metabolize several carbon sources has been also used as a host for *de novo* resveratrol from different sugars like the monosaccharides: glucose (237.6 mg/L), fructose (204.6 mg/L), galactose (170.6 mg/L), xylose (248.6 mg/L) or maltose (206.9 mg/L) and the disaccharides cellobiose (529.8 mg/L) and sucrose (668.6 mg/L), with 50 g/L of initial sugar concentration in all of them [110]. The authors introduced essential genes for resveratrol biosynthesis, namely *TAL* from *Herpetosiphon aurantiacus*, *At4CL2*, and *VvVST1*, alongside a feedback-insensitive allele of chorismate mutase (*SsARO7^{G139S}*). Using this same resveratrol biosynthetic pathway, Kumokita et al. [111] reported a production of 452 mg/L of resveratrol from 20 g/L of glycerol using *Pichia pastoris* as host.

Regarding the use of non-conventional yeasts, *Yarrowia lipolytica* has been gaining attention for resveratrol production processes. Sáez-Sáez et al. [5]

reported the production of 12.4 g/L of resveratrol in fed-batch fermentation from glucose (Table 3). The authors expressed 5 copies of the resveratrol synthetic pathway comprising *TAL* from *Flavobacterium johnsoniae*, alongside *YIARO4^{K221L}*/*YIARO7^{G139S}*, mutated feedback-insensitive versions of DAHP synthase and chorismate mutase, respectively. The highest resveratrol titer reported in the literature was also attained using a *Y. lipolytica* strain, expressing multiple copies of a resveratrol biosynthetic pathway comprising *FjTAL*, *Pc4CL1* (from *Petroselinum crispum*), and *VvSTS*, together with engineering of the shikimic acid pathway and diverting glycolytic flux toward erythrose-4-phosphate. The final strain was able to produce 22.5 g/L of resveratrol from glucose using a fed-batch strategy [112]. This is the highest yield reported so far (65.5 mg of resveratrol per gram of glucose), corresponding to 18.5% of the maximum theoretical yield. While there is still a

Table 3. Successful cases of microbial production of resveratrol using different non-conventional yeasts as hosts.

Microbial host	Relevant genotype	Substrate/precursor	Titer (mg/L)	Ref.
<i>P. pastoris</i>	<i>TAL</i> (<i>H. aurantiacus</i>) <i>4CL</i> (<i>A. thaliana</i>) <i>VST</i> (<i>V. vinifera</i>) <i>ARO4^{K229L}</i> (<i>S. cerevisiae</i>) <i>ARO7^{G141S}</i> (<i>S. cerevisiae</i>)	Glycerol	452	[111]
<i>O. polymorpha</i>	<i>TAL</i> (<i>H. aurantiacus</i>) <i>4CL</i> (<i>A. thaliana</i>) <i>STS</i> (<i>V. vinifera</i>)	Tyrosine	97.23	[124]
<i>S. stipitis</i>	<i>TAL</i> (<i>H. aurantiacus</i>) <i>4CL2</i> (<i>A. thaliana</i>) <i>VST</i> (<i>V. vinifera</i>) <i>ARO7^{G139S}</i> (<i>S. stipitis</i>)	Glucose	237.6	[110]
		Fructose	204.6	
		Galactose	170.6	
		Xylose	248.6	
		Maltose	206.9	
		Cellobiose	529.8	
<i>S. venezuelae</i>	<i>STS</i> (<i>A. hypogaea</i>) <i>4CL</i> (<i>S. coelicolor</i>) Δpks	Sucrose	668.6	[122]
		<i>p</i> -Coumaric acid	0.4	
<i>Y. lipolytica</i>	<i>TAL</i> (<i>R. toruloides</i>) <i>4CL</i> (<i>P. crispum</i>) <i>VST</i> (<i>V. vinifera</i>) <i>ARO4^{br}</i> (<i>S. cerevisiae</i>) ^a	Glucose	12.67	[138]
<i>Y. lipolytica</i>	<i>4CL</i> (<i>N. tabacum</i>) <i>STS</i> (<i>A. hypogaea</i>) <i>PEX10</i> , <i>ACC1</i> (<i>Y. lipolytica</i>)	<i>p</i> -Coumaric acid	48.7	[139]
<i>Y. lipolytica</i>	<i>TAL</i> (<i>F. johnsoniae</i>) <i>PAL</i> (<i>V. vinifera</i>) <i>C4H</i> , <i>4CL1</i> (<i>A. thaliana</i>) <i>VST</i> (<i>V. vinifera</i>)	Glycerol	430	[140]
<i>Y. lipolytica</i>	<i>TAL</i> (<i>F. johnsoniae</i>) <i>4CL</i> (<i>A. thaliana</i>) <i>VST</i> (<i>V. vinifera</i>) <i>ARO4^{br}</i> , <i>ARO7^{br}</i> (<i>Y. lipolytica</i>) ^a	Glucose	12,355	[5]
<i>Y. lipolytica</i>	<i>TAL</i> (<i>F. johnsoniae</i>) <i>4CL</i> (<i>P. crispum</i>) <i>VST</i> (<i>V. vinifera</i>) ^a	Glucose	22,500	[112]

^aMultiple modifications, refer to the original research article.

large room for improvement, the first reports on *de novo* resveratrol production were below 1 g/L with yields up to 2.5% [87]. This means that in less than a decade a dramatic rise in resveratrol yields and titers was observed, which accentuates the feasibility and profitability of resveratrol's biosynthesis, supported by recent LCA studies [108, 109].

Bacteria as resveratrol producers

Among bacteria, *E. coli* is one of the most employed hosts for the introduction of resveratrol biosynthetic pathways (Table 4). In parallel with the aforesaid integration of *Nt4CL2* and *VvSTS* in *S. cerevisiae*, Beekwilder et al. [77] introduced the same genes in *E. coli* and achieved a production of 16 mg/L of resveratrol in the same rich medium (with 820.8 mg/L *p*-coumaric acid). In another study, by expression *At4CL1* and *AhSTS* in an *E. coli* strain, 104.5 mg/L of resveratrol was obtained on a mineral medium supplemented with 164 mg/L *p*-coumaric acid [113]. Lim et al. [114] reported the highest titer in literature for *E. coli* fermentation, achieving 2.3 g/L of resveratrol from 2.46 g/L *p*-coumaric acid supplemented with 0.05 mM cerulenin, to reduce carbon loss from the malonyl-CoA to fatty acid production pathway. Other studies reported resveratrol production in *E. coli* from tyrosine by using either PAL [115] or TAL [116], achieving similar titers of 37 and 35 mg/L of resveratrol from 540 and 544 mg/L tyrosine, respectively, while coupling them with different additional modifications. Resveratrol production in *E. coli* from glucose was first reported through site-specific integration of *RgTAL*, *Pc4CL*, and *VvSTS* into the loci of the genes *tyrR*, a transcriptional regulatory gene, and *trpED*, encoding anthranilate synthetase. The inactivation of these genes enhances the expression levels of several genes in the shikimate pathway and abolishes the competing production of L-tryptophan, respectively. The authors reported a resveratrol concentration of 4.612 mg/L using the final recombinant strain [117]. Wu et al. [118] extensively engineered an *E. coli* strain by constructing a *de novo* resveratrol synthetic pathway from tyrosine (*TAL* from *Trichosporon cutaneum*, *Pc4CL* and *VvSTS*) and engineering the availability of malonyl-CoA using CRISPR interference (CRISPRi) and rational engineering of tyrosine ammonia lyase through the reduction of mRNA secondary structure in its 5' region. Altogether, the final strain was able to produce 304.5 mg/L of resveratrol from 5 g/L glucose, though the strain was unable to consume all the glucose in the medium. Modular engineering strategies have also been reported using *E. coli*-*E. coli* consortia. Resveratrol production from glycerol [119] and glucose

[120] was reported, with titers of 22.6 and 55.7 mg/L of resveratrol, respectively. Both studies employed similar strategies relying on the division of the biosynthetic pathway in two strains, where the first produced *p*-coumaric acid and the second converted the excreted *p*-coumaric acid into resveratrol. More recently, another *E. coli*-*E. coli* co-culture was used to produce resveratrol from glucose and arabinose, using a combination of CRISPR/Cas9 and CRISPRi technologies, where the first module was engineered to have deficient arabinose utilization, producing *p*-coumaric acid from glucose, and the second consumed arabinose, glucose and the excreted *p*-coumaric acid, leading to a resveratrol titer of 204.8 mg/L [121].

