



Microbial Production of Naringenin

Daniela Gomes, Joana L. Rodrigues, and Ligia R. Rodrigues

Contents

Introduction	2
Naringenin as a Relevant Natural Compound	3
Biosynthetic Pathway	4
Plant Extraction Versus Chemical Synthesis Versus Heterologous Production	8
Advances on the Construction of Microbial Chassis Able to Produce Naringenin	9
<i>Escherichia coli</i> as Microbial Cell Factory to Produce Naringenin	17
<i>Saccharomyces cerevisiae</i> as Microbial Cell Factory to Produce Naringenin	20
Non-model Organisms as Alternative Chassis to Produce Naringenin	22
Challenges on the Efficient Heterologous Production of Naringenin	
Using Microbial Chassis	24
Conclusion	27
References	28

Abstract

Naringenin is a plant natural compound that belongs to the flavonoid subclass of the polyphenolic compounds. It has several recognized health benefits, namely, antioxidant, anti-inflammatory, anticancer, and antiviral. Given these reported health benefits, an increasing interest in its utilization and incorporation by the pharmaceutical and nutraceutical industries in medicines and food supplements has emerged. As other secondary metabolites, this compound is present in low amounts within plants, which limits its extraction and further utilization. For this reason, it is mandatory to find efficient, green, and cost-effective new strategies for its production. In the last years, the use of microbial cell factories as chassis

D. Gomes

CEB-Centre of Biological Engineering, Universidade do Minho, Braga, Portugal

J. L. Rodrigues · L. R. Rodrigues (✉)

CEB-Centre of Biological Engineering, Universidade do Minho, Braga, Portugal

LABBELS- Associate Laboratory, Braga/Guimarães, Portugal

e-mail: joanarodrigues@deb.uminho.pt; lrnr@deb.uminho.pt

for naringenin production has been exploited. Different microorganisms have been engineered and used for its heterologous production, namely, *Escherichia coli*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Streptomyces* spp. In this chapter, the advances that have been made to achieve the microbial heterologous production of naringenin were summarized. Despite these efforts, obtaining industrially relevant amounts of naringenin to meet its industrial demand remains challenging. Therefore, the main limitations to reach higher production levels of naringenin, as well as relevant strategies to overcome them, are also highlighted in this chapter.

Keywords

Naringenin · Heterologous production · Microbial chassis · Metabolic engineering · Synthetic biology

Introduction

For many years, natural compounds have been used in traditional medicine. Nowadays, the pharmaceutical and the nutraceutical industries have been reinforcing their interest in using these compounds and incorporating them in new drugs or food supplements (Najmi et al. 2022; Nasim et al. 2022). Plants are a natural source of several compounds with interesting properties and biological activities. These compounds are usually produced as secondary metabolites. For this reason, they are present only in residual amounts in the plant which limits their extraction and, consequently, their industrial use (Gomes et al. 2022a). Polyphenols are secondary metabolites that have recently gained special attention due to their wide range of possible utilizations and recognized properties. These compounds are divided into several subclasses, being the flavonoids considered the largest subclass (Gomes et al. 2022a). Naringenin is considered the core compound for the synthesis of more complex flavonoids since they are produced through several modifications on the structure of this compound (Tariq et al. 2023). Beyond that, naringenin is also considered a potential pharmaceutical and nutraceutical compound due to its several reported health benefits (Tariq et al. 2023). Therefore, an increasing interest in finding new strategies to produce this compound in relevant amounts to meet industrial demands has emerged. Moreover, it is also mandatory to develop a cost-effective and environmentally friendly production processes. During the last years, the use of several microorganisms as microbial cell factories to produce naringenin has been exploited (Braga et al. 2019). However, despite those efforts, relevant production levels to attend the industrial needs have still not been possible. Hence, find the key points of the pathway that are impairing the improvement of the production levels to better engineer the microbial chassis is crucial (Braga et al. 2019; Gomes et al. 2022a; Tariq et al. 2023).

In this chapter, the relevance of naringenin as a pharmaceutical and nutraceutical compound will be summarized, and a detailed description of the biosynthetic

pathways involved in its production will be presented. Moreover, recent studies reporting the advances in the construction of this biosynthetic pathway using various microbial chassis, as well as the challenges toward the microbial production of naringenin at an industrial scale, will be discussed.

Naringenin as a Relevant Natural Compound

Flavonoids, including naringenin, share a core structure represented by 15 carbons. These carbons are distributed by two benzene rings that are linked together through one heterocyclic pyran ring (Fig. 1a). Naringenin belongs to the flavanones subclass and contains three hydroxyl groups (OH) on the carbons 4', 5, and 7, as well as a carbonyl group (=O) on the carbon 4 of the C ring (Fig. 1b).

Naringenin is naturally present in several citrus fruits, such as grapefruits, oranges, limes, tomatoes, and tart cherries. Moreover, this compound can also be found in other sources in lower quantities, namely, in beans, cocoa, tea, coffee, and bergamot orange (Amin et al. 2020). As other polyphenolic compounds, naringenin has a wide range of recognized interesting biological activities, namely, antioxidant, antiviral, anticancer, and anti-inflammatory (Motallebi et al. 2022).

Regarding antioxidant activity, naringenin is recognized to have a strong free radical-scavenging activity, being able to neutralize several types of radicals, namely, hydroxyl, nitric oxide, and hydrogen peroxide radicals. Moreover, it was also shown to be able to reduce lipid peroxidation (Rashmi et al. 2017). Naringenin has also shown positive effects in vitro on the progression of the autoimmune disease *Pemphigus vulgaris* and on protecting keratinocytes cells, as it was able to reduce reactive oxygen species (ROS) production (Liang et al. 2017). Additionally, naringenin also showed positive effects on the pretreatment of Alzheimer's disease by decreasing lipid peroxidation in the hippocampus of A β -injected rat model (Ghofrani et al. 2015).

Additionally, naringenin has been reported as an antiviral compound. Recently, it was verified that this compound has potential to exert therapeutic effects against porcine epidemic diarrhea virus since it interacts with the viral replicase proteins 3CLpro and PLP-2 affecting viral replication (Xia et al. 2023). Naringenin was

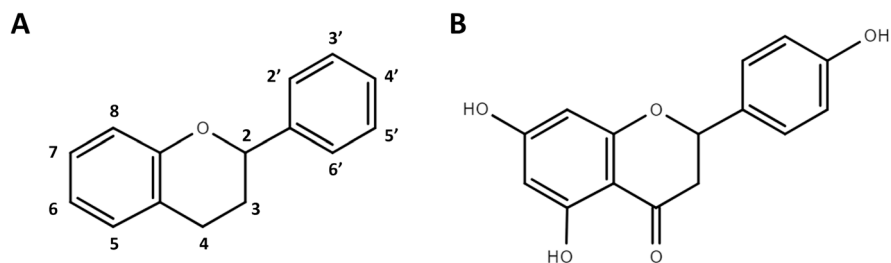


Fig. 1 Schematic representation of flavonoids' core structure (a) and naringenin structure (b)

reported to have in vitro antiviral activity against Zika virus. Cataneo et al. (2019) showed a decrease in the viral replication and the assembly of viral particles through the interaction of naringenin with the protease domain of the NS2B-NS3 viral protease protein (Cataneo et al. 2019).

Regarding the anticancer activity, naringenin has potential to be used on the treatment of several types of cancer since it is able to inhibit the cancer progression via several mechanisms (Motallebi et al. 2022). For example, naringenin has been shown to decrease the expression of B-cell lymphoma-2-like 1 and inactivate the phosphatidylinositol 3-kinase/protein kinase B pathway and, consequently, inhibit the cell proliferation and induce the apoptosis of ovarian cancer cells (Xu et al. 2022). Moreover, naringenin was reported to strongly bind to human cyclin-dependent kinase 6 (CDK6), inhibiting its expression. CDK6 overexpression is usually related to the development and progression of tumors. For this reason, this binding between naringenin and CDK6 showed positive effects on reducing cancer cells' viability and colonization ability, as well as apoptosis induction (Yousuf et al. 2022).

Beyond all these biological activities, naringenin has also been reported as a compound with anti-inflammatory activity. This anti-inflammatory response is associated with the downregulation or inactivation of several inflammatory factors like nuclear factor κ B (NF- κ B) and pro-inflammatory cytokines and leukocyte recruitment. For example, Liu et al. (2016) reported that naringenin can decrease the inflammatory response in murine endotoxemia model by decreasing the expression of tumor necrosis factor- α (TNF- α), interleukin (IL) 6, cyclo-oxygenase-2, NADPH oxidase-2, inducible NO synthase, and NF- κ B. This modulation on the Toll-like receptor 4 signaling pathway leads to an increase of cell survival and protection against murine endotoxemia (Liu et al. 2016). The effect of treating a hypertrophic scar mouse model with naringenin was also tested, and a significant inhibition of hypertrophic scars was observed after treatment. This inhibition was achieved due to fibroblast inactivation and downregulation of TNF- α , IL-1 β , IL-6, and transforming growth factor- β 1 that are involved in the inflammatory response (Shan et al. 2017).

Beyond that, naringenin is also the core precursor of other more complex flavonoids, namely, eriodictyol, taxifolin, and luteolin, that also have relevant therapeutic potential (Marienhagen and Bott 2013). Considering the interesting biological activities associated with naringenin and an increasing interest on its exploitation as pharmaceutical and nutraceutical compound, as well as its function as precursor of other interesting molecules, its market size is projected to accomplish USD 230.9 million by 2031 with a compound annual growth rate of 4.6% (Growth Market Reports 2022).

Biosynthetic Pathway

Naringenin is naturally produced in plants through the action of several enzymes. The biosynthetic pathway responsible for its production can be divided into two parts, namely, the shikimate pathway and the phenylpropanoid pathway (Braga et al. 2019; Gomes et al. 2022a, b).

The production of *L*-tyrosine (Tyr) and *L*-phenylalanine (Phe) aromatic amino acids is achieved by the shikimate pathway (Fig. 2). These amino acids are the major precursors of naringenin and other flavonoids (Milke et al. 2018). The synthesis of these two aromatic amino acids starts with the conversion of glucose into erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) through the glycolysis and pentose phosphate (PP) pathway, respectively (Averesch and Krömer 2018; Tohge et al. 2013). Then, E4P and PEP are used by 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) synthase (DAHPS) to produce DAHP. Then, 3-dehydroquinate (DHQ) synthase (DHQS) converts DAHP into DHQ that is further converted into shikimate by the DHQ dehydratase/shikimate dehydrogenase (DHQD/SDH) bifunctional enzyme. The next steps correspond to the phosphorylation of shikimate into shikimate 3-phosphate (S3P) by shikimate kinase (SK). S3P is further condensed with other molecules of PEP to produce 5-enolpyruvylshikimate-3-phosphate (EPSP) by the action of EPSP synthase (EPSPS). Finally, chorismate (CHO) is produced by dephosphorylation of EPSP by chorismate synthase (CS) (Marchiosi et al. 2020). CHO can be finally converted into Tyr and Phe by two different routes. The first step for both routes is catalyzed by CHO mutase (CM) that converts CHO into prephenate (Gomes et al. 2022a). On the Phe-producing pathway, prephenate is converted by prephenate aminotransferase (PAT) or prephenate dehydratase (PDT) into aroenate and phenylpyruvate, respectively. Then, aroenate dehydratase (ADT) or aromatic amino acid aminotransferase (AAT) catalyzes the final conversion of the respective compounds into Phe. On the other hand, for Tyr production, prephenate dehydrogenase (PDH) can alternatively convert prephenate into *p*-hydroxyphenylpyruvate that is then converted by the aromatic amino acid aminotransferase (AAT) into Tyr. As an alternative, aroenate dehydrogenase (ADH) can also perform the conversion of aroenate into this aromatic amino acid (Tzin and Galili 2010; Yoo et al. 2013).

After the production of the aromatic amino acids Phe and Tyr through the shikimate pathway, both amino acids can be used as precursors of the phenylpropanoid pathway to achieve the synthesis of naringenin (Fig. 3) (Gomes et al. 2022b). When Phe is used as a precursor, this compound is converted by the action of phenylalanine ammonia-lyase (PAL) into cinnamic acid that is further converted into *p*-coumaric acid via a hydroxylation step catalyzed by cinnamate-4-hydroxylase (C4H) (Pandey et al. 2016). Using Tyr as an aromatic amino acid precursor, only the action of tyrosine ammonia-lyase (TAL) is required to obtain *p*-coumaric acid (Watts et al. 2006). Then, 4-coumarate-CoA ligase (4CL) converts *p*-coumaric acid into *p*-coumaroyl-CoA that is converted into naringenin chalcone by the action of the type III polyketide synthase enzyme chalcone synthase (CHS) using three molecules of malonyl-CoA as extender substrate (Liou et al. 2018). Finally, chalcone isomerase (CHI) catalyzes the intramolecular cyclization of the chalcone ring converting naringenin chalcone into naringenin (Jez and Noel 2002).

