Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Review

Medical properties, market potential, and microbial production of golden polyketide curcumin for food, biomedical, and cosmetic applications☆

Selma Beganovic and Christoph Wittmann

Curcumin, a potent plant polyketide in turmeric, has gained recognition for its outstanding health benefits, including anti-inflammatory, antioxidant, and anticancer effects. Classical turmeric farming, which is widely used to produce curcumin, is linked to deforestation, soil degradation, excessive water use, and reduced biodiversity. In recent years, the microbial synthesis of curcumin has been achieved and optimized through novel strategies, offering increased safety, improved sustainability, and the potential to revolutionize production. Here, we discuss recent breakthroughs in microbial engineering and fermentation techniques, as well as their capacity to increase the yield, purity, and cost-effectiveness of curcumin production. The utilization of microbial systems not only addresses supply chain limitations but also helps meet the growing demand for curcumin in various industries, including pharmaceuticals, foods, and cosmetics.

Address
Institute of Systems Biotechnology, Saarland University, Germany

Corresponding author: Wittmann, Christoph (christoph.wittmann@uni-saarland.de)

Current Opinion in Biotechnology 2024, **87**:103112

This review comes from a themed issue on **Food Biotechnology**

Edited by **Christoph Wittmann** and **Ken-ichi Yoshida**

Available online xxxx

https://doi.org/10.1016/j.copbio.2024.103112

Introduction

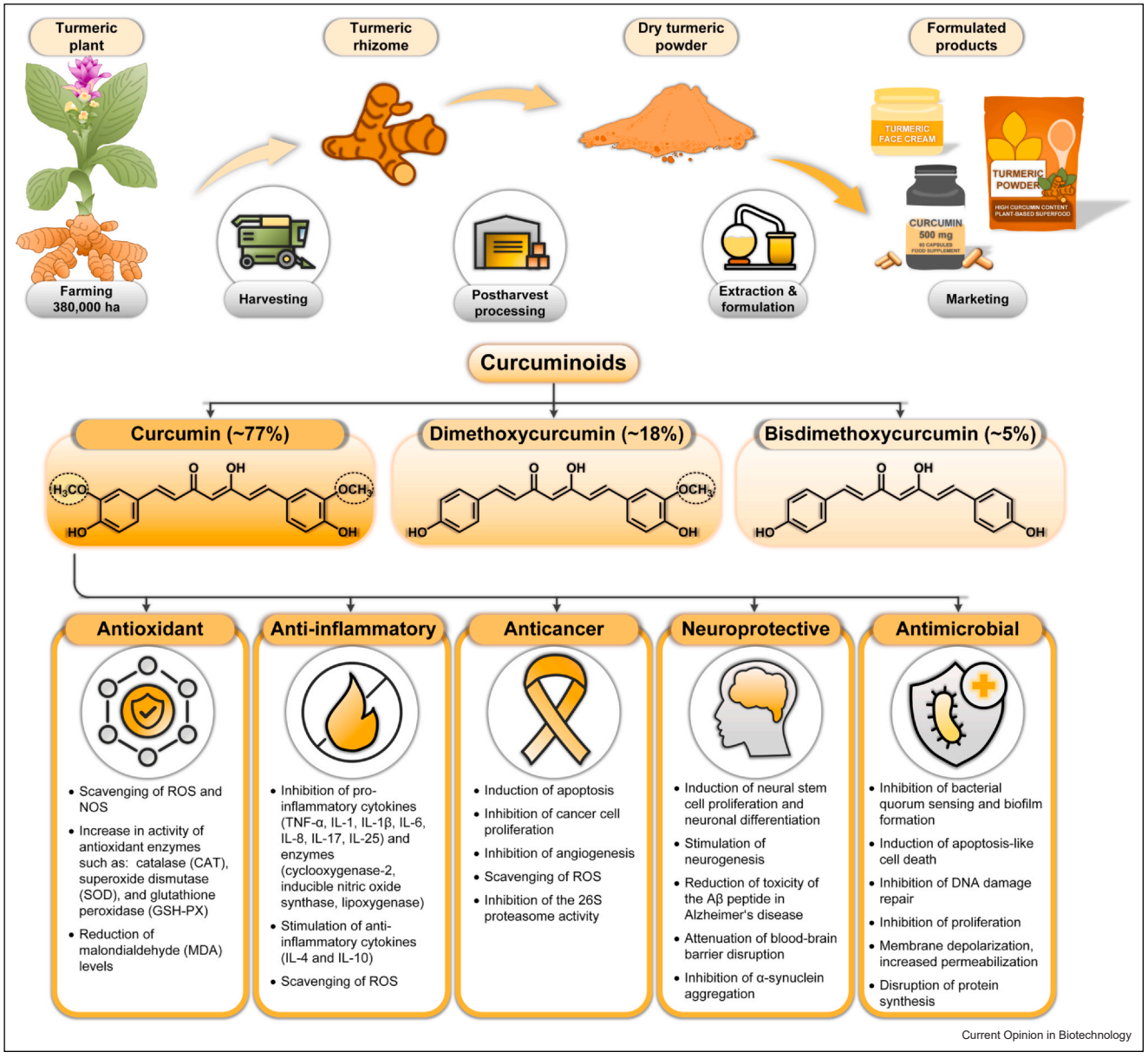
Curcumin is the principal curcuminoid produced from the popular Indian spice turmeric (*Curcuma longa* L.), a member of the ginger family [1,2]. Chemically, the molecule contains two phenolic rings connected by a seven-carbon linker, which is responsible for the antioxidant properties of curcumin (Figure 1). Methoxy groups are attached to the rings, which contribute to the compound's solubility and influence its overall reactivity. As demonstrated by recent studies, curcumin has multiple benefits [3•]. In regard to human health, curcumin limits the growth of food pathogens [4], allows [3•] individuals to monitor food spoilage through color changes [5], and provides a bright yellow color to various foods [6,7]. In addition, numerous studies have reported that curcumin exhibits antioxidant [8], anti-inflammatory [9], anticancer [10], neuroprotective [11], and antimicrobial [12] effects (Figure 1).

Economically, curcumin has commercial value in the pharmaceutical, food, and cosmetics industries, as demonstrated by the US$ 70 million in market value in 2022. The market is expected to more than double over the next 8 years, underlining that importance of curcumin will substantially increase (https://www.gminsights.com/industry-analysis/curcumin-market). Today, curcumin is almost exclusively obtained from turmeric plants through classical farming, followed by extraction and purification of the compound from the powdered turmeric rhizome (Figure 1). The global production of fresh turmeric rhizomes is estimated to reach 1.1 million metric tons per year, and India represents 80% of the global market [14]. Fresh rhizome contains 2–5% curcumin (depending on the growing season) [13], leading to an annual production volume of approximately 22,000–55,000 tons of curcumin.