Other hosts have been used for the biosynthesis of resveratrol either from precursors such as *p*-coumaric acid/tyrosine or from carbon sources like glucose or glycerol, namely: *Streptomyces venezuelae* [122], *Lactococcus lactis* [123], *Ogataea polymorpha* [124], *Corynebacterium glutamicum* [125, 126] and *Rhodotorula toruloides* [127].

Major advances and short-term challenges

Over the last two decades, research has significantly advanced the shift from fossil fuels to biotechnological methods for producing compounds like resveratrol. While the first years were mostly spent evaluating the conversion of *p*-coumaric acid into resveratrol and understanding the fitness of microorganisms to accumulate this type of molecules, the heterologous expression of the resveratrol biosynthetic pathway enabling its *de novo* production from carbon sources *via* tyrosine or phenylalanine was the major breakthrough of this period. The evolution of bioinformatic tools and genomic engineering, particularly CRISPR/Cas9, has expedited the identification and evaluation of new production targets at a fast pace, thereby substantially increasing resveratrol yields and titers in the recent decade.

Nevertheless, there remain a lot of short-term challenges in the quest for improved yields and titers. Future research should focus on selective (rather than extensive) engineering of the resveratrol biosynthetic pathway and its precursors, and on optimizing the fermentation process for better industrial-scale production. These should be tackled together for a more effective bioprocess, which requires a suitable microbe, and vice-versa.

The shikimate pathway is critical for resveratrol production, beginning with the condensation of erythrose-4-phosphate and phosphoenolpyruvate to form phenylpyruvate. Future studies should investigate strategies to boost levels of these precursors and

Table 4. Successful cases of microbial production of resveratrol using different bacteria hosts.

Microbial host	Relevant genotype	Substrate/precursor	Titer (mg/L)	Ref.
<i>C. glutamicum</i>	STS (<i>A. hypogaea</i>) 4CL (<i>P. crispum</i>) ^a	<i>p</i> -Coumaric acid	158	[125]
<i>C. glutamicum</i>	TAL (<i>F. johnsoniae</i>) 4CL (<i>P. crispum</i>) STS (<i>A. hypogaea</i>) aroH (<i>E. coli</i>) ^a	Glucose	12	[126]
<i>E. coli</i>	4CL (<i>N. tabacum</i>) STS (<i>V. vinifera</i>)	<i>p</i> -Coumaric acid	16	[77]
<i>E. coli</i>	4CL (<i>A. thaliana</i>) STS (<i>A. hypogaea</i>)	<i>p</i> -Coumaric acid	105	[113]
<i>E. coli</i>	4CL (<i>L. erythrorhizon</i>) STS (<i>A. hypogaea</i>)	<i>p</i> -Coumaric acid	171	[115]
<i>E. coli</i>	ACC (<i>C. glutamicum</i>) PAL (<i>R. rubra</i>) 4CL (<i>L. erythrorhizon</i>) STS (<i>A. hypogaea</i>) ACC (<i>C. glutamicum</i>)	Tyrosine	37	[141]
<i>E. coli</i>	TAL (<i>S. espanaensis</i>) 4CL (<i>S. coelicolor</i>) STS (<i>A. hypogaea</i>)	<i>p</i> -Coumaric acid	1.4	[142]
<i>E. coli</i>	4CL (<i>A. thaliana</i>) STS (<i>V. vinifera</i>)	<i>p</i> -Coumaric acid	2300	[114]
<i>E. coli</i>	TAL (<i>R. glutinis</i>) 4CL (<i>P. crispum</i>) STS (<i>V. vinifera</i>) matB, matC (<i>R. trifolii</i>)	Tyrosine	35.02	[116]
<i>E. coli</i>	4CL (<i>A. thaliana</i>) STS (<i>V. vinifera</i>) ^a	<i>p</i> -Coumaric acid	1600	[143]
<i>E. coli</i>	TAL (<i>S. espanaensis</i>) 4CL (<i>S. coelicolor</i>) STS (<i>A. hypogaea</i>)	Glucose	5.2	[144]
<i>E. coli</i>	4CL (<i>P. crispum</i>) STS (<i>V. vinifera</i>) fabD downregulation	<i>p</i> -Coumaric acid	268.2	[145]
<i>E. coli</i>	4CL::STS (<i>A. thaliana</i> , <i>A. hypogaea</i>)	<i>p</i> -Coumaric acid	80.5	[146]
<i>E. coli</i>	TAL (<i>S. espanaensis</i>) 4CL (<i>A. thaliana</i>) STS (<i>A. hypogaea</i>)	Tyrosine	114.4	[147]
<i>E. coli</i>	4CL (<i>A. thaliana</i>) STS (<i>A. hypogaea</i>)	<i>p</i> -Coumaric acid	160	[148]
<i>E. coli</i>	TAL (<i>R. glutinis</i>) 4CL (<i>P. crispum</i>) STS (<i>V. vinifera</i>)	Glucose	4.612	[117]
<i>E. coli</i>	Δ tyrR and Δ trpED TAL (<i>T. cutaneum</i>) 4CL (<i>P. crispum</i>) STS (<i>V. vinifera</i>) matB, matC (<i>R. trifolii</i>) ^a	Glucose	304.5	[118]
<i>E. coli</i>	TAL (<i>Saccharothrix espanaensis</i>) 4CL (<i>Ocimum sanctum</i>) STS (<i>V. vinifera</i>) ^a	Glycerol	80.4	[149]
<i>E. coli</i> - <i>E. coli</i> (co-culture)	TAL (<i>R. glutinis</i>) tktA ^{ibr} , aroG ^{ibr} (<i>E. coli</i>) Δ pheA	Glycerol	22.6	[119]
<i>E. coli</i> - <i>E. coli</i> (co-culture)	4CL (<i>Streptomyces coelicolor</i>) STS (<i>V. vinifera</i>) TAL (<i>R. glutinis</i>) tktA ^{ibr} , aroG ^{ibr} (<i>E. coli</i>) Δ pheA, Δ pgi	<i>p</i> -Coumaric (excreted)		
<i>E. coli</i> - <i>E. coli</i> (co-culture)	4CL (<i>Petroselinum crispum</i>) STS (<i>A. hypogaea</i>) ACC (<i>C. glutamicum</i>) Δ zwf	Glucose	55.7	[120]
<i>E. coli</i> - <i>E. coli</i> (co-culture)	TAL (<i>Phanerochaete chrysosporium</i>) ^a 4CL (<i>A. thaliana</i>) STS (<i>V. vinifera</i>) ^a	<i>p</i> -Coumaric (excreted)		
<i>L. lactis</i>	TAL, 4CL, STS, ACC (multiple sources)	Glucose	204.80	[121]
<i>R. toruloides</i>	C4H, 4CL, ATR2 (<i>A. thaliana</i>) VISTS (<i>Vitis labrusca</i>) At4CL::VISTS CYB5 (<i>R. toruloides</i>)	Glucose	1.27	[123]
		Glucose	125.2	[127]

^aMultiple modifications, refer to the original research article.

optimize other shikimate pathway reactions to enhance resveratrol titers. Tuning pyruvate kinase activity, by using alternative variants [128], and suppressing competing pathways can also have a positive impact on aromatic amino acid formation or resveratrol production. Bringing enzymes closer in multi-step pathways, *via* fusion, scaffolding, or compartmentalization [129], can increase resveratrol titers, as demonstrated previously with 4CL ligase and STS fusion [80], and the same rationale could be applied to other enzymes of the resveratrol biosynthetic pathway. Further, increase in the number of copies of the resveratrol biosynthetic pathway expressed in a given microbe can be valuable, as it was shown to be relevant in several studies [5, 21, 87].

Even though there is a lot to accomplish regarding the fine-tuning of the resveratrol biosynthetic pathway, developing efficient fermentation processes by thoroughly addressing the operation mode is mandatory in an industrial context. The implementation of fed-batch strategies has been associated with the highest resveratrol titers reported in the literature [5, 21, 87]. An interesting strategy for increased resveratrol titers should encompass the use of mixed feeding strategies, such as glucose/ethanol, balancing yeast growth, which preferably occurs in glucose cultivation, and resveratrol production from ethanol, which is known to lead to higher resveratrol yields. This has already been reported to have a positive impact on the production of other compounds like geranylgeraniol [130], also favored by ethanol feed. Harnessing on the insolubility of resveratrol in water, exploiting configurations such as aqueous two-phase systems for an extractive fermentation can also be relevant, by coupling the aqueous fermentation medium with an organic phase, immiscible in water, with a high affinity for resveratrol. This was already reported to be beneficial for the biosynthesis of other chemicals like limonene [131] or vitamin A [132], and would potentially solve various problems at once: possible cell toxicity associated with high resveratrol concentrations, product inhibition of fermentation process, and downstream processing, by simultaneous extracting the end product.

Conclusion

Resveratrol is gaining prominence for its nutritional and therapeutic potential. Over the last decades, a significant and, most importantly, constant rise in studies on resveratrol has been observed, with almost 3000 articles being published in 2022 alone. These studies focus not only on its production, but also on other forms of synthesis/extraction and purification, and comprehension of its underlying mechanisms of action

and its effects on humans. This goes hand-in-hand with the increase in clinical trials completed or on-going, which are filling in on the previous lack of information at the beginning of the century. As more knowledge is generated, more steps are taken toward the integration and benefit from resveratrol's known beneficial properties. This review article does not simply compile the studies on resveratrol bioproduction but rather focuses on the potential of this molecule, showing at the same the feasibility of this process itself. The rising demand for high-value chemicals associated with the ever-growing environmental concern correlated with fossil resource consumption is driving the fast expansion and establishment of biobased processes. And while there is still a lot to achieve to maximize yields and titers, the current scenario is already fit for implementation, driving a greener circular bioeconomy through bioprocess engineering.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of [UIDB/04469/2020] unit. Carlos E. Costa acknowledges the FCT through the ESSEntial project [PTDC/BII-BTI/1858/2021]. Aloia Romaní acknowledges the Ministry of Science and Innovation (MICIN) through the grant [RYC2020-030690-I].

ORCID

Carlos E. Costa  <http://orcid.org/0000-0001-6842-8511>

References

- [1] Tang K, Zhan JC, Yang HR, et al. Changes of resveratrol and antioxidant enzymes during UV-induced plant defense response in peanut seedlings. *J Plant Physiol.* 2010;167:95–102. doi: [10.1016/j.jplph.2009.07.011](https://doi.org/10.1016/j.jplph.2009.07.011).
- [2] Takaoka M. Resveratrol, a new phenolic compound, from *Veratrum grandiflorum*. *J Chem Soc Jpn.* 1939;60:1090–1100.
- [3] Pezzuto JM. Resveratrol: twenty years of growth, development and controversy. *Biomol Ther.* 2019;27:1–14. doi: [10.4062/biomolther.2018.176](https://doi.org/10.4062/biomolther.2018.176).
- [4] Nonomura S, Kanagawa H, Makimoto A. Chemical constituents of Polygonaceous plants. I. Studies on the components of Ko-J O-Kon. *Yakugaku Zasshi.* 1963;83:988–990. doi: [10.1248/yakushi1947.83.10_988](https://doi.org/10.1248/yakushi1947.83.10_988).
- [5] Sáez-Sáez J, Wang G, Marella ER, et al. Engineering the oleaginous yeast *Yarrowia lipolytica* for high-level resveratrol production. *Metab Eng.* 2020;62:51–61. doi: [10.1016/j.ymben.2020.08.009](https://doi.org/10.1016/j.ymben.2020.08.009).