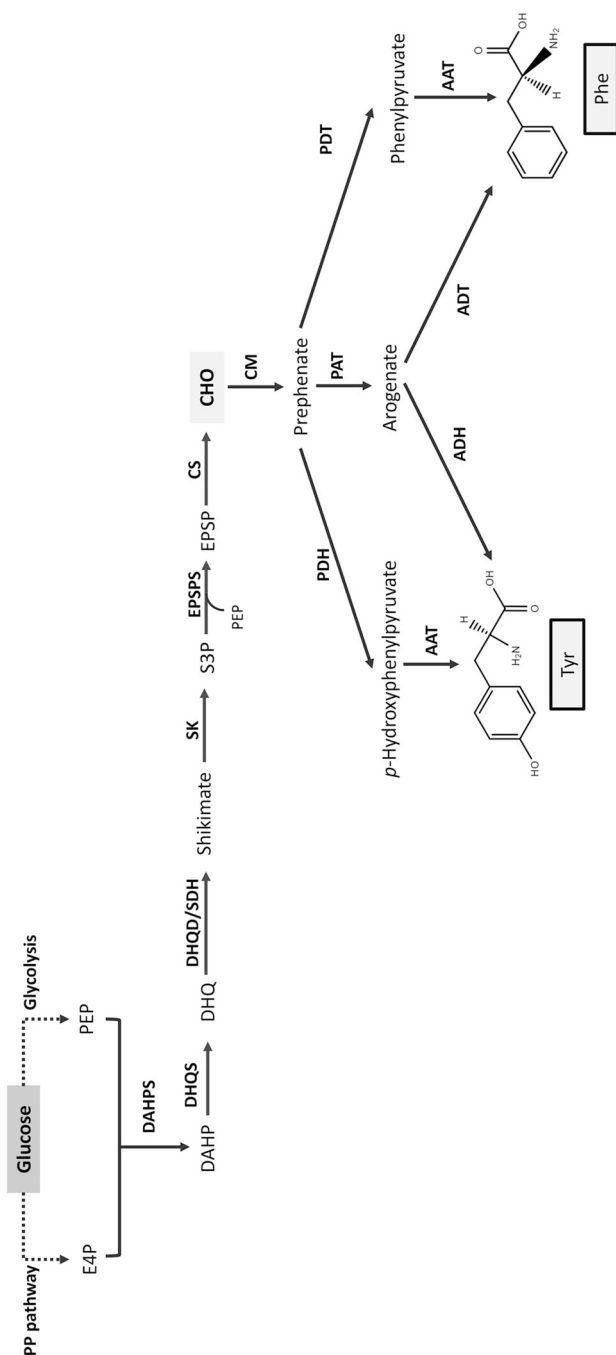


Fig. 2 Schematic representation of shikimate pathway that is responsible for the synthesis of *L*-tyrosine (Tyr) and *L*-phenylalanine (Phe) in plants. Erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) are obtained from glucose through the action of pentose phosphate (PP) pathway and glycolysis, respectively. The other main intermediaries of the represented biosynthetic pathway are 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP), 5-enolpyruvylshikimate-3-phosphate (EPSP), arogenate, chorismate (CHO), *p*-hydroxyphenylpyruvate, phenylpyruvate, shikimate, and shikimate 3-phosphate (S3P). The enzymes involved in the biosynthetic pathway are aromatic amino acid aminotransferase (AAT), arogenate dehydratase (ADT), arogenate dehydrogenase (ADH), chorismate synthase (CS), chorismate mutase (CM), DAHP synthase (DAHPS), DHQ dehydratase/shikimate dehydrogenase (DHQD/SDH), DHQ synthase (DHQS), EPSP synthase (EPSPS), prephenate aminotransferase (PAT), prephenate dehydrogenase (PDH), and prephenate dehydratase (PDT)

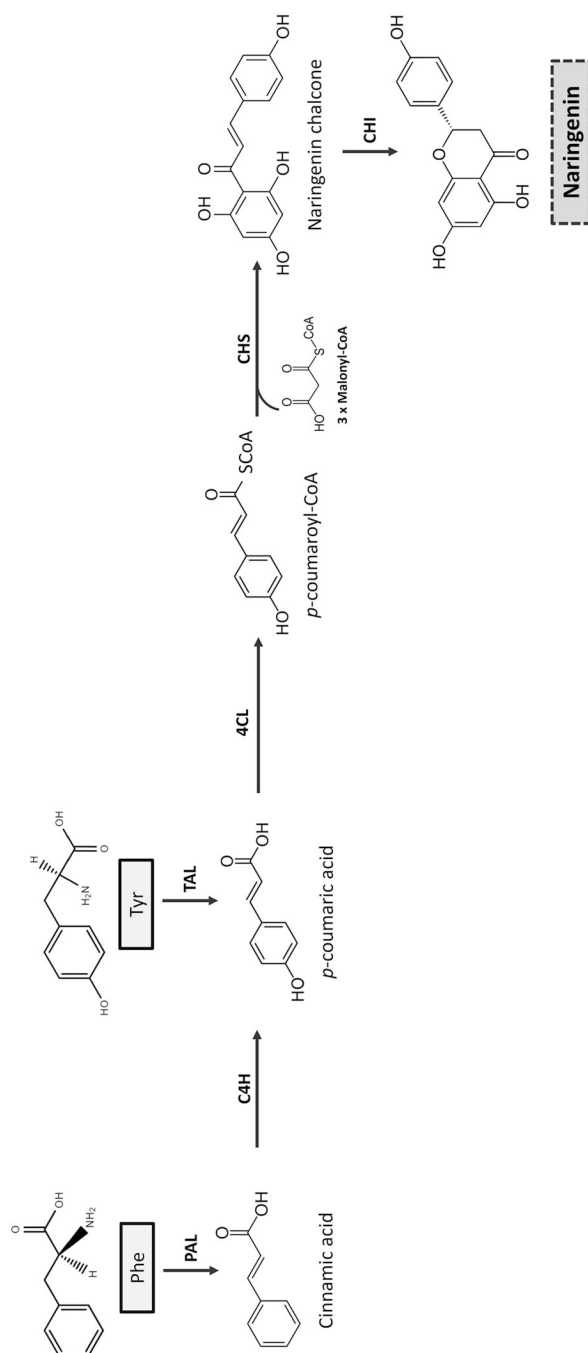


Fig. 3 Schematic representation of the naringenin biosynthetic pathway from the aromatic amino acids *L*-tyrosine (Tyr) and *L*-phenylalanine (Phe). In this pathway, three molecules of malonyl-CoA are used as extender substrate for the synthesis of naringenin chalcone from *p*-coumaroyl-CoA. The enzymes involved in the pathway are 4-coumarate-CoA ligase (4CL), chalcone isomerase (CHI), chalcone synthase (CHS), cinnamic acid 4-hydroxylase (C4H), phenylalanine ammonia-lyase (PAL), and tyrosine ammonia-lyase (TAL)

Plant Extraction Versus Chemical Synthesis Versus Heterologous Production

As a plant secondary metabolite, naringenin is accumulated only in residual quantities in plants. The production and accumulation of these compounds vary greatly among the different plant species and plant tissues, and it is also affected by the geographical and seasonal variations (Rodrigues et al. 2015b). The plant extraction is considered a difficult process being characterized by very low yields (Gomes et al. 2022a; Li et al. 2020b; Rainha et al. 2020a; Rodrigues and Rodrigues 2020; Sheng et al. 2020; Wang et al. 2016). Moreover, complex and high energy downstream processing is also required to obtain pure compounds increasing the costs to obtain the compound of interest (Najmi et al. 2022). The chemical synthesis has emerged as an alternative way to obtain these compounds. However, this process requires the utilization of toxic solvents and expensive substrates, and it involves complex reaction cascades and hard downstream purification processes. For this reason, the chemical synthesis is considered an expensive and non-green process (Liu et al. 2017; Milke et al. 2018).

Obtaining relevant amounts of naringenin using a cost-effective and green production process is crucial for satisfying the compound demand and to allow a deeper understanding of its biological activities and health effects, as well as its further incorporation into pharmaceutical and nutraceutical compounds. The heterologous production of this compound is considered an interesting solution since it is more environmentally friendly methodology. The heterologous production using plants as chassis can be advantageous since the genes of the phenylpropanoid pathway are natively present on the plant. For this reason, only few genes of the pathway need to be introduced into the plant chassis. Nevertheless, the plant possesses competing pathways that can convert this compound into others, leading to a decrease in the production yields. Moreover, the production of complex mixtures of compounds results in difficult downstream processing and purification processes. The use of plants as heterologous chassis has other limitations like the long growing periods and the growth dependence of the right weather conditions and on the fertile land availability and occupation. Moreover, the genetic manipulation of plants is still considered a challenging process, and their reported genomic instability can lead to the loss of gene clusters (Ebrahimi and Mokhtari 2017; Rainha et al. 2020a; Wilson and Roberts 2012). The use of plant cell cultures instead of the whole plant as chassis can be an alternative solution since it has the advantages of being independent of seasonal, environmental, and geographical variations since the cultures are manipulated in controlled environments (Smetanska 2008; Wilson and Roberts 2012). Despite these advantages, the use of plant cell cultures has also some limitations such as the low growth rates and physiological heterogeneity, low metabolic content, variable yields, and genetic instability. Moreover, the cultures are more susceptible to stress and aggregation. Besides, operational limitations also affect the use of these cell cultures, namely, the wall adhesion, shear sensitivity, mixing, and light requirements (Rainha et al. 2020a; Smetanska 2008).

The use of microorganisms as chassis for the heterologous production of plant naturally occurring compounds has emerged in the last years as an interesting alternative and efficient solution to produce these compounds (Amorim et al. 2022; Braga et al. 2022; Krivoruchko and Nielsen 2015; Milke et al. 2018; Rainha et al. 2022a; Rodrigues et al. 2022, 2020, 2015a; Rodrigues and Rodrigues 2022). The use of microbial chassis has several advantages compared to the plant extraction and chemical synthesis. Microorganisms are easy to grow, and their fast growth cycles lead to short production times, enabling the production of the same or higher amounts of the desired compounds in less time compared to plants (Gomes et al. 2022a; Krivoruchko and Nielsen 2015; Lussier et al. 2012). Microorganisms are also able to produce the precursors (the aromatic amino acids Phe and Tyr) and the extender substrate (malonyl-CoA) used on the biosynthetic pathway responsible to produce polyphenols and, specifically, naringenin. Moreover, the construction and introduction of the complete biosynthetic pathway and its efficient expression and the production of functional enzymes are required (Milke et al. 2018). The introduction of numerous heterologous genes in a microbial host can be challenging. However, over the last years, genetic manipulation of microorganisms has significantly improved, and various methodologies have been developed and reported, becoming much easier to construct efficient microbial cell factories (Gomes et al. 2022a; Rainha et al. 2020a, b; Rodrigues et al. 2017). Moreover, several biotechnology processes have been developed to use renewable feedstocks as substrates for growth and production of the compounds of interest, contributing to the circular economy. Additionally, several efforts have been made to scale up the production processes using microbial chassis from a laboratory scale to industrial bioreactors. All these advantages contribute to establishing microbial cell factories as an easier, cost-effective, and environmentally friendly production process (Braga et al. 2019; Gomes et al. 2022a, b; Rodrigues and Rodrigues 2020; Wang et al. 2016).

Advances on the Construction of Microbial Chassis Able to Produce Naringenin

Among the microorganisms that have been exploited as microbial chassis to produce naringenin, *Escherichia coli* and *Saccharomyces cerevisiae* are the most commonly used. Both model microorganisms are well characterized, with their genome already sequenced, and several molecular approaches and synthetic biology and genome engineering tools are well established (Cho et al. 2022). Moreover, they are easy to grow and manipulate, being highly adapted to the laboratorial conditions. Furthermore, several optimized fermentation conditions and scale-up strategies have been already established for both microorganisms (Adams 2016; Yang et al. 2020).

E. coli has been widely used as microbial chassis for the synthesis of several polyphenols, including naringenin (Dunstan et al. 2020; Fowler et al. 2009; Leonard et al. 2007, 2008; Liu et al. 2021; Santos et al. 2011; Wu et al. 2014, 2015; Xu et al. 2011; Zhou et al. 2020a, b). *E. coli* is a prokaryotic organism that is characterized by fast growth rates, short doubling period, and high expression levels of heterologous

genes. These features are crucial to achieve an efficient production process (Yang et al. 2020). The disadvantages of using *E. coli* for the production of plant-derived compounds are the lack of ability of this microorganism to perform post-translational modifications and the lack of intracellular organelles (similar to the ones present on plant cells) which can limit the efficient and functional expression of the insoluble and membrane-bound plant enzymes of the pathway (Xu et al. 2020).

Regarding *S. cerevisiae*, this microorganism is considered generally recognized as safe (GRAS) which facilitates the use of the produced compounds by the pharmaceutical and nutraceutical companies. Compared to *E. coli*, *S. cerevisiae* has higher doubling times and slower growth rates. In addition, it has been widely used as microbial chassis due to its high metabolic ability. *S. cerevisiae* has the advantage to perform post-translational modifications. Moreover, this microorganism contains intracellular compartments that mimic those present in plant cells, being useful for the efficient expression of eukaryotic cytochrome P450 enzymes (C4H and C3H) required in polyphenols biosynthetic pathways (Chen et al. 2020; Krivoruchko and Nielsen 2015; Liu et al. 2022; Rainha et al. 2022b; Rodrigues and Rodrigues 2022). Therefore, over the last years, this eukaryotic organism has also been already engineered to achieve the production of naringenin (Koopman et al. 2012; Li et al. 2021; Lyu et al. 2017, 2019; Mao et al. 2023; Rodriguez et al. 2017; Trantas et al. 2009; Zhang et al. 2021).

The production of naringenin was also already achieved in the non-model organisms *Yarrowia lipolytica* and *Streptomyces* spp. (Lv et al. 2019a, b; Palmer et al. 2020; Wei et al. 2020; Ye et al. 2023). Despite not being model organisms, those organisms offer advantages for metabolic engineering purposes. Both strains are genetically well characterized, and synthetic biology and metabolic engineering tools have been developed for their use (Cobb et al. 2015; Larroude et al. 2020; Ledesma-Amaro and Nicaud 2016; Liu et al. 2022; Tao et al. 2018). The eukaryotic organism *Y. lipolytica* has a high metabolic flux toward the tricarboxylic acid (TCA) cycle, which is useful to produce acetyl-CoA and, consequently, malonyl-CoA that is required as extender substrate of the biosynthetic pathway. This strain can also tolerate harsh conditions of pH and salt and high concentrations of organic compounds. Moreover, its GRAS status makes this organism an attractive microbial chassis to produce compounds for incorporation into the nutraceutical and pharmaceutical markets (Bilal et al. 2021). Despite this advantages, *Y. lipolytica* showed lack of plasmid stability which can be difficult for the expression of heterologous genes (Liu et al. 2022). *Streptomyces* spp. have the advantage of being able to naturally produce several complex secondary metabolites, including antibiotics and other polyketides. This Gram-positive prokaryote also contains a high metabolic flux, thereby providing high amounts of the precursors required for the biosynthetic pathway. However, some *Streptomyces* sp. strains are hard to genetically modify, and their genetic manipulation times are longer. Compared to *E. coli* and *S. cerevisiae*, this microorganism also has longer cell doubling periods and slower growth rates, resulting in extended production processes (Hwang et al. 2021; Liu et al. 2018, 2022). In this section, the advances made to produce naringenin in these microbial chassis will be presented. The production levels obtained in each microbial chassis throughout the last years are summarized in Tables 1, 2 and 3.

Table 1 Heterologous production of naringenin in *Escherichia coli*. The genes of the biosynthetic pathway, improvements on precursors' availability, substrate, conditions of fermentation, and production titers obtained are listed

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate	Conditions	Production (mg/L)	References
<i>Escherichia coli</i>	BL21Star	<i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	–	<i>p</i> -Coumaric acid (4.9 g/L)	Shake flasks 36 h	119	Leonard et al. (2007)
	BL21Star	<i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve malonyl-CoA: ↓ <i>fabB</i> and <i>fabF</i>	<i>p</i> -Coumaric acid (4.9 g/L)	Shake flasks 36 h	186	Leonard et al. (2008)
	BL21Star	<i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve malonyl-CoA: ↑ <i>acc</i> and <i>bpl</i> ; ↓ <i>sdhA</i> , <i>citE</i> , <i>brnQ</i> , and <i>adhE</i>	<i>p</i> -Coumaric acid (2.46 g/L)	Shake flasks 38 h	215	Fowler et al. (2009)
	BL21 Star™ (DE3)	<i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve malonyl-CoA: ↑ <i>acc</i> , <i>pdh</i> , and <i>pgk</i> ; ↓ <i>fumC</i> and <i>sucC</i>	<i>p</i> -Coumaric acid (4.03 g/L)	Shake flasks 24 h	474	Xu et al. (2011)
	BL21 (DE3)	<i>RgTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve malonyl-CoA: ↓ <i>fabB</i> , <i>fabF</i> , <i>fumC</i> , <i>sucC</i> ; and <i>adhE</i>	Tyrosine (5.43 g/L)	Shake flasks 48 h	421.6	Wu et al. (2015)
	K12 (MG1655)	<i>RgTAL</i> , <i>Sc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve malonyl-CoA: Addition of cerulenin	Glucose (5 g/L)	Shake flasks 48 h	84	Santos et al. (2011)
	BL21 (DE3)	<i>RgTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aroG</i> and <i>tyrA</i> Improve malonyl-CoA: ↑ <i>matB</i> and <i>matC</i> from <i>Rhizobium trifolii</i>	Glucose (5 g/L)	Shake flasks 48 h	100.64	Wu et al. (2014)

(continued)

Table 1 (continued)

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate	Conditions	Production (mg/L)	References
	DH5α	<i>F₁TAL</i> , <i>Gm4CL</i> , <i>AtCHS</i> , <i>AtCHI</i>	Improve aromatic amino acids: ↑ <i>ppsA</i> and feedback inhibition-resistant versions of <i>aroG</i> and <i>tyr4</i> ; ↓ <i>tyrR</i> gene and <i>pheL4</i> Improve malonyl-CoA: Addition of cerulenin	Glycerol (4 g/L)	Deep well plates 48 h	484	Dunstan et al. (2020)
		<i>TcTAL</i> , <i>Pc4CL</i> , <i>MsCHI</i> , <i>PhCHS</i>	Improve malonyl-CoA: Dynamic regulation biosensor	Glucose (10 g/L)	Shake flask 27 h	251.4	Zhou et al. (2020a)
		<i>TcTAL</i> , <i>Pc4CL</i> , <i>MsCHI</i> , <i>PhCHS</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aroG</i> and <i>tyr4</i> Improve malonyl-CoA: Dynamic regulation biosensor	Glucose (15 g/L) + glycerol (2.5 g/L) Glucose (10 g/L) + 500 g/l glucose (30 mL of feeding solution) at 60 h, 72 h, 90 h, and 96 h	Shake flask 72 h Fed-batch bioreactor 84 h	485 588	Zhou et al. (2020b)

^a**Genes:** *4CL* 4-coumarate-CoA ligase, *C4H* cinnamate-4-hydroxylase, *CHI* chalcone isomerase, *CHS* chalcone synthase, *CPR* cytochrome P450 reductase, *PAL* phenylalanine ammonia lyase, *TAL* tyrosine ammonia lyase. Gene source: *At Arabidopsis thaliana*, *Gm Glycine max*, *Ha Hypericum androsaemum*, *His Huperzia serrata*, *Ms Medicago sativa*, *Ni Nicotiana tabacum*, *Pc Petroselinum crispum*, *Ph Poplar hybrid*, *Ps Paeonia suffruticosa*, *Rs Rhododendron simsii*, *Rt Rhodotorula toruloides*, *Se Salmonella enterica*, *Tc Trichosporon cutaneum*
^b↑ means “expression”; ↓ means “repression”; *acc* acetyl-CoA carboxylase, *acs* acetyl-CoA synthase, *adhE* acetaldehyde dehydrogenase, *aroG*, *aroI*, *aro7*, and *aro4* 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHPS) synthase (DAHPS) genes, *bpl* biotin ligase, *brnQ* amino acid transporter, *citE* and *sdhA* citrate cycle genes, *E4P* erythrose-4-phosphate, *fabB* and *fabF* fatty acids synthases, *fumC* fumarate, *matB* and *matC* malonate assimilation pathway, *pheL4* and *tyrA* chorismate mutase/prephenate dehydrogenase, *pdc*, *pdc5*, and *aro10* phenylpyruvate decarboxylase (PDC) genes, PEX10 peroxisome biogenesis factor gene, *PEX11* fatty acid β-oxidation gene, *sucC* succinyl-CoA synthetase, *tyrR* transcriptional regulator of aromatic amino acid biosynthesis gene

Table 2 Heterologous production of naringenin in *Saccharomyces cerevisiae*. The genes of the biosynthetic pathway, improvements on precursors' availability, substrate, conditions of fermentation, and production titers obtained are listed

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate ^c	Conditions	Production (mg/L)	References
<i>S. cerevisiae</i>	YPH499	<i>PhPAL</i> , <i>Gm4CL</i> , <i>GmCHS</i> , <i>GmCHI</i>	–	Phenylalanine (1.7 g/L)	Shake flask 150 h	8.9	Trantas et al. (2009)
				Phenylalanine (1.7 g/L) + <i>p</i> -coumaric acid (1.64 g/L)	Shake flasks 100 h	15.6	
	CENPK2-1C	<i>AtPAL</i> , <i>AtCHI</i> , <i>AtCHS</i> , <i>At4CL</i> , <i>RcTAL</i> , <i>AtC4H</i> , <i>AtCPR</i> , <i>AtCHS</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aroG</i> ; <i>lfdc</i>	Glucose (20 g/L)	Shake flasks 150 h	54.5	Koopman et al. (2012)
					Batch bioreactor 38 h	108.9	
	CENPK102-5B	<i>FjTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve aromatic amino acids: ↑ <i>aro4</i> and <i>aro7</i> ; <i>lfdc5</i> and <i>aro10</i>	Synthetic fed-batch medium containing glucose polymer	Microtiter plates 72 h	1.55	Rodriguez et al. (2017)
	BY4741	<i>FjTAL</i> , <i>At4CL</i> , <i>HcCHS</i> , <i>PhCHI</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aro4</i> ; <i>lfdc5</i> and <i>aro10</i>	Glucose (20 g/L)	Shake flasks 72 h	90	Lyu et al. (2017)
	BY4741	<i>FjTAL</i> , <i>At4CL</i> , <i>HcCHS</i> , <i>PhCHI</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aro4</i> ; <i>lfdc5</i> and <i>aro10</i>	Glucose (20 g/L)	Shake flasks 72 h	220	Lyu et al. (2019)

(continued)

Table 2 (continued)

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate ^c	Conditions	Production (mg/L)	References
	CEN.PK2-1D	<i>FjTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aro4</i> ; ↓ <i>pdcs</i> and <i>aro10</i>	Glucose (20 g/L)	Deep well plates 72 h	149.8	Li et al. (2021)
	CEN.PK2-1D	<i>FjTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve aromatic amino acids: ↑ feedback inhibition-resistant versions of <i>aro7</i> and <i>aro4</i> ; ↓ <i>pdcs</i> and <i>aro10</i> Improve malonyl-CoA: ↑ PEX11 gene	Glucose (20 g/L) + 400 g/L glucose (500 mL of feeding solution) at a constant rate of 6 mL/h	Fed-batch bioreactor 108 h	1129.4	Zhang et al. (2021)
	IMX581	<i>AtPAL</i> , <i>AtC4H</i> , <i>At4CL</i> , <i>RsCHS</i> , <i>PcCHI</i>	Improve malonyl-CoA: ↑ <i>matB</i> and <i>matC</i> from <i>R. trifolii</i> ; dynamic regulation biosensor	Glucose (30 g/L)	Shake flasks 96 h	563	Mao et al. (2023)
				Glucose (30 g/L) + 200 g/L glucose solution with a feeding rate that was exponentially increased	Fed-batch bioreactor 120 h	2050	

^a**Genes:** *4CL* 4-coumarate-CoA ligase, *C4H* cinnamate-4-hydroxylase, *CHI* chalcone synthase, *CHS* chalcone synthase, *CPR* cytochrome P450 reductase, *PAL* phenylalanine ammonia lyase, *TAL* tyrosine ammonia lyase. Gene source: *At Arabidopsis thaliana*, *Gm Glycine max*, *Ha Hypericum androsaemum*, *His Huperzia serrata*, *Ms Medicago sativa*, *Nt Nicotiana tabacum*, *Pc Petroselinum crispum*, *Ph Poplar hybrid*, *Ps Paeonia suffruticosa*, *Rs Rhododendron simsii*, *Ri Rhodotorula toruloides*, *Se Salmonella enterica*, *Tc Trichosporon cutaneum*

^b↑ means “expression”; ↓ means “repression”; *acc* acetyl-CoA carboxylase, *acs* acetyl-CoA synthase, *adhE* acetaldehyde dehydrogenase, *aroG*, *aroI*, *aro7*, and *aro4* 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) synthase (DAHPS) genes, *bpl* biotin ligase, *brnQ* amino acid transporter, *citE* and *sdhA* citrate cycle genes, *E4P* erythrose-4-phosphate, *fabB* and *fabF* fatty acids synthases, *fumC* fumarate, *matB* and *matC* malonate assimilation pathway, *pheLA* and *tyrA* chorismate mutase/prephenate dehydrogenase, *pdcs*, *pdcs*, and *aro10* phenylpyruvate decarboxylase (PDC) genes, PEX10 peroxisome biogenesis factor gene, PEX11 fatty acid β-oxidation gene, *sucC* succinyl-CoA synthetase, *tyrR* transcriptional regulator of aromatic amino acid biosynthesis gene

^cGlucose polymer (undefined concentration) is converted into glucose monomers by releasing enzymes during the fermentation

Table 3 Heterologous production of naringenin in *Yarrowia lipolytica*, and *Streptomyces albidoflavus*. The genes of the biosynthetic pathway, improvements on precursors' availability, substrate, conditions of fermentation, and production titers obtained are listed

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate	Conditions	Production (mg/L)	References
<i>Y. lipolytica</i>	PO1f	<i>RtTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	–	Glucose (40 g/L)	Shake flasks 144 h	71.2	Lv et al. (2019a)
	PO1f	<i>RtTAL</i> , <i>Pc4CL</i> , <i>PhCHS</i> , <i>MsCHI</i>	Improve aromatic amino acids: ↑ <i>aroI</i> Improve malonyl-CoA: ↑ <i>acc</i> and <i>acs</i>	Glucose (40 g/L)	Shake flasks 220 h	252.4	Lv et al. (2019b)
	PO1f	<i>RgTAL</i> , <i>A4CL</i> , <i>AtCHS</i> , <i>AtCHI</i>	Improve aromatic amino acids: ↑ xylose assimilation pathway to increase E4P supply	Glucose (20 g/L) + xylose (60 g/L)	Shake flasks 144 h	715.3	Wei et al. (2020)
	PO1f	<i>SeTAL</i> , <i>Nt4CL</i> , <i>HsCHS</i>	Improve aromatic amino acids: ↑ feedback inhibition resistant versions of <i>aro4</i> ; improve malonyl-CoA: ↑ PEX10 gene	Glucose (80 g/L)	Batch bioreactor 196 h	357	Palmer et al. (2020)
				Glucose (80 g/L) + 400 g/L glucose (200 mL of feeding solution) at 120 h + 400 g/L glucose (100 mL of feeding solution) at 192 h and 240 h	Fed-batch bioreactor 312 h	898	

(continued)

Table 3 (continued)

Microorganism	Background strain	Genes ^a	Improvements on precursors availability ^b	Substrate	Conditions	Production (mg/L)	References
<i>Streptomyces albidoflavus</i>	J1074	TAL, 4CL, CHS, CHI (unknown origins)	Improve malonyl-CoA: ↑ <i>matB</i> and <i>matC</i> from <i>rhizobium trifolii</i>	Tyrosine (3.6 g/L) + <i>p</i> -coumaric acid (0.82 g/L)	Shake flasks 192 h	22.4	Ye et al. (2023)

^a**Genes:** *4CL* 4-coumarate-CoA ligase, *C4H* cinnamate-4-hydroxylase, *CHI* chalcone isomerase, *CHS* chalcone synthase, *CPR* cytochrome P450 reductase, *PAL* phenylalanine ammonia lyase, *TAL* tyrosine ammonia lyase. Gene source: *At Arabidopsis thaliana*, *Gm Glycine max*, *Ha Hypericum androsaemum*, *Hs Hyperzia serrata*, *Ms Medicago sativa*, *Nt Nicotiana tabacum*, *Pc Petroselinum crispum*, *Ph Poplar hybrid*, *Ps Paeonia suffruticosa*, *Rs Rhododendron simsii*, *Rt Rhodotorula toruloides*, *Se Salmonella enterica*, *Tc Trichosporon cutaneum*
^b↑ means “expression”; ↓ means “repression”; *acc* acetyl-CoA carboxylase, *acs* acetyl-CoA synthase, *adhE* acetaldehyde dehydrogenase, *aroG*, *aroI*, *aro7*, and *aro4* 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHPS) genes, *bpl* biotin ligase, *bmiQ* amino acid transporter, *citE* and *sdhA* citrate cycle genes, *E4P* erythrose-4-phosphate, *fabB* and *fabF* fatty acids synthases, *fumC* fumarase, *matB* and *matC* malonate assimilation pathway, *pheLA* and *tyrA* chorismate mutase/prephenate dehydrogenase, *pdc*, *pdc5*, and *aro10* phenylpyruvate decarboxylase (PDC) genes, *PEX10* peroxisome biogenesis factor gene, *PEX11* fatty acid β-oxidation gene, *sucC* succinyl-CoA synthetase, *tyrR* transcriptional regulator of aromatic amino acid biosynthesis gene

***Escherichia coli* as Microbial Cell Factory to Produce Naringenin**

The first reports of naringenin production in engineered *E. coli* strains were performed using *p*-coumaric acid as substrate (Fowler et al. 2009; Leonard et al. 2008; Xu et al. 2011). *p*-Coumaric acid is the hydroxycinnamic acid precursor on the naringenin biosynthetic pathway. The supplementation of these precursors to the production media incurs a high cost, posing a significant drawback for scaling up the production experiments and for further implementation of a cost-effective industrial production process. In the first approach, Leonard et al. (2007) reported the production of 119 mg/L of naringenin by expressing the 4CL gene from *Petroselinum crispum* (*Pc4CL*), CHS from *Petunia hybrida* (*PhCHS*), and CHI from *Medicago sativa* (*MsCHI*). Later, the authors made efforts to optimize this strain to be able to produce higher amounts of naringenin. This optimization was performed by improving the flux toward malonyl-CoA. The malonyl-CoA availability inside of the cells is considered low, and this compound is primarily used by the central metabolism to produce phospholipids and fatty acids. Considering that, only very residual amounts are still available to be used on the production of secondary metabolite compounds (Milke and Marienhagen 2020). Since malonyl-CoA is the extender substrate of the pathway and three molecules are required to achieve the final synthesis of naringenin, the authors repressed the fatty acid synthases (FAS), specifically FabB and FabF, inhibiting the competing pathway responsible for the synthesis of fatty acids. This optimized strain was able to produce 186 mg/L of naringenin (Leonard et al. 2008). One year later, Fowler et al. (2009) also reported a strain able to produce naringenin with the supplementation of the hydroxycinnamic acid to the culture media. In this study, the authors also made modifications to drive the metabolic flux for a higher production of malonyl-CoA. The best optimized strain was constructed by overexpressing acetyl-CoA carboxylase (ACC) and biotin ligase (BPL) in combination with the repression of the competing citrate cycle gene *sdhA* and *citE*, the amino acid transporter gene *brnQ*, and the pyruvate consumer gene *adhE*. By expressing the same 4CL, CHS, and CHI genes from the studies performed by Leonard et al. (2007) and Leonard et al. (2008), the authors achieved a production of 215 mg/L of naringenin in the optimized strain (Fowler et al. 2009). Afterward, Xu et al. (2011) also constructed an *E. coli* strain able to produce naringenin using *p*-coumaric acid as substrate. The biosynthetic pathway responsible to produce naringenin was also composed by *Pc4CL*, *PhCHS*, and *MsCHI*. In this study, the authors also optimized the *E. coli* strain toward the improvement of the metabolic flux for the synthesis of malonyl-CoA. The highest production of naringenin was achieved when the native genes ACC, pyruvate dehydrogenase (PDH), and phosphoglycerate kinase (PGK) were overexpressed and the fumarase (*fumC*) and succinyl-CoA synthetase (*sucC*) genes were knocked out. The production of naringenin achieved on this strain was 474 mg/L (Xu et al. 2011).

As alternative to the supplementation of hydroxycinnamic acids to the media, the supplementation of Tyr has also been considered by several authors as an alternative to achieve higher production levels of plant natural compounds. Wu et al. (2015) tested the production of naringenin by supplementing Tyr to the production media.

In this study, the authors also performed genome modifications in the *E. coli* strain to improve the metabolic flux toward malonyl-CoA. Using the clustered regularly interspaced short palindromic repeats interference (CRISPRi) system, the expression of identified gene targets from the competing TCA cycle and glycolysis was fine-tuned. The constructed *E. coli* strain, with a downregulation on the *fabB*, *fabF*, *fumC*, *sucC*, and acetaldehyde dehydrogenase (*adhE*) genes, exhibited significantly higher levels of malonyl-CoA. The biosynthetic pathway responsible to produce naringenin production composed by TAL from *Rhodotorula glutinis* (RgTAL), Pc4CL, PhCHS, and MsCHI was expressed on this optimized strain, and the final production of 421.6 mg/L of naringenin was achieved. This production level matches the titers previously achieved by Xu et al. (2011) with supplementation of *p*-coumaric acid.

As the supplementation of hydroxycinnamic acids and aromatic amino acids in the production media results in commercially unfavorable and expensive production processes due to the high market prices of these compounds, several efforts have been made to produce naringenin from simple carbon sources, such as glucose. De novo production of naringenin was firstly reported by Santos et al. (2011). In this work, the complete biosynthetic pathway responsible for naringenin production from the aromatic amino acid Tyr was assembled, and naringenin was produced using glucose as substrate. Genes from different origins were tested to find the best combination to achieve higher productions of naringenin. The highest production of this compound was achieved when RgTAL, 4CL from *Streptomyces coelicolor* (Sc4CL), PhCHS, and MsCHI were expressed. The production experiments were performed with and without the supplementation of cerulenin. Cerulenin is a fatty acid inhibitor that is used to improve the malonyl-CoA flux. Using this inhibitor, the production of naringenin from glucose increased from 29 to 84 mg/L (Santos et al. 2011). In fact, the amount of Tyr that is naturally produced by *E. coli* using glucose as substrate can be limited since this compound is used by the primary metabolism for the synthesis of several key metabolic compounds. For this reason, improving the flux toward the synthesis of this aromatic amino acid can be mandatory to overcome this limiting factor and achieve higher production levels of the desired compound. Taking this into account, Wu et al. (2014) identified potential targets to improve the Tyr flux and constructed a tyrosine-overproducing *E. coli* strain by overexpressing feedback inhibition-resistant versions of DAHPS gene (*aroG*) and chorismate mutase/prephenate dehydrogenase gene (*tyrA*). Contrary to the native enzymes, these resistant versions of the enzymes are not affected by the amount of the aromatic amino acids that are produced. For this reason, the aromatic amino acids' synthesis is not inhibited, and the metabolic flux toward their synthesis is constant. Beyond these modifications, the heterologous genes *matB* and *matC* from *Rhizobium trifolii* were also expressed. These genes are involved in the conversion of malonate into malonyl-CoA, improving the metabolic flux toward the synthesis of this compound without the need of adding the expensive inhibitor compound cerulenin. When RgTAL, Pc4CL, PhCHS, and MsCHI were expressed in this optimal constructed strain, it was able to de novo produce 100.64 mg/L of naringenin (Wu et al. 2014). Some years later, Dunstan et al. (2020) also constructed a strain able to produce naringenin from simple carbon sources. In the first approach, the authors tested TAL

and 4CL from different origins in combination with CHS from *Arabidopsis thaliana* (*AtCHS*) and CHI also from *A. thaliana* (*AtCHI*). Among the tested pathways, only four were able to produce significant amounts of naringenin. The expression of these four pathways was tested in different *E. coli* strains, and *E. coli* DH5 α was selected as the best chassis. Moreover, operational optimizations were performed to select the optimal production media and determine the most effective carbon source (glucose vs glycerol) for achieving higher production levels. A titer of 466 mg/L of naringenin was obtained when terrific broth (TB) media containing 0.4% (w/v) of glycerol was used. The authors also attempted to increase this production levels by engineering the *E. coli* strain toward the improvement of Tyr metabolic flux. The best naringenin-producing chassis held a double knockout on the transcriptional regulator of aromatic amino acid biosynthesis (*tyrR*) gene and on the chorismate mutase/prephenate dehydratase (*pheLA*) bifunctional gene. Moreover, the expression of a plasmid holding feedback insensitive versions of *aroG* and *tyrA*, as well as the PEP synthase gene (*ppsA*), was also tested in this strain. This final optimized strain was able to produce 484 mg/L of naringenin. However, in this study, the authors supplemented the production media with cerulenin which represents a big limitation since it significantly increases the associated production costs (Dunstan et al. 2020). In the same year, Zhou et al. (2020a) designed an interesting strategy to improve the malonyl-CoA flux in *E. coli* and, consequently, improve the production of naringenin. The authors designed a dynamic regulation network composed by three modules (A, B, and C). Module A was composed by the naringenin biosynthetic pathway holding TAL from *Trichosporon cutaneum* (*TcTAL*), *Pc4CL*, *PhCHS*, and *MsCHI* genes. Module B inhibited the competing pathways involved into malonyl-CoA consumption, being activated by the presence of naringenin. Regarding module C, this regulated and improved the synthesis of malonyl-CoA being activated in the presence of *p*-coumaric acid. Afterward, random mutations using the commercial GeneMorph II random mutagenesis kit were performed in modules B and C, and the optimal constructed strain (Mut-17) was able to produce 251.4 mg/L. Later, the same authors used the constructed strain Mut-17 as platform strain to perform modifications to also improve the metabolic flux toward Tyr. By expressing the feedback inhibition-resistant version of the *aroG* and *tyrA* genes, and knocking out the *tyrR* repressor gene, the authors constructed the best-performing chassis able to produce naringenin. Using this strain, 485 mg/L of naringenin was produced in shake flask experiments. When the production process was set at a bioreactor scale (5 L), 588 mg/L of naringenin was produced using a fed-batch operation mode with constant pulses of glucose, with a productivity of the production process of 7 mg/L/h. This titer represents the highest value reported to date for the production of naringenin using *E. coli* as microbial chassis (Zhou et al. 2020b). While a significant progress has been achieved using *E. coli* as microbial chassis for naringenin production, there is still substantial work required for this chassis to be considered ideal for implementing a large-scale industrial production process. To achieve that, the production titers, yields, and productivities need to be significantly improved.

***Saccharomyces cerevisiae* as Microbial Cell Factory to Produce Naringenin**

As occurred with *E. coli*, the first reports of production of naringenin exploiting *S. cerevisiae* as chassis were also achieved with supplementation of hydroxycinnamic acid (*p*-coumaric acid) or aromatic amino acid (Phe or Tyr) precursors. Firstly, Trantas et al. (2009) reported the construction of a *S. cerevisiae* strain holding the naringenin biosynthetic pathway composed by PAL from *Poplar hybrid* (*Ph*PAL), 4CL from *Glycine max* (*Gm*4CL), C4H from *G. max* (*Gm*C4H), cytochrome P450 reductase (CPR) from *P. hybrid* (*Ph*CPR), CHS from *G. max* (*Gm*CHS), and CHI from *G. max* (*Gm*CHI). The authors tested the expression of the heterologous *Ph*CPR to try to enhance the activity of the C4H gene. When *Ph*CPR was expressed, *p*-coumaric acid production was fourfold improved showing that the yeast-native CPR activity is not enough to support the flux required by C4H enzyme. This strain was able to produce 8.9 mg/L and 15.6 mg/L of naringenin with Phe and *p*-coumaric acid supplementation, respectively (Trantas et al. 2009).

Later, several efforts were made to use simple carbon sources such as glucose as substrate. Koopman et al. (2012) reported de novo production of naringenin in *S. cerevisiae* for the first time. The constructed strain contained PAL from *A. thaliana* (*At*PAL), C4H from *A. thaliana* (*At*C4H), CPR from *A. thaliana* (*At*CPR), 4CL from *A. thaliana* (*At*4CL), *At*CHS, and *At*CHI. In the first shake flask experiment, only 1.47 mg/L of naringenin was produced. Since this production level was considered residual, the authors engineered the *S. cerevisiae* strain toward increasing the production of the aromatic amino acid Phe. In this strain, the DAHPS was replaced for a feedback-insensitive version of the gene to maintain a constant synthesis of the aromatic amino acids. Moreover, the authors also deleted the phenylpyruvate decarboxylase (PDC) gene. This gene is involved on the conversion of phenylpyruvate into phenylacetaldehyde. Since phenylpyruvate is the precursor of Phe, the deletion of the PDC gene prevents the pathway flux deviation. Moreover, the authors have also increased the number of copies of the *At*CHS gene and expressed TAL from *Rhodobacter capsulatus* (*Rc*TAL) to improve the catalytic efficiency of the strain. This final strain has shown larger production of naringenin compared to the non-optimized chassis. In shake flask cultures, 54.5 mg/L of naringenin was produced. In batch reactors (aerated and pH controlled), 108.9 mg/L of naringenin was produced (Koopman et al. 2012). Later, Rodriguez et al. (2017) also optimized a *S. cerevisiae* strain to improve the flux toward the synthesis of aromatic amino acids. The authors improved the aromatic amino acid synthesis by overexpressing the DAHPS and CM genes involved in their synthesis and by deleting the PDC genes (*PDC5* and *Aro10*) used in the competing pathway responsible for phenylacetaldehyde synthesis. Despite these modifications, this strain was only able to produce 1.55 mg/L of naringenin. However, this strain was further used for the synthesis of more complex flavonoids, like liquiritigenin, fisetin, kaempferol, resokaempferol, fisetin, and quercetin (Rodriguez et al. 2017). On the same year, Lyu et al. (2017) also constructed a modified *S. cerevisiae* strain for de novo production of naringenin. This strain was also modified to improve the metabolic flux for the

amino acid synthesis and prevent the pathway deviation. Deletions on the PDC5 and Aro10 genes as well as the integration of a feedback-insensitive version of the DAHPS gene (Aro4) were performed. By expressing TAL from *Flavobacterium johnsoniae* (FjTAL), At4CL, CHS from *Hypericum androsaemum* (HaCHS), and MsCHI, 90 mg/L of naringenin was obtained (Lyu et al. 2017). Later, the same authors attempted to optimize the culture operation conditions, and they were able to improve the naringenin production to 220 mg/L when Tween 80 was added to the production media. Tween 80 is a non-ionic surfactant compound that is reported to increase the enzymatic activity and cell biomass growth when added to the production media. Moreover, this compound can interact with the produced compound due to its hydrophobic parts and facilitates the transportation to the extracellular media (Lyu et al. 2019). Later, Li et al. (2021) integrated multiple copies of the complete naringenin biosynthetic pathway composed by FjTAL, Pc4CL, PhCHS, and MsCHI into the yeast genome using the repetitive ribosomal DNA (rDNA) clusters as the integration sites. The best constructed strain was able to produce 149.8 mg/L of naringenin (Li et al. 2021).

Beyond improving the flux toward the synthesis of the aromatic amino acid production, it is also important to improve the titers of the natively produced malonyl-CoA in *S. cerevisiae* since this compound is primarily required to perform the extension step and, consequently, produce naringenin. Taking this into account, Zhang et al. (2021) made several efforts to improve the malonyl-CoA intracellular concentration by increasing the acetyl-CoA supply using the fatty acid β -oxidation pathway as target and tested its effect on the naringenin production. Firstly, the authors investigated the impact of the supplementation of fatty acids on the naringenin production and verified that only unsaturated fatty acids lead to improvements on the production levels. Due to the high costs associated with supplying fatty acids to the media, the authors tested the use of soybean oil as the source of unsaturated fatty acids in combination with the expression of lipases. Soybean oil is considered a cheap substrate that can be hydrolyzed by lipases to produce fatty acids. The overexpression of a lipase from *S. cerevisiae* and soybean oil supplementation leads to an improvement on naringenin production by enhancing the fatty acid β -oxidation. Moreover, key genes from the fatty acid β -oxidation (PEX11, FOX1, FOX2, FOX3) were also overexpressed. The best-performing strain was obtained when only PEX11 gene was overexpressed. Using this strain, 1129.4 mg/L of naringenin was produced in a 5-L bioreactor using a fed-batch operation mode (Zhang et al. 2021). More recently, Mao et al. (2023) also developed a *S. cerevisiae* strain with several modifications to improve the malonyl-CoA flux, as well as to perform a dynamic regulation between *p*-coumaric acid and naringenin productions. Firstly, the authors proved that high extracellular concentrations of *p*-coumaric acid and low concentrations of native malonyl-CoA can limit the production of naringenin. Considering that, the authors attempted to decrease the accumulation of *p*-coumaric acid by testing the PAL expression under the control of different promoters. Moreover, a mutant version of the ACC gene with improved activity was expressed in combination with the alternative pathway composed by *matB* and *matC* genes from *R. trifolii* for the conversion of malonate into malonyl-

CoA to improve this compound availability. Afterward, a dynamic control strategy in combination with RNA interference (RNAi) was designed to adjust the expression of the competing pathway responsible for fatty acid biosynthesis. In this strategy, the FAS1 was downregulated, and, consequently, the malonyl-CoA was directed for naringenin synthesis resulting in an improvement of the naringenin production levels. In addition, the authors tested the effect of the expression of cellular transporters on the production of naringenin. Beyond the different transporters tested, the expression of the transporter of PDR12, which is reported to increase the tolerance of the cells to aromatic amino acids, leads to an improvement on the naringenin production levels. The combination of all these strategies allowed the construction of a strain able to produce 563 mg/L of naringenin on shake flask mimicking fed-batch conditions. When this strain was used at a bioreactor scale also using a fed-batch cultivation mode, 2.05 g/L of naringenin was produced. This production level corresponds to the highest de novo production of naringenin reported to date and represents a step forward to achieve the implementation of a production process at an industrial scale (Mao et al. 2023).

Non-model Organisms as Alternative Chassis to Produce Naringenin

Y. lipolytica was firstly engineered as microbial chassis for the production of naringenin and other flavonoids also with relevant therapeutic potential (eriodictyol and taxifolin) by Lv et al. (2019a). Since genetic instability and variations in copy number are reported for this microorganism, the authors integrated the biosynthetic pathways responsible for flavonoid production into the yeast genome. Cre-loxP system was used to perform multi-copy integration of the pathway genes on the 26 rDNA sequences of the genome. These 26 rDNA sequences are repetitive sequences present on the genome, and they were used as regions of homology for the integration of the foreign DNA on the *Y. lipolytica* genome in multiple copies. The foreign DNA cassette was composed by the genes of the pathway in combination with the URA3 marker containing the loxP flanking sequence. The presence of the loxP flanking sequences allowed the selection marker excision through the expression of Cre recombinase. Cre recombinase recognizes loxP sequences and performs its excision being a useful strategy since few auxotrophic markers are available on this host. The authors selected TAL from *Rhodotorula toruloides* (RtTAL), Pc4CL, PhCHS, and MsCHI as the genes of the biosynthetic pathway for naringenin production. The production of naringenin was evaluated, and up to 71.2 mg/L of naringenin was produced using the rich media yeast extract-peptone-dextrose (YPD) in shake flask experiments (Lv et al., 2019a). Afterward, the naringenin production levels were improved by optimizing the copy number of the genes that represent a bottleneck on the pathway and by increasing the supply of the pathway precursors. In the first approach, the authors found a significant improvement on the production levels by expressing five copies of the PhCHS gene in combination with only one copy of the other pathway genes compared to the expression of only one copy of PhCHS and of the other genes of the pathway.