The average yield of the spice is approximately 3.8 tons ha^{-1}, and 10% of the harvest must be stored as a seed material for the next farming season [15]. Unfortunately, turmeric farming raises environmental concerns, as the plants occupy valuable agricultural land (380 000 ha) and require intensive watering, which can lead to water scarcity in arid areas. Furthermore, these large monocultures can be infected by plant-parasitic nematodes [16], fungi [17], bacteria [18], and insects [19], necessitating massive use of pesticides and, inter alia, posing high risks of harvest loss.

☆ Christoph Wittmann had no involvement in the peer review of the article and has no access to information regarding its peer review. Full responsibility for the editorial process of this article was delegated to Ken-ichi Yoshida.

Figure 1



From nature to applications: the curcumin value chain. Curcumin compounds are obtained from farmed turmeric plants and are extracted from the dried rhizome and formulation. Conventional extraction methods, such as solid–liquid and Soxhlet extraction [92], are easily performed but suffer from low efficiency, time exhaustion, and nonselectivity [20,93]. New methods that rely on ultrasound [94], electromagnetic waves [95], enzymes [96], supercritical fluids [97], pressurized liquids [98], and green solvents [99] offer more ecofriendly and efficient curcumin purification methods but remain in their infancy. Medically, curcumin is known to modulate various cellular pathways, and its mode of action has been intensively studied; curcumin molecules perform relevant interactions with transcription factors, proteins, cytokines, and inflammatory mediators, thereby providing protection against oxidation, inflammation, cancer, neurodegenerative diseases, and infections [100].

Curcumin can be chemically synthesized, but the yields are low; in addition, the products are contaminated with organic reagents, complicating their safe use in food and therapy [20].

Classical manufacturing involves obvious drawbacks, including the need for alternative routes that are more efficient and sustainable. In this work, after updating the applications and market potential of curcumin, we review novel strategies that harness synthetic biology and systems metabolic engineering [21–23] to breed microbial cell factories for curcumin synthesis. Building upon our present knowledge of the underlying biochemistry and metabolic pathways involved, we showcase several

successful examples of curcumin synthesis in bacteria, fungi, and yeasts and anticipate a more sustainable future for curcumin manufacturing.

Market potential and applications of curcumin

Curcumin was awarded the generally regarded as safe (GRAS) status by the United States Food and Drug Administration and is used in various applications in the food, medical, and cosmetic industries (Figure 1). Extensive research efforts in recent years have led to tremendous growth in terms of market applications and commercial value of curcumin. In the food industry, curcumin is marketed as Natural Yellow 3, diferuloylmethane, CI 75300, or E100 [24]. Due to its pronounced yellow color, curcumin is a common dye for baked goods, dairy products, mustard, beverages, ice cream, and salad dressings [25]; thus, curcumin offers a beneficial replacement to synthetic yellow dyes that are unsafe [26]. Due to its pH-dependent color change [27], curcumin has been applied as a pH biosensor for monitoring food spoilage [28].

Owing to its antimicrobial and antioxidant activities, curcumin is also used to preserve green food [29] and has gained increasing attention for its ability to extend the shelf life of food [30]. Notably, when excited with visible light within 400 to 500 nm, curcumin is photoactivated and drives the formation of reactive oxygen species, such as singlet oxygen, superoxide anions, and hydroxyl radicals [31,32]. These factors, in turn, reduce the growth of prevalent food pathogens, including *Staphylococcus aureus* [33], *Listeria monocytogenes* [34], *Listeria innocua* [35], *Escherichia coli* O157:H7 [35], and *Salmonella typhimurium* [36], in fresh meat. Moreover, curcumin limits the growth of food-borne fungi, such as *Aspergillus flavus* in maize kernels [37], *Penicillium expansum* [38], and *Botrytis cinerea* in apples [39], and inactivates enzymes responsible for browning and deteriorating fruits [40,41].

Medically, curcumin has a range of health benefits and promotes well-being (Figure 1). Based on recent findings, curcumin suppresses cancer growth [10,42,43], protects against inflammation [9,44], and fights and prevents infectious and noninfectious diseases, including malaria, tuberculosis [45–47], diabetes [48,49], Alzheimer's disease [50,51], and Parkinson's disease [52,53]. Furthermore, curcumin actively supports wound healing [54,55] and helps achieve appropriate immune system modulation [56,57], leading to a wide range of therapeutic applications. As reported in the PubMed database, 268 clinical trials involving curcumin (February 2024) have been conducted since 2019 and have demonstrated the numerous health benefits of curcumin, highlighting the great potential of the compound. For example, oral administration of curcumin significantly reduced the progression of prostate-specific antigen, a biomarker for prostate cancer [58]; knee injury and osteoarthritis outcome scores [59]; and serum levels of islet amyloid polypeptide, a hallmark of insulin resistance [60]. Therefore, curcumin exhibits anticancer, anti-inflammatory, and antidiabetic properties in humans.