- [6] Langcake P, Pryce RJ. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol Plant Pathol*. 1976;9:77–86. doi: [10.1016/0048-4059\(76\)90077-1](https://doi.org/10.1016/0048-4059(76)90077-1).
- [7] Siemann EH, Creasy LL. Concentration of the phytoalexin resveratrol in wine. *Am J Enol Vitic*. 1992;43:49–52. doi: [10.5344/ajev.1992.43.1.49](https://doi.org/10.5344/ajev.1992.43.1.49).
- [8] Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992;339:1523–1526. doi: [10.1016/0140-6736\(92\)91277-f](https://doi.org/10.1016/0140-6736(92)91277-f).
- [9] Klatsky AL. Alcohol and cardiovascular diseases. *Expert Rev Cardiovasc Ther*. 2009;7:499–506. doi: [10.1586/erc.09.22](https://doi.org/10.1586/erc.09.22).
- [10] Catalgol B, Batirel S, Taga Y, et al. Resveratrol: French paradox revisited. *Front Pharmacol*. 2012;3:141. doi: [10.3389/fphar.2012.00141](https://doi.org/10.3389/fphar.2012.00141).
- [11] Jang M, Cai L, Udeani GO, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*. 1997;275:218–220. doi: [10.1126/science.275.5297.218](https://doi.org/10.1126/science.275.5297.218).
- [12] Salehi B, Mishra AP, Nigam M, et al. Resveratrol: a double-edged sword in health benefits. *Biomedicines*. 2018;6:91. doi: [10.3390/biomedicines6030091](https://doi.org/10.3390/biomedicines6030091).
- [13] Bertelli AAA, Das DK. Grapes, wines, resveratrol, and heart health. *J Cardiovasc Pharmacol*. 2009;54:468–476. doi: [10.1097/FJC.0b013e3181bfaff3](https://doi.org/10.1097/FJC.0b013e3181bfaff3).
- [14] Adhikari B, Dhungana SK, Ali MW, et al. Resveratrol, total phenolic and flavonoid contents, and antioxidant potential of seeds and sprouts of Korean peanuts. *Food Sci Biotechnol*. 2018;27:1275–1284. doi: [10.1007/s10068-018-0364-7](https://doi.org/10.1007/s10068-018-0364-7).
- [15] Aggarwal BB, Bhardwaj A, Aggarwal RS, et al. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res*. 2004;24:2783–2840.
- [16] Tian B, Liu J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. *J Sci Food Agric*. 2020;100:1392–1404. doi: [10.1002/jsfa.10152](https://doi.org/10.1002/jsfa.10152).
- [17] Karacabey E, Mazza G. Optimization of solid-liquid extraction of resveratrol and other phenolic compounds from milled grape canes (*Vitis vinifera*). *J Agric Food Chem*. 2008;56:6318–6325. doi: [10.1021/jf800687b](https://doi.org/10.1021/jf800687b).
- [18] Wang DG, Liu WY, Chen GT. A simple method for the isolation and purification of resveratrol from *Polygonum cuspidatum*. *J Pharm Anal*. 2013;3:241–247. doi: [10.1016/j.jpha.2012.12.001](https://doi.org/10.1016/j.jpha.2012.12.001).
- [19] Fan E, Zhang K, Zhu M, et al. Obtaining resveratrol: from chemical synthesis to biotechnological production. *MROC*. 2010;7:272–281. doi: [10.2174/157019310792246454](https://doi.org/10.2174/157019310792246454).
- [20] Mei YZ, Liu RX, Wang DP, et al. Biocatalysis and biotransformation of resveratrol in microorganisms. *Biotechnol Lett*. 2015;37:9–18. doi: [10.1007/s10529-014-1651-x](https://doi.org/10.1007/s10529-014-1651-x).
- [21] Li M, Kildegaard KR, Chen Y, et al. *De novo* production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. *Metab Eng*. 2015;32:1–11. doi: [10.1016/j.ymben.2015.08.007](https://doi.org/10.1016/j.ymben.2015.08.007).
- [22] Pirola L, Fröjdö S. Resveratrol: one molecule, many targets. *IUBMB Life*. 2008;60:323–332. doi: [10.1002/iub.47](https://doi.org/10.1002/iub.47).
- [23] Soares DG, Andreazza AC, Salvador M. Sequestering ability of butylated hydroxytoluene, propyl gallate, resveratrol, and vitamins C and E against ABTS, DPPH, and hydroxyl free radicals in chemical and biological systems. *J Agric Food Chem*. 2003;51:1077–1080. doi: [10.1021/jf020864z](https://doi.org/10.1021/jf020864z).
- [24] Riccio BVF, Fonseca-Santos B, Ferrari PC, et al. Characteristics, biological properties and analytical methods of *trans*-resveratrol: a review. *Crit Rev Anal Chem*. 2020;50:339–358. doi: [10.1080/10408347.2019.1637242](https://doi.org/10.1080/10408347.2019.1637242).
- [25] Nour V, Trandafir I, Muntean C. Ultraviolet irradiation of *trans*-resveratrol and HPLC determination of *trans*-resveratrol and *cis*-resveratrol in romanian red wines. *J Chromatogr Sci*. 2012;50:920–927. doi: [10.1093/chromsci/bms091](https://doi.org/10.1093/chromsci/bms091).
- [26] Frémont L. Biological effects of resveratrol. *Life Sci*. 2000;66:663–673. doi: [10.1016/s0024-3205\(99\)00410-5](https://doi.org/10.1016/s0024-3205(99)00410-5).
- [27] Camont L, Cottart C-H, Rhayem Y, et al. Simple spectrophotometric assessment of the *trans*-/*cis*-resveratrol ratio in aqueous solutions. *Anal Chim Acta*. 2009;634:121–128. doi: [10.1016/j.aca.2008.12.003](https://doi.org/10.1016/j.aca.2008.12.003).
- [28] Feng C, Chen J, Ye W, et al. Synthetic biology-driven microbial production of resveratrol: advances and perspectives. *Front Bioeng Biotechnol*. 2022;10:833920. doi: [10.3389/fbioe.2022.833920](https://doi.org/10.3389/fbioe.2022.833920).
- [29] Gambini J, Inglés M, Olaso G, et al. Properties of resveratrol: *in vitro* and *in vivo* studies about metabolism, bioavailability, and biological effects in animal models and humans. *Oxid Med Cell Longev*. 2015;2015:837042–13. doi: [10.1155/2015/837042](https://doi.org/10.1155/2015/837042).
- [30] Chen X, He H, Wang G, et al. Stereospecific determination of *cis*- and *trans*-resveratrol in rat plasma by HPLC: application to pharmacokinetic studies. *Biomed Chromatogr*. 2007;21:257–265. doi: [10.1002/bmc.747](https://doi.org/10.1002/bmc.747).
- [31] Trela BC, Waterhouse AL. Resveratrol: isomeric molar absorptivities and stability. *J Agric Food Chem*. 1996;44:1253–1257. doi: [10.1021/jf9504576](https://doi.org/10.1021/jf9504576).
- [32] Robinson K, Mock C, Liang D. Pre-formulation studies of resveratrol. *Drug Dev Ind Pharm*. 2015;41:1464–1469. doi: [10.3109/03639045.2014.958753](https://doi.org/10.3109/03639045.2014.958753).
- [33] Chung IM, Kim JJ, Lim JD, et al. Comparison of resveratrol, SOD activity, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress. *Environ Exp Bot*. 2006;56:44–53. doi: [10.1016/j.envexpbot.2005.01.001](https://doi.org/10.1016/j.envexpbot.2005.01.001).
- [34] Chen S, Han Y, Jian L, et al. Fabrication, characterization, physicochemical stability of zein-chitosan nanocomplex for co-encapsulating curcumin and resveratrol. *Carbohydr Polym*. 2020;236:116090. doi: [10.1016/j.carbpol.2020.116090](https://doi.org/10.1016/j.carbpol.2020.116090).
- [35] Delmas D, Aires V, Limagne E, et al. Transport, stability, and biological activity of resveratrol. *Ann N Y Acad Sci*. 2011;1215:48–59. doi: [10.1111/j.1749-6632.2010.05871.x](https://doi.org/10.1111/j.1749-6632.2010.05871.x).
- [36] Tang H, Xiang S, Li X, et al. Preparation and *in vitro* performance evaluation of resveratrol for oral self-microemulsion. Xu B, editor. *PLoS One*. 2019;14:e0214544. doi: [10.1371/journal.pone.0214544](https://doi.org/10.1371/journal.pone.0214544).
- [37] Balata GF, Essa EA, Shamardl HA, et al. Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol. *Drug Des Devel Ther*. 2016;10:117–128. doi: [10.2147/DDDT.S95905](https://doi.org/10.2147/DDDT.S95905).
- [38] Davidov-Pardo G, McClements DJ. Resveratrol encapsulation: designing delivery systems to overcome solubility, stability and bioavailability issues. *Trends Food Sci Technol*. 2014;38:88–103. doi: [10.1016/j.tifs.2014.05.003](https://doi.org/10.1016/j.tifs.2014.05.003).
- [39] Murador DC, de Souza Mesquita LM, Vannuchi N, et al. Bioavailability and biological effects of bioactive compounds extracted with natural deep eutectic solvents

- and ionic liquids: advantages over conventional organic solvents. *Curr Opin Food Sci.* 2019;26:25–34. doi: [10.1016/j.cofs.2019.03.002](https://doi.org/10.1016/j.cofs.2019.03.002).
- [40] Sakuragi M, Yoshimura H, Kusakabe K. Effects of structures of microemulsions containing a deep eutectic solvent on the entrapment amount and the skin permeation of resveratrol. *Jpn J Appl Phys.* 2020;59:035002. doi: [10.35848/1347-4065/ab6b74](https://doi.org/10.35848/1347-4065/ab6b74).
- [41] Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov.* 2006;5:493–506. doi: [10.1038/nrd2060](https://doi.org/10.1038/nrd2060).
- [42] Singh AP, Singh R, Verma SS, et al. Health benefits of resveratrol: evidence from clinical studies. *Med Res Rev.* 2019;39:1851–1891. doi: [10.1002/med.21565](https://doi.org/10.1002/med.21565).
- [43] Walle T, Hsieh F, DeLegge MH, et al. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos.* 2004;32:1377–1382. doi: [10.1124/dmd.104.000885](https://doi.org/10.1124/dmd.104.000885).
- [44] Muñoz O, Bustamante S. Pharmacological properties of resveratrol. A pre-clinical and clinical review. *Biochem Pharmacol (Los Angel)*. 2015;04 :1000184. doi: [10.4172/2167-0501.1000184](https://doi.org/10.4172/2167-0501.1000184).
- [45] Berman AY, Motechin RA, Wiesenfeld MY, et al. The therapeutic potential of resveratrol: a review of clinical trials. *NPJ Precis Oncol.* 2017;1:1–9. doi: [10.1038/s41698-017-0038-6](https://doi.org/10.1038/s41698-017-0038-6).
- [46] Howells LM, Berry DP, Elliott PJ, et al. Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases—safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev Res.* 2011;4:1419–1425. doi: [10.1158/1940-6207.CAPR-11-0148](https://doi.org/10.1158/1940-6207.CAPR-11-0148).
- [47] Athar M, Back JH, Tang X, et al. Resveratrol: a review of preclinical studies for human cancer prevention. *Toxicol Appl Pharmacol.* 2007;224:274–283. doi: [10.1016/j.taap.2006.12.025](https://doi.org/10.1016/j.taap.2006.12.025).
- [48] Brown VA, Patel KR, Viskaduraki M, et al. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res.* 2010;70:9003–9011. doi: [10.1158/0008-5472.CAN-10-2364](https://doi.org/10.1158/0008-5472.CAN-10-2364).
- [49] Almeida L, Vaz-da-Silva M, Falcão A, et al. Pharmacokinetic and safety profile of *trans*-resveratrol in a rising multiple-dose study in healthy volunteers. *Mol Nutr Food Res.* 2009;53: s 7–15.
- [50] Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature.* 2006;444:337–342. doi: [10.1038/nature05354](https://doi.org/10.1038/nature05354).
- [51] Howitz KT, Bitterman KJ, Cohen HY, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature.* 2003;425:191–196. doi: [10.1038/nature01960](https://doi.org/10.1038/nature01960).
- [52] Baxter RA. Anti-aging properties of resveratrol: review and report of a potent new antioxidant skin care formulation. *J Cosmet Dermatol.* 2008;7:2–7. doi: [10.1111/j.1473-2165.2008.00354.x](https://doi.org/10.1111/j.1473-2165.2008.00354.x).
- [53] Sakuragi M, Yano R, Hasnol SBM, et al. Evaluation of microemulsions containing a large amount of a deep eutectic solvent as a potential transdermal carrier of resveratrol. *Jpn J Appl Phys.* 2020;59:095004. doi: [10.35848/1347-4065/abaa94](https://doi.org/10.35848/1347-4065/abaa94).
- [54] de Vries K, Strydom M, Steenkamp V. A Brief Updated Review of Advances to Enhance Resveratrol's Bioavailability. *Molecules.* 2021;26:4367. doi: [10.3390/molecules26144367](https://doi.org/10.3390/molecules26144367).
- [55] Pillai S, Mahajan MN, Granger SP, et al. Cosmetic compositions containing resveratrol and retinoids. Patent US 6358517 B1. 2002.
- [56] Zeng G, Zhong F, Li J, et al. Resveratrol-mediated reduction of collagen by inhibiting proliferation and producing apoptosis in human hypertrophic scar fibroblasts. *Biosci Biotechnol Biochem.* 2013;77:2389–2396. doi: [10.1271/bbb.130502](https://doi.org/10.1271/bbb.130502).
- [57] Fabbrocini G, Staibano S, De Rosa G, et al. Resveratrol-containing gel for the treatment of acne vulgaris: a single-blind, vehicle-controlled, pilot study. *Am J Clin Dermatol.* 2011;12:133–141. doi: [10.2165/11530630-000000000-00000](https://doi.org/10.2165/11530630-000000000-00000).
- [58] Gokce EH, Tuncay Tanrıverdi S, Eroglu I, et al. Wound healing effects of collagen-laminin dermal matrix impregnated with resveratrol loaded hyaluronic acid-DPPC microparticles in diabetic rats. *Eur J Pharm Biopharm.* 2017;119:17–27. doi: [10.1016/j.ejpb.2017.04.027](https://doi.org/10.1016/j.ejpb.2017.04.027).
- [59] Shen WW, Zeng Z, Zhu WX, et al. MiR-142-3p functions as a tumor suppressor by targeting CD133, ABCG2, and Lgr5 in colon cancer cells. *J Mol Med.* 2013;91:989–1000. doi: [10.1007/s00109-013-1037-x](https://doi.org/10.1007/s00109-013-1037-x).
- [60] Papat R, Plesner T, Davies F, et al. A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. *Br J Haematol.* 2013;160:714–717. doi: [10.1111/bjh.12154](https://doi.org/10.1111/bjh.12154).
- [61] Movahed A, Nabipour I, Lieben Louis X, et al. Antihyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. *Evid Based Complement Alternat Med.* 2013;2013:1–11. doi: [10.1155/2013/851267](https://doi.org/10.1155/2013/851267).
- [62] Hirst JA, Farmer AJ, Ali R, et al. Quantifying the effect of metformin treatment and dose on glycemic control. *Diabetes Care.* 2012;35:446–454. doi: [10.2337/dc11-1465](https://doi.org/10.2337/dc11-1465).
- [63] Brasnyó P, Molnár GA, Mohás M, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. *Br J Nutr.* 2011;106:383–389. doi: [10.1017/S0007114511000316](https://doi.org/10.1017/S0007114511000316).
- [64] Bashmakov YK, Assaad-Khalil SH, Abou Seif M, et al. Resveratrol promotes foot ulcer size reduction in type 2 diabetes patients. *ISRN Endocrinol.* 2014;2014:816307–816308. doi: [10.1155/2014/816307](https://doi.org/10.1155/2014/816307).
- [65] Wong RHX, Raederstorff D, Howe PRC. Acute resveratrol consumption improves neurovascular coupling capacity in adults with type 2 diabetes mellitus. *Nutrients.* 2016;8:425. doi: [10.3390/nu8070425](https://doi.org/10.3390/nu8070425).
- [66] Arzola-Paniagua MA, García-Salgado López ER, Calvo-Vargas CG, et al. Efficacy of an orlistat-resveratrol combination for weight loss in subjects with obesity: a randomized controlled trial. *Obesity.* 2016;24:1454–1463. doi: [10.1002/oby.21523](https://doi.org/10.1002/oby.21523).
- [67] Tomé-Carneiro J, Larrosa M, González-Sarriás A, et al. Resveratrol and clinical trials: the crossroad from *in vitro* studies to human evidence. *Curr Pharm Des.* 2013;19:6064–6093. doi: [10.2174/13816128113199990407](https://doi.org/10.2174/13816128113199990407).
- [68] Tomé-Carneiro J, González M, Larrosa M, et al. Consumption of a grape extract supplement containing resveratrol decreases oxidized LDL and ApoB in patients undergoing primary prevention of cardiovascular disease: a triple-blind, 6-month follow-up,

- placebo-controlled, randomized trial. *Mol Nutr Food Res*. 2012;56:810–821. doi: [10.1002/mnfr.201100673](https://doi.org/10.1002/mnfr.201100673).
- [69] Zamora-Ros R, Urpi-Sarda M, Lamuela-Raventós RM, et al. High urinary levels of resveratrol metabolites are associated with a reduction in the prevalence of cardiovascular risk factors in high-risk patients. *Pharmacol Res*. 2012;65:615–620. doi: [10.1016/j.phrs.2012.03.009](https://doi.org/10.1016/j.phrs.2012.03.009).
- [70] Magyar K, Halmosi R, Palfi A, et al. Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. *Clin Hemorheol Microcirc*. 2012;50:179–187. doi: [10.3233/CH-2011-1424](https://doi.org/10.3233/CH-2011-1424).
- [71] Molino S, Dossena M, Buonocore D, et al. Polyphenols in dementia: from molecular basis to clinical trials. *Life Sci*. 2016;161:69–77. doi: [10.1016/j.lfs.2016.07.021](https://doi.org/10.1016/j.lfs.2016.07.021).
- [72] Witte AV, Kerti L, Margulies DS, et al. Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. *J Neurosci*. 2014;34:7862–7870. doi: [10.1523/JNEUROSCI.0385-14.2014](https://doi.org/10.1523/JNEUROSCI.0385-14.2014).
- [73] Liu Z, Zhou T, Ziegler AC, et al. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. *Oxid Med Cell Longev*. 2017;2017:2525967. doi: [10.1155/2017/2525967](https://doi.org/10.1155/2017/2525967).
- [74] Turner RS, Thomas RG, Craft S, et al. A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. *Neurology*. 2015;85:1383–1391. doi: [10.1212/WNL.0000000000002035](https://doi.org/10.1212/WNL.0000000000002035).
- [75] Future Market Insights. Global resveratrol market snapshot (2023–2033). 2023.
- [76] Future Market Insights. Market Insights on Resveratrol covering sales outlook, demand forecast & up-to-date key trends. 2021. p. 384
- [77] Beekwilder J, Wolswinkel R, Jonker H, et al. Production of resveratrol in recombinant microorganisms. *Appl Environ Microbiol*. 2006;72:5670–5672. doi: [10.1128/AEM.00609-06](https://doi.org/10.1128/AEM.00609-06).
- [78] Becker JW, Armstrong GO, van der Merwe MJ, et al. Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine-related antioxidant resveratrol. *FEMS Yeast Res*. 2003;4:79–85. doi: [10.1016/S1567-1356\(03\)00157-0](https://doi.org/10.1016/S1567-1356(03)00157-0).
- [79] Sydor T, Schaffer S, Boles E. Considerable increase in resveratrol production by recombinant industrial yeast strains with use of rich medium. *Appl Environ Microbiol*. 2010;76:3361–3363. doi: [10.1128/AEM.02796-09](https://doi.org/10.1128/AEM.02796-09).
- [80] Zhang Y, Li S-Z, Li J, et al. Using unnatural protein fusions to engineer resveratrol biosynthesis in yeast and mammalian cells. *J Am Chem Soc*. 2006;128:13030–13031. doi: [10.1021/ja0622094](https://doi.org/10.1021/ja0622094).
- [81] Wang Y, Halls C, Zhang J, et al. Stepwise increase of resveratrol biosynthesis in yeast *Saccharomyces cerevisiae* by metabolic engineering. *Metab Eng*. 2011;13:455–463. doi: [10.1016/j.ymben.2011.04.005](https://doi.org/10.1016/j.ymben.2011.04.005).
- [82] Jiang H, Wood KV, Morgan JA. Metabolic engineering of the phenylpropanoid pathway in *Saccharomyces cerevisiae*. *Appl Environ Microbiol*. 2005;71:2962–2969. doi: [10.1128/AEM.71.6.2962-2969.2005](https://doi.org/10.1128/AEM.71.6.2962-2969.2005).
- [83] Shin SY, Jung SM, Kim MD, et al. Production of resveratrol from tyrosine in metabolically engineered *Saccharomyces cerevisiae*. *Enzyme Microb Technol*. 2012;51:211–216. doi: [10.1016/j.enzmictec.2012.06.005](https://doi.org/10.1016/j.enzmictec.2012.06.005).
- [84] Trantas E, Panopoulos N, Ververidis F. Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. *Metab Eng*. 2009;11:355–366. doi: [10.1016/j.ymben.2009.07.004](https://doi.org/10.1016/j.ymben.2009.07.004).
- [85] Vos T, de la Torre Cortés P, van Gulik WM, et al. Growth-rate dependency of *de novo* resveratrol production in chemostat cultures of an engineered *Saccharomyces cerevisiae* strain. *Microb Cell Fact*. 2015;14:133. doi: [10.1186/s12934-015-0321-6](https://doi.org/10.1186/s12934-015-0321-6).
- [86] Katz MP, Durhuus T, Smits HP, et al. Production of metabolites. US Patent 13/700,011 (US20130209613 A1). 2011. p. 121.
- [87] Li M, Schneider K, Kristensen M, et al. Engineering yeast for high-level production of stilbenoid antioxidants. *Sci Rep*. 2016;6:36827. doi: [10.1038/srep36827](https://doi.org/10.1038/srep36827).
- [88] Meng L, Diao M, Wang Q, et al. Efficient biosynthesis of resveratrol via combining phenylalanine and tyrosine pathways in *Saccharomyces cerevisiae*. *Microb Cell Fact*. 2023;22:46. doi: [10.1186/s12934-023-02055-9](https://doi.org/10.1186/s12934-023-02055-9).
- [89] Liu Q, Yu T, Li X, et al. Rewiring carbon metabolism in yeast for high level production of aromatic chemicals. *Nat Commun*. 2019;10:4976. doi: [10.1038/s41467-019-12961-5](https://doi.org/10.1038/s41467-019-12961-5).
- [90] Shin SY, Han NS, Park YC, et al. Production of resveratrol from *p*-coumaric acid in recombinant *Saccharomyces cerevisiae* expressing 4-coumarate:coenzyme A ligase and stilbene synthase genes. *Enzyme Microb Technol*. 2011;48:48–53. doi: [10.1016/j.enzmictec.2010.09.004](https://doi.org/10.1016/j.enzmictec.2010.09.004).
- [91] Wang Y, Yu O. Synthetic scaffolds increased resveratrol biosynthesis in engineered yeast cells. *J Biotechnol*. 2012;157:258–260. doi: [10.1016/j.jbiotec.2011.11.003](https://doi.org/10.1016/j.jbiotec.2011.11.003).
- [92] Yuan SF, Yi X, Johnston TG, et al. *De novo* resveratrol production through modular engineering of an *Escherichia coli*-*Saccharomyces cerevisiae* co-culture. *Microb Cell Fact*. 2020;19:143. doi: [10.1186/s12934-020-01401-5](https://doi.org/10.1186/s12934-020-01401-5).
- [93] Sun X, Shen X, Jain R, et al. Synthesis of chemicals by metabolic engineering of microbes. *Chem Soc Rev*. 2015;44:3760–3785. doi: [10.1039/c5cs00159e](https://doi.org/10.1039/c5cs00159e).
- [94] Costa CE, Møller-Hansen I, Romani A, et al. Resveratrol production from hydrothermally pretreated eucalyptus wood using recombinant industrial *Saccharomyces cerevisiae* strains. *ACS Synth Biol*. 2021;10:1895–1903. doi: [10.1021/acssynbio.1c00120](https://doi.org/10.1021/acssynbio.1c00120).
- [95] Costa CE, Romani A, Teixeira JA, et al. Resveratrol production for the valorisation of lactose-rich wastes by engineered industrial *Saccharomyces cerevisiae*. *Bioresour Technol*. 2022;359:127463. doi: [10.1016/j.biortech.2022.127463](https://doi.org/10.1016/j.biortech.2022.127463).
- [96] Costa CE, Romani A, Møller-Hansen I, et al. Valorisation of wine wastes by *de novo* biosynthesis of resveratrol using a recombinant xylose-consuming industrial *Saccharomyces cerevisiae* strain. *Green Chem*. 2022;24:9128–9142. doi: [10.1039/D2GC02429B](https://doi.org/10.1039/D2GC02429B).
- [97] Baptista SL, Costa CE, Cunha JT, et al. Metabolic engineering of *Saccharomyces cerevisiae* for the production of top value chemicals from biorefinery carbohydrates. *Biotechnol Adv*. 2021;47:107697. doi: [10.1016/j.biotechadv.2021.107697](https://doi.org/10.1016/j.biotechadv.2021.107697).
- [98] Cunha JT, Romani A, Costa CE, et al. Molecular and physiological basis of *Saccharomyces cerevisiae* tolerance to adverse lignocellulose-based process

- conditions. *Appl Microbiol Biotechnol.* 2019;103:159–175. doi: [10.1007/s00253-018-9478-3](https://doi.org/10.1007/s00253-018-9478-3).
- [99] Liu L, Ma X, Bilal M, et al. Toxicity and inhibition mechanism of gallic acid on physiology and fermentation performance of *Escherichia coli*. *Bioresour Bioprocess.* 2022;9:76. doi: [10.1186/s40643-022-00564-w](https://doi.org/10.1186/s40643-022-00564-w).
- [100] Pereira FB, Romani A, Ruiz HA, et al. Industrial robust yeast isolates with great potential for fermentation of lignocellulosic biomass. *Bioresour Technol.* 2014;161:192–199. doi: [10.1016/j.biortech.2014.03.043](https://doi.org/10.1016/j.biortech.2014.03.043).
- [101] Cunha JT, Aguiar TQ, Romani A, et al. Contribution of *PRS3*, *RPB4* and *ZWF1* to the resistance of industrial *Saccharomyces cerevisiae* CCUG53310 and PE-2 strains to lignocellulosic hydrolysate-derived inhibitors. *Bioresour Technol.* 2015;191:7–16. doi: [10.1016/j.biortech.2015.05.006](https://doi.org/10.1016/j.biortech.2015.05.006).
- [102] Costa CE, Romani A, Cunha JT, et al. Integrated approach for selecting efficient *Saccharomyces cerevisiae* for industrial lignocellulosic fermentations: importance of yeast chassis linked to process conditions. *Bioresour Technol.* 2017;227:24–34. doi: [10.1016/j.biortech.2016.12.016](https://doi.org/10.1016/j.biortech.2016.12.016).
- [103] Baptista SL, Cunha JT, Romani A, et al. Xylitol production from lignocellulosic whole slurry corn cob by engineered industrial *Saccharomyces cerevisiae* PE-2. *Bioresour Technol.* 2018;267:481–491. doi: [10.1016/j.biortech.2018.07.068](https://doi.org/10.1016/j.biortech.2018.07.068).
- [104] Cunha JT, Soares PO, Baptista SL, et al. Engineered *Saccharomyces cerevisiae* for lignocellulosic valorization: a review and perspectives on bioethanol production. *Bioengineered.* 2020;11:883–903. doi: [10.1080/21655979.2020.1801178](https://doi.org/10.1080/21655979.2020.1801178).
- [105] Arias A, Costa CE, Moreira MT, et al. Resveratrol-based biorefinery models for favoring its inclusion along the market value-added chains: a critical review. *Sci Total Environ.* 2024;908:168199. doi: [10.1016/j.scitotenv.2023.168199](https://doi.org/10.1016/j.scitotenv.2023.168199).
- [106] Cunha JT, Costa CE, Ferraz L, et al. *HAA1* and *PRS3* overexpression boosts yeast tolerance towards acetic acid improving xylose or glucose consumption: unravelling the underlying mechanisms. *Appl Microbiol Biotechnol.* 2018;102:4589–4600. doi: [10.1007/s00253-018-8955-z](https://doi.org/10.1007/s00253-018-8955-z).
- [107] Carvalho P, Costa CE, Baptista SL, et al. Yeast cell factories for sustainable whey-to-ethanol valorisation towards a circular economy. *Biofuel Res J.* 2021;8:1529–1549. doi: [10.18331/BRJ2021.8.4.4](https://doi.org/10.18331/BRJ2021.8.4.4).
- [108] Arias A, Costa CE, Feijoo G, et al. Process modeling, environmental and economic sustainability of the valorization of whey and eucalyptus residues for resveratrol biosynthesis. *Waste Manag.* 2023;172:226–234. doi: [10.1016/j.wasman.2023.10.030](https://doi.org/10.1016/j.wasman.2023.10.030).
- [109] Arias A, Costa CE, Moreira MT, et al. Environmental and techno-economic assessment on the valorization of vine-side streams to produce resveratrol. *J Clean Prod.* 2023;429:139622. doi: [10.1016/j.jclepro.2023.139622](https://doi.org/10.1016/j.jclepro.2023.139622).
- [110] Kobayashi Y, Inokuma K, Matsuda M, et al. Resveratrol production from several types of saccharide sources by a recombinant *Scheffersomyces stipitis* strain. *Metab Eng Commun.* 2021;13:e00188. doi: [10.1016/j.mec.2021.e00188](https://doi.org/10.1016/j.mec.2021.e00188).
- [111] Kumokita R, Yoshida T, Shirai T, et al. Aromatic secondary metabolite production from glycerol was enhanced by amino acid addition in *Pichia pastoris*. *Appl Microbiol Biotechnol.* 2023;107:7391–7401. doi: [10.1007/s00253-023-12798-5](https://doi.org/10.1007/s00253-023-12798-5).
- [112] Liu M, Wang C, Ren X, et al. Remodelling metabolism for high-level resveratrol production in *Yarrowia lipolytica*. *Bioresour Technol.* 2022;365:128178. doi: [10.1016/j.biortech.2022.128178](https://doi.org/10.1016/j.biortech.2022.128178).
- [113] Watts KT, Lee PC, Schmidt-Dannert C. Biosynthesis of plant-specific stilbene polyketides in metabolically engineered *Escherichia coli*. *BMC Biotechnol.* 2006;6:22. doi: [10.1186/1472-6750-6-22](https://doi.org/10.1186/1472-6750-6-22).
- [114] Lim CG, Fowler ZL, Hueller T, et al. High-yield resveratrol production in engineered *Escherichia coli*. *Appl Environ Microbiol.* 2011;77:3451–3460. doi: [10.1128/AEM.02186-10](https://doi.org/10.1128/AEM.02186-10).
- [115] Katsuyama Y, Funa N, Horinouchi S. Precursor-directed biosynthesis of stilbene methyl ethers in *Escherichia coli*. *Biotechnol J.* 2007;2:1286–1293. doi: [10.1002/biot.200700098](https://doi.org/10.1002/biot.200700098).
- [116] Wu J, Liu P, Fan Y, et al. Multivariate modular metabolic engineering of *Escherichia coli* to produce resveratrol from L-tyrosine. *J Biotechnol.* 2013;167:404–411. doi: [10.1016/j.jbiotec.2013.07.030](https://doi.org/10.1016/j.jbiotec.2013.07.030).
- [117] Liu X, Lin J, Hu H, et al. De novo biosynthesis of resveratrol by site-specific integration of heterologous genes in *Escherichia coli*. *FEMS Microbiol Lett.* 2016;363:fnw061. doi: [10.1093/femsle/fnw061](https://doi.org/10.1093/femsle/fnw061).
- [118] Wu J, Zhou P, Zhang X, et al. Efficient *de novo* synthesis of resveratrol by metabolically engineered *Escherichia coli*. *J Ind Microbiol Biotechnol.* 2017;44:1083–1095. doi: [10.1007/s10295-017-1937-9](https://doi.org/10.1007/s10295-017-1937-9).
- [119] Camacho-Zaragoza JM, Hernández-Chávez G, Moreno-Avitia F, et al. Engineering of a microbial co-culture of *Escherichia coli* strains for the biosynthesis of resveratrol. *Microb Cell Fact.* 2016;15:163. doi: [10.1186/s12934-016-0562-z](https://doi.org/10.1186/s12934-016-0562-z).
- [120] Hong J, Im DK, Oh MK. Investigating *E. coli* coculture for resveratrol production with ¹³C metabolic flux analysis. *J Agric Food Chem.* 2020;68:3466–3473. doi: [10.1021/acs.jafc.9b07628](https://doi.org/10.1021/acs.jafc.9b07628).
- [121] Li J, Qiu Z, Zhao GR. Modular engineering of *E. coli* coculture for efficient production of resveratrol from glucose and arabinose mixture. *Synth Syst Biotechnol.* 2022;7:718–729. doi: [10.1016/j.synbio.2022.03.001](https://doi.org/10.1016/j.synbio.2022.03.001).
- [122] Park SR, Yoon JA, Paik JH, et al. Engineering of plant-specific phenylpropanoids biosynthesis in *Streptomyces venezuelae*. *J Biotechnol.* 2009;141:181–188. doi: [10.1016/j.jbiotec.2009.03.013](https://doi.org/10.1016/j.jbiotec.2009.03.013).
- [123] Gaspar P, Dudnik A, Neves AR, et al. Engineering *Lactococcus lactis* for stilbene production. 28th International Conference on Polyphenols 2016. 2016.
- [124] Wang L, Deng A, Zhang Y, et al. Efficient CRISPR-Cas9 mediated multiplex genome editing in yeasts. *Biotechnol Biofuels.* 2018;11:277. doi: [10.1186/s13068-018-1271-0](https://doi.org/10.1186/s13068-018-1271-0).
- [125] Kallscheuer N, Vogt M, Stenzel A, et al. Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2S)-flavanones. *Metab Eng.* 2016;38:47–55. doi: [10.1016/j.ymben.2016.06.003](https://doi.org/10.1016/j.ymben.2016.06.003).
- [126] Braga A, Oliveira J, Silva R, et al. Impact of the cultivation strategy on resveratrol production from glucose in engineered *Corynebacterium glutamicum*. *J Biotechnol.* 2018;265:70–75. doi: [10.1016/j.jbiotec.2017.11.006](https://doi.org/10.1016/j.jbiotec.2017.11.006).

- [127] Zhang M, Gao Q, Liu Y, et al. Metabolic engineering of *Rhodotorula toruloides* for resveratrol production. *Microb Cell Fact*. 2022;21:270. doi: [10.1186/s12934-022-02006-w](https://doi.org/10.1186/s12934-022-02006-w).
- [128] Hassing EJ, de Groot PA, Marquenie VR, et al. Connecting central carbon and aromatic amino acid metabolisms to improve *de novo* 2-phenylethanol production in *Saccharomyces cerevisiae*. *Metab Eng*. 2019;56:165–180. doi: [10.1016/j.ymben.2019.09.011](https://doi.org/10.1016/j.ymben.2019.09.011).
- [129] Li M, Borodina I. Application of synthetic biology for production of chemicals in yeast *Saccharomyces cerevisiae*. *FEMS Yeast Res*. 2015;15:1–12. doi: [10.1111/1567-1364.12213](https://doi.org/10.1111/1567-1364.12213).
- [130] Tokuhiko K, Muramatsu M, Ohto C, et al. Overproduction of geranylgeraniol by metabolically engineered *Saccharomyces cerevisiae*. *Appl Environ Microbiol*. 2009;75:5536–5543. doi: [10.1128/AEM.00277-09](https://doi.org/10.1128/AEM.00277-09).
- [131] Cheng S, Liu X, Jiang G, et al. Orthogonal engineering of biosynthetic pathway for efficient production of limonene in *Saccharomyces cerevisiae*. *ACS Synth Biol*. 2019;8:968–975. doi: [10.1021/acssynbio.9b00135](https://doi.org/10.1021/acssynbio.9b00135).
- [132] Sun L, Kwak S, Jin YS. Vitamin A production by engineered *Saccharomyces cerevisiae* from xylose via two-phase *in situ* extraction. *ACS Synth Biol*. 2019;8:2131–2140. doi: [10.1021/acssynbio.9b00217](https://doi.org/10.1021/acssynbio.9b00217).
- [133] Amri A, Chaumeil JC, Sfar S, et al. Administration of resveratrol: what formulation solutions to bioavailability limitations? *J Control Release*. 2012;158:182–193. doi: [10.1016/j.jconrel.2011.09.083](https://doi.org/10.1016/j.jconrel.2011.09.083).
- [134] Yen CC, Chang CW, Hsu MC, et al. Self-nanoemulsifying drug delivery system for resveratrol: enhanced oral bioavailability and reduced physical fatigue in rats. *Int J Mol Sci*. 2017;18:1853. doi: [10.3390/ijms18091853](https://doi.org/10.3390/ijms18091853).
- [135] Shamseddin A, Crauste C, Durand E, et al. Resveratrol formulated with a natural deep eutectic solvent inhibits active matrix metalloproteinase-9 in hormetic conditions. *Eur J Lipid Sci Tech*. 2017;119:1700171. doi: [10.1002/ejlt.201700171](https://doi.org/10.1002/ejlt.201700171).
- [136] Sun P, Liang J-L, Kang L-Z, et al. Increased resveratrol production in wines using engineered wine strains *Saccharomyces cerevisiae* EC1118 and relaxed antibiotic or auxotrophic selection. *Biotechnol Prog*. 2015;31:650–655. doi: [10.1002/btpr.2057](https://doi.org/10.1002/btpr.2057).
- [137] Villa-Ruano N, Rivera A, Rubio-Rosas E, et al. Comparative activity of six recombinant stilbene synthases in yeast for resveratrol production. *Appl Sci*. 2020;10:4847. doi: [10.3390/app10144847](https://doi.org/10.3390/app10144847).
- [138] Gu Y, Ma J, Zhu Y, et al. Engineering *Yarrowia lipolytica* as a chassis for *de novo* synthesis of five aromatic-derived natural products and chemicals. *ACS Synth Biol*. 2020;9:2096–2106. doi: [10.1021/acssynbio.0c00185](https://doi.org/10.1021/acssynbio.0c00185).
- [139] Palmer CM, Miller KK, Nguyen A, et al. Engineering 4-coumaroyl-CoA derived polyketide production in *Yarrowia lipolytica* through a β -oxidation mediated strategy. *Metab Eng*. 2020;57:174–181. doi: [10.1016/j.ymben.2019.11.006](https://doi.org/10.1016/j.ymben.2019.11.006).
- [140] He Q, Szczepańska P, Yuzbashev T, et al. *De novo* production of resveratrol from glycerol by engineering different metabolic pathways in *Yarrowia lipolytica*. *Metab Eng Commun*. 2020;11:e00146. doi: [10.1016/j.mec.2020.e00146](https://doi.org/10.1016/j.mec.2020.e00146).
- [141] Katsuyama Y, Funa N, Miyahisa I, et al. Synthesis of unnatural flavonoids and stilbenes by exploiting the plant biosynthetic pathway in *Escherichia coli*. *Chem Biol*. 2007;14:613–621. doi: [10.1016/j.chembiol.2007.05.004](https://doi.org/10.1016/j.chembiol.2007.05.004).
- [142] Choi O, Wu CZ, Kang SY, et al. Biosynthesis of plant-specific phenylpropanoids by construction of an artificial biosynthetic pathway in *Escherichia coli*. *J Ind Microbiol Biotechnol*. 2011;38:1657–1665. doi: [10.1007/s10295-011-0954-3](https://doi.org/10.1007/s10295-011-0954-3).
- [143] Bhan N, Xu P, Khalidi O, et al. Redirecting carbon flux into malonyl-CoA to improve resveratrol titers: proof of concept for genetic interventions predicted by OptForce computational framework. *Chem Eng Sci*. 2013;103:109–114. doi: [10.1016/j.ces.2012.10.009](https://doi.org/10.1016/j.ces.2012.10.009).
- [144] Kang S-Y, Lee JK, Choi O, et al. Biosynthesis of methylated resveratrol analogs through the construction of an artificial biosynthetic pathway in *E. coli*. *BMC Biotechnol*. 2014;14:67. doi: [10.1186/1472-6750-14-67](https://doi.org/10.1186/1472-6750-14-67).
- [145] Yang Y, Lin Y, Li L, et al. Regulating malonyl-CoA metabolism via synthetic antisense RNAs for enhanced biosynthesis of natural products. *Metab Eng*. 2015;29:217–226. doi: [10.1016/j.ymben.2015.03.018](https://doi.org/10.1016/j.ymben.2015.03.018).
- [146] Zhang E, Guo X, Meng Z, et al. Construction, expression, and characterization of *Arabidopsis thaliana* 4CL and *Arachis hypogaea* RS fusion gene 4CL::RS in *Escherichia coli*. *World J Microbiol Biotechnol*. 2015;31:1379–1385. doi: [10.1007/s11274-015-1889-z](https://doi.org/10.1007/s11274-015-1889-z).
- [147] Wang S, Zhang S, Xiao A, et al. Metabolic engineering of *Escherichia coli* for the biosynthesis of various phenylpropanoid derivatives. *Metab Eng*. 2015;29:153–159. doi: [10.1016/j.ymben.2015.03.011](https://doi.org/10.1016/j.ymben.2015.03.011).
- [148] Afonso MS, Ferreira S, Domingues FC, et al. Resveratrol production in bioreactor: assessment of cell physiological states and plasmid segregational stability. *Biotechnol Rep*. 2015;5:7–13. doi: [10.1016/j.btre.2014.10.008](https://doi.org/10.1016/j.btre.2014.10.008).
- [149] Park JY, Lim JH, Ahn JH, et al. Biosynthesis of resveratrol using metabolically engineered *Escherichia coli*. *Appl Biol Chem*. 2021;64:20. doi: [10.1186/s13765-021-00595-5](https://doi.org/10.1186/s13765-021-00595-5).