Regarding the improvement of the precursor supply, the authors overexpressed the pentafunctional enzyme from *Y. lipolytica* (ARO1) that catalyzes several steps involved in the conversion of CHO into Tyr. Moreover, the authors also tested the expression of ACC and acetyl-CoA synthase (ACS) genes from different organisms to boost malonyl-CoA supply and found that the overexpression of both genes from *Y. lipolytica* yielded better results. Additionally, optimizations of the fermentation operational conditions, namely, on the C/N ratio and pH, were also performed. Under the best conditions, the engineered *Y. lipolytica* produced 252.4 mg/L of naringenin. This titer resulted in an optimization in the production levels of 3.5-fold compared to the previous study performed by the authors (Lv et al. 2019b). Due to the need to construct a cost-effective production process, the source for low-cost substrates to be used as substrates in heterologous production processes became a priority. For example, agricultural residues are a source of sugars with a lot of potential to be used on this process. Beyond the available sugars, xylose is one of the sugars that is present in higher amounts in agricultural residues and in hardwood hemicellulose. Taking this into account, Wei et al. (2020) evaluated the use of a mixture of glucose and xylose to produce naringenin. Since the *Y. lipolytica* wild-type strain is not able to naturally metabolize xylose, the authors engineered the strain by introducing into the yeast genome a xylose-inducible module able to activate the genes of the xylose utilization pathway. Using this strategy, xylose is successfully converted to E4P that is used as a precursor on the shikimate pathway that is required to achieve the final production of aromatic amino acids. The genes of the biosynthetic pathway responsible to produce naringenin (*RgTAL*, *At4CL*, *AtCHS*, and *AtCHI*) were also integrated into the *Y. lipolytica* genome using the CRISPR-Cas9 strategy. The authors tested the production of naringenin using a strain containing only the naringenin biosynthetic pathway (control strain) and using the constructed strain with the naringenin pathway and the xylose-inducible utilization module. The control strain was able to produce 239.1 mg/L of naringenin. On the other hand, the strain containing the xylose utilization module produced 715.3 mg/L of naringenin. These production levels were achieved in shake flask experiments using YPD media supplemented also with xylose (60 g/L). This titer represents a threefold increase in naringenin production, demonstrating the potential of using the xylose utilization pathway as an alternative to enhance the supply of shikimate pathway precursors. This approach can further boost the production levels of the target compound using a low-cost and eco-friendly substrate (Wei et al. 2020). Palmer et al. (2020) also constructed a *Y. lipolytica* strain to produce naringenin by integrating the genes responsible for the naringenin biosynthetic pathway into random sites of the yeast genome. The authors also optimized the strain to boost the precursor supply. Specifically, the DAHPS gene was replaced for a feedback-insensitive mutant version to improve Tyr supply, and the peroxisome biogenesis factor gene (PEX10), known to be involved in the peroxisome synthesis and in the improvement of the acetyl-CoA synthesis, was overexpressed. Additionally, since CHS is considered the bottleneck step of the pathway, the authors also integrated an additional copy of this gene into the yeast genome. The strain ability to produce naringenin was evaluated in a 3-L bioreactor scale, and 357 mg/L and 898 mg/L of

naringenin were produced using batch and fed-batch operational modes, respectively. The production level achieved in the fed-batch bioreactor strategy corresponds to the highest production of naringenin reported so far in *Y. lipolytica*.

More recently, *Streptomyces albidoflavus*, which belongs to the *Streptomyces* sp. family, has also been explored to produce naringenin. In this initial work, the authors integrated one copy of the naringenin pathway (TAL, 4CL, CHS, and CHI from an unknown origin) into the strain's genome and examined the effect of several production media on the naringenin production levels. After identifying the most effective media for the production experiments, the authors tested the expression of *matB* and *matC* genes to enhance the malonyl-CoA supply and integrated an additional copy of the biosynthetic pathway into the strain genome, resulting in the production of 22.4 mg/L of naringenin with supplementation of malonate, Tyr, and *p*-coumaric acid to the media (Ye et al. 2023). Since this is the first study reporting the heterologous production of naringenin using *S. albidoflavus*, there is a potential to further optimize the production levels. These strategies may include the design of genetic engineering strategies to improve Tyr-native production and the exploration of alternative methods to increase the malonyl-CoA supply. Despite that, a big effort will be needed to reach the production levels already reported using the other microbial chassis.

Challenges on the Efficient Heterologous Production of Naringenin Using Microbial Chassis

Despite recent advances in the heterologous production of naringenin, further improvements are needed to establish an efficient, cost-effective, and competitive process. The main bottlenecks and limitations in achieving higher production levels are mainly related to the availability of the required precursors and extended substrates, the efficient expression of the enzymes involved in the biosynthetic pathway, and the difficulties in exporting the compound to the extracellular media (Gomes et al. 2022a; Rainha et al. 2020a; Rodrigues and Rodrigues 2020).

During the last years, the availability of the precursors and extender substrates of the pathway has been identified as the main bottleneck to achieve higher production levels of the desired compounds. As shown throughout the previous topic focused on the advances on the construction of microbial chassis able to produce naringenin, several efforts have been made by the researchers to improve the native supply of both aromatic amino acids and malonyl-CoA in all the microorganisms that were herein reviewed. Most of the strategies used to improve the precursors and extender substrate supply rely on the overexpression of genes responsible for their synthesis or on the deletion of genes of competing pathways that also use these compounds preventing the pathway flux deviation (Dickey et al. 2021). Regarding the improvement of aromatic amino acids (Tyr or Phe), the most used strategies rely on the replacement of the DAHPS and CM genes of the shikimate pathway by mutant feedback-insensitive version of the enzymes and on the prevention of the deviation of the aromatic amino acids' precursors for competing pathways. These strategies

allowed a continuous flux toward the synthesis of aromatic amino acids and higher production levels of the target compound (Dunstan et al. 2020; Koopman et al. 2012; Lyu et al. 2017; Palmer et al. 2020; Rodriguez et al. 2017; Wu et al. 2014; Zhou et al. 2020a). The improvement of the malonyl-CoA supply is also mandatory. Strategies like the overexpression of the ACC and ACS enzymes involved on the production of malonyl-CoA, as well as the downregulation of targets of the fatty acid competing pathway, have been extensively exploited to improve the malonyl-CoA supply and, consequently, improve the production of naringenin (Dunstan et al. 2020; Fowler et al. 2009; Lv et al. 2019b; Mao et al. 2023; Santos et al. 2011; Xu et al. 2011; Zhou et al. 2020b). Additionally, the construction of an alternative biosynthetic pathway for the synthesis of malonyl-CoA from malonate by expressing *matB* and *matC* genes from *R. trifolii* has also been evaluated by several researchers (Mao et al. 2023; Wu et al. 2014; Ye et al. 2023). Despite the potential of these strategies, several efforts are still required to find the proper metabolic flux toward the synthesis of these crucial pathway compounds. Considering that, the development and construction of biosensors have recently emerged as an interesting strategy to find a right metabolic flux since it allows a dynamic regulation of the metabolic process, as well as the high-throughput screening and monitoring of the target metabolites due to the presence of reporter genes in the biosensor module (Fig. 4). This strategy has been

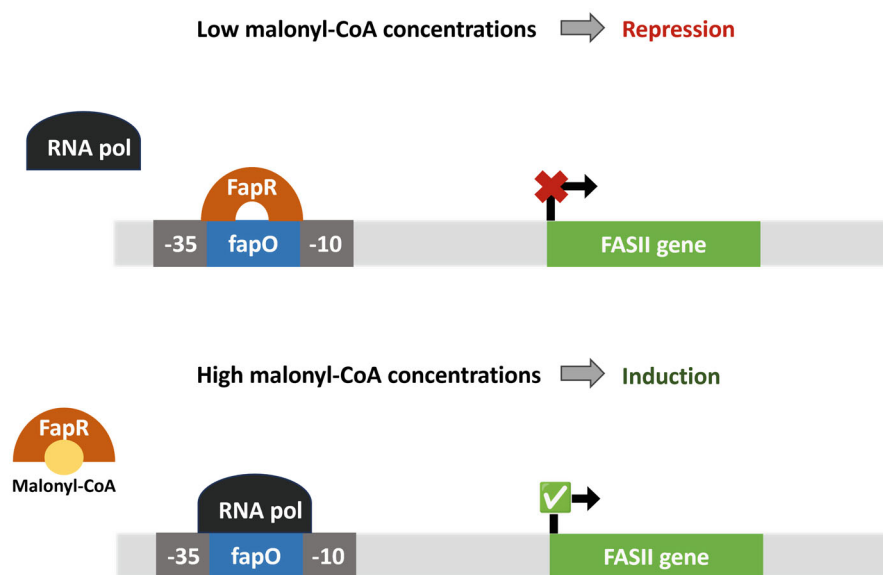


Fig. 4 Design of the malonyl-CoA biosensor that allows a dynamic regulation of the metabolic flux. When the intracellular malonyl-CoA level is low, the repressor molecule FapR binds to the operator fapO forming the FapR-fapO complex. With the formation of this complex, the RNA polymerase (RNA pol) is not able to bind to fapO, and the transcription of the fatty acid synthase (FASII) gene is repressed. When the intracellular malonyl-CoA concentration is high, malonyl-CoA binds to the C-terminal of FapR disrupting the formation of the FapR-fapO complex. Consequently, the operator fapO is free and the RNA pol can attach, and the transcription of FASII gene is induced

already studied in *E. coli* and *S. cerevisiae* and involves the construction of a synthetic regulatory module that is naturally present in some Gram-positive bacteria, namely, in *Bacillus subtilis* (Johnson et al. 2017; Mao et al. 2023; Milke and Marienhagen 2020; Zhou et al. 2020a). This module is composed of a transcriptional repressor, FapR, that inhibits the fatty acid synthesis by interacting with the operator fapO module. This interaction forms a FapR-fapO complex, and the RNA polymerase is repressed, preventing the transcription of the genes involved on the fatty acid synthesis. When high levels of malonyl-CoA are accumulated, this molecule interacts with the C-terminal of the FapR module, and the repression system stops. Consequently, the FapR-fapO complex is disrupted, and the fapO operator is free to interact with the RNA polymerase allowing the transcription of the fatty acids' synthesis genes. The engineering of this dynamic regulation module ensures the proper balance and metabolic flux between all the compounds (Johnson et al. 2017).

In fact, Mao et al. (2023) evaluated a malonyl-CoA biosensor based on this strategy combined with RNAi to regulate also the genes of fatty acids' biosynthesis pathway. This approach led to the highest reported production level of naringenin (2.05 g/L) in any microorganism, demonstrating the potential of dynamic regulation biosensors for enhancing production levels.

Besides the availability of the substrates of the pathway, the choice of the right enzymes for the biosynthetic pathway and their efficient expression is one of the primary steps that should be considered to construct an efficient microbial cell factory. Firstly, the metabolic balance between all the intermediaries of the biosynthetic pathway should be ensured by testing different enzyme combinations and their effect on the final production levels. Some of the steps of this biosynthetic pathway are characterized by their poor substrate specificity and low catalytic activity (Liu et al. 2017). Taking this into account, the use of enzyme engineering tools like rational design or directed evolution should be considered to improve the enzyme performance and, consequently, improve the flux toward the production of the compound of interest. Considering that all the enzymatic steps of the naringenin biosynthetic pathway are already well characterized, the rational design can be considered an interesting approach to be used since it allows the full design of a functional enzyme (Li et al. 2020a; Xu et al. 2020). However, directed evolution can also be considered as an interesting strategy since it performs random mutagenesis of the gene and mutant enzymes with improved activities can be obtained. This strategy has the advantage of not requiring previously knowledge about the protein structure or activity (Ko et al. 2020). Moreover, since the biosynthetic pathway responsible to produce naringenin is catalyzed by several steps, it is also relevant to consider the use of multifunctional enzymes that can catalyze more than just one of the steps of the biosynthetic pathway. For example, the use of TAL to achieve *p*-coumaric acid production instead of PAL and C4H is useful to decrease the metabolic burden of the cells that possibly impairs the production levels (Rainha et al. 2020a). Moreover, this strategy is also advantageous since the expression of the C4H enzyme is not required. Since this enzyme is a membrane-bound enzyme, it can be very difficult to be expressed in prokaryotic organisms, like *E. coli* and *Streptomyces* spp., because they do not contain intracellular compartments (Gomes et al. 2022b; Rodrigues and

Rodrigues 2020). Additionally, the native cytochrome P450 activity in heterologous hosts, namely, in *S. cerevisiae*, was already proven to not be enough to support the C4H activity being required the additional expression of CPR enzymes (Koopman et al. 2012; Trantas et al. 2009). Moreover, it is advisable to consider codon optimization of the heterologous genes taking into account the codon usage of the microorganism used as chassis to prevent the expression of non-functional proteins due to possible translation errors, premature translational termination, or even frameshifting events (Rainha et al. 2020a; Rodrigues and Rodrigues 2017).

Another issue associated with the production of heterologous compounds, inclusively naringenin, is its transportation to the extracellular media. As other polyphenols, naringenin possesses antimicrobial activity. For this reason, the accumulation of this compound in large amounts inside the cells can be toxic for the host, resulting in decreased cell growth and productivity. Considering that, the engineering of transporters can be a useful strategy to increase the productivity of the production process by improving the release of the produced compound to the extracellular media. This is also advantageous for an industrial production process since it eliminates the need for an additional and time-consuming extraction process, which is also associated with increased costs in the production process (Liu et al. 2017; Lv et al. 2016). The use of transporter engineering was already exploited by Mao et al. (2023) to improve the production of naringenin in *S. cerevisiae*. In fact, the highest de novo production of naringenin was achieved in this study, demonstrating the relevance of exploiting the use of transporters to increase the production titers and yields (Mao et al. 2023). Therefore, the use of this strategy in other microbial chassis should be considered. However, deep studies on polyphenol transport are required to fully understand the structure and mechanism of action of several transporters to be further exploited on these microbial cell factories.

Conclusion

The utilization of natural compounds, particularly polyphenols like naringenin, has gained substantial attention over the last years in the pharmaceutical and nutraceutical industries. Several challenges have arisen to obtain these compounds from plant sources due to their limited availability, being required efficient extraction methods. Considering the wide range of applications and health benefits of polyphenols, including naringenin, it is mandatory to explore a cost-effective and environmentally friendly production process to meet the industrial demand for this compound. Microorganisms, serving as microbial cell factories, present a promising strategy for the heterologous production of naringenin. *E. coli* and *S. cerevisiae* have been widely exploited as microbial chassis to produce naringenin. The highest production level achieved using *E. coli* as microbial chassis was 588 mg/L in a fed-batch reactor experiment (Zhou et al. 2020b). More recently, Mao et al. (2023) achieved the highest de novo production of naringenin (2.05 g/L) reported so far exploiting *S. cerevisiae* as chassis in a fed-batch reactor experiment (Mao et al. 2023). Considering that, this strain has a great potential to be exploited in an industrial

production process. Other alternative chassis, like *Y. lipolytica* and *S. albidoflavus*, were recently exploited. The production levels obtained in *S. albidoflavus* are far from becoming competitive. However, the production obtained in *Y. lipolytica* has reached 898 mg/L representing also a chassis with an industrial potential (Palmer et al. 2020). Despite the potential of these constructed strains and the advances that were made over the last years to achieve the heterologous production of naringenin, reaching industrially relevant production levels remains a challenge. To enhance microbial production, addressing key bottlenecks is still imperative. Overcoming precursors' limitations, specifically aromatic amino acids, and malonyl-CoA, sorting to genetic engineering manipulations, have been widely exploited in these microbial chassis and proved to be efficient to increase the obtained production levels. However, the combination of the different strategies and the exploitation of bio-sensors for a dynamic regulation of the metabolic flux should be considered to achieve better results. Beyond the improvement of the availability of the pathway substrates and precursors, it is crucial to ensure the efficient pathway expression. The optimal enzyme selection, involving rational design or directed evolution, the use of multifunctional enzymes, and codon optimization, can contribute to improved performance and higher productivities. Moreover, transporter engineering is proven to be crucial for efficient compound export, mitigating intracellular toxicity and simplifying the downstream processing. Considering that, finding new transporters and studying their mechanism of action are crucial. With the emergence and establishment of metabolic engineering and synthetic biology powerful tools, some of these challenges are now easier to overcome. The combination of all these presented strategies sorting to the most recent synthetic biology technologies can be the key point to obtain a robust and highly efficient microbial chassis able to produce naringenin and, finally, achieve a viable and sustainable alternative method for its production at an industrial scale.

Acknowledgments 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, with DOI 10.54499/UIDB/04469/2020. DG acknowledges FCT for her grant SFRH/BD/04433/2020.

References

- Adams BL. The next generation of synthetic biology chassis: moving synthetic biology from the laboratory to the field. *ACS Synth Biol*. 2016;5(12):1328–30.
- Amin I, Majid S, Farooq A, Wani Ahmad H, Noor F, Kha R, et al. Naringenin (4,5,7-trihydroxyflavanone) as a potent neuroprotective agent : From chemistry to medicine. In: Atta-ur-Rahman, editor. *Studies in natural products chemistry*. Amsterdam: Elsevier B.V; 2020. p. 271–300.
- Amorim C, Rodrigues JL, Braga A, Gomes D, Rainha J, Silvério SC, Cardoso BB, et al. Engineering *Saccharomyces cerevisiae* for the one-step production of a functional sweetening mixture towards food applications. *Food Bioprod Process*. 2022;2022(135):123–34.
- Averesch NJH, Krömer JO. Metabolic engineering of the shikimate pathway for production of aromatics and derived compounds-present and future strain construction strategies. *Front Bioeng Biotechnol*. 2018;6(32):1–19.

- Bilal M, Xu S, Iqbal HMN, Cheng H. *Yarrowia lipolytica* as an emerging biotechnological chassis for functional sugars biosynthesis. *Crit Rev Food Sci Nutr*. 2021;61(4):535–52.
- Braga A, Rocha I, Faria N. Microbial hosts as a promising platform for polyphenol production. In: Akhtar M, Swamy M, Sinniah U, editors. *Natural bio-active compounds: vol 1, Production and applications*. Singapore: Springer; 2019. p. 71–103.
- Braga A, Gomes D, Rainha J, Cardoso BB, Amorim C, Silvério SC, et al. Tailoring fructooligosaccharides composition with engineered *Zymomonas mobilis* ZM4. *Appl Microbiol Biotechnol*. 2022;106(12):4617–26.
- Cataneo AHD, Kuczera D, Koishi AC, Zanluca C, Silveira GF, de Arruda TB, et al. The citrus flavonoid naringenin impairs the in vitro infection of human cells by Zika virus. *Sci Rep*. 2019;9(1):1–15.
- Chen R, Yang S, Zhang L, Zhou YJ. Advanced strategies for production of natural products in yeast. *iScience*. 2020;23(3):100879.
- Cho JS, Kim GB, Eun H, Moon CW, Lee SY. Designing microbial cell factories for the production of chemicals. *JACS Au*. 2022;2(8):1781–99.
- Cobb RE, Wang Y, Zhao H. High-efficiency multiplex genome editing of *Streptomyces* species using an engineered CRISPR/Cas system. *ACS Synth Biol*. 2015;4(6):723–8.
- Dickey RM, Forti AM, Kunjapur AM. Advances in engineering microbial biosynthesis of aromatic compounds and related compounds. *Bioresour Bioproc*. 2021;8(91):1–17.
- Dunstan MS, Robinson CJ, Jervis AJ, Yan C, Carbonell P, Hollywood KA, et al. Engineering *Escherichia coli* towards de novo production of gatekeeper (2S)-flavanones: naringenin, pinocembrin, eriodictyol and homoeriodictyol. *Synth Biol*. 2020;5(1):1–11.
- Ebrahimi M, Mokhtari A. Engineering of secondary metabolites in tissue and cell culture of medicinal plants: an alternative to produce beneficial compounds using bioreactor technologies. In: Abdullah SNA, Chai-Ling H, Wagstaff C, editors. *Crop improvement*. Cham: Springer; 2017. p. 137–67.
- Fowler ZL, Gikandi WW, Koffas MAG. Increased malonyl coenzyme A biosynthesis by tuning the *Escherichia coli* metabolic network and its application to flavanone production. *Appl Environ Microbiol*. 2009;75(18):5831–9.
- Ghohrani S, Joghataei MT, Mohseni S, Baluchnejadmojarad T, Bagheri M, Khamse S, et al. Naringenin improves learning and memory in an Alzheimer's disease rat model: insights into the underlying mechanisms. *Eur J Pharmacol*. 2015;2015(764):195–201.
- Gomes D, Rainha J, Rodrigues LR, Rodrigues JL. Yeast synthetic biology approaches for the production of valuable polyphenolic compounds. In: Harzevili FD, editor. *Synthetic biology of yeast*. Cham: Springer Nature; 2022a. p. 119–56.
- Gomes D, Rodrigues LR, Rodrigues JL. Perspectives on the design of microbial cell factories to produce prenylflavonoids. *Int J Food Microbiol*. 2022b;367:109588.
- Growth Market Reports. Naringenin, neohesperidin and phloretin market 2023–2031. [cited 2023 Dec 19]. 2022. <https://growthmarketreports.com/report/naringenin-neohesperidin-dc-and-phloretin-market-global-industry-analysis>
- Hwang S, Lee Y, Kim JH, Kim G, Kim H, Kim W, et al. *Streptomyces* as microbial chassis for heterologous protein expression. *Front Bioeng Biotechnol*. 2021;9(804295):1–23.
- Jez JM, Noel JP. Reaction mechanism of chalcone isomerase: pH dependence, diffusion control, and product binding differences. *J Biol Chem*. 2002;277(2):1361–9.
- Johnson AO, Gonzalez-Villanueva M, Wong L, Steinbüchel A, Tee KL, Xu P, et al. Design and application of genetically-encoded malonyl-CoA biosensors for metabolic engineering of microbial cell factories. *Metab Eng*. 2017;2017(44):253–64.
- Ko YS, Kim JW, Lee JA, Han T, Kim GB, Park JE, et al. Tools and strategies of systems metabolic engineering for the development of microbial cell factories for chemical production. *Chem Soc Rev R Soc Chem*. 2020;49(14):4615–36.
- Koopman F, Beekwilder J, Crimi B, van Houwelingen A, Hall RD, Bosch D, et al. *De novo* production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. *Microb Cell Factories*. 2012;11:1–15.

- Krivoruchko A, Nielsen J. Production of natural products through metabolic engineering of *Saccharomyces cerevisiae*. *Curr Opin Biotechnol*. 2015;35:7–15.
- Larroude M, Trabelsi H, Nicaud JM, Rossignol T. A set of *Yarrowia lipolytica* CRISPR/Cas9 vectors for exploiting wild-type strain diversity. *Biotechnol Lett*. 2020;42(5):773–85.
- Ledesma-Amaro R, Nicaud JM. *Yarrowia lipolytica* as a biotechnological chassis to produce usual and unusual fatty acids. *Prog Lipid Res*. 2016;2016(61):40–50.
- Leonard E, Lim KH, Saw PN, Koffas MAG. Engineering central metabolic pathways for high-level flavonoid production in *Escherichia coli*. *Appl Environ Microbiol*. 2007;73(12):3877–86.
- Leonard E, Yan Y, Fowler ZL, Li Z, Lim CG, Lim KH, et al. Strain improvement of recombinant *Escherichia coli* for efficient production of plant flavonoids. *Mol Pharm*. 2008;5(2):257–65.
- Li C, Zhang R, Wang J, Wilson LM, Yan Y. Protein engineering for improving and diversifying natural products biosynthesis. *Trends Biotechnol*. 2020a;38(7):729–44.
- Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol Biochem*. 2020b;148:80–9.
- Li H, Gao S, Zhang S, Zeng W, Zhou J. Effects of metabolic pathway gene copy numbers on the biosynthesis of (2S)-naringenin in *Saccharomyces cerevisiae*. *J Biotechnol*. 2021;2021(325):119–27.
- Liang J, Halipu Y, Hu F, Yakeya B, Chen W, Zhang H, et al. Naringenin protects keratinocytes from oxidative stress injury via inhibition of the NOD2-mediated NF- κ B pathway in pemphigus vulgaris. *Biomed Pharmacother*. 2017;92:796–801.
- Liou G, Chiang YC, Wang Y, Weng JK. Mechanistic basis for the evolution of chalcone synthase catalytic cysteine reactivity in land plants. *J Biol Chem*. 2018;293(48):18601–12.
- Liu X, Wang N, Fan S, Zheng X, Yang Y, Zhu Y, et al. The citrus flavonoid naringenin confers protection in a murine endotoxaemia model through AMPK-ATF3-dependent negative regulation of the TLR4 signalling pathway. *Sci Rep*. 2016;6:1–14.
- Liu X, Ding W, Jiang H. Engineering microbial cell factories for the production of plant natural products: from design principles to industrial-scale production. *Microb Cell Fact BioMed Central*. 2017;16(1):1–9.
- Liu R, Deng Z, Liu T. *Streptomyces* species: ideal chassis for natural product discovery and overproduction. *Metab Eng*. 2018;2018(50):74–84.
- Liu X, Li L, Zhao GR. Systems metabolic engineering of *Escherichia coli* coculture for *de novo* production of genistein. *ACS Synth Biol*. 2021;11(5):1746–57.
- Liu J, Wang X, Dai G, Zhang Y, Bian X. Microbial chassis engineering drives heterologous production of complex secondary metabolites. *Biotechnol Adv*. 2022;2022(59):107966.
- Lussier F-X, Colatrinano D, Wiltshire Z, Page JE, Martin VJJ. Engineering microbes for plant polyketide biosynthesis. *Comput Struct Biotechnol J*. 2012;3(4):e201210020.
- Lv H, Li J, Wu Y, Garyali S, Wang Y. Transporter and its engineering for secondary metabolites. *Appl Microbiol Biotechnol*. 2016;100(14):6119–30.
- Lv Y, Edwards H, Zhou J, Xu P. Combining 26s rDNA and the Cre-loxP system for iterative gene integration and efficient marker curation in *Yarrowia lipolytica*. *ACS Synth Biol Am Chem Soc*. 2019a;8(3):568–76.
- Lv Y, Marsafari M, Zhou J, Xu P. Optimizing oleaginous yeast cell factories for flavonoids and hydroxylated flavonoids biosynthesis. *ACS Synth Biol*. 2019b;8:2514–23.
- Lyu X, Ng KR, Lee JL, Mark R, Chen WN. Enhancement of naringenin biosynthesis from tyrosine by metabolic engineering of *Saccharomyces cerevisiae*. *J Agric Food Chem*. 2017;65(31):6638–46.
- Lyu X, Zhao G, Ng KR, Mark R, Chen WN. Metabolic engineering of *Saccharomyces cerevisiae* for *de novo* production of kaempferol. *J Agric Food Chem*. 2019;67(19):5596–606.
- Mao J, Mohedano MT, Fu J, Li X, Liu Q, Nielsen J, et al. Fine-tuning of p-coumaric acid synthesis to increase (2S)-naringenin production in yeast. *Metab Eng*. 2023;79:192–202.
- Marchiosi R, dos Santos WD, Constantin RP, de Lima RB, Soares AR, Finger-Teixeira A, et al. Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochem Rev*. 2020;19(4):865–906.

- Marienhagen J, Bott M. Metabolic engineering of microorganisms for the synthesis of plant natural products. *J Biotechnol.* 2013;163(2):166–78.
- Milke L, Marienhagen J. Engineering intracellular malonyl-CoA availability in microbial hosts and its impact on polyketide and fatty acid synthesis. *Appl Microbiol Biotechnol.* 2020;104(14):6057–65.
- Milke L, Aschenbrenner J, Marienhagen J, Kallscheuer N. Production of plant-derived polyphenols in microorganisms: current state and perspectives. *Appl Microbiol Biotechnol.* 2018;102:1575–85.
- Motallebi M, Bhia M, Rajani HF, Bhia I, Tabarraei H, Mohammadkhani N, et al. Naringenin: a potential flavonoid phytochemical for cancer therapy. *Life Sci.* 2022;2022(305):120752.
- Najmi A, Javed SA, Al Bratty M, Alhazmi HA. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules.* 2022;27(2):1–26.
- Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus.* 2022;65(3):399–411.
- Palmer CM, Miller KK, Nguyen A, Alper HS. Engineering 4-coumaroyl-CoA derived polyketide production in *Yarrowia lipolytica* through a β -oxidation mediated strategy. *Metab Eng.* 2020;2020(57):174–81.
- Pandey RP, Parajuli P, Koffas MAG, Sohng JK. Microbial production of natural and non-natural flavonoids: pathway engineering, directed evolution and systems/synthetic biology. *Biotechnol Adv.* 2016;34(5):634–62.
- Rainha J, Gomes D, Rodrigues LR, Rodrigues JL. Synthetic biology approaches to engineer *Saccharomyces cerevisiae* towards the industrial production of valuable polyphenolic compounds. *Life.* 2020a;10(5):56.
- Rainha J, Rodrigues JL, Rodrigues LR. CRISPR-Cas9: a powerful tool to efficiently engineer *Saccharomyces cerevisiae*. *Life.* 2020b;11(1):1–16.
- Rainha J, Rodrigues JL, Faria C, Rodrigues LR. Curcumin biosynthesis from ferulic acid by engineered *Saccharomyces cerevisiae*. *Biotechnol J.* 2022a;17(3):1–8.
- Rainha J, Rodrigues LR, Rodrigues JL. Microbial production of curcumin. In: Jafari SM, Harzevilli FD, editors. *Microbial production of food bioactive compounds*. Cham: Springer Nature; 2022b. p. 1–35.
- Rashmi R, Magesh SB, Ramkumar KM, Suryanarayanan S, SubbaRao MV. Antioxidant potential of naringenin helps to protect liver tissue from streptozotocin-induced damage. *Rep Biochem Mol Biol.* 2017;7(1):76–84.
- Rodrigues JL, Rodrigues LR. Synthetic biology: perspectives in industrial biotechnology. In: Pandey A, Teixeira J, editors. *Current developments in biotechnology and bioengineering. Foundations of biotechnology and bioengineering*. Amsterdam: Elsevier; 2017. p. 239–69.
- Rodrigues JL, Rodrigues LR. Biosynthesis and heterologous production of furanocoumarins: perspectives and current challenges. *Nat Prod Rep.* 2020;38:869–79.
- Rodrigues JL, Rodrigues LR. Microbial production of caffeic acid. In: Jafari SM, Harzevilli FD, editors. *Microbial production of food bioactive compounds*. Cham: Springer Nature; 2022. p. 1–34.
- Rodrigues JL, Araújo RG, Prather KLJ, Kluskens LD. Production of curcuminoids from tyrosine by a metabolically engineered *Escherichia coli* using caffeic acid as an intermediate. *Biotechnol J.* 2015a;10(4):1–27.
- Rodrigues JL, Prather KLJ, Kluskens LD, Rodrigues LR. Heterologous production of curcuminoids. *Microbiol Mol Biol Rev.* 2015b;79(1):39–60.
- Rodrigues JL, Ferreira D, Rodrigues LR. Synthetic biology strategies towards the development of new bioinspired technologies for medical applications. In: Rodrigues LR, Mota M, editors. *Bioinspired materials for medical applications*. Oxford: Elsevier; 2017. p. 451–97.
- Rodrigues JL, Gomes D, Rodrigues LR. A combinatorial approach to optimize the production of curcuminoids from tyrosine in *Escherichia coli*. *Front Bioeng Biotechnol.* 2020;8(59):1–15.

- Rodrigues JL, Gomes D, Rodrigues LR. Challenges in the heterologous production of furanocoumarins in *Escherichia coli*. *Molecules*. 2022;27(21):1–20.
- Rodriguez A, Strucko T, Stahlhut SG, Kristensen M, Svenssen DK, Forster J, et al. Metabolic engineering of yeast for fermentative production of flavonoids. *Bioresour Technol*. 2017;245:1645–54.
- Santos CNS, Koffas M, Stephanopoulos G. Optimization of a heterologous pathway for the production of flavonoids from glucose. *Metab Eng*. 2011;13(4):392–400.
- Shan S, Zhang Y, Wu M, Yi B, Wang J, Li Q. Naringenin attenuates fibroblast activation and inflammatory response in a mechanical stretch-induced hypertrophic scar mouse model. *Mol Med Rep*. 2017;16(4):4643–9.
- Sheng H, Sun X, Yan Y, Yuan Q, Wang J, Shen X. Metabolic engineering of microorganisms for the production of flavonoids. *Front Bioeng Biotechnol*. 2020;8:1–15.
- Smetanska I. Advances in biochemical engineering science. In: Stahl U, Donalies UEB, Nevoigt E, editors. *Food biotechnology*. Berlin/Heidelberg: Springer; 2008. p. 188–228.
- Tao W, Yang A, Deng Z, Sun Y. CRISPR/Cas9-based editing of *Streptomyces* for discovery, characterization, and production of natural products. *Front Microbiol*. 2018;9(1660):1–8.
- Tariq H, Asif S, Andleeb A, Hano C, Abbasi BH. Flavonoid production: current trends in plant metabolic engineering and *de novo* microbial production. *Meta*. 2023;13(124):1–26.
- Tohge T, Watanabe M, Hoefgen R, Fernie AR. Shikimate and phenylalanine biosynthesis in the green lineage. *Front Plant Sci*. 2013;4:1–13.
- 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(6):355–66.
- Tzin V, Galili G. The biosynthetic pathways for shikimate and aromatic amino acids in *Arabidopsis thaliana*. *Arab B*. 2010;8(i):e0132.
- Wang J, Guleria S, Koffas MAG, Yan Y. Microbial production of value-added nutraceuticals. *Curr Opin Biotechnol*. 2016;37:97–104.
- Watts KT, Mijts BN, Lee PC, Manning AJ, Schmidt-Dannert C. Discovery of a substrate selectivity switch in tyrosine ammonia-lyase, a member of the aromatic amino acid lyase family. *Chem Biol*. 2006;13(12):1317–26.
- Wei W, Zhang P, Shang Y, Zhou Y, Ye BC. Metabolically engineering of *Yarrowia lipolytica* for the biosynthesis of naringenin from a mixture of glucose and xylose. *Bioresour Technol*. 2020;2020(314):123726.
- Wilson SA, Roberts SC. Recent advances towards development and commercialization of plant cell culture processes for synthesis of biomolecules. *Plant Biotechnol J*. 2012;10(3):249–68.
- Wu J, Zhou T, Du G, Zhou J, Chen J. Modular optimization of heterologous pathways for *de novo* synthesis of (2S)-naringenin in *Escherichia coli*. *PLoS One*. 2014;9(7):1–9.
- Wu J, Du G, Chen J, Zhou J. Enhancing flavonoid production by systematically tuning the central metabolic pathways based on a CRISPR interference system in *Escherichia coli*. *Sci Rep*. 2015;5(13477):1–14.
- Xia X, Chen D, Ren Y. Antiviral activity of chrysin and naringenin against porcine epidemic diarrhea virus infection. *Front Vet Sci*. 2023;10:1278997.
- Xu P, Ranganathan S, Fowler ZL, Maranas CD, Koffas MAG. Genome-scale metabolic network modeling results in minimal interventions that cooperatively force carbon flux towards malonyl-CoA. *Metab Eng*. 2011;13(5):578–87.
- Xu X, Liu Y, Du G, Ledesma-Amaro R, Liu L. Microbial chassis development for natural product biosynthesis. *Trends Biotechnol*. 2020;38(7):779–96.
- Xu J, Guo Z, Yuan S, Li H. BCL2L1 is identified as a target of naringenin in regulating ovarian cancer progression. *Mol Cell Biochem*. 2022;477(5):1541–53.
- Yang D, Park SY, Park YS, Eun H, Lee SY. Metabolic engineering of *Escherichia coli* for natural product biosynthesis. *Trends Biotechnol*. 2020;38(7):745–65.

- Ye S, Magadán-Corpas P, Pérez-Valero Á, Villar CJ, Lombó F. Metabolic engineering strategies for naringenin production enhancement in *Streptomyces albidoflavus* J1074. *Microb Cell Factories*. 2023;22(167):1–12.
- Yoo H, Widhalm JR, Qian Y, Maeda H, Cooper BR, Jannasch AS, et al. An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine:phenylpyruvate aminotransferase. *Nat Commun*. 2013;4(2833):1–11.
- Yousuf M, Shamsi A, Khan S, Khan P, Shahwan M, Elsbali AM, et al. Naringenin as a potential inhibitor of human cyclin-dependent kinase 6: molecular and structural insights into anti-cancer therapeutics. *Int J Biol Macromol*. 2022;2022(213):944–54.
- Zhang Q, Yu S, Lyu Y, Zeng W, Zhou J. Systematically engineered fatty acid catabolite pathway for the production of (2S)-naringenin in *Saccharomyces cerevisiae*. *ACS Synth Biol*. 2021;10(5):1166–75.
- Zhou S, Yuan S-F, Nair P, Alper H, Deng Y, Zhou J. Development of a growth coupled dynamic regulation network balancing malonyl-CoA node to enhance (2S)-naringenin synthesis in *E. coli*. *BioRxiv*. 2020a;7:1–29.
- Zhou S, Hao T, Zhou J. Fermentation and metabolic pathway optimization to *de novo* synthesize (2S)-naringenin in *Escherichia coli*. *J Microbiol Biotechnol*. 2020b;30(10):1574–82.