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Simple phenylpropanoids: recent advances in biological activities, biosynthetic pathways, and microbial production†

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Simple phenylpropanoids are a large group of natural products with primary C6–C3 skeletons. They are not only important biomolecules for plant growth but also crucial chemicals for high-value industries, including fragrances, nutraceuticals, biomaterials, and pharmaceuticals. However, with the growing global demand for simple phenylpropanoids, direct plant extraction or chemical synthesis often struggles to meet current needs in terms of yield, titre, cost, and environmental impact. Benefiting from the rapid development of metabolic engineering and synthetic biology, microbial production of natural products from inexpensive and renewable sources provides a feasible solution for sustainable supply. This review outlines the biological activities of simple phenylpropanoids, compares their biosynthetic pathways in different species (plants, bacteria, and fungi), and summarises key research on the microbial production of simple phenylpropanoids over the last decade, with a focus on engineering strategies that seem to hold most potential for further development. Moreover, constructive solutions to the current challenges and future perspectives for industrial production of phenylpropanoids are presented.

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1. Introduction

Simple phenylpropanoids are a group of secondary metabolites comprising a phenyl ring linked with a three-carbon side chain (C6–C3). In plants, they are primarily derived from the aromatic amino acid L-phenylalanine (L-Phe).[1] These compounds can be classified into several subcategories associated with changes in the substituent on the benzene ring and the position of the propenyl double bond, such as phenylpropanoic acids (cinnamic and hydroxycinnamic acids), phenylpropanoic aldehydes, phenylpropanols, phenylpropene and simple coumarins.[2] By serving as biogenetic precursors of various

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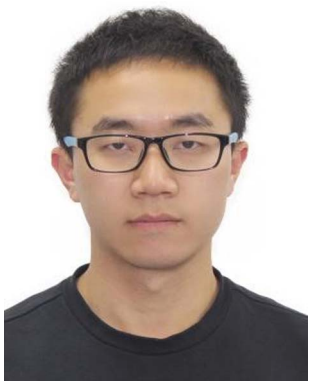
complex phenylpropanoids and other downstream metabolites, simple phenylpropanoids are essential for structural support, pigmentation, defence, and signalling throughout the plant kingdom.[3,4] They also show a wide range of biological activities and are useful as raw materials applicable to high value-added chemical products, which have received considerable attention from agriculture, cosmetics, biofuel, biomaterials, and pharmaceutical industries.[5,6]

Plants synthesize approximately 10 gigatons of phenylpropanoid molecules each year, which constitute approximately 20% of the total carbon in the terrestrial biosphere.[7] Simple phenylpropanoids are mainly obtained from natural raw materials, particularly from millions of tons of agricultural wastes and forest litter produced per annum.[8] Access to a sustainable supply of simple phenylpropanoids is restricted by a variety of factors, including sluggish growth and accumulation, variable synthesis fluctuations caused by climatic and environmental changes, challenging extraction and purification methods, as well as time-consuming and contaminating procedures.[9,10] Development of more nature-friendly production methods has long been the subject of research owing to the intensifying contradiction between the increasing demand for simple phenylpropanoids and the urgent need for ecological conservation. Compared to plant extraction and chemical synthesis, microbial production is a promising alternative, not only for its much shorter production periods but also for being safer, low-cost, and environmentally friendly. Recent advances in the design-build-test-learn cycle associated with metabolic engineering, synthetic biology, systems biology, bioinformatics, and other advanced technologies have accelerated the development of microbial cell factories, resulting in the accumulation of simple phenylpropanoids from inexpensive and renewable sources.[11–13] However, despite the tremendous progress made, several urgent challenges remain in advancing microbial engineering as a general approach for the biosynthesis of simple phenylpropanoids, including (1) non-specificity and inefficient cofactor supply of enzymes, (2) unbalanced metabolic flux between glycolysis and pentose phosphate pathway (PPP), (3) low cell performance, and (4) metabolic promiscuity in single cells.

In this review, we first summarize the extensive biological activities of simple phenylpropanoids, and emphasize the importance in food, cosmetic, nutraceutical, chemical, and, especially, the pharmaceutical industries. In addition, we outline biosynthetic pathways of simple phenylpropanoids in different species (plants, bacteria, and fungi), which provides inspiration for route design and optimization of microbial cell factories for heterologous synthesis. Next, we summarize effective engineering strategies for the microbial production of simple phenylpropanoids that are based on the reprogramming the metabolic flux toward aromatic amino acids (AAAs), and efficient microbial production of simple phenylpropanoids. Finally, we here discuss in detail the potential challenges for further improving titers of simple phenylpropanoids in microbial cell factories under the concept of the four-dimensional metabolic engineering. We also present some perspectives and constructive solutions to the current challenges.

Zhanpin Zhu earned her master's degree in Biomedical Engineering from the Chinese University of Hong Kong under the supervision of Prof. Waye Mary Miu Yee. After working for 5 years in pharmacy, she joined the group of Prof. Lei Zhang and worked at the Naval Medical University. Her current research is focused on the improvement of phenylpropanoids in microbial cell factories through metabolic engineering and synthetic biology approaches.



Ruibing Chen studied Pharmacy at the Naval Medical University, China, where he received PhD degree in 2018. He has completed postdocs at the Dalian Institute of Chemical Physics, CAS, China (2021). He currently works as associate professor of Pharmacognosy at the Naval Medical University. His research interests focus on developing modular genetic platforms for programming metabolic process in plants and microorganisms, resulting in efficient technologies for engineering and manipulating biological systems, which is further used for elucidating, reconstructing, and optimizing the biosynthesis of natural products, resulting in modified organism for synthesizing and producing active molecules.

Prof. Lei Zhang is dedicated to develop integrated omics-based approach toward understanding biosynthesis pathway of natural active products in medicinal plant and then successfully genetic engineering plant (yeast) cell factories to produce designer secondary metabolites. He has published more than 180 articles in Nat. Chem. Biol., Nat. Commun., PNAS, Mol. Plant, Plant Biotechnol., New Phytologist, APSB etc. as the corresponding author and total more than 2800 cited times by high-ranking journal.

2. Biological activities

Simple phenylpropanoids are widely found in fruits, vegetables, cereals, coffee, tea, and some traditional herbs.[14–16] They possess various biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, neuroprotective, and anticancer properties.[17–20] In addition to their pharmacological activities, simple phenylpropanoids are core precursors to many complex natural products, such as flavonoids, lignans, and polyphenols. Therefore, simple phenylpropanoids play a significant role in food, cosmetic, nutraceutical, chemical, and, especially, the pharmaceutical industries (Fig. 1).

2.1. Bioactivities of simple phenylpropanoids

2.1.1. Ultraviolet protection. Due to their perfuming, antioxidant and ultraviolet (UV) protection properties, *trans*-cinnamic acid (*t*-CA) and its derivatives, such as cinnamyl aldehyde, cinnamyl alcohol, cinnamyl alcohol, have been widely used in cosmetics for decades.[21] It is well known that UV

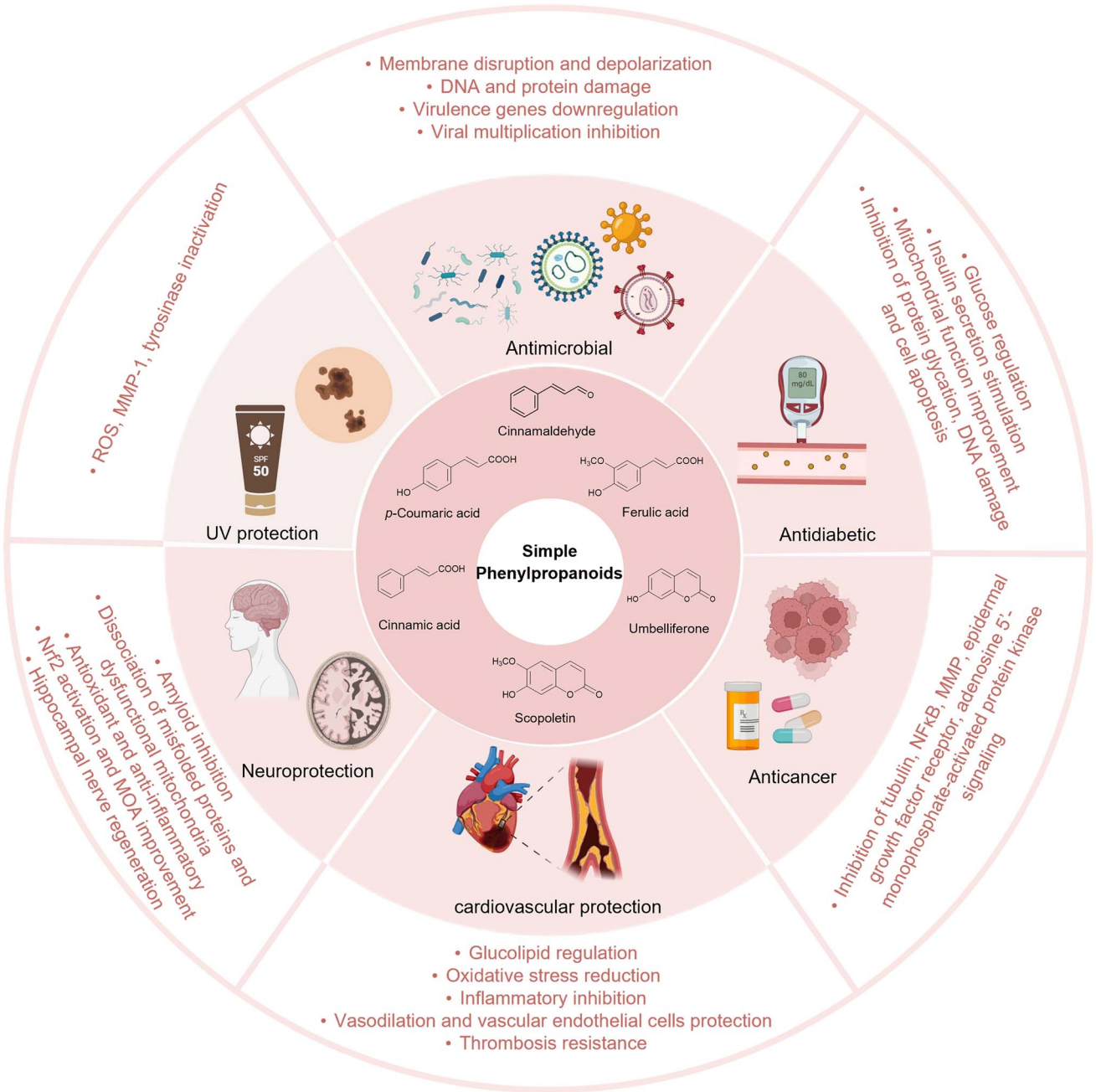


Fig. 1 Biological activities and pharmacological functions of simple phenylpropanoids. Abbreviations: NFκB, nuclear factor κB; MMP, matrix metalloproteinase; Nrf2, NF-E2-related factor 2; MOA, monoamine oxidase A; ROS, reactive oxygen species. Figure created with https://Biorender.com.

irradiation of skin can lead to the increased expression of matrix metalloproteinase-1 (MMP-1) and tyrosinase, which are responsible for collagen breakdown and pigmentation, and reactive oxygen species (ROS) production. Caffeic acid (CaA) and sinapic acid can inhibit MMP-1 expression, ROS generation and collagen degradation *in vitro* through the inactivation of mitogen-activated protein kinases and nuclear factor κB (MAPKs/NFκB) signalling pathways.[22] Therefore, these compounds are effective in the prevention and treatment of UV irradiation-induced skin photoaging. Tyrosinase is the rate-limiting step in melanogenesis in human skin. Because of their structural similarity to tyrosine, *t*-CA and cinnamyl aldehyde show anti-tyrosinase activities and can be used to treat dermatological disorders by blocking the process of melanogenesis, such as occurs during pigmentation and melanoma.[23] Cinnamic acid-derived *p*-coumaric acid (*p*-HCA) is a drug candidate that has been extensively tested *in vitro* and *in vivo* for the treatment of hyperpigmentation.[24]

2.1.2. Antimicrobial. Simple phenylpropanoids, such as *t*-CA, cinnamyl alcohol, have been utilized as functional ingredients for natural food preservatives because of their antimicrobial activities.[25,26] Cinnamyl aldehyde is the most potent antimicrobial substance in cinnamon, and is active against multiple bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella anatum*, as well as many fungi.[27] *trans*-Cinnamyl aldehyde is identified as Generally Recognized as Safe by the United States Food and Drug Administration, and has not only served as a fungicide in agriculture, but is also added into edible antimicrobial films for inhibition of foodborne pathogens.[28] There are many mechanisms involved in the antimicrobial activity of simple phenylpropanoids, which may differ for specific pathogens. CaA has been shown to relieve chronic infections induced by microorganisms (bacteria, fungi, and viruses), mainly through membrane disruption and depolarisation, by damaging DNA and protein structures, by downregulating virulence genes, and by inhibiting cellular proliferation.[29] Interestingly, *t*-CA has been observed to generate multiple-target or synergistic effects in combination with classical antimalarial drugs.[30] These results have inspired the exploration of the application of simple phenylpropanoids combined with other natural antimicrobials or medicinal antibiotics, which may revitalise conventional drugs and address antibiotic resistance issues. Recently, a mouse experiment evaluated the antiviral effects of *t*-CA against Zika virus.[31] These plant-derived compounds have the potential to be used as a nutraceutical or drug candidate against pandemics.

2.1.3. Antidiabetic. Diabetes, a metabolic disorder characterised by insulin resistance and hyperglycaemia, is associated with oxidative stress and increased inflammation. The inability to regulate blood glucose levels leads to multiple complications, including retinopathy and vascular lesions.[32] *t*-CA and its derivatives have beneficial effects on diabetes and its complications. It regulates glucose metabolism by improving glucose tolerance and stimulating insulin secretion. Cinnamic aldehydes are effective in improving insulin sensitivity and mitochondrial function.[33] Elevated plasma concentrations of methylglyoxal are associated with the onset and progression of diabetes, but ferulic acid (FA) can inhibit methylglyoxal-induced DNA damage, protein glycation, and cell apoptosis in pancreatic β-cells.[34] Umbelliferone has antioxidant and anti-glycation activities in diabetic mice, and can delay diabetic nephropathy by attenuating ferroptosis *via* activation of the Nrf-2/HO-1 pathway.[35]

2.1.4. Anticancer. The ubiquitous presence of α,β-unsaturated acid groups in simple phenylpropanoids makes these derivatives potentially therapeutic in the treatment of cancer through different mechanisms. *t*-CA has anticancer effects by inhibiting a wide range of targets in cancer cells, including NFκB,[36] tubulin,[37] adenosine 5′-monophosphate-activated protein kinase signalling,[38] epidermal growth factor receptor, and matrix metalloproteinase.[39] *p*-HCA is known to display favourable inhibitory effects on lung cancer, breast cancer, liver cancer, colon adenocarcinoma and neuroblastoma, as it has apoptotic and antiproliferative effects.[40] Treatment with CaA has been reported to selectively remove induced pluripotent stem cells (iPSCs) without affecting normal and iPSC-derived differentiated cells, indicating that it can be used as a safe and economical anti-teratoma agent in iPSCs-based therapy.[41] Moreover, CaA can play multiple roles in different stages of chemotherapy. Pre-treatment can regulate chemoresistance, meaning that CaA and caffeic acid phenethyl ester enhance the sensitivity of cancer cells to chemotherapy.[42] Co-administration with other antitumour drugs can display synergistic effects.[43] CaA can also prevent cancer recurrence by directly targeting cancer stem cells.[44] Through studies on network pharmacology and molecular docking, EGFR, BRAF, and AKT1 have been proven to be key targets of scopoletin against non-small cell lung cancer.[45] Drug combination has been a popular strategy for the discovery and development of novel drugs. CaA and FA bound to resveratrol have shown stronger inhibitory effects on the proliferation of human colorectal cancer cells (HCT116).[46] Accordingly, it is conceivable that simple phenylpropanoid-based hybrids can provide new ideas for discovering novel anticancer agents to improve therapeutic efficiency, reduce side effects, and overcome multidrug resistance.

2.1.5. Neuroprotection. Cognitive impairment is an increasingly important public health issue in modern society. Aβ aggregation, mitochondrial dysfunction and oxidative stress are major factors associated with neurodegeneration in Alzheimer's disease (AD). Hydroxycinnamic acids can show neuroprotective and pro-cognitive effects by modulating oxidant machinery and inflammatory status.[18] Hydroxycinnamic acids can also activate autophagy and limit brain damage by breaking down misfolded proteins and dysfunctional mitochondria.[47] Due to their activities as antioxidants, anti-inflammatories and for their ability to inhibit amyloid formation, FA has served as a scaffold to develop many potential analogues for AD therapy.[48,49] As the α,β-unsaturated carbonyl group serving as an activator of NF-E2-related factor 2 (Nrf2), coniferaldehyde was screened from cinnamaldehyde analogues and was found to greatly attenuate AD-like pathology and preserve brain function in the APP/PS1 AD mouse model by activating Nrf2.[50] By combining *t*-CA and a D-amino acid, a unique anti-neuroinflammatory compound was

synthesised with some efficacy against AD, and was found to be able to bind strongly the proinflammatory cytokine interleukin-1β (IL-1β).[51] Recently, a number of non-clinical trials have supported antidepressant effects of phenylpropanoids. FA can achieve significant alleviation of depression in animal models by enhancing monoamine oxidase A (MOA) activity, promoting hippocampal nerve regeneration, anti-oxidative stress, and anti-inflammatory activity, and activating the protein kinase B/collapsin response mediator protein 2 (AKT/CRMP2) signalling pathway.[52]

2.1.6. Cardiovascular protection. Abnormal blood lipid levels are implicated in the pathogenesis of cardiovascular disease. The analysis of hyperlipidemic subjects revealed that FA could improve lipid profiles, and reduce levels of the oxidative stress biomarker and the inflammatory markers (hs-CRP).[53] FA has antithrombotic activity and appears protective to vascular endothelial cells and is expected to be useful in preventing coronary heart disease and atherosclerosis.[54] The mechanisms of *t*-CA and cinnamic aldehyde on ameliorating glucolipid metabolism are similar in mouse experiments and can improve mitochondrial function, reduce serotonin content and upregulate autophagy-mediated lipid clearance.[55] Caffeic acid phenethyl ester, which is isolated from propolis, is the most studied CaA derivative. It has vasorelaxant, antihypertensive, anti-atherosclerotic and anti-angiogenic activities and is the most promising compound for clinical application.[56] Because of their antioxidant and vasodilator effects, coumarins can be used as lipid-lowering agents. Umbelliferone has been shown to be an antidiabetic and antihyperlipidemic agent in hyperglycaemic rat models and exerts protective effects on the liver and kidney to reduce diabetic complications.[57] A separate study on isoproterenol-induced myocardial injury showed that umbelliferone effectively protected normal cardiac function from oxidative and inflammatory responses and cell death by upregulating the Nrf2/HO-1 signalling pathway.[58]

2.2. Bioactivities of complex phenylpropanoids

In addition to serving as versatile phytochemicals, simple phenylpropanoids are key precursors of valuable complex natural products. The lignan compound podophyllotoxin has significant antitumor activity and is the raw material used in synthesis of etoposide, a clinically important chemotherapy drug.[59] Silybin, a flavonolignan, exerts a therapeutic effect on hepatic disease.[60] Icaritin is extracted from the Chinese *Herba Epimedii* and has been developed as a clinical drug for the treatment of liver cancer in China. Icaritin shows anti-inflammatory and immunomodulatory effects.[61] Salvianolic acid B is the major bioactive water-soluble polyphenolic acid of *Salvia miltiorrhiza*, exhibits anti-inflammatory, anticancer, and cardioprotective activities, and has been clinically utilised to treat cardio- and cerebrovascular disorders.[62,63]

Current efforts have developed novel forms such as nanoparticles to enhance bioavailability, and many artificial derivatives based on simple phenylpropanoid scaffolds have been synthesised to further expand applications.[64]

3. Biosynthetic pathways

The diversity and conservatism of simple phenylpropanoids are the result of efficient modification and amplification towards the fundamental "C6–C3" structure through an orchestrated cascade of enzymes, including oxygenases, reductases, ligases and transferases.[65] The biosynthesis of simple phenylpropanoids are derived from the aromatic amino acids (AAAs) L-Phe and L-tyrosine (L-Tyr) in plants (Fig. 2). As nodes connecting primary and secondary metabolism, L-Phe and L-Tyr are synthesised from chorismate (CHA) *via* the AAA pathway. The condensation of two core precursors, phosphoenolpyruvate (PEP), derived from glycolysis, and erythrose 4-phosphate (E4P), derived from the pentose phosphate pathway (PPP), leads to a metabolic flux into the shikimate pathway for CHA synthesis. Therefore, the biosynthetic pathways of simple phenylpropanoids can be divided into primary metabolism and plant-specific phenylpropanoid metabolism. Except for plant-specific CO_2 fixation (shown in green in Fig. 2), the AAA biosynthetic pathway is relatively conserved in plants and microorganisms (shown in red in Fig. 2). Biosynthetic pathways of simple phenylpropanoids and their derived complex phenylpropanoids usually exist in plants.

Plants absorb carbon dioxide to produce sugar, whereas glucose is the most direct carbon source for cellular biomass in bacteria and fungi. Central carbon metabolism converts glucose to PEP and E4P *via* glycolysis and nonoxidative steps in the PPP, respectively. Notably, PPP is related to the redox cofactor NADPH, which is critical for several downstream enzymes and the normal cellular energy metabolism.[66,67] The pathway connecting central carbon metabolism and the AAA network is the shikimate pathway, which comprises seven enzymatic reactions and is ubiquitous in plants, fungi and bacteria.[68] As the first committed step, studies have shown that the aldol condensation of PEP and E4P to produce 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) in a reaction catalysed by DAHP synthase is a rate limiting reaction.[69–71]

The following steps to generate the final product of the shikimate pathway, chorismate (CHA), are typically supported by individual monofunctional enzymes in plants and *E. coli*, including 3-dehydroquinate synthase (Dhps), 3-dehydroquinate dehydratase (Dqd), shikimate dehydrogenase (Sdh), shikimate kinase (Sk), 5-enolpyruvate-shikimate-3-phosphate synthase (Epsp synthase) and chorismate synthase (CS), whereas in *Saccharomyces cerevisiae*, a penta-functional protein (Arom) can directly catalyse the reaction of DAHP to 5-enolpyruvylshikimate-3-phosphate (EPSP).[72,73] As a key branching point of the primary metabolic pathway to make aromatic amino acids, CHA firstly forms prephenate (PPA) in a reaction catalysed by chorismate mutase (Cm). The subsequent conversion to Phe and Tyr occurs *via* two routes in plants. One is conserved in both plants and microorganisms, where PPA catalyses the synthesis of L-Phe by prephenate dehydratase (Pdt) and phenylpyruvate aminotransferase (Ppy-at) or L-Tyr by prephenate dehydrogenase (Pdh) and 4-hydroxyphenylpyruvate aminotransferase (Hpp-at). PPA is converted to L-arogenate (AGN) by prephenate aminotransferase

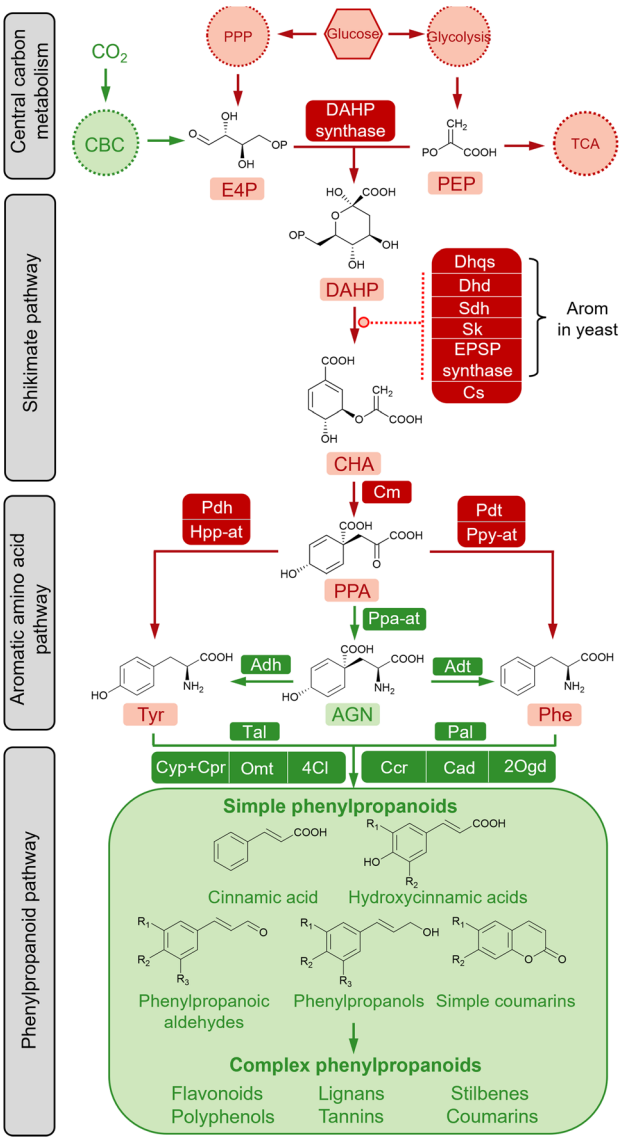


Fig. 2 The schematic diagram of simple phenylpropanoids biosynthesis. The conserved part of the pathway in plants and microorganisms (including glycolysis, pentose phosphate pathway (PPP), shikimate pathway, and aromatic amino acid pathway) and the specific part in plants (including CO_2 fixation and simple phenylpropanoid pathway) are shown in red and green, respectively. Abbreviations: CO_2, carbon dioxide; CBC, Calvin–Benson cycle, TCA, tricarboxylic acid cycle; PPP, pentose phosphate pathway; E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; CHA, chorismate; PPA, prephenate; AGN, L-arogenate. Enzymes: Dhqs, 3-dehydroquinate synthase; Dhd, 3-dehydroquinate dehydratase; Sdh, shikimate dehydrogenase; Sk, shikimate kinase; EPSP synthase, 5-enolpyruvate-shikimate-3-phosphate synthase; Cs, chorismate synthase; Cm, chorismate mutase; Pdh, prephenate dehydrogenase; Hpp-at, 4-hydroxyphenylpyruvate aminotransferase; Pdt, prephenate dehydratase; Ppy-at, Phenylpyruvate aminotransferase; Ppa-at, prephenate aminotransferase; Adh, arogenate dehydrogenase; Adt, arogenate dehydratase; Pal, phenylalanine ammonia-lyase; Tal, tyrosine ammonia-lyase; Cyp, cytochrome P450-dependent monooxygenase; Cpr, Cytochrome P450 reductase; Omt, *O*-methyltransferase; 4Cl, 4-coumarate-CoA ligase; Ccr, cinnamoyl-CoA reductase; Cad, cinnamyl alcohol dehydrogenase; 2Ogd, 2-oxoglutarate-dependent dioxygenase.

(Ppa-at), which is then converted in reactions catalysed by arogenate dehydratase (Adt) or arogenate dehydrogenase (Adh) to L-Phe or L-Tyr, respectively.[74,75]

The biosynthetic pathway producing simple phenylpropanoids that occurs in plants has been heterologously established in microorganisms—the specific pathways involved are described in the following section. The conversion of L-Phe to *t*-CA is catalysed by phenylalanine ammonia-lyase (Pal). Studies have demonstrated that the broad substrate spectrum of Pal includes L-Phe and L-Tyr, and that the reverse reaction to L-Phe can also be catalysed by Pal in the presence of ammonia.[76] *t*-CA is modified by the hydroxylation and methylation of the aromatic ring, giving rise to hydroxycinnamic acids. Among these, *p*-HCA can also be directly formed from the deamination of L-Tyr by the bifunctional Phe/Tyr ammonia-lyase (PTal) enzyme from monocot grasses of the Poaceae family, or from some bacterial and fungal species.[77] The enzyme 4-coumarate-CoA ligase (4Cl) catalyses the conversion of phenylpropanoic acids to the coenzyme A (CoA) thioesters, which are then converted to the corresponding aldehydes by cinnamoyl-CoA reductase (Ccr), and subsequently to phenylpropanols by cinnamyl alcohol dehydrogenase (Cad), and simple coumarins by 2-oxoglutarate-dependent dioxygenase (2Ogd). Furthermore, simple phenylpropanoids are considered basic building blocks for downstream decorations to produce large quantities of secondary metabolites, such as flavonoids, stilbenes, lignans, polyphenols, and condensed tannins.[65] These complex phenylpropanoids and their derivatives have various biological and commercial values and are being intensively evaluated.[78]

4. Microbial production

As the compounds connecting the microbial innate pathway and the plant allogenic pathway, L-Phe and L-Tyr obtain carbon fluxes from the central carbon metabolic and shikimate pathways for the synthesis of simple phenylpropanoids (Fig. 2). Reprogramming the primary metabolic flux to AAAs (L-Phe and L-Tyr) is the basis for the high-level synthesis of simple phenylpropanoids. Therefore, the construction of a microbial cell factory for the efficient synthesis of simple phenylpropanoids requires two steps: construction of an AAA high-producing chassis (Fig. 3) and introduction and optimization of simple phenylpropanoid pathways (Fig. 4).

4.1. Efficient microbial production of AAAs

Multilevel metabolic engineering strategies have been studied to overproduce AAAs (L-Phe and L-Tyr), such as rewiring central carbon metabolism, relieving negative regulation, eliminating by-product formation, and engineering transport processes (Fig. 3). To rewire the central carbon flux towards the AAA pathway, an improved supply of PEP and E4P is required. The PPP is the exclusive pathway for glucose conversion to E4P, and its optimization is crucial for E4P supply. Since *S. cerevisiae* tends to quickly introduce metabolic flux into glycolysis for ethanol fermentation under anaerobic conditions and limits PPP, the deficiency of E4P is a major problem.[79] In addition, E4P has been

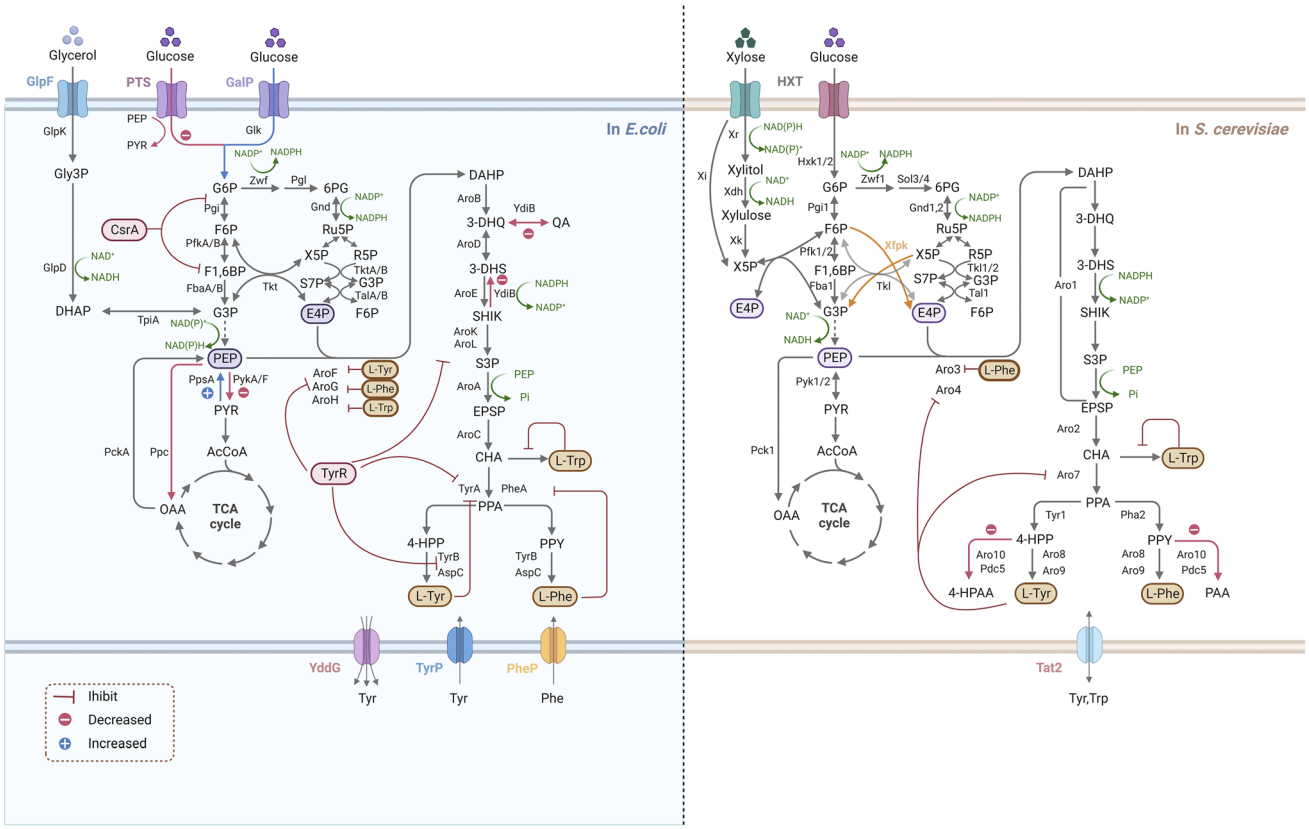


Fig. 3 Overview of metabolic engineering strategies for efficient microbial production of AAAs in *E. coli* and *S. cerevisiae*. Endogenous AAA pathways are represented by the solid black arrows, the dashed arrows indicate multiple reactions, while the alternative pathway that have been evaluated are represented by yellow arrows. The blue arrows represent the increased fluxes by overexpressing genes, the red arrows represent the decreased fluxes by deletion of genes. The indicator arrows (red) show inhibitions by isoenzymes allosteric regulatory circuits and the transcriptional regulatory factors. Abbreviations: (a) in *E. coli*: GlpF, glycerol uptake facilitator; GlpK, glycerol kinase; Gly-3P, sn-glycerol-3-phosphate; GlpD, glycerol 3-phosphate dehydrogenase; TpiA, triosephosphate isomerase; PTS, phosphoenolpyruvate phosphotransferase system; GalP, galactose permease; Glk, glucokinase; G6P, glucose 6-phosphate; Pgi, phosphoglucose isomerase; F6P, fructose 6-phosphate; PfkA/B, phosphofructokinase; F1, 6BP, fructose-1,6-bisphosphate; FbaA/B, fructose 1,6-bisphosphate aldolase; G3P, glyceraldehyde 3-phosphate; Zwf, glucose-6-phosphate dehydrogenase; Pgl, 6-phosphogluconolactonase; 6PG, 6-phosphogluconate; Gnd, 6-phosphogluconate dehydrogenase; Ru5P, ribulose 5-phosphate; X5P, xylulose-5-phosphate; R5P, ribose 5-phosphate; S7P, sedoheptulose 7-phophate; F6P, fructose 6-phosphate; TalA/B, transaldolase; TktA/B, transketolase; E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; PpsA, PEP synthase; PykA/F, pyruvate kinase; PckA, PEP carboxykinase; Ppc, PEP carboxylase; PYR, pyruvate; AcCoA, acetyl-CoA; OAA, oxaloacetate; TCA, tricarboxylic acid cycle; 3-DHQ, 3-dehydroquinate; 3-DHS, 3-dehydroshikimate; SHIK, shikimate; S3P, shikimate 3-phosphate; EPSP, 5-enolpyruvate-shikimate-3-phosphate; CHA, chorismate; PPA, prephenate; 4-HPP, 4-hydroxyphenylpyruvate; PPY, phenylpyruvate. 4-HPAA, 4-hydroxyphenylacetaldehyde; PAA, phenylacetaldehyde; AroF/G/H, DAHP synthases; AroB, 3-dehydroquinate synthase; AroD, 3-dehydroquinate dehydratase; AroE, shikimate dehydrogenase; YdiB, quinate/shikimate dehydrogenase; AroK/L, shikimate kinase; AroA, Epsp synthase; AroC, chorismate synthase, PheA/TyrA, chorismite mutase/prephenate dehydratase; TyrB, aromatic amino acid aminotransferase; AspC, aspartate aminotransferase; CsrA and TyrR, transcriptional repressors; YddG, TyrP, PheP, transporters of aromatic amino acids (b) in *S. cerevisiae*: Xr, xylose reductase; Xdh, xylitol dehydrogenase; Xi, xylose isomerase; Xk, xylulokinase; Hxk1/2, hexokinase; Pgi1, phosphoglucose isomerase; Pfk1/2, phosphofructokinase; Fba1, fructose 1,6-bisphosphate aldolase; Zwf1, glucose-6-phosphate dehydrogenase; Sol3/4,6-phosphogluconolactonase; Gnd1/2,6-phosphogluconate dehydrogenase; Tal1, transaldolase; Tkl1/2, transketolase; Xfpk, phosphoketolase; Pyk1/2, pyruvate kinase; Pck1, PEP carboxykinase; Aro3/4, DAHP synthases; Aro1, a penta-functional protein catalyzing DAHP to EPSP; Aro2, chorismate synthase; Aro7, chorismate mutase holoenzyme; Pha2, prephenate dehydratase; Tyr1, prephenate dehydrogenase. Aro8/9, aromatic amino transferases; Aro10, phenylpyruvate decarboxylase; Pdc5, pyruvate decarboxylase; Tat2: tryptophan and tyrosine permease. Figure created with https://Biorender.com.

reported to be the primary limiting substrate for AAA biosynthesis in some bacteria, such as *E. coli*, *B. subtilis*, and *Corynebacterium glutamicum*.[80–82] In *S. cerevisiae*, the optimization strategy for PPP depends on cell culture conditions because the upstream (Zwf and Gnd) and downstream (Tkl and Tal) enzymes of PPP are rate-limiting steps for E4P synthesis under high- and low-glucose concentrations, respectively.[83] Therefore, different strategies should be used for PPP optimization to accommodate the use of high- and low-glucose-induced promoters.[83–85] A novel PHK pathway consisting of phosphoketolase (Xfpk) can split xylose 5-phosphate (X5P) and/or fructose 6-phosphate (F6P) into acetyl-phosphate and glyceraldehyde-3-phosphate (G3P)/E4P, which is critical for rewiring the carbon metabolism of glycolysis into E4P.[86] Therefore, simultaneous expression of Xfpk, Tal, and Tkl can significantly increase the metabolic flux towards E4P in *S. cerevisiae*.[83] Although *S. cerevisiae* is not able to use xylose as

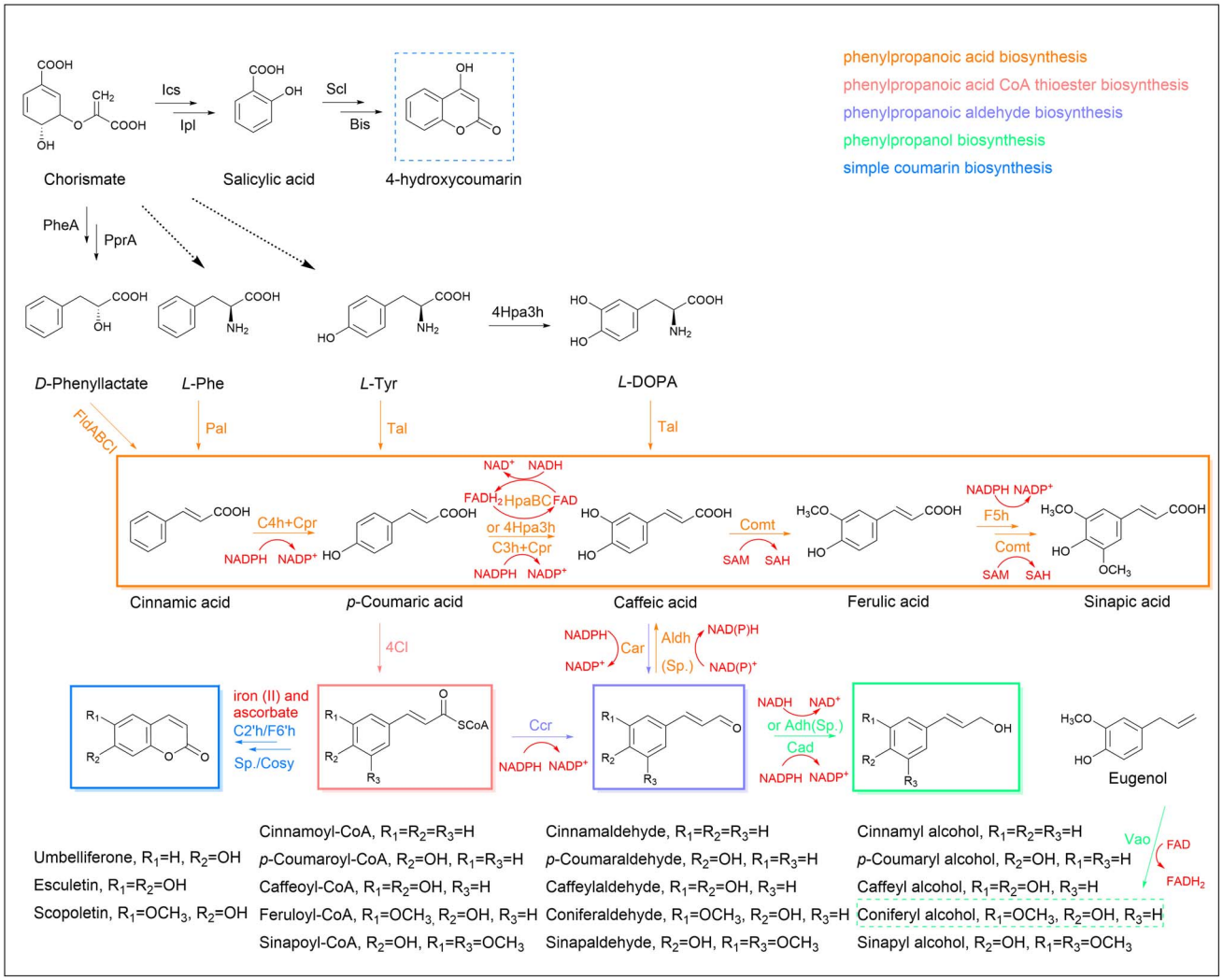


Fig. 4 Biosynthesis of simple phenylpropanoids. The multiple reactions are represented by the dashed arrow. Abbreviations: Ics, isochorismate synthase; Ipl, isochorismate pyruvate lyase; Scl, salicylate-CoA ligase; Bis, biphenyl synthases; PheA, chorismate mutase; PprA, phenylpyruvate reductase; FldABCI phenyllactate dehydratase; Pal, phenylalanine ammonia-lyase; Tal, tyrosine ammonia-lyase; C4h/C3h, cytochrome P450-dependent monooxygenase; Cpr, Cytochrome P450 reductase; 4Hpa3h, 4-hydroxyphenylacetic acid 3-hydroxylase; HpaBC, *p*-hydroxyphenylacetate 3-hydroxylase; Comt, caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferase; F5h, ferulate 5-hydroxylase; 4Cl, 4-coumarate-CoA ligase; Ccr, cinnamoyl-CoA reductase; Cad, cinnamyl alcohol dehydrogenase; Car, carboxylic acid reductase; Aldh/Adh, endogenous aldehyde/alcohol dehydrogenase; C2′h, coumaroyl-CoA 2′-hydroxylase; F6′h, feruloyl-CoA 6′-hydroxylase. Cosy, coumarin synthase; Sp., spontaneous reactions.

a sole carbon source, it is clear that metabolic flux enters the PPP faster for E4P synthesis when xylose is used, which may be useful in further improving the E4P supply in yeast.[87] In contrast with E4P, carbon flow towards PEP is usually sufficient because of its important position in glycolysis. Construction of a non-PTS route in *E. coli* has been proven to avoid PEP loss for glucose transport, (>50%), thereby supporting the synthesis of downstream AAA.[88] The availability of PEP and E4P can also be improved by adjusting the activity of precursor pathway enzymes, such as by deletion of pyruvate kinases (encoded by *PYKA* and *PYKF*), or by over-expression of PEP synthase (encoded by *PPSA*), transketolase (encoded by *TKTA*) and/or transaldolase (encoded by *TALB*).[89–91] It is reasonable that the carbon flux between PEP and E4P is significantly different, thus it is essential to balance the availability of E4P and PEP (increase E4P and decrease PEP) to maintain high productivity. Promoter engineering by fine-tuning the strength of glycolysis and PEP alleviated this limitation in *S. cerevisiae*.[92,93]

Since bypass inevitably diverts the flow of the target pathway, it is necessary to control the pathway by weakening its branches

to facilitate product synthesis. In *E. coli*, the bifunctional enzyme (YdiB) was replaced by the monofunctional enzyme (AroE) to reduce the by-product formation of quinate in the shikimate pathway.[94] In *S. cerevisiae*, increased flux in the AAA biosynthetic pathway results in the accumulation of undesired fusel alcohol/acids *via* the Ehrlich pathway, which can be relieved by a double knock-out of phenylpyruvate decarboxylase (Aro10) and pyruvate decarboxylase (Pdc5).[95] Interestingly, a recent report has found that the deletion of a novel gene HTZ1 significantly increased the tyrosine-producing capacity of yeast cells, although the underlying mechanism remains to be elaborated.[96] Primary metabolism is strictly regulated by microorganisms, including yeasts. At the enzyme activity level, metabolic flux is controlled mainly by AAA-mediated feedback inhibition in the first and post-chorismate steps in the above-mentioned biosynthetic pathways. By protein structure analysis with site directed mutagenesis, the feedback-resistant (fbr) variants such as aro4^{K229L} and aro7^{G141S} have been widely utilized in *S. cerevisiae* to eliminate the allosteric regulation for the enhancement of the corresponding AAAs and derived metabolites.[71,97,98] *CSRA*, encoding the carbon-storage regulator of *E. coli*, regulates enzymes in glycolysis, and over 2- fold increase in yield of L-Phe were achieved when CsrA was disrupted.[99] TyrR is the tyrosine repressor that negatively regulate several genes transcription of Tyr-producing pathway in *E. coli*, and inactivation of TyrR led to an increase in the L-Tyr biosynthesis.[100,101] Transport engineering contributes to strain development by addressing substrate uptake and product output, as well as loss of intermediates. The tyrosine-specific transporters TyrP and TyrR were mutated in *E. coli* overexpressing aroG$^{\Delta fbr}$, aroL and tyrC, leading to a maximum L-Tyr titre of 43.14 g L^{-1} by fed-batch fermentation.[102]

Notably, the regulation of cell growth and highly tailored production should be carried out in an optimal and sustainable manner. A synthetic RNA (sRNA) strategy was employed in *E. coli* to fine-tune target gene expression, which could coordinate the flux balance between L-Tyr production and biomass accumulation.[103] Adaptive laboratory evolution could also be useful as a way of enhancing the robustness of engineered strains. A *Pseudomonas putida* S12 mutant strain highly accumulating L-Phe was successfully screened by a combination of random mutagenesis and selection of toxic analogs.[104] Recently, with the aid of biosensors as a high-throughput screening tool, recombinant strains with the desired properties for enhanced AAAs are likely to be identified in high efficiency.[105,106] Researchers have explored the potential of non-conventional microbial chassis to generate AAAs, such as *Yarrowia lipolytica* and *C. glutamicum*, which have been an attractive platform optimized for aromatic compounds production.[107,108]

4.2. Efficient microbial production of simple phenylpropanoids

4.2.1. Phenylpropanoic acids

4.2.1.1. Cinnamic acid. Cinnamic acid belongs to a class of auxins that regulate cell growth and differentiation. Owing to its broad applicability, the microbial production of cinnamic acid, which is a relatively facile and green technology, has been actively explored over the last few decades. Cinnamic acid is the non-oxidative deamination product of L-Phe in a reaction catalysed by Pal and mostly occurs in the *trans* configuration (*t*-CA) (Fig. 4). Pal is the starting and limiting enzyme in the simple phenylpropanoids biosynthetic pathway, hence much effort has been made in identifying high-efficiency alternative enzymes for functional expression in heterologous microbes.[109,110] The production titre of *t*-CA reached 6.9 g L^{-1} by heterologous expression of an efficient *Sm*Pal from *Streptomyces maritimus* in an engineered L-Phe-overproducing *E. coli*, together with cultivation optimization and casamino acid supplementation in a 2 litre bioreactor.[111] In order to further optimize the catalytic function of *Sm*Pal, the combination of a *Sm*Pal-based whole-cell biocatalyst in *C. glutamicum* and a crossflow membrane-based cell recycling system was designed to produce *t*-CA from L-Phe with a yield of 75% (0.75 mol mol^{-1}).[112] An alternative pathway harbouring a phenyllactate dehydratase encoded by FLDABCI genes from *Clostridium sporogenes* was developed for additional *t*-CA production from D-phenyllactate (Fig. 4), which exceeded the Pal-dependent pathway to achieve an 11-fold increase in *t*-CA yield.[113] Most recently, a high-density culture system of the cyanobacterium *Synechocystis* sp. PCC 6803 successfully synthesized approximately 0.8 g L^{-1} *t*-CA from CO_2 as the carbon source,[114] which indicates that exploiting the primary metabolism of cyanobacteria has great potential for the green and sustainable production of phenylpropanoids. As the cytotoxicity of *t*-CA impairs cell proliferation, researchers have found that near-neutral culture pH and alcoholic carbon source might be useful to improve the cellular tolerance to *t*-CA inhibition.[115]

4.2.1.2. Coumaric acid. Coumaric acid, also known as hydroxycinnamic acid, naturally exists as three isomers (*ortho*-, *meta*-, and *para*-) with different substitution positions for the active hydroxyl group on the benzene ring. Among these, *para*-coumaric acid (*p*-HCA) occurs more often in nature.[116] With advances in metabolic engineering, microbial production has become a promising alternative for obtaining large-scale yields of *p*-HCA. Starting with aromatic amino acids, L-Tyr can be directly converted to *p*-HCA by Tal, which is typically found in bacteria. Alternatively, the deamination product *t*-CA of L-Phe can be further hydroxylated to *p*-HCA by membrane bound cinnamate-4-hydroxylase (C4h) in plants.[117] The Tal-dependent one-step biosynthesis is more convenient and economical; also, the Pal-C4h pathway requires plant-derived Cpr and cofactor NADPH to form the electron transport chain to complete the hydroxylation reaction (Fig. 4). Therefore, the one-step reaction catalysed by Tal is favoured for application in *p*-HCA production from microbial cell factories.[118–120] However, this conversion is often limited by the poor activity of the heterologous Tal enzyme and the competitive inhibition of *p*-HCA on Tal and Pal. Therefore, it is necessary to identify novel enzymes with higher and more stable activities from different sources. The substrate specificity and activity of enzymes can be altered by rationally designing active amino acid residues. By the combination of directed evolution and high-throughput screening, a Tal variant from *Rhodotorula glutinis* with higher selectivity for L-Tyr and superior catalytic efficiency was

screened, producing a *p*-HCA titre that was 2.2-fold higher than that of the control strain.[121] Systematic bioinformatics analysis and enzyme characterization have been applied to identify Tals most suitable for *p*-HCA production in *E. coli* and yeast.[122,123] Interestingly, when the *Fj*Tal protein was anchored to the yeast vacuole, *p*-HCA production was significantly enhanced.[124] Although C4h as a membrane-bound Cyp is not amenable to expression in *E. coli*, a novel C4h from *Lycoris aurea* was identified and functionally expressed in *E. coli*, and when combined with the optimization of NADPH regeneration and Cpr expression enabled a *p*-HCA titre of 25.62 mg L^{-1}.[125] In order to greatly enlarge the capacity of yeast in producing *p*-HCA, carbon metabolic allocation is optimized towards aromatic amino acid biosynthesis, which, together with the microbial Tal and introduction of a plant Pal-C4h, led to a high-level production of *p*-HCA (12.5 g L^{-1}) in fed-batch fermentation in *S. cerevisiae*.[126] To eliminate the Crabtree effect, xylose as the carbon source for *p*-HCA production was investigated in *S. cerevisiae* and finally achieved a 45-fold increase in *p*-HCA production compared to the use of glucose as the carbon source under the same conditions. In recent years, the *de novo* synthesis of *p*-HCA in *Synechocystis* sp. PCC 6803 has been achieved. Using the fixation of CO_2 by solar energy as the carbon source, the highest total titre was up to 0.4 g L^{-1} in a high-density cultivation system.[127,128] To eliminate the genetic and production instability of strains using episomal plasmid expression in the fermentation, a high *p*HCA-yielding *S. cerevisiae* was built through POT1-mediated delta integration, after which the titre and gene copy number remained stable for more than one hundred generations.[129] Transporters are responsible for the efflux of small product molecules, and have showed significance in strain improvement. The deletion of a specific transporter (TAT1), which is responsible for transport of L-Tyr and L-Trp, led to a 50% increase in *p*-HCA titre.[130] Upregulation of the aromatic acid transporter ESBP6 promoted increases in the *p*-HCA titre, as well as improvements in the yeast tolerance to toxicity.[131] Although the microbial production of *p*-HCA has already achieved on a g L^{-1} scale, microbial cell factories still need to be further optimized for high-level synthesis, such as by targeting product degradation and inhibition, cofactor imbalance, and transcriptional regulation.[132,133]

4.2.1.3. Caffeic acid. CaA is a phytonutrient frequently found in plants, including vegetables. The biosynthesis of CaA involves the *ortho*-hydroxylation of *p*-HCA through the Cyp enzyme *p*-coumarate 3-hydroxylase (C3h) (Fig. 4), which is traditionally difficult to perform in bacteria.[134] Therefore, identifying different C3h isozymes from other organisms is crucial for the synthesis of CaA in engineered cells. A microbial C3h encoded by SAM5 from *Saccharothrix espanaensis* and a site-directed mutant of CYP199A2 from *Rhodopseudomonas palustris* were shown to have activity when expressed in bacteria to synthesise CaA from *p*-HCA.[135,136] The *de novo* biosynthesis of CaA in *E. coli* was also achieved by expressing endogenous 4-hydroxyphenylacetic acid 3-hydroxylase (4Hpa3h) that can hydroxylate both *p*-HCA and L-Tyr to generate CaA and L-3,4-dihydroxyphenylalanine (L-DOPA), respectively, while L-Dopa was further converted to CaA by Tal from *Rhodobacter capsulatus*.[91] An alternative pathway catalysed by bacteria-coupled enzymes HpaB (FAD-dependent 4-hydroxyphenylacetate-3-monooxygenase) and HpaC (NADH-flavin oxidoreductase) has been shown to be the most preferred strategy for CaA synthesis in both bacterial and fungal systems.[137,138] In this pathway, the direct electron donor is $FADH_2$, rather than NADPH, and $FADH_2$-dependent enzymes are usually more adapted in microorganisms.[139] The yield of CaA also depends on the compatibility of HpaB and HpaC in cell factories. Through orthogonal assays, the combination of HpaB from *Pseudomonas aeruginosa* and HpaC from *Salmonella enterica* was regarded as the best.[140] By expressing this enzyme pair, combined with the cofactor optimization strategy, the yield of CaA in *S. cerevisiae* reached 5.5 g L^{-1}.[83] This strategy using suitable cofactor engineering provides fundamental insights for future research in other natural product biosynthesis. Apart from enzyme screening and cofactor engineering, control of transport proteins has also been proven to be an effective strategy for reducing cytotoxicity and promoting CaA synthesis. By overexpressing the putative sugar ABC transporter permease (YcjP), which was identified through transcriptome data mining, the production of CaA was further enhanced to about 7.9 g L^{-1} in *E. coli* with the integration of other optimized factors. This is the highest known titre so far.[141] Recently, the multi-copy integration expression strategies based on delta sites were applied to integrate the genes of the CaA pathway into yeast and resulted in an increase of CaA production by 50 times compared to that produced by the initial multi-copy-plasmid expression.[142] Some other carbon sources and the whole-cell biocatalyst strategy have also been successfully used for CaA synthesis.[143,144] By introducing the xylose assimilation pathway into *Candida glycerinogenes*, the advantage of using mixed sugars as carbon sources showed that the optimized strain eventually obtained 1.2-fold higher CaA than that using glucose.[145] In addition, the generation of CaA can proceed in four steps, from L-Tyr through *p*-HCA, coumaroyl-CoA and caffeoyl-CoA, as CoA thioesterase can convert caffeoyl-CoA to CaA, although this pathway is inefficient.[146,147]

4.2.1.4. Ferulic acid. FA (*p*-hydroxy-3-methoxycinnamic acid), a phytomolecule crosslinked with lignin and hemicellulose in plant cell walls, is abundant in certain cereal raw materials and medicinal herbs.[148] Caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferase (Comt) catalyses the methylation of the 3-hydroxyl group of CaA to generate FA, which can be further converted to sinapic acid (Fig. 4). SA has multiple pharmaceutical applications,[149] but limited data are available on its microbial production. Most efforts have been devoted to constructing FA synthetic pathways in microbial hosts to produce FA using glucose or L-Tyr as substrates.[150,151] For example, a recombinant *E. coli* harbouring plasmids with the pathway genes (TAL, C3H and COMT) produced 257.3 mg L^{-1} FA from L-Tyr.[152] In another example, after the optimization of gene expression by changes to the promoter strength and copy number, coupled with enhanced NADPH levels to improve the conversion towards CaA, and overexpression of a methionine kinase, 212 mg L^{-1} of FA was produced in shake flask cultures.[153] More recently, an engineered yeast achieved a yield

of 3.8 g L^{-1} of FA in a fed-batch fermentation through cofactor engineering of that accelerated the methyl cycle and SAM regeneration.[83] To address the insufficient supply of HpaBC-dependent cofactor $FADH_2$, a NAD(P)H-flavin reductase (Fre) was introduced to activate $FADH_2$ regeneration, which promoted an 8.1-fold increase in efficiency of hydroxylation. Combined with the efforts on a L-Tyr overproducer and SAM reactivation, the total titre of FA reached 5.09 g L^{-1} in *E. coli* under the fed-batch condition.[154] FA and SA can also be synthesized from the oxidation of coniferyl aldehyde and sinapoyl aldehyde, respectively, by the corresponding aldehyde dehydrogenase (Aldh), which indirectly detoxify the reactive aldehydes in cellular metabolic processes.[155,156] Alternatively, the biotransformation of eugenol to FA was also established in a recombinant *S. cerevisiae* by expressing vanillyl-alcohol oxidase (*Ps*Vao) from *Penicillium simplicissimum*, and 16.9 g L^{-1} of FA was produced from eugenol feeding in a fermentor.[157] Of special interest, the Vao family of enzymes involve many reactions with phenolic substrates and the FAD cofactor. Considering that eugenol is an inexpensive and readily available substrate, its bioconversion to FA is likely to expand to industrial levels. Notably, an alternative one-step production method based on microbial fermentation is of great interest, using feruloyl esterase to prepare FA from agro-industrial wastes (*e.g.*, wheat bran and brewery spent grain) with microbial fermentation.[158–160] Feruloyl esterase is expected to be a catalyst for the production of biofuels from biomass.

4.2.2. Phenylpropanoic aldehydes. Simple phenylpropanoic aldehydes include cinnamaldehyde, *p*-coumaraldehyde, caffeoylaldehyde, coniferaldehyde, and sinapaldehyde. Phenylpropanoic aldehydes are produced from phenylpropanoic acids by the actions of two enzymes, 4Cl and Ccr, in which the CoA thioester of the 4Cl products is converted to an aldehyde group using NADPH (Fig. 4). The co-expression of three plant enzymes Pal, 4Cl, and Ccr in *E. coli* achieved cinnamaldehyde production from L-Phe.[161] The fusion of 4Cl1-Ccr was functionally overexpressed in *E. coli* to construct a metabolic channel for improved production of *p*-coumaraldehyde, caffealdehyde and coniferaldehyde from feedstocks consisting of the corresponding phenylpropanoic acids.[162] In addition, a novel microbial pathway consisting of an aryl carboxylic acid reductase (Car) and a phosphopantetheinyl transferase (EntD) were shown to synthesize cinnamaldehyde from *t*-CA without the presence of plant 4Cl. The *de novo* biosynthesis of cinnamaldehyde was carried out in L-Phe-overproducing *S. cerevisiae* using glucose as a substrate.[163] Although the *de novo* biosynthesis of cinnamaldehyde was achieved in a strain of *S. cerevisiae* producing high levels of L-Phe, cell growth was affected by the concentration of cinnamaldehyde, and some unanticipated metabolites such as cinnamyl alcohol were detected in the fermentation broth. The toxicity of aldehyde chemicals is harmful to the growth and metabolism of microbes, which provides a reasonable explanation for the absence of natural accumulation of aldehydes in microorganisms.[164] In contrast, the endogenous Aldh and alcohol dehydrogenase (Adh) in *S. cerevisiae* are thought to be spontaneously involved in the reversible conversion of aldehydes to alcohols and acids and are associated with sugar metabolism.[165] Therefore, the spontaneous conversion from cinnamaldehyde to cinnamyl alcohols may be explained by a cellular Adh/Aldh-related detoxification mechanism, which could be an important target for further optimization to increase the production of aldehydes. By knocking out 10 endogenous Adh and Aldh enzymes and using some necessary engineering strategies, such as changes to cofactor supply, enzyme expression enhancement, auto-induction systems, and engineered *E. coli*, a maximum cinnamaldehyde titre of 3.8 g L^{-1} was achieved using glucose.[166]

4.2.3. Phenylpropanoids. Phenylpropanoids, also known as monolignols, are mainly synthesised through the monolignol pathway, in which cinnamyl alcohol dehydrogenase (Cad) catalyses the NADPH-mediated reversible conversion of aldehydes to their corresponding alcohols (Fig. 4).

The biotechnological production of natural phenylpropanols has been achieved by reconstructing the plant monolignol pathway in microbial hosts. Although the synthesis of cinnamyl alcohol and hydrocinnamyl alcohol has been achieved in *S. cerevisiae* and *E. coli* by expressing Cad or Ccr enzymes from different species,[167–169] severe product inhibition is the key limiting factor in cinnamyl alcohol biosynthesis. Product inhibition was successfully removed using a dibutyl phthalate/water biphasic system, which constantly separated and concentrated cinnamyl alcohol synthesised by *E. coli* from *t*-CA into the organic phase.[170] Coniferyl alcohol, a precursor of silybin and other natural pharmaceuticals, is the most abundant monolignol in plants. The co-expression of 4Cl, Ccr, and Cad from *Arabidopsis thaliana* in a FA producer achieved *de novo* synthesis of coniferyl alcohol with titres of 187.7 mg L^{-1} and 201.1 mg L^{-1} in *E. coli* and *S. cerevisiae* through fed-batch fermentation, respectively.[171,172] Moreover, it is noteworthy that these enzymes responsible for the monolignol biosynthesis are known to be promiscuous on the substrate specificity, which is double-edged for the production of phenylpropanols.[173,174] A study applied a co-culture strategy to the engineered *E. coli* hosts, which minimized the effect of promiscuous HpaBC catalysing the side reaction of L-Tyr to produce L-Dopa, reaching 534 mg L^{-1} caffeyl alcohol and 124.9 mg L^{-1} coniferyl alcohol.[137] Additionally, a study used the *Ps*Vao to convert eugenol to coniferyl alcohol in *E. coli*, together with catalase (Cta1) from *S. cerevisiae* to avoid over-oxidation of coniferyl alcohol, which reached the final coniferyl alcohol titre of 53.9 g L^{-1} in a 5 L bioreactor with a conversion rate of 86.72%.[175] In another study, *E. coli* was demonstrated to synthesize non-natural phenylpropanols through feeding of different precursors, such as 5-bromoconiferyl alcohol and 2-nitroconiferyl alcohol.[176]

4.2.4. Simple coumarins. Simple coumarins have a core skeleton of fused benzene and α-pyrone rings, and include compounds such as basic coumarin, umbelliferone (7-hydroxycoumarin), 4-hydroxycoumarin, esculetin (6,7-dihydroxycoumarin), and scopoletin (7-hydroxy-6-methoxycoumarin). They are derived from the respective cinnamates by three consecutive reactions: an *ortho*-hydroxylation of the aromatic ring is catalysed by coumaroyl-CoA 2′-hydroxylase (C2′h) or feruloyl-CoA 6′-hydroxylase (F6′h) to form *o*-hydroxycin-namoyl-

CoA thioesters, which is followed by spontaneous reactions (a *trans–cis*-isomerisation and a lactonisation) (Fig. 4).[177,178] A new coumarin synthase (Cosy) was discovered recently with the capability to accomplish the final reactions and bypass the spontaneous steps, which may be helpful in enhancing coumarin production in the future.[179] Multiple P450 enzymes are involved in the pathway and the *ortho*-hydroxylation step is considered to be a key stage for simple coumarin biosynthesis due to its irreversibility.[180,181] The last two reactions take place partially spontaneously in the presence of the CoA group and *ortho*-hydroxyl group, catalysed by light at room temperature. By co-expressing Tal, 4Cl and C2′h, 2.43 mg L^{-1} umbelliferone was biosynthesized in *E. coli* without extra addition of 4-coumarate. Similarly, *de novo* synthesis of scopoletin was achieved in *E. coli* from glucose and glycerol by expressing a pathway containing Tal, HpaBC, 4Cl, Caffeoyl-CoA *O*-methyltransferase (CCoAomt) and F6′h.[182] A recombinant *E. coli* strain harbouring F6′h and 4Cl genes was grown in culture supplemented with *p*-HCA, CaA, and FA, yielding 82.9 mg L^{-1} of umbelliferone, 79.5 mg L^{-1} of scopoletin, and 52.3 mg L^{-1} of esculetin.[183]

To prevent the degradation of 4Cl-producing thioester intermediates, a predicted acyl-CoA thioesterase (YbgC) was deleted for higher production of esculetin and umbelliferone from glucose.[183] A systematic study that overcame the limitation of 4Cl through protein engineering improved the supply of L-Tyr through metabolic flow remodelling and optimized fermentation conditions, obtaining 356.59 mg L^{-1} umbelliferone from L-Tyr.[184] To overcome the inefficient spontaneous reactions, researchers established a novel artificial pathway condensing malonyl-CoA and salicylic acid by a biphenyl synthase (Bis) that was introduced into *E. coli* for the *de novo* synthesis of 4-hydroxycoumarin from glycerol. The resulting titre was 483.1 mg L^{-1} in 24 h.[185] Furthermore, the production was enhanced to 935 mg L^{-1} in shake flasks by alleviating the thioesterase-mediated degradation of salicoyl-CoA.[186] Nowadays, the use of lignin hydrolysate to produce valuable chemicals with benzene rings has attracted much interest in biotransformation technology. An engineered budding yeast expressing necessary enzymes to generate scopoletin from lignin hydrolysate was recently reported.[187] The scopoletin production reached 4.79 mg L^{-1}, suggesting that this approach may offer new opportunities for improved biosynthesis of coumarins from renewable sources.

5. Discussion and future perspectives

Driven by the important applications of simple phenylpropanoids, either because of their wide bioactivity or as precursors for the synthesis of complex natural product molecules, great progress has been achieved in the microbial production of simple phenylpropanoids (Table S1 provided as an attachment).† Although many simple phenylpropanoids have been synthesised at the g L^{-1} level in microorganisms with considerable engineering endeavours over the past 20 years, their titre, yield, and productivity (TYP) still require improvement to achieve industrial production. Some challenges that limit the high-level synthesis of simple phenylpropanoids must be addressed. Based on the three-dimensional metabolic engineering strategy proposed in the previous study,[11] here we map these challenges to an upgraded four-dimensional metabolic engineering strategy (*i.e.*, "point-line-plane-system"), and suggest a potential technical proposal to meet these challenges (Fig. 5).

(i) The "Point" level: the unsatisfied enzyme activity and cofactor utilization.

Enzymes are the most basic elements that fundamentally determine the synthetic efficiency in a cell factory through specific activities and cofactor supply. The catalytic promiscuity and low cofactor utilisation of enzymes in the simple phenylpropanoid pathway are key issues hindering synthetic efficiency. 4Cl is a rate-limiting enzyme with a broad substrate spectrum that generates CoA thioester intermediates from phenylpropanoic acids (Fig. 4), which may result in unexpected disruption of metabolic flux. For instance, when 4Cl is designed to react with FA, it preferentially reacts with upstream phenylpropanoic acids (*i.e.*, *t*-CA, *p*-HCA, and CaA), thereby blocking the metabolic flux towards FA. Given that several 4CL protein crystal structures have been resolved (UniProt Q42524, Q9SMT7, and Q94M3), and there have been some advances in changing the 4Cl substrate preference.[188] It is expected that specific unnatural variants of 4Cl can be designed through classical structural biology-based protein rational modification. The continued evolution of machine-learning-assisted models will provide feasible clues for the virtual screening and prediction of key enzymes.[189,190] More importantly, the latest advances in deep learning-inspired language models may enable the *de novo* design of enzymes, which, in the case of ProGen, can be adapted to generate artificial protein sequences that are functionally identical to natural proteins.[191] Comt is the key enzyme in FA synthesis, and its low turnover of the methyl donor SAM results in low catalytic efficiency for CaA. Although the conversion rate of CaA to FA was increased from 28% to 64% through the metabolic engineering strategy of expressing an efficient form of Comt and by optimising the methyl cycle,[83] more than 36% of CaA still failed to synthesise FA, and the catalytic problem of Comt itself has not been solved. Biosensors are an ideal strategy for high-throughput screening of enzymes based on intuitive phenotypic changes. A FAR_{ON} switch system derived from a phenolic acid decarboxylase regulator (aPadR) was designed to respond to FA in mammalian cells.[192] In addition, a sensitive synchronous fluorometric method based on the oxidation of FA with Ce(IV) in a sulfuric acid medium was used to detect FA *in vitro*. Therefore, optimization of the FAR_{ON} biosensor and the Ce(IV)-dependent sensor to improve the cofactor turnover of Comt through directed evolution is expected to further increase FA production.

(ii) The "Line" level: the imbalance of multi-pathway metabolic fluxes.

Simple phenylpropanoids are derived from two AAAs (L-Phe and L-Tyr) condensed from two endogenous intermediates, *i.e.*, E4P from PPP and PEP from glycolysis. Therefore, an equal supply of E4P and PEP is advantageous for the synthesis of simple phenylpropanoids. Although glycolysis and PPP are the two fundamental routes for catabolizing glucose in most living

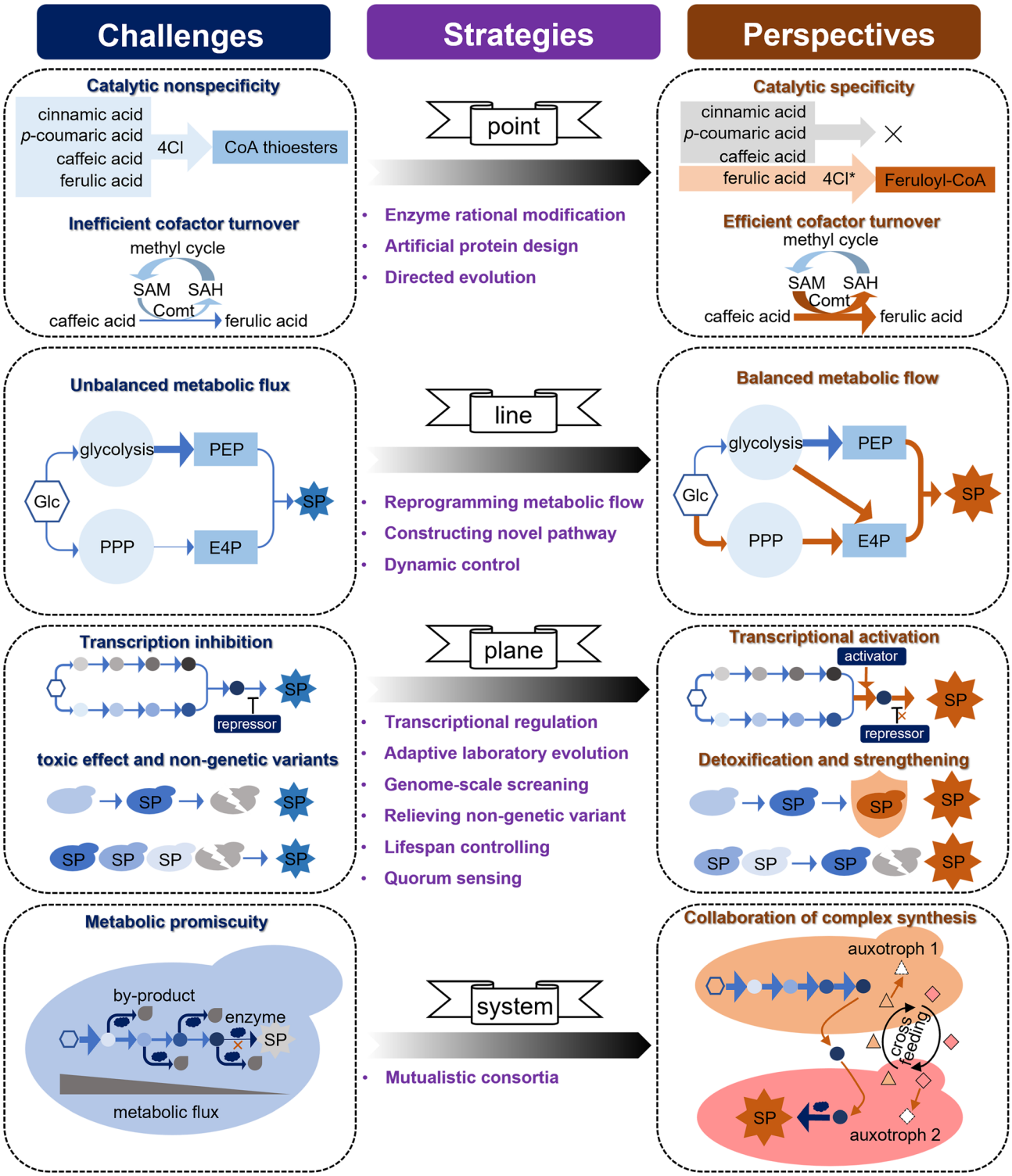


Fig. 5 Challenges and perspectives of microbial synthesis of simple phenylpropanoids. Potential challenges, corresponding strategies and expected results are mapped in the four-dimensional metabolic engineering strategy (*i.e.*, "point-line-plane-system"). Arrows represent multi-step reactions and different colored circles represent metabolites in the pathway. 4Cl, 4-coumarate-CoA ligase; 4Cl*, mutated 4-coumarate-CoA ligase; Comt, caffeic acid *O*-methyltransferase; SAM, *S*-adenosyl-*l*-methion; SAH, *S*-adenosy-*l*-homocysteine; Glc, glucose; PPP, pentose phosphate pathway; PEP, phosphoenolpyruvate; E4P, erythrose-4-phosphate; SP, simple phenylpropanoids.

cells, E4P has been reported to be the primary limiting substrate for simple phenylpropanoid biosynthesis in many microorganisms.[80,193] To maintain the intracellular balance of NADH/NAD^+ and a fast growth rate, *S. cerevisiae* tends to introduce metabolic flux into glycolysis for ethanol fermentation and limits PPP when consuming sugars under anaerobic

conditions.[79] Some attempts to control heterologous expression under glucose-limited conditions, stretching PPP fluxes and weakening glycolysis have successfully increased the E4P supply and simple phenylpropanoid production in *S. cerevisiae*.[83] Although yeast central metabolism has been extensively rewired to improve the supply of E4P,[126] it is challenging to balance metabolic flux because of the metabolic competition between PPP and glycolysis. Considering that *S. cerevisiae* can be modified from an ethanol-producing to an oil-producing yeast, this strong metabolic plasticity implies the possibility of further improvement in the metabolic flux towards E4P through metabolic reprogramming.[194] Furthermore, a novel PHK pathway consisting of phosphoketolase (Xfpk) and phosphotransacetylase (Pta) was introduced to channel more carbon flux towards E4P from F6P derived from glycolysis.[86] Metabolic flux analysis indicated that the availability of E4P and the unbalanced supply of E4P and PEP remain key issues. To further alleviate this limitation, the introduction of an efficient xylose pathway may be a viable option to improve E4P supply.[195] In addition, it may be possible to construct a self-adjusting system[196,197] or optogenetic regulation[198] to allow the dynamic control of metabolic flux towards glycolysis and PPP.

(iii) The "plane" level: the weakness of cell performance.

Even for well-studied microorganisms such as *E. coli* and *S. cerevisiae*, it is often not easy to accurately understand and correct the negative effects of metabolic engineering on whole cells. These negative effects may be caused by transcriptional regulation, accumulation of toxic products, and unknown mechanisms. Ric1 is a transcriptional repressor of multiple genes in the aromatic amino acid biosynthetic pathway in *S. cerevisiae*, and inhibition of Ric1 has been shown to increase the yield of shikimic acid, a precursor of simple phenylpropanoids.[199,200] Although the identified targets can be used to optimize the synthesis levels, most targets for the global optimization of microbial synthesis performance are unknown. Aromatic compounds are generally toxic to microorganisms, which makes their high-level production in microbial hosts challenging. Adaptive laboratory evolution is a powerful tool used in the improvement of microbial cell factories at the entire cell level (the "plane" level), which emphasizes the importance of "collaboration" between scientists' deliberate choices and microorganisms' initiative to achieve design goals, and compensates for the lack of comprehensive understanding of host strains. Coordination between adaptive laboratory evolution and whole-genome sequencing revealed that Esbp6 is an important transporter for the secretion of *p*-HCA and tolerance to aromatic acids, which can be used to optimize the production of simple phenylpropanoids.[131] Inspired by this, we believe that evaluating the growth rate on a medium with high concentrations of aromatic acids in a genome-scale collection of *S. cerevisiae* gene-deletion strains will uncover novel strategies for improving aromatic acid tolerance.

During microbial fermentation, although the genetic information of all individual cells in a microbial population is identical, their contributions to compound synthesis and single-cell biosynthetic performance may vary greatly (up to a 10-fold difference) owing to nongenetic cell-to-cell variation, which could have a significant impact on group performance.[201,202] Nongenetic cell-to-cell variations may be due to different epigenetic modifications and variation in the regulation of expression due to differences in developmental history (uneven cell division and different parent cells) or exposure to environmental factors (differences in the concentrations of various substances in the local medium). Understanding the key mechanisms affecting nongenetic variations requires long-term efforts that do not provide reliable short-term engineering strategies. In one study, a minority (15%) of the total cell population produced more than half of the total free fatty acids, and the majority of cells performed very weakly in the fermentation of a fatty acid-producing *E. coli*. A quality control (PopQC) system was constructed to continuously select and kill low-performing nongenetic strains and maintain high-performing nongenetic variants for production.[203] Since such genetic circuits for screening often rely on specific regulatory elements, biosensors that respond to simple phenylpropanoid concentrations should first be developed. Although this strategy alleviates the problems caused by nongenetic variants to some extent, it may inadvertently kill young cells with synthetic potential. Conversely, regulating cellular processes such as autophagy, apoptosis, and replication can extend the lifespan and the effective time available for product synthesis, thereby increasing the yield.[204–206] Quorum sensing is a widespread bacterial mechanism for cell-to-cell communication that synchronises gene expression and has been successfully implemented to improve the synthetic performance of 4-hydroxycoumarin, flavonoids, and simple chemicals in bacteria.[207–209] Although autoinducer-2 has been successfully used as a 'universal signal' for interspecies communication to improve CaA synthesis in *S. cerevisiae*,[83,210] its mechanism and universality require further exploration. As an important mechanism of intercellular communication, an in-depth study of exosomes is expected to provide opportunities to improve the overall performance of cell factories.[211]

(iv) The "system" level:

As multicellular organisms, plants often complete the oriented synthesis of natural products through the division of labour and metabolite transport of multiple cells, tissues, organs, and organelles.[212] The synthesis of aromatic acids involves the cooperation of multiple organelles, which is important for ensuring an optimal catalytic environment and avoiding metabolic promiscuity.[213] Although compartmentalisation strategies can significantly improve synthesis efficiency by the modularized expression of heterologous pathways in different organelles, it sometimes comes at the expense of an optimal reaction environment for catalytic enzymes. For example, to avoid side reactions and waste of metabolic flux caused by the catalytic promiscuity of 4Cl, 4Cl can be considered as a node to separate the upstream and downstream pathways for the synthesis of ferulate CoA, a key precursor of many phenylpropanoids. However, both the upstream and downstream pathways need to be expressed in the cytosol to ensure the necessary reaction environment, such as NADPH and SAM cofactors. Therefore, building a stable and mutualistic microbial consortium system that cooperates with plant cells

could reasonably confer wider metabolic capabilities and achieve more complex synthesis, which is difficult to achieve in a single cell. In addition, mutualistic consortia also endow microorganisms with higher synthetic titres through unique abilities, such as balancing metabolic flow, relieving metabolic burden, optimising resource utilisation, enriching the cellular environment and cofactors, and adapting to fluctuating environments. Considering these advantages, microbial consortium strategies have been extended to construct bacterial, yeast, and yeast systems for the synthesis of terpenoids,[214] phenylpropanoids,[215,216] and polyketones,[217] and alkaloids.[218] However, most are simply mixed cultures formed by changing the initial inoculation ratio,[219] which makes it difficult to form a stable community, and this instability is further amplified in large-scale fermentation. Efforts to construct a mutualistic relationship for the members of microbial consortia to form stable mutualistic consortia is a viable approach to improve these strategies.[216,220]

Overall, based on the current gram-scale synthesis of simple phenylpropanoids in microorganisms, the integration of multidisciplinary technologies and tools is expected to further break the bottlenecks and increase production levels. These strategies will provide a framework for the synthesis of complex phenylpropanoids and other types of natural products.

6. Author contributions

Z. P. Zhu: conceptualisation, visualisation, writing – original draft, writing – review & editing. R. B. Chen: conceptualisation, founding acquisition, writing – original draft, writing – review & editing. L. Zhang: conceptualisation, supervision, founding acquisition, writing – review & editing.

7. Conflicts of interest

There are no conflicts to declare.

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9. References

1 T. Vogt, *Mol. Plant*, 2010, **3**, 2–20.
2 S. Wang, S. Alseekh, A. R. Fernie and J. Luo, *Mol. Plant*, 2019, **12**, 899–919.
3 W. Biała and M. Jasiński, *Front. Plant Sci.*, 2018, **9**, 1610.
4 N. Q. Dong and H. X. Lin, *J. Integr. Plant Biol.*, 2021, **63**, 180–209.
5 N. Redhu, A. Khatkar and K. K. Sharma, *Crit. Rev. Food Sci. Nutr.*, 2020, **60**, 2655–2675.
6 R. J. Sharifi, M. N. Cruz, J. P. Lopez, E. P. Lopez, N. Harun, B. Yeskaliyeva, A. Beyatli, O. Sytar, S. Shaheen, F. Sharopov, Y. Taheri, A. O. Docea, D. Calina and W. C. Cho, *Oxid. Med. Cell. Longevity*, 2021, **2021**, 6492346.
7 D. Peters, *Adv. Biochem. Eng. Biotechnol.*, 2007, **105**, 1–30.
8 A. Tilay, M. Bule, J. Kishenkumar and U. Annapure, *J. Agric. Food Chem.*, 2008, **56**, 7644–7648.
9 A. Nieter, S. Kelle, D. Linke and R. G. Berger, *Bioresour. Technol.*, 2016, **220**, 38–46.
10 C. J. Paddon, P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D. R. Polichuk, K. H. Teoh, D. W. Reed, T. Treynor, J. Lenihan, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K. W. Ellens, S. Fickes, J. Galazzo, S. P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, H. Jiang, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L. F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievense, P. S. Covello, J. D. Keasling, K. K. Reiling, N. S. Renninger and J. D. Newman, *Nature*, 2013, **496**, 528–532.
11 R. Chen, S. Yang, L. Zhang and Y. J. Zhou, *iScience*, 2020, **23**, 100879.
12 A. Cravens, J. Payne and C. D. Smolke, *Nat. Commun.*, 2019, **10**, 2142.
13 J. Nielsen and J. D. Keasling, *Cell*, 2016, **164**, 1185–1197.
14 F. Destani, A. Cassano, A. Fazio, J.-P. Vincken and B. Gabriele, *J. Food Eng.*, 2013, **117**, 263–271.
15 M. G. S. Palmieri, L. T. Cruz, F. S. Bertges, H. M. Húngaro, L. R. Batista, S. S. da Silva, M. J. V. Fonseca, M. P. Rodarte, F. M. P. Vilela and M. d. P. H. do Amaral, *Biocatal. Agric. Biotechnol.*, 2018, **16**, 43–48.
16 C. G. Schmidt, L. M. Gonçalves, L. Prietto, H. S. Hackbart and E. B. Furlong, *Food Chem.*, 2014, **146**, 371–377.
17 L. Zhao, X. Jin, Z. Xiong, H. Tang, H. Guo, G. Ye, D. Chen, S. Yang, Z. Yin and H. Fu, *Int. J. Mol. Sci.*, 2022, **23**, 11119.
18 G. Caruso, J. Godos, A. Privitera, G. Lanza, S. Castellano, A. Chillemi, O. Bruni, R. Ferri, F. Caraci and G. Grosso, *Nutrients*, 2022, **14**, 819.
19 L. Liu, W. R. Hudgins, S. Shack, M. Q. Yin and D. Samid, *Int. J. Cancer*, 1995, **62**, 345–350.
20 P. Khare, S. Jagtap, Y. Jain, R. K. Baboota, P. Mangal, R. K. Boparai, K. K. Bhutani, S. S. Sharma, L. S. Premkumar and K. K. Kondepudi, *Biofactors*, 2016, **42**, 201–211.
21 T. R. E. Panel, D. Belsito, D. Bickers, M. Bruze, P. Calow, H. Greim, J. Hanifin, A. Rogers, J. Saurat and I. Sipes, *Food Chem. Toxicol.*, 2007, **45**, S1–S23.
22 J. Jeon, J. Sung, H. Lee, Y. Kim, H. S. Jeong and J. Lee, *J. Food Biochem.*, 2019, **43**, e12701.
23 M. Vaezi, *J. Biomol. Struct. Dyn.*, 2023, **41**, 4798–4810.
24 Y. Seo, S. Kim, Y. Boo, J. Baek, S. Lee and J. Koh, *Clin. Exp. Dermatol.*, 2011, **36**, 260–266.
25 A. M. Awad, P. Kumar, M. R. Ismail-Fitry, S. Jusoh, M. F. Ab Aziz and A. Q. Sazili, *J. Food Process. Preserv.*, 2022, **46**, e16796.
26 L. Yue, D. Sun, I. M. Khan, X. Liu, Q. Jiang and W. Xia, *Food Chem.*, 2020, **309**, 125513.

27 I. b. Jantan, B. A. Karim Moharam, J. Santhanam and J. A. Jamal, *Pharm. Biol.*, 2008, **46**, 406–412.
28 M. Friedman, *J. Agric. Food Chem.*, 2017, **65**, 10406–10423.
29 F. Khan, N. I. Bamunuarachchi, N. Tabassum and Y.-M. Kim, *J. Agric. Food Chem.*, 2021, **69**, 2979–3004.
30 A. T. Silva, C. M. Bento, A. C. Pena, L. M. Figueiredo, C. Prudêncio, L. Aguiar, T. Silva, R. Ferraz, M. S. Gomes, C. Teixeira and P. Gomes, *Molecules*, 2019, **25**, 66.
31 Y. Chen, Z. Li, P. Pan, Z. Lao, J. Xu, Z. Li, S. Zhan, X. Liu, Y. Wu and W. Wang, *Antiviral Res.*, 2021, **192**, 105117.
32 Y. Zheng, S. H. Ley and F. B. Hu, *Nat. Rev. Endocrinol.*, 2018, **14**, 88–98.
33 S. Adisakwattana, *Nutrients*, 2017, **9**, 163.
34 W. Sompong, H. Cheng and S. Adisakwattana, *J. Physiol. Biochem.*, 2017, **73**, 121–131.
35 T. Jin and C. Chen, *Food Chem. Toxicol.*, 2022, **163**, 112892.
36 S. Kumar, B. K. Singh, A. K. Prasad, V. S. Parmar, S. Biswal and B. Ghosh, *Eur. J. Pharmacol.*, 2013, **700**, 32–41.
37 Y. Luo, K.-M. Qiu, X. Lu, K. Liu, J. Fu and H.-L. Zhu, *Bioorg. Med. Chem.*, 2011, **19**, 4730–4738.
38 C. Kopp, S. P. Singh, P. Regenhard, U. Müller, H. Sauerwein and M. Mielenz, *Int. J. Mol. Sci.*, 2014, **15**, 2906–2915.
39 S. Li and S. Hu, *Trop. J. Pharm. Res.*, 2020, **19**, 957–963.
40 K. Pei, J. Ou, J. Huang and S. Ou, *J. Sci. Food Agric.*, 2016, **96**, 2952–2962.
41 A. Kim, S.-Y. Lee and S.-K. Chung, *Phytomedicine*, 2022, **102**, 154144.
42 S. Mirzaei, M. H. Gholami, A. Zabolian, H. Saleki, M. V. Farahani, S. Hamzehlou, F. B. Far, S. O. Sharifzadeh, S. Samarghandian and H. Khan, *Pharmacol. Res.*, 2021, **171**, 105759.
43 A. Koraneekit, T. Limpaiboon, A. Sangka, P. Boonsiri, S. Daduang and J. Daduang, *Oncol. Lett.*, 2018, **15**, 7397–7402.
44 S.-R. Park, S.-R. Kim, I.-S. Hong and H.-Y. Lee, *Front. Cell Dev. Biol.*, 2020, **8**, 585987.
45 C. Yuan, M.-H. Wang, F. Wang, P.-Y. Chen, X.-G. Ke, B. Yu, Y.-F. Yang, P.-T. You and H.-Z. Wu, *Life Sci.*, 2021, **270**, 119105.
46 Y. Sawata, T. Matsukawa, S. Doi, T. Tsunoda, N. Arikawa, N. Matsunaga, K. Ohnuki, S. Shirasawa and Y. Kotake, *Mol. Cell. Biochem.*, 2019, **462**, 25–31.
47 J. M. Brimson, M. I. Prasanth, D. S. Malar, P. Thitilertdecha, A. Kabra, T. Tencomnao and A. Prasansuklab, *Pharmaceuticals*, 2021, **14**, 982.
48 A. Sgarbossa, D. Giacomazza and M. Di Carlo, *Nutrients*, 2015, **7**, 5764–5782.
49 Y. P. Singh, H. Rai, G. Singh, G. K. Singh, S. Mishra, S. Kumar, S. Srikrishna and G. Modi, *Eur. J. Med. Chem.*, 2021, **215**, 113278.
50 Y. Dong, T. Stewart, L. Bai, X. Li, T. Xu, J. Iliff, M. Shi, D. Zheng, L. Yuan and T. Wei, *Theranostics*, 2020, **10**, 179.
51 S. Huang, W. Liu, Y. Li, K. Zhang, X. Zheng, H. Wu and G. Tang, *ACS Chem. Neurosci.*, 2021, **12**, 419–429.
52 X. Dong and D. Zhao, *CNS Neurosci. Ther.*, 2023, **29**, 2397–2412.
53 A. Bumrungpert, S. Lilitchan, S. Tuntipopipat, N. Tirawanchai and S. Komindr, *Nutrients*, 2018, **10**, 713.
54 D. Li, Y. X. Rui, S. D. Guo, F. Luan, R. Liu and N. Zeng, *Life Sci.*, 2021, **284**, 119921.
55 Y. Wu, M. H. Wang, T. Yang, T. T. Qin, L. L. Qin, Y. M. Hu, C. C. Zhang, B. B. Sun, L. Ding and L. L. Wu, *Front. Nutr.*, 2022, **8**, 794841.
56 H. Silva and N. M. F. Lopes, *Front. Physiol.*, 2020, **11**, 595516.
57 B. Ramesh, P. Viswanathan and K. V. Pugalendi, *Eur. J. Pharmacol.*, 2007, **566**, 231–239.
58 O. Y. Althunibat, M. S. Abduh, M. H. Abukhalil, S. H. Aladaileh, H. Hanieh and A. M. Mahmoud, *Biomed. Pharmacother.*, 2022, **149**, 112900.
59 S. Shen, Y. Tong, Y. Luo, L. Huang and W. Gao, *Nat. Prod. Rep.*, 2022, **39**, 1856–1875.
60 V. Křen and K. Valentová, *Nat. Prod. Rep.*, 2022, **39**, 1264–1281.
61 Z. Bi, W. Zhang and X. Yan, *Biomed. Pharmacother.*, 2022, **151**, 113180.
62 Y. Fan, Q. Luo, J. Wei, R. Lin, L. Lin, Y. Li, Z. Chen, W. Lin and Q. Chen, *Brain Res.*, 2018, **1679**, 125–133.
63 T. Qin, A. Rasul, A. Sarfraz, I. Sarfraz, G. Hussain, H. Anwar, A. Riaz, S. Liu, W. Wei and J. Li, *Int. J. Biol. Sci.*, 2019, **15**, 2256–2264.
64 F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini and A. Pinto, *Int. J. Mol. Sci.*, 2020, **21**, 4618.
65 H. Deng, Q. Xu, H.-Y. Guo, X. Huang, F. Chen, L. Jin, Z.-S. Quan and Q.-K. Shen, *Phytochemistry*, 2022, **206**, 113532.
66 M. Jiang and H. Zhang, *Curr. Opin. Biotechnol.*, 2016, **42**, 1–6.
67 J. Zhang, A. ten Pierick, H. M. van Rossum, R. M. Seifar, C. Ras, J.-M. Daran, J. J. Heijnen and S. A. Wahl, *Sci. Rep.*, 2015, **5**, 12846.
68 Y. Deng and S. Lu, *Crit. Rev. Plant Sci.*, 2017, **36**, 257–290.
69 K. M. Herrmann and L. M. Weaver, *Annu. Rev. Plant Biol.*, 1999, **50**, 473–503.
70 R. Patnaik and J. C. Liao, *Appl. Environ. Microbiol.*, 1994, **60**, 3903–3908.
71 M. Luttik, Z. Vuralhan, E. Suir, G. Braus, J. Pronk and J. Daran, *Metab. Eng.*, 2008, **10**, 141–153.
72 A. R. Hawkins, J. D. Moore and H. K. Lamb, *Biochem. Soc. Trans.*, 1993, **21**, 181–186.
73 K. Huang, M. Li, Y. Liu, M. Zhu, G. Zhao, Y. Zhou, L. Zhang, Y. Wu, X. Dai and T. Xia, *Front. Plant Sci.*, 2019, **10**, 1268.
74 M.-H. Cho, O. R. Corea, H. Yang, D. L. Bedgar, D. D. Laskar, A. M. Anterola, F. A. Moog-Anterola, R. L. Hood, S. E. Kohalmi and M. A. Bernards, *J. Biol. Chem.*, 2007, **282**, 30827–30835.
75 H. Yoo, J. R. Widhalm, Y. Qian, H. Maeda, B. R. Cooper, A. S. Jannasch, I. Gonda, E. Lewinsohn, D. Rhodes and N. Dudareva, *Nat. Commun.*, 2013, **4**, 2833.
76 A. Gloge, J. Zoń, Á. Kövári, L. Poppe and J. Rétey, *Chem. – Eur. J.*, 2000, **6**, 3386–3390.

77 C. B. Jendresen, S. G. Stahlhut, M. Li, P. Gaspar, S. Siedler, J. Förster, J. Maury, I. Borodina and A. T. Nielsen, *Appl. Environ. Microbiol.*, 2015, **81**, 4458–4476.
78 B. R. Albuquerque, S. A. Heleno, M. B. P. P. Oliveira, L. Barros and I. C. F. R. Ferreira, *Food Funct.*, 2021, **12**, 14–29.
79 G. M. Walker and R. S. Walker, *Adv. Appl. Microbiol.*, 2018, **105**, 87–129.
80 N. Flores, J. Xiao, A. Berry, F. Bolivar and F. Valle, *Nat. Biotechnol.*, 1996, **14**, 620–623.
81 R. Patnaik and J. C. Liao, *Appl. Environ. Microbiol.*, 1994, **60**, 3903–3908.
82 S. Liu, J.-Z. Xu and W.-G. Zhang, *World J. Microbiol. Biotechnol.*, 2022, **38**, 22.
83 R. Chen, J. Gao, W. Yu, X. Chen, X. Zhai, Y. Chen, L. Zhang and Y. J. Zhou, *Nat. Chem. Biol.*, 2022, **18**, 520–529.
84 K. I. Minard and L. McAlister-Henn, *J. Biol. Chem.*, 2005, **280**, 39890–39896.
85 M. Deaner and H. S. Alper, *Metab. Eng.*, 2017, **40**, 14–22.
86 A. Bergman, V. Siewers, J. Nielsen and Y. Chen, *AMB Express*, 2016, **6**, 115.
87 G. M. Borja, A. Rodriguez, K. Campbell, I. Borodina, Y. Chen and J. Nielsen, *Microb. Cell Fact.*, 2019, **18**, 1–14.
88 P. P. Lin, A. J. Jaeger, T. Y. Wu, S. C. Xu, A. S. Lee, F. Gao, P. W. Chen and J. C. Liao, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 3538–3546.
89 G. M. Santangelo, *Microbiol. Mol. Biol. Rev.*, 2006, **70**, 253–282.
90 G. Gosset, J. Yong-Xiao and A. Berry, *J. Ind. Microbiol.*, 1996, **17**, 47–52.
91 Y. Lin and Y. Yan, *Microb. Cell Fact.*, 2012, **11**, 1–9.
92 L. Guo, S. Ding, Y. Liu, C. Gao, G. Hu, W. Song, J. Liu, X. Chen and L. Liu, *Biotechnol. Bioeng.*, 2022, **119**, 983–993.
93 A. Rodriguez, K. R. Kildegaard, M. Li, I. Borodina and J. Nielsen, *Metab. Eng.*, 2015, **31**, 181–188.
94 D. Juminaga, E. E. Baidoo, A. M. Redding-Johanson, T. S. Batth, H. Burd, A. Mukhopadhyay, C. J. Petzold and J. D. Keasling, *Appl. Environ. Microbiol.*, 2012, **78**, 89–98.
95 F. Koopman, J. Beekwilder, B. Crimi, A. van Houwelingen, R. D. Hall, D. Bosch, A. J. van Maris, J. T. Pronk and J.-M. Daran, *Microb. Cell Fact.*, 2012, **11**, 1–15.
96 M. Cai, Y. Wu, H. Qi, J. He, Z. Wu, H. Xu and M. Qiao, *ACS Synth. Biol.*, 2021, **10**, 49–62.
97 M. Reifenrath and E. Boles, *Metab. Eng.*, 2018, **45**, 246–254.
98 M. Li, K. R. Kildegaard, Y. Chen, A. Rodriguez, I. Borodina and J. Nielsen, *Metab. Eng.*, 2015, **32**, 1–11.
99 M. Tatarko and T. Romeo, *Curr. Microbiol.*, 2001, **43**, 26–32.
100 J. Pittard, H. Camakaris and J. Yang, *Mol. Microbiol.*, 2005, **55**, 16–26.
101 T. Lütke-Eversloh and G. Stephanopoulos, *Appl. Microbiol. Biotechnol.*, 2007, **75**, 103–110.
102 B. Kim, R. Binkley, H. U. Kim and S. Y. Lee, *Biotechnol. Bioeng.*, 2018, **115**, 2554–2564.
103 D. Na, S. M. Yoo, H. Chung, H. Park, J. H. Park and S. Y. Lee, *Nat. Biotechnol.*, 2013, **31**, 170–174.
104 K. Nijkamp, N. van Luijk, J. A. de Bont and J. Wery, *Appl. Microbiol. Biotechnol.*, 2005, **69**, 170–177.
105 J. Abatemarco, M. F. Sarhan, J. M. Wagner, J.-L. Lin, L. Liu, W. Hassouneh, S.-F. Yuan, H. S. Alper and A. R. Abate, *Nat. Commun.*, 2017, **8**, 332.
106 Y. Liu, Y. Zhuang, D. Ding, Y. Xu, J. Sun and D. Zhang, *ACS Synth. Biol.*, 2017, **6**, 837–848.
107 M. Larroude, J. M. Nicaud and T. Rossignol, *Microb. Biotechnol.*, 2021, **14**, 2420–2434.
108 E. Kurpejović, A. Burgardt, G. M. Bastem, N. Junker, V. F. Wendisch and B. S. Akbulut, *J. Biotechnol.*, 2023, **363**, 8–16.
109 I. Limem, E. Guedon, A. Hehn, F. Bourgaud, L. C. Ghedira, J.-M. Engasser and M. Ghoul, *Process Biochem.*, 2008, **43**, 463–479.
110 F. Zhang, J. Ren and J. Zhan, *Appl. Biochem. Biotechnol.*, 2021, **193**, 1099–1115.
111 H. B. Bang, K. Lee, Y. J. Lee and K. J. Jeong, *Process Biochem.*, 2018, **68**, 30–36.
112 J. Son, J. H. Jang, I. H. Choi, C. G. Lim, E. J. Jeon, H. Bae Bang and K. J. Jeong, *Microb. Cell Factories*, 2021, **20**, 1–12.
113 S. Masuo, Y. Kobayashi, K.-I. Oinuma and N. Takaya, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 8701–8709.
114 K. Kukil and P. Lindberg, *Microb. Cell Fact.*, 2022, **21**, 1–16.
115 Z. Qian, J. Yu, X. Chen, Y. Kang, Y. Ren, Q. Liu, J. Lu, Q. Zhao and M. Cai, *ACS Synth. Biol.*, 2022, **11**, 1600–1612.
116 N. Schultheiss, M. Roe and S. X. Boerrigter, *CrystEngComm*, 2011, **13**, 611–619.
117 L. Achnine, E. B. Blancaflor, S. Rasmussen and R. A. Dixon, *Plant Cell*, 2004, **16**, 3098–3109.
118 T. Vannelli, W. W. Qi, J. Sweigard, A. A. Gatenby and F. S. Sariaslani, *Metab. Eng.*, 2007, **9**, 142–151.
119 K. Nijkamp, R. M. Westerhof, H. Ballerstedt, J. A. De Bont and J. Wery, *Appl. Microbiol. Biotechnol.*, 2007, **74**, 617–624.
120 Y. Kawai, S. Noda, C. Ogino, Y. Takeshima, N. Okai, T. Tanaka and A. Kondo, *Microb. Cell Fact.*, 2013, **12**, 1–9.
121 Y. Huo, F. Wu, G. Song, R. Tu, W. Chen, E. Hua and Q. Wang, *Chin. J. Biotech.*, 2020, **36**, 2367–2376.
122 Y. Brack, C. Sun, D. Yi and U. T. Bornscheuer, *ChemBioChem*, 2022, **23**, e202200062.
123 Y. Huang, X. Jiang, W. Chen, G. Zhang and Q. Wang, *Chin. J. Biotech.*, 2022, **38**, 4553–4566.
124 S. Zhang, J. Zhou, G. Zhang and J. Chen, *Chin. J. Biotech.*, 2020, **36**, 1838–1848.
125 Y. Li, J. Li, B. Qian, L. Cheng, S. Xu and R. Wang, *Molecules*, 2018, **23**, 3185.
126 Q. Liu, T. Yu, X. Li, Y. Chen, K. Campbell, J. Nielsen and Y. Chen, *Nat. Commun.*, 2019, **10**, 4976.
127 L. F. Brey, A. J. Włodarczyk, J. F. B. Thøfner, M. Burow, C. Crocoll, I. Nielsen, A. J. Z. Nielsen and P. E. Jensen, *Metab. Eng.*, 2020, **57**, 129–139.
128 E.-B. Gao, K. Kyere-Yeboah, J. Wu and H. Qiu, *Algal Res.*, 2021, **54**, 102180.
129 H. Qi, Y. Li, M. Cai, J. He, J. Liu, X. Song, Z. Ma, H. Xu and M. Qiao, *J. Appl. Microbiol.*, 2022, **133**, 707–719.
130 A. Rodriguez, Y. Chen, S. Khoomrung, E. Özdemir, I. Borodina and J. Nielsen, *Metab. Eng.*, 2017, **44**, 265–272.

131 R. Pereira, E. T. Mohamed, M. S. Radi, M. J. Herrgård, A. M. Feist, J. Nielsen and Y. Chen, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 27954–27961.
132 J. Combes, E. C. Rivera, T. Clément, C. Fojcik, V. Athes, M. Moussa and F. Allais, *Sep. Purif. Technol.*, 2021, **259**, 118170.
133 P. Zhang, W. Wenping, Z. Ying and Y. Bangce, *Synth. Biol. J.*, 2021, **2**, 778.
134 Y. Li, J. Mao, Q. Liu, X. Song, Y. Wu, M. Cai, H. Xu and M. Qiao, *ACS Synth. Biol.*, 2020, **9**, 756–765.
135 M. Berner, D. Krug, C. Bihlmaier, A. Vente, R. Müller and A. Bechthold, *J. Bacteriol.*, 2006, **188**, 2666–2673.
136 T. Furuya, Y. Arai and K. Kino, *Appl. Environ. Microbiol.*, 2012, **78**, 6087–6094.
137 Z. Chen, X. Sun, Y. Li, Y. Yan and Q. Yuan, *Metab. Eng.*, 2017, **39**, 102–109.
138 K. Sakae, D. Nonaka, M. Kishida, Y. Hirata, R. Fujiwara, A. Kondo, S. Noda and T. Tanaka, *Enzyme Microb. Technol.*, 2023, 110193.
139 T. M. Louie, X. S. Xie and L. Xun, *Biochemistry*, 2003, **42**, 7509–7517.
140 L. Liu, H. Liu, W. Zhang, M. Yao, B. Li, D. Liu and Y. Yuan, *Engineering*, 2019, **5**, 287–295.
141 L. Wang, N. Li, S. Yu and J. Zhou, *Bioresour. Technol.*, 2023, **368**, 128320.
142 H. Qi, L. Yu, Y. Li, M. Cai, J. He, J. Liu, L. Hao, H. Xu and M. Qiao, *Front. Microbiol.*, 2022, **13**, 851706.
143 T. Furuya and K. Kino, *Appl. Microbiol. Biotechnol.*, 2014, **98**, 1145–1154.
144 H. Kawaguchi, Y. Katsuyama, D. Danyao, P. Kahar, S. Nakamura-Tsuruta, H. Teramura, K. Wakai, K. Yoshihara, H. Minami and C. Ogino, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 5279–5290.
145 X.-H. Wang, C. Zhao, X.-Y. Lu, H. Zong and B. Zhuge, *ACS Synth. Biol.*, 2022, **11**, 900–908.
146 Z. Zhuang, F. Song, H. Zhao, L. Li, J. Cao, E. Eisenstein, O. Herzberg and D. Dunaway-Mariano, *Biochemistry*, 2008, **47**, 2789–2796.
147 H. Zhang and G. Stephanopoulos, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 3333–3341.
148 D. M. De Oliveira, A. Finger-Teixeira, T. Rodrigues Mota, V. H. Salvador, F. C. Moreira-Vilar, H. B. Correa Molinari, R. A. Craig Mitchell, R. Marchiosi, O. Ferrarese-Filho and W. Dantas dos Santos, *Plant Biotechnol. J.*, 2015, **13**, 1224–1232.
149 A. Pandi and V. M. Kalappan, *Mol. Biol. Rep.*, 2021, **48**, 3733–3745.
150 O. Choi, C.-Z. Wu, S. Y. Kang, J. S. Ahn, T.-B. Uhm and Y.-S. Hong, *J. Ind. Microbiol. Biotechnol.*, 2011, **38**, 1657–1665.
151 K. Sun-Young, C. Oksik, L. Jae, H. Bang, U. Tai-Boong and H. Young-Soo, *Microb. Cell Fact.*, 2012, **11**, 153.
152 J. L. Rodrigues, D. Gomes and L. R. Rodrigues, *Front. Bioeng. Biotechnol.*, 2020, **8**, 59.
153 H. Lv, Y. Zhang, J. Shao, H. Liu and Y. Wang, *Bioresour. Bioprocess.*, 2021, **8**, 1–12.
154 Z. Zhou, X. Zhang, J. Wu, X. Li, W. Li, X. Sun, J. Wang, Y. Yan, X. Shen and Q. Yuan, *Metab. Eng.*, 2022, **73**, 247–255.
155 M. Tylichová, D. Kopečný, S. Moréra, P. Briozzo, R. Lenobel, J. Snégaroff and M. Šebela, *J. Mol. Biol.*, 2010, **396**, 870–882.
156 V. Vasiliou, A. Pappa and D. R. Petersen, *Chem. Biol. Interact.*, 2000, **129**, 1–19.
157 F. Lambert, J. Zucca, F. Ness and M. Aigle, *Flavour Fragrance J.*, 2014, **29**, 14–21.
158 S. Liu, L. Soomro, X. Wei, X. Yuan, T. Gu, Z. Li, Y. Wang, Y. Bao, F. Wang and B. Wen, *Bioresour. Technol.*, 2021, **332**, 124967.
159 J. Zhang, S. Liu, H. Sun, Z. Jiang, Z. Zhou, X. Han, Y. Zhou, H. Sun, W. Zhou and J. Mao, *Foods*, 2021, **10**, 2577.
160 H. K. Sibhatu, S. A. Jabasingh, A. Yimam and S. Ahmed, *LWT–Food Sci. Technol.*, 2021, **135**, 110009.
161 H. B. Bang, Y. H. Lee, S. C. Kim, C. K. Sung and K. J. Jeong, *Microb. Cell Fact.*, 2016, **15**, 1–12.
162 S. Liu, Q. Qi, N. Chao, J. Hou, G. Rao, J. Xie, H. Lu, X. Jiang and Y. Gai, *Microb. Cell Fact.*, 2015, **14**, 1–14.
163 M. Gottardi, J. D. Knudsen, L. Prado, M. Oreb, P. Branduardi and E. Boles, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 4883–4893.
164 L. N. Jayakody and Y. S. Jin, *Appl. Microbiol. Biotechnol.*, 2021, **105**, 2675–2692.
165 O. De Smidt, J. C. Du Preez and J. Albertyn, *FEMS Yeast Res.*, 2008, **8**, 967–978.
166 H. B. Bang, J. Son, S. C. Kim and K. J. Jeong, *Metab. Eng.*, 2023, **76**, 63–74.
167 K. Venkatesan and K. Srinivasan, *Arkivoc*, 2008, **16**, 302–310.
168 C. Zhang, Y. Zang, P. Liu, Z. Zheng and J. Ouyang, *J. Biotechnol.*, 2019, **302**, 92–100.
169 E. Klumbys, Z. Zebec, N. J. Weise, N. J. Turner and N. S. Scrutton, *Green Chem.*, 2018, **20**, 658–663.
170 C. Zhang, Q. Xu, H. Hou, J. Wu, Z. Zheng and J. Ouyang, *Microb. Cell Factories*, 2020, **19**, 163.
171 S. Y. Park, D. Yang, S. H. Ha and S. Y. Lee, *Biotechnol. Bioeng.*, 2022, **119**, 946–962.
172 J. Yang, J. Liang, L. Shao, L. Liu, K. Gao, J.-L. Zhang, Z. Sun, W. Xu, P. Lin and R. Yu, *Metab. Eng.*, 2020, **59**, 44–52.
173 J.-L. Ferrer, M. Austin, C. Stewart Jr and J. Noel, *Plant Physiol. Biochem.*, 2008, **46**, 356–370.
174 J. P. Wang, B. Liu, Y. Sun, V. L. Chiang and R. R. Sederoff, *Front. Plant Sci.*, 2019, **9**, 1942.
175 H. Zhou, S. Gao, W. Zeng and J. Zhou, *Biochem. Eng. J.*, 2021, **168**, 107953.
176 J. Aschenbrenner, P. Marx, J. Pietruszka and J. Marienhagen, *ChemBioChem*, 2019, **20**, 949–954.
177 F. Bourgaud, A. Hehn, R. Larbat, S. Doerper, E. Gontier, S. Kellner and U. Matern, *Phytochem. Rev.*, 2006, **5**, 293–308.
178 B.-I. Shimizu, *Front. Plant Sci.*, 2014, **5**, 549.
179 R. Vanholme, L. Sundin, K. C. Seetso, H. Kim, X. Liu, J. Li, B. De Meester, L. Hoengenaert, G. Goeminne and K. Morreel, *Nat. Plants*, 2019, **5**, 1066–1075.

180 G. Vialart, A. Hehn, A. Olry, K. Ito, C. Krieger, R. Larbat, C. Paris, B. i. Shimizu, Y. Sugimoto and M. Mizutani, *Plant J.*, 2012, **70**, 460–470.
181 K. Kai, M. Mizutani, N. Kawamura, R. Yamamoto, M. Tamai, H. Yamaguchi, K. Sakata and B. i. Shimizu, *Plant J.*, 2008, **55**, 989–999.
182 Y. Lin, X. Sun, Q. Yuan and Y. Yan, *Metab. Eng.*, 2013, **18**, 69–77.
183 S.-M. Yang, G. Y. Shim, B.-G. Kim and J.-H. Ahn, *Microb. Cell Fact.*, 2015, **14**, 1–12.
184 Y. Zhao, X. Jian, J. Wu, W. Huang, C. Huang, J. Luo and L. Kong, *J. Biol. Eng.*, 2019, **13**, 1–13.
185 Y. Lin, X. Shen, Q. Yuan and Y. Yan, *Nat. Commun.*, 2013, **4**, 2603.
186 X. Shen, M. Mahajani, J. Wang, Y. Yang, Q. Yuan, Y. Yan and Y. Lin, *Metab. Eng.*, 2017, **42**, 59–65.
187 C. H. Zhao, R. K. Zhang, B. Qiao, B. Z. Li and Y. J. Yuan, *Biochem. Eng. J.*, 2020, **160**, 107634.
188 S. Gao, H. N. Yu, R. X. Xu, A. X. Cheng and H. X. Lou, *Phytochemistry*, 2015, **111**, 48–58.
189 A. D. Shrivastava and D. B. Kell, *Molecules*, 2021, **26**, 2065.
190 F. Li, L. Yuan, H. Lu, G. Li, Y. Chen, M. K. Engqvist, E. J. Kerkhoven and J. Nielsen, *Nat. Catal.*, 2022, **5**, 662–672.
191 A. Madani, B. Krause, E. R. Greene, S. Subramanian, B. P. Mohr, J. M. Holton, J. L. Olmos Jr, C. Xiong, Z. Z. Sun, R. Socher, J. S. Fraser and N. Naik, *Nat. Biotechnol.*, 2023, **41**, 1099–1106.
192 Y. Wang, S. Liao, N. Guan, Y. Liu, K. Dong, W. Weber and H. Ye, *Sci. Adv.*, 2020, **6**, eabb9484.
193 M. Ikeda, *Appl. Microbiol. Biotechnol.*, 2006, **69**, 615–626.
194 T. Yu, Y. J. Zhou, M. Huang, Q. Liu, R. Pereira, F. David and J. Nielsen, *Cell*, 2018, **174**, 1549–1558.
195 S. Kwak, J. H. Jo, E. J. Yun, Y. S. Jin and J. H. Seo, *Biotechnol. Adv.*, 2019, **37**, 271–283.
196 D. Liu, M. S. Sica, J. Mao, L. F. Chao and V. Siewers, *ACS Synth. Biol.*, 2022, **11**, 3228–3238.
197 P. Xu, L. Li, F. Zhang, G. Stephanopoulos and M. Koffas, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 11299–11304.
198 E. M. Zhao, Y. Zhang, J. Mehl, H. Park, M. A. Lalwani, J. E. Toettcher and J. L. Avalos, *Nature*, 2018, **555**, 683–687.
199 E. S. Bensen, B. G. Yeung and G. S. Payne, *Mol. Biol. Cell*, 2001, **12**, 13–26.
200 M. Suástegui, C. Yu Ng, A. Chowdhury, W. Sun, M. Cao, E. House, C. D. Maranas and Z. Shao, *Metab. Eng.*, 2017, **42**, 134–144.
201 H. Vashistha, M. Kohram and H. Salman, *Elife*, 2021, **10**, e64779.
202 Y. Taniguchi, P. J. Choi, G. W. Li, H. Chen, M. Babu, J. Hearn, A. Emili and X. S. Xie, *Science*, 2010, **329**, 533–538.
203 Y. Xiao, C. H. Bowen, D. Liu and F. Zhang, *Nat. Chem. Biol.*, 2016, **12**, 339–344.
204 Z. Zhou, Y. Liu, Y. Feng, S. Klepin, L. S. Tsimring, L. Pillus, J. Hasty and N. Hao, *Science*, 2023, **380**, 376–381.
205 L. Guo, M. Qi, C. Gao, C. Ye, G. Hu, W. Song, J. Wu, L. Liu and X. Chen, *Metab. Eng.*, 2022, **73**, 235–246.
206 L. Guo, W. Diao, C. Gao, G. Hu, Q. Ding, C. Ye, X. Chen, J. Liu and L. Liu, *Nat. Catal.*, 2020, **3**, 307–318.
207 C. Ge, Z. Yu, H. Sheng, X. Shen, X. Sun, Y. Zhang, Y. Yan, J. Wang and Q. Yuan, *Nat. Commun.*, 2022, **13**, 2182.
208 E. X. Wang, Y. Liu, Q. Ma, X. T. Dong, M. Z. Ding and Y. J. Yuan, *Biotechnol. Lett.*, 2019, **41**, 951–961.
209 C. V. Dinh and K. L. J. Prather, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 25562–25568.
210 K. B. Xavier and B. L. Bassler, *Curr. Opin. Microbiol.*, 2003, **6**, 191–197.
211 R. Kalluri and V. S. LeBleu, *Science*, 2020, **367**, eaau6977.
212 N. Ozber, J. L. Watkins and P. J. Facchini, *J. Ind. Microbiol. Biotechnol.*, 2020, **47**, 815–828.
213 H. Maeda and N. Dudareva, *Annu. Rev. Plant Biol.*, 2012, **63**, 73–105.
214 K. Zhou, K. Qiao, S. Edgar and G. Stephanopoulos, *Nat. Biotechnol.*, 2015, **33**, 377–383.
215 Z. Li, X. Wang and H. Zhang, *Metab. Eng.*, 2019, **54**, 1–11.
216 S. M. Brooks, C. Marsan, K. B. Reed, S. F. Yuan, D. D. Nguyen, A. Trivedi, G. Altin-Yavuzarslan, N. Ballinger, A. Nelson and H. S. Alper, *Nat. Commun.*, 2023, **14**, 4448.
217 Y. Liu, X. Tu, Q. Xu, C. Bai, C. Kong, Q. Liu, J. Yu, Q. Peng, X. Zhou, Y. Zhang and M. Cai, *Metab. Eng.*, 2018, **45**, 189–199.
218 M. Urui, Y. Yamada, Y. Ikeda, A. Nakagawa, F. Sato, H. Minami and N. Shitan, *Microb. Cell Fact.*, 2021, **20**, 200.
219 R. Wang, S. Zhao, Z. Wang and M. A. Koffas, *Curr. Opin. Biotechnol.*, 2020, **62**, 65–71.
220 S. K. Aulakh, L. Sellés Vidal, E. J. South, H. Peng, S. J. Varma, L. Herrera-Dominguez, M. Ralser and R. Ledesma-Amaro, *Nat. Chem. Biol.*, 2023, **19**, 951–961.