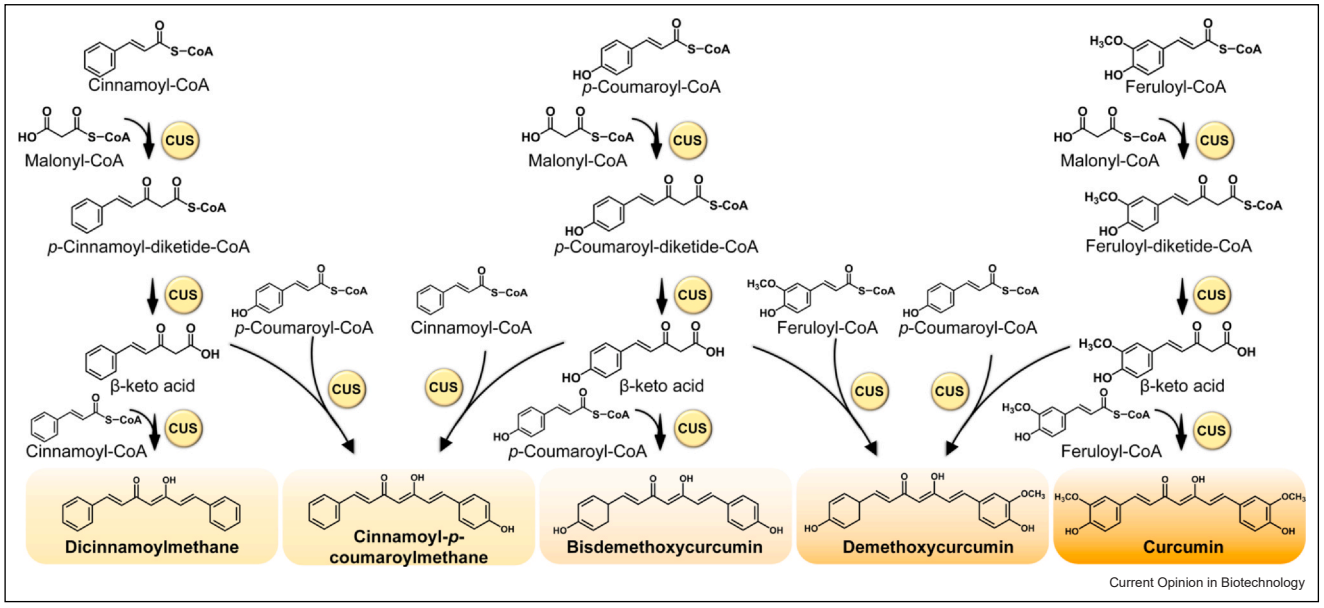
Biochemistry of curcumin synthesis in plants

As a polyketide, curcumin and its derivatives are synthesized from CoA thioesters, namely, one molecule of malonyl-CoA and two molecules of aromatic CoA [61•] (Figure 2). The latter compounds are formed by CoA-mediated activation of hydroxycinnamate intermediates of the phenylpropanoid pathway [62]; normally, this pathway supplies precursors for the biosynthesis of lignin, a major structural component of the cell wall in higher terrestrial plants [63,64], and other metabolites, such as flavonoids [65].

The biosynthetic route exhibits promiscuity and accepts different aromatic CoA thioesters. Curcumin is formed from two molecules of feruloyl-CoA, whereas partial or exclusive incorporation of *p*-coumaroyl-CoA results in the demethoxy analogs demethoxycurcumin and bisdemethoxycurcumin. The signature enzyme of the pathway is a type III polyketide synthase (PKS), which catalyzes the decarboxylative condensation of the aromatic CoA thioester with malonyl-CoA as the extender unit. CUS was originally discovered in rice (*Oryza sativa* L.), although the plant tissue does not contain curcuminoids *in vitro* [66] or *in vivo* [67]. Notably, *Os*CUS exhibits differential affinities for cinnamoyl-CoA, *p*-coumaroyl-CoA, and feruloyl-CoA; in contrast, *Os*CUS strongly prefers *p*-coumaroyl-CoA as a substrate, strongly impacting the curcuminoid spectrum [42].

Inspired by the findings for rice, researchers subsequently revealed the biosynthesis of curcuminoids in turmeric (*C. longa*), revealing a striking difference (Figure 3a). In turmeric, the following type III PKSs are collaboratively involved in the biosynthesis of curcuminoids: diketide-CoA synthase (DCS; BAH56225) and curcumin synthase (CURS) [68]. The DCS enzyme drives the formation of the diketide-CoA intermediate from malonyl-CoA and either *p*-coumaroyl-CoA or feruloyl-CoA. CURSs perform dual functions, as they catalyze the hydrolysis of diketide-CoA to β-keto acid and perform condensation with the second hydroxycinnamoyl-CoA thioester. Further studies of native curcuminoid pathways have revealed three different variants (CURS1, 2, and 3; BAH56226, BAH85780, and BAH85781, respectively) [69]. CURS1 exhibits a preference for feruloyl-CoA, although it accepts *p*-coumaroyl-CoA [68]; CURS2 exhibits a preference for feruloyl-CoA; and CURS3 exhibits similar affinities for feruloyl-CoA and *p*-coumaroyl-CoA [69]. In principle, differential expression of the three CURSs and

Figure 2



In vitro biosynthesis of curcumin and curcuminoid derivatives by curcuminoid synthase (CUS) from rice (*Oryza saiva*, *Os*). The gene *os07g17010,* encoding CUS from rice (*Os*CUS), was identified while researchers screened for putative type III PKS activity in rice. The enzyme catalyzes a three-step reaction from a phenylpropanoid-derived aromatic CoA thioester via diketide-CoA and β-keto acid intermediates to the corresponding curcuminoid molecule. Functional studies of a putative type III PKS, encoded in the rice genome, surprisingly revealed the synthesis of curcumin and bisdemethoxycurcumin, as well as related derivatives from CoA-based precursors [66] (Figure 2). Given its activity, the enzyme was named curcuminoid synthase (CUS). Rice-based enzymes (*Os*CUSs) function in a nontraditional 'head-to-head' fashion [101], thereby forming diketide-CoA. Subsequently, the intermediate is hydrolyzed to the corresponding β-ketoacid in a reaction catalyzed by the same enzyme [66]. In addition to other PKSs, *Os*CUS uses β-ketoacid as a second extender substrate and condenses the compound with another phenylpropanoid-derived CoA thioester [101]. In this manner, a single enzyme catalyzes the correspondingly 'one-pot' synthesis of curcuminoids [72]. *In vitro* feeding of malonyl-CoA and different aromatic CoA thioesters enabled the production of the natural curcuminoids curcumin, demethoxycurcumin, and bisdemethoxycurcumin; in addition, new, more unpolar derivatives were generated, such as dicinnamoylmethane and cinnamoyl-*p*-coumaroylmethane. Regarding selectivity, *Os*CUS exhibits a preference for *p*-coumaroyl-CoA but also accepts cinnamoyl-CoA and feruloyl-CoA, enabling the synthesis of asymmetrical curcuminoids (demethoxycurcumin and cinnamoyl-*p*-coumaroylmethane). When *Os*CUS was subsequently expressed in *E. coli* BLR (DE3), it enabled the formation of all curcuminoids *in vivo*.

availability of the building blocks define the curcuminoid spectrum (Figure 3b) [44,69,70].

Upstream of the biosynthetic pathway, feruloyl-CoA and *p*-coumaroyl-CoA are derived from ferulate and *p*-coumarate, respectively, by the enzyme *p*-coumaroyl-CoA ligase (4CL) [61]. Ferulate is formed by the enzymatic modification of *p*-coumarate via caffeate as an intermediate [71••]. Notably, *p*-coumarate is synthesized directly via deamination of L-tyrosine or hydroxylation of cinnamate, which is produced by deamination of L-phenylalanine (Figure 3a) [72].

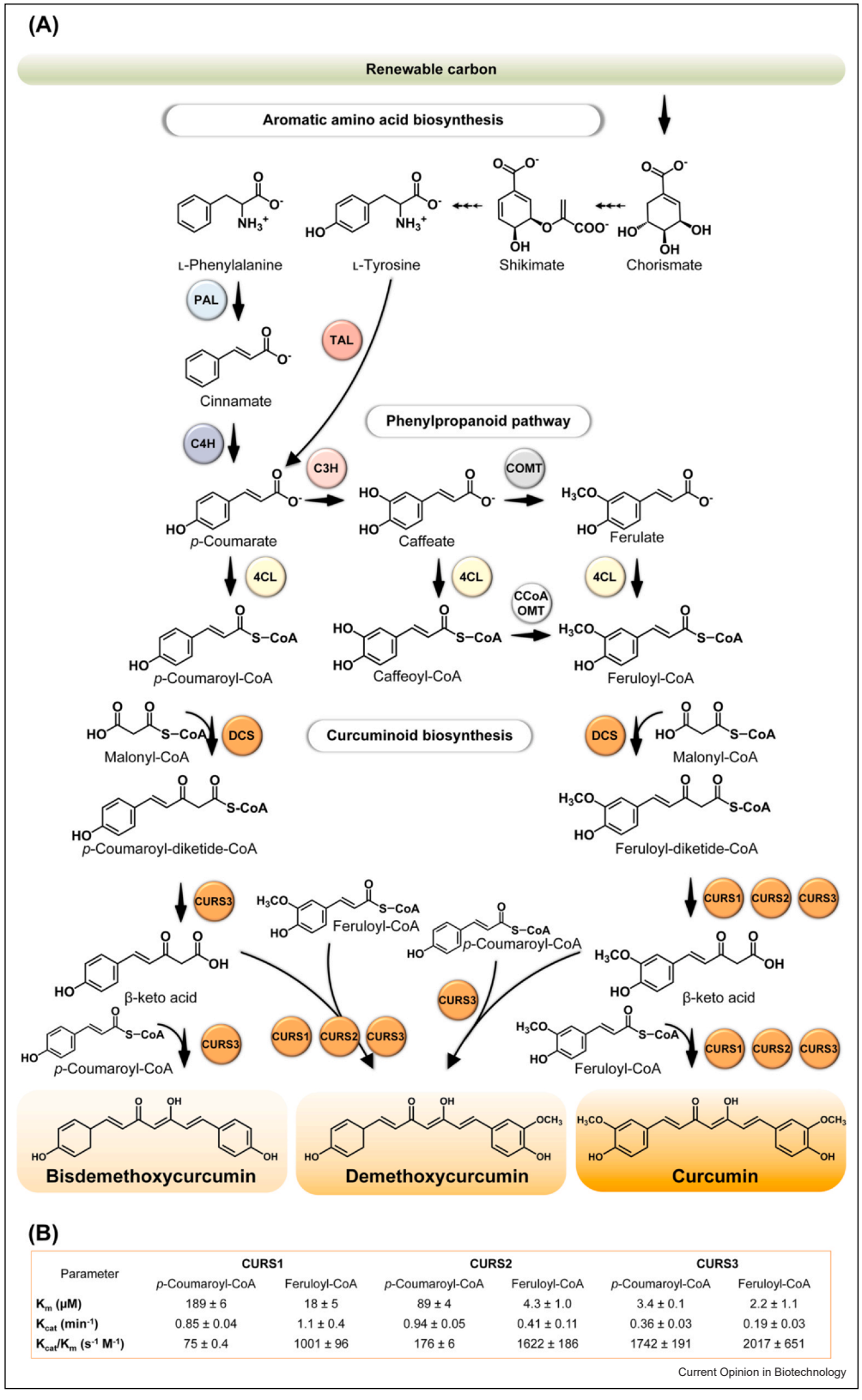
Briefly, *in vitro* studies of chalcone synthase from ginger (*Zingiber officinale* L.; GenBank: DQ486012.1) using feruloyl-CoA, *p*-coumaroyl-CoA, and cinnamoyl-CoA yielded curcumin, bisdemethoxycurcumin, and dicinnamoylmethane, respectively [73] (Figure 2). However, further testing has not been performed with the enzyme [74].

Pioneering synthesis of curcumin in *Escherichia coli* using rice-and turmeric-based heterologous pathways

The identification of curcuminoid synthase activity in rice (*Os*CUS) and the demonstration of its functional operation *in vitro* [66,67] set the stage for heterologous curcumin production in microbes (Figure 4). To this end, *E. coli* BLR (DE3) was constructed with a plasmid-based heterologous pathway to convert the precursor ferulate into curcumin. The designed artificial biosynthesis-related gene cluster included four genes, including *os07g17010* from rice, which encodes *Os*CUS; *4cl1*, which encodes 4-coumarate-CoA ligase 1 (from *Lithospermum erythrorhizon*, purple gromwell); *accBC*; *dtsR1*, which encodes acetyl-CoA carboxylase subunit α; and a homolog of the β subunit (from *Corynebacterium glutamicum*).

The recombinant *E. coli* BLR (DE3) strain that expresses these genes was tested in a two-step culture process. After large amounts of biomass were formed in

Figure 3



Parameter	CURS1		CURS2		CURS3	
	p-Coumaroyl-CoA	Feruloyl-CoA	*p*-Coumaroyl-CoA	Feruloyl-CoA	*p*-Coumaroyl-CoA	Feruloyl-CoA
K_m (μM)	189 ± 6	18 ± 5	89 ± 4	4.3 ± 1.0	3.4 ± 0.1	2.2 ± 1.1
K_{cat} (min^{-1})	0.85 ± 0.04	1.1 ± 0.4	0.94 ± 0.05	0.41 ± 0.11	0.36 ± 0.03	0.19 ± 0.03
K_{cat}/K_m (s^{-1} M^{-1})	75 ± 0.4	1001 ± 96	176 ± 6	1622 ± 186	1742 ± 191	2017 ± 651

Metabolic pathways associated with the heterologous production of curcumin and related curcuminoids in microbes. The figure illustrates the native biosynthetic machinery from the turmeric plant **(a)** and the corresponding kinetic properties of the CURS1, CURS2, and CURS3 enzymes **(b)**. The phenylpropanoid pathway is initiated by deamination of the aromatic amino acids L-phenylalanine and L-tyrosine by the enzymes PAL and TAL, yielding cinnamate and *p*-coumarate, respectively. Additionally, *p*-coumarate can be synthetized from L-phenylalanine via cinnamate by PAL and C4H. The final product of the phenylpropanoid pathway is ferulate, which is synthesized from *p*-coumarate via caffeate by C3H and COMT. The first committed reaction of the curcuminoid biosynthetic pathway is catalyzed by 4CL, which produces CoA-activated forms of phenylpropanoids. Subsequently, DCS elongates feruloyl-CoA or *p*-coumaroyl-CoA to form one acetate unit from malonyl-CoA, resulting in the formation of β-keto acids. Finally, CURS catalyzes the condensation of β-keto acids with one additional molecule of feruloyl-CoA or *p*-coumaroyl-CoA. Depending on the combination of starter and elongation units, curcumin, demethoxycurcumin, or bisdemethoxycurcumin can be produced. Abbreviations: C4H: cinnamate 4-hydroxylase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; CURS: curcumin synthase [70]; K_m: Michaelis–Menten constant; K_{cat}: turnover number; K_{cat}/K_m: catalytic efficiency.

complex nutrient broth, the cells were transferred to mineral media. The setup contained 40 g l^{-1} glucose (as a growth substrate) and 1 mM ferulate (as a biotransformation precursor). Within 60 hours, the recombinant strain formed 113 mg l^{-1} of curcumin in a yield of 0.61 mol (mol ferulate)$^{-1}$ [67] (Figure 4). Subsequently, the artificial pathway was extended further expressing phenylalanine ammonia-lyase (PAL) from the yeast *Rhodotorula rubra*, which enabled isopropyl β-D-1-thiogalactopyranoside-inducible production from L-phenylalanine and L-tyrosine in the corresponding four-plasmid mutant (Figure 4). The same study produced curcumin from rice bran pitch, a ferulate-rich agricultural waste that is generated during rice milling [67].

Subsequently, the researchers induced the functional expression of the *dcs* and *curs1* genes from turmeric with *E. coli* K-12 using isopropyl β-D-1-thiogalactopyranoside-inducible medium- and high-copy-number plasmids [75] (Figure 4). Additionally, *4cl1* from tale cress (*Arabidopsis thaliana* (L.), Heyn.) was expressed on a low-copy-weight plasmid, and the three-plasmid host formed 69 mg l^{-1} curcumin in a yield of 0.18 mol (mol ferulate)$^{-1}$. A two-stage process was used for production. Elevated levels of glucose (40 g l^{-1}) and ferulate (2 mM) were contained in the medium during the production phase. Additional studies suggested that the turmeric-based pathway, which consists of 4CL1 from *A. thaliana* and DCS and CURS1 from *C. longa* [75], exhibited greater efficiency than that of the rice-based pathway described previously, comprising 4CL from *L. erythrorhizon*, CUS, and the overexpression of acetyl-CoA carboxylase from *C. glutamicum* [67].

Strikingly, the chosen host substantially affected production (Figure 4). Recombinant *E. coli* BL21 (DE3) accumulated 301 mg l^{-1} of curcumin in a ferulate-supplemented one-step process in terrific broth (TB), far more than the K-12 MG1655 (DE3) and K-12 JM109 (DE3) strains that expressed the same genes on the same vectors [76]. In contrast to the K-12 lineage, the BL21 (DE3) strain (as with other strains from the B lineage) lacks two major proteases, Lon and OmpT; thus, the strain can better express foreign proteins from cloned genes [77]. Furthermore, *E. coli* BL21 (DE3) exhibited a mutation in the $hsdS_B$ gene, reducing plasmid degradation [78]. Furthermore, the host benefited from streamlined metabolism because it exhibited an increased capacity to re-metabolize secreted acetate; as a result, the host tolerated higher glucose levels and grew to higher cell concentrations than those of *E. coli* K-12 [79,80]. This metabolic property was linked to the upregulation of the tricarboxylic acid cycle and the glyoxylate shunt, which is involved in acetate metabolism, at the transcriptional level.

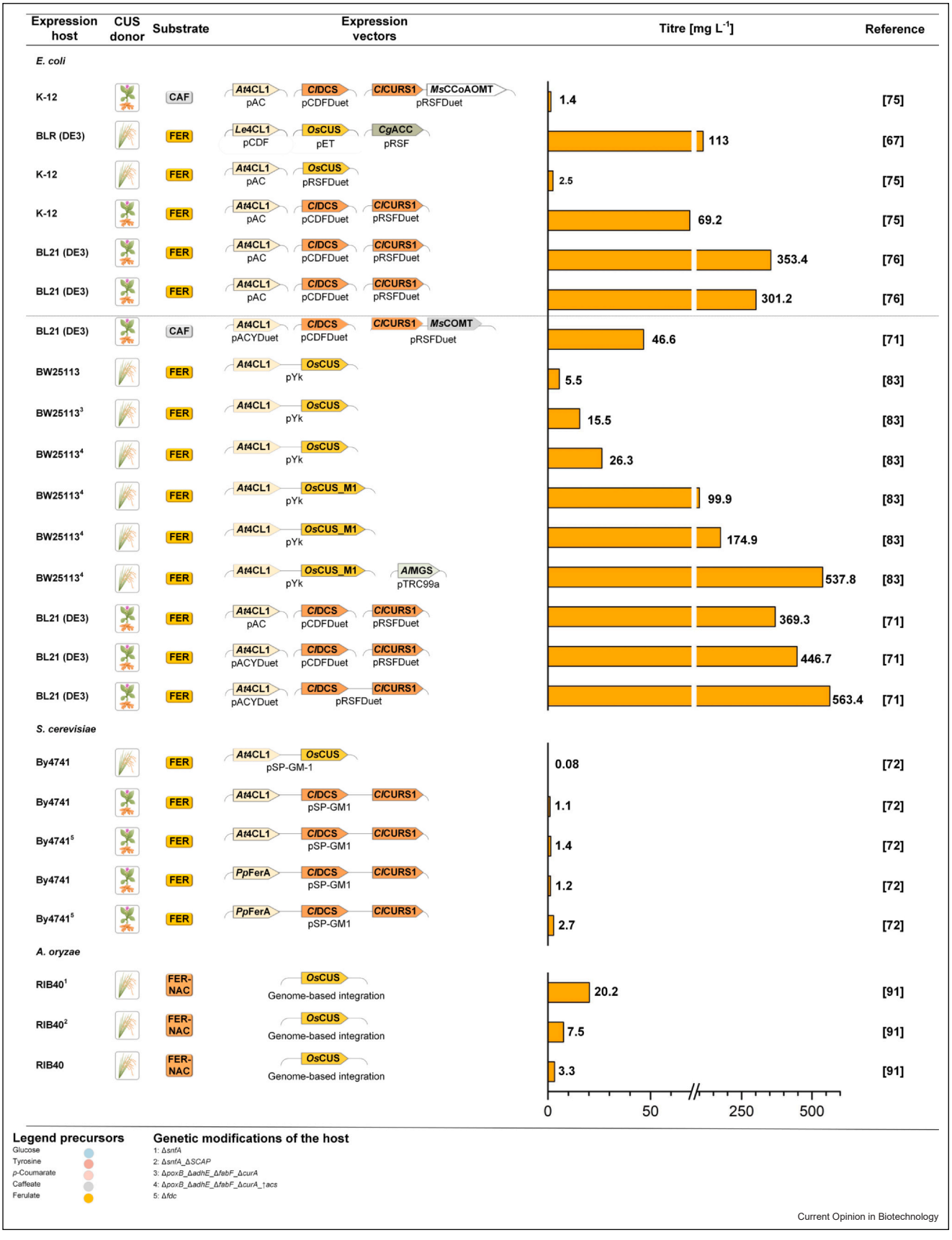
A range of medium tests were performed to explore the effects caused by nutrient availability and buffer capacity. This led to a two-step fermentation process (cell growth in Luria-Bertani (LB) and curcumin production in M9), which yielded 353 mg l^{-1} curcumin at a yield of 0.95 mol (mol ferulate)$^{-1}$ [76] (Figure 4).

Furthermore, curcuminoids were synthesized from L-tyrosine via the caffeate pathway, although at substantially lower levels than those of the two-step fermentation [75] (Figure 4). This synthesis involved the utilization of tyrosine ammonia lyase (TAL) from the yeast *Rhodotorula glutinis* and 4-coumarate 3-hydroxylase (C3H) from the soil bacterium *Saccharothrix espanaensis* to produce caffeate from L-tyrosine. Subsequently, caffeoyl-CoA 3-*O*-methyltransferase (COMT) from lucerne (*Medicago sativa* L.) was employed to convert caffeoyl-CoA into feruloyl-CoA, a critical step in the production process.

Increased curcumin production in recombinant *Escherichia coli* using fine-tuned and extended biosynthetic pathways and novel coculture concepts

Research on microbial curcumin synthesis has intensified over the past 5 years. For example, in a range of studies, researchers explored new concepts to increase the production of curcumin in recombinant *E. coli* to a new level. Impressively, curcumin was generated from glucose through microbial *de novo* synthesis first time using the genomic expression of six genes in the tyrosine-overproducing derivative of *E. coli* C41; in this process, the need for antibiotics was eliminated [81••] (Figure 4). The biosynthetic genes were organized into two separate operons (modules). The ferulate-producing module (encompassing *tal* and *c3h* from *S. espanaensis* and *comt* from *M. sativa*) was integrated into the *bioC* locus of the host, while the curcumin-producing module

Figure 4


Expression host
CUS donor
Substrate
Expression vectors
Titre [mg L-1]
Reference
E. coli
K-12 CAF At4CL1 pAC C/DCS pCDFDuet C/CURS1 MsCCoAOMT pRSFDuet 1.4 [75]
BLR (DE3) FER Le4CL1 pCDF OsCUS pET CgACC pRSF 113 [67]
K-12 FER At4CL1 pAC OsCUS pRSFDuet 2.5 [75]
K-12 FER At4CL1 pAC C/DCS pCDFDuet C/CURS1 pRSFDuet 69.2 [75]
BL21 (DE3) FER At4CL1 pAC C/DCS pCDFDuet C/CURS1 pRSFDuet 353.4 [76]
BL21 (DE3) FER At4CL1 pAC C/DCS pCDFDuet C/CURS1 pRSFDuet 301.2 [76]
BL21 (DE3) CAF At4CL1 pACYDuet C/DCS pCDFDuet C/CURS1 MsCOMT pRSFDuet 46.6 [71]
BW25113 FER At4CL1 OsCUS pYk 5.5 [83]
BW25113[3] FER At4CL1 OsCUS pYk 15.5 [83]
BW25113[4] FER At4CL1 OsCUS pYk 26.3 [83]
BW25113[4] FER At4CL1 OsCUS_M1 pYk 99.9 [83]
BW25113[4] FER At4CL1 OsCUS_M1 pYk 174.9 [83]
BW25113[4] FER At4CL1 OsCUS_M1 pYk AtMGS pTRC99a 537.8 [83]
BL21 (DE3) FER At4CL1 pAC C/DCS pCDFDuet C/CURS1 pRSFDuet 369.3 [71]
BL21 (DE3) FER At4CL1 pACYDuet C/DCS pCDFDuet C/CURS1 pRSFDuet 446.7 [71]
BL21 (DE3) FER At4CL1 pACYDuet C/DCS C/CURS1 pRSFDuet 563.4 [71]
S. cerevisiae
By4741 FER At4CL1 OsCUS pSP-GM-1 0.08 [72]
By4741 FER At4CL1 C/DCS C/CURS1 pSP-GM1 1.1 [72]
By4741[5] FER At4CL1 C/DCS C/CURS1 pSP-GM1 1.4 [72]
By4741 FER PpFerA C/DCS C/CURS1 pSP-GM1 1.2 [72]
By4741[5] FER PpFerA C/DCS C/CURS1 pSP-GM1 2.7 [72]
A. oryzae
RIB40[1] FER-NAC OsCUS Genome-based integration 20.2 [91]
RIB40[2] FER-NAC OsCUS Genome-based integration 7.5 [91]
RIB40 FER-NAC OsCUS Genome-based integration 3.3 [91]
0 50 250 500
Legend precursors
Glucose
Tyrosine
p-Coumarate
Caffeate
Ferulate
Genetic modifications of the host
1: ΔsnfA
2: ΔsnfA_ΔSCAP
3: ΔpoxB_ΔadhE_ΔfabF_ΔcurA
4: ΔpoxB_ΔadhE_ΔfabF_ΔcurA_↑acs
5: Δfdc
Current Opinion in Biotechnology

Overview of the microbial-based bioprocesses used to produce curcumin from caffeate, ferulate, and feruloyl-*N*-acetylcysteamine. The data are displayed regarding pioneering studies (above the dashed line) and recent work from the past 5 years (below the dashed line). From top to bottom the following hosts were used: *E. coli* K12 producing from *p*-coumarate (*P*-COU) and caffeate (CAF) via the turmeric pathway [75], *E. coli* BLR (DE3) producing from ferulate (FER) via the rice-based pathway [67], *E. coli* K12 from FER via the rice-based pathway and the turmeric pathway [75], *E. coli* BL21 (DE3) from FER via the turmeric route [76], *E. coli* BL21 (DE3) from CAF via the turmeric pathway [71], *E. coli* BW25113 from FER via the rice-based route [83••], *E. coli* BL21 (DE3) from FER via the turmeric route [71], *S. cerevisiae* By4741 from FER via the turmeric route [61], and *A. oryzae* RIB40 from FER-NAC via the rice-based pathway [91]. Given that curcumin production by *A. oryzae* was carried out on agar plate cultures [91], we estimated the achieved titer by assuming a volume of agar of 20 ml, which is routinely applied to grow such microbes. In established bioprocesses, curcumin is typically extracted from acidified whole-cell broth (pH = 3) using polar solvents, such as ethyl acetate, followed by drying. The recovered curcumin is subsequently resuspended in solvents, such as dimethyl sulfoxide [67], acetonitrile [71,75,76], and methanol [81,91], and analyzed via high-performance liquid chromatography via ultraviolet detection at 425 nm. Abbreviations: CAF: caffeate; FER: ferulate; FER-NAC: feruloyl-*N*-acetylcysteamine; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; CUS: curcuminoid synthase; CURS: curcumin synthase; ACC: acetyl-CoA carboxylase; *Al*: *A. laidlawii*; *At*: *A. thaliana*; *Cl*: *C. longa*; *Ms*: *Medicago sativa*; *Nt*: *Nicotiana tabacum*: *Os*: *O. sativa*; *Pp*: *P. paucimobilis*; *Rg*: *R. glutinis*; *Se*: *S. espanaensis; Cg: C. glutamicum.* [1]: *ΔsnfA*; [2]: *ΔsnfA ΔSCAP*; [3]: *ΔpoxB ΔadhE ΔfabF ΔcurA*; [4]: *ΔpoxB ΔadhE ΔfabF ΔcurA ↑acs*; [5]: *Δfdc*. For details about curcumin production from glucose, L-tyrosine, and *p*-coumarate, refer to Figure S1.

(containing the *4cl2* from *Nicotiana tabacum* L., *dcs* and *curs1* from *C. longa*) was inserted into the *lacZ* locus. Each gene was flanked by a ribosomal binding site and a T7 terminator sequence and expressed under the control of a viral T7 promoter. The authors were able to appropriately balance the expression levels [82] within the six-enzyme pathway by comprehensively screening a library that contained 5′-untranslated region sequence mutants obtained using multiplexed automated genome engineering. Among the variants generated, one mutant called 6M08rv exhibited a remarkable 38.2-fold improvement in curcumin production compared with that of the original parental strain. Notably, this improvement was achieved even though the calculated expression levels of two critical enzymes within the pathway (4CL and DCS) were significantly lower in 6M08rv than in the parental strain. Although the final curcumin titer ($3.8\ mg\ l^{-1}$) was much lower than that obtained from ferulate-based processes, this approach is inspiring and provides an excellent starting point for creating streamlined *de novo* synthetic strains.

Following their previous work on *E. coli* BL21 (DE3), Rodrigues et al. systematically fine-tuned and extended the biosynthetic route to curcumin [71••] (Figure 4). Various plasmid combinations were tested, and the conversion of ferulate to feruloyl-CoA was optimized (module 1). Subsequently, the conversion of the latter compound into curcumin was evaluated (module 2). The best genetic layout included the combined expression of *dcs* and *curs1* from turmeric on a high-copy-number plasmid plus the expression of *4cl1 from A. thaliana* from a low-copy-weight plasmid and the production of $563\ mg\ l^{-1}$ curcumin within 63 hours, which is the highest titer reported thus far. When both modules were combined in one single host, curcumin was obtained from L-tyrosine; however, the level attained was much lower ($6.4\ mg\ l^{-1}$), suggesting that imbalances remain, and further engineering efforts are needed.

An interesting solution to this problem was provided in the same work [71••]. Using a novel approach, the synthesis of curcumin was performed by two different producers (Figure 4). The strategy was based on a co-culture in which one recombinant host carried synthetic module 1 to synthesize ferulate from L-tyrosine, whereas the second recombinant host expressed module 2 and used ferulate as a substrate to produce curcumin (Fig. S1).

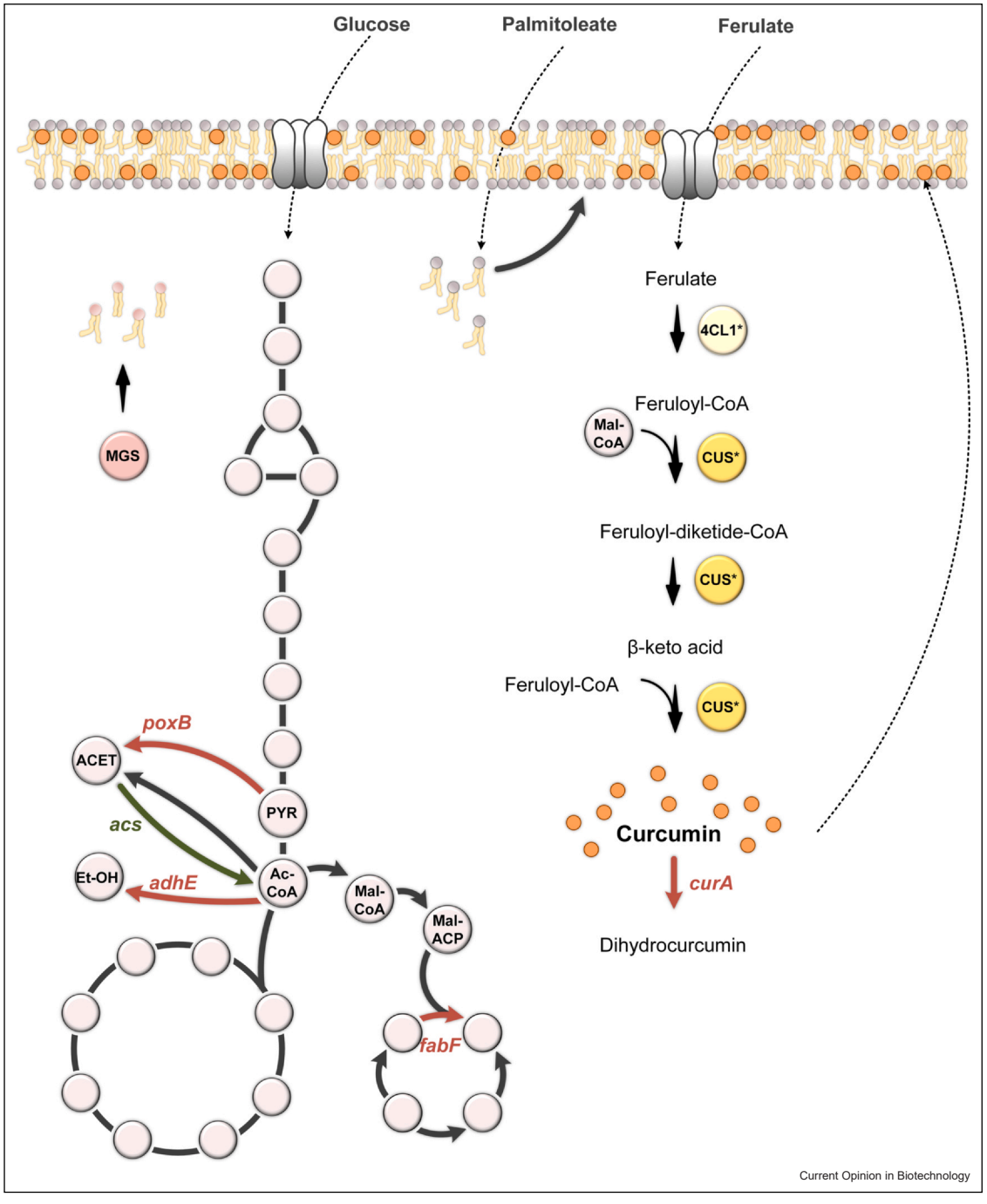
For this purpose, the first host expressed *tal* from *R. glutinis*, *c3h* from *S. espanaensis*, and *comt* from *A. thaliana*, while the second host expressed *4cl1* from *A. thaliana* as well as *dcs* and *curs1* from *C. longa*. In this manner, the number of heterologous genes in each strain was reduced from six to three, ultimately resulting in the formation of $15.9\ mg\ l^{-1}$ curcumin, the highest known titer of curcumin produced from L-tyrosine.

Increased membrane fluidity supports high-level production in *Escherichia coli* BW25113

Another study yielded a remarkable titer of $537\ mg\ l^{-1}$ and successfully expanded the panel of producers using *E. coli* BW25113 as a host [83••] (Figure 5). *E. coli* BW25113 is a derivative of *E. coli* K-12 that has become the parent strain for the single-gene deletion Keio collection and is extensively employed for systematic phenotypic surveys and synthetic biology efforts. This study utilized the rice-based route and ferulate as a biotransformation precursor. Another important finding of this work was that increasing membrane fluidity provided a beneficial effect on cell production cells, which reduced the toxic effects caused by the product.

Given its pronounced hydrophobic nature, curcumin may be anchored within the phospholipid bilayer, affecting its fluidity [84]. The exposure of *E. coli* to curcumin resulted in reduced membrane fluidity [83••]. Interestingly, this drawback could be partially reversed by pretreating the cells with unsaturated fatty acids, such as palmitoleate and oleate, which simultaneously promoted the production of curcumin [83••]. In contrast, adverse effects were observed when saturated fatty acids were added. The available knowledge was

Figure 5



System-level engineering of *E. coli* to produce curcumin. The comprehensive strategy used comprised (1) directed evolution and expression of the improved rice-based *cus* gene, (2) inactivation of *poxB*, *adhE*, *fabF*, and *curA*, (3) overexpression of acetyl-CoA-forming *acs*, (4) supplementation of unsaturated palmitoleate, and (5) enlargement of the membrane surface by overexpression of MGS from *A. laidlawii* [83••]. Abbreviations: ACET: acetate; Et-OH: ethanol; PYR: pyruvate; Ac-CoA: acetyl-CoA; Mal-CoA: malonyl-CoA; Mal-ACP: malonyl-ACP; CUS: curcuminoid synthase.

subsequently merged into a comprehensive strategy that involved the following methods: (1) performing directed evolution and expressing the improved rice-based *cus* gene; (2) inactivating pyruvate dehydrogenase (*poxB*), alcohol dehydrogenase (*adhE*), 3-oxoacyl-[acyl carrier protein] synthase 2 (*fabF*), and *curA* (curcumin reductase, which catalyzes the reduction of curcumin to dihydrocurcumin); (3) overexpressing acetyl-CoA synthase (*acs*), which results in the formation of acetyl-CoA; (4) adding unsaturated palmitoleate; and (5) enlarging the membrane surface and storage capacity of curcumin through overexpressing monoglucosyl-diacylglycerol synthase (MGS) from *Acholeplasma laidlawii* (Figure 5). After an incubation was performed with 4 mM ferulate as a biotransformation precursor in minimal medium, the titer of the metabolically engineered strain reached 537 mg l^{-1}. The accumulation of curcumin exerts toxic effects on the host, limiting overall production performance; this limitation is a relevant challenge in industrial bioproduction [85–87]. Here, membrane engineering may offer a valuable tool for overcoming storage bottlenecks.

Yeast and fungi fail as alternative hosts in the competitive production of curcumin

E. coli has become a prominent host for microbial curcumin production, similar to many other plant-based metabolites [88]. However, recent studies have aimed to implement alternative, GRAS microbes, which are attractive for addressing industrial markets in food, cosmetics, and medicine. Prominent candidates included *Aspergillus oryzae* [89] and *Saccharomyces cerevisiae* [90].

A metabolically engineered *A. oryzae* mutant that genomically expressed *cus* from rice but lacked the genes *snfA* and *SCAP* successfully formed curcumin when grown on MPY-based agar plates in the presence of feruloyl-*N*-acetylcysteamine, an analog of feruloyl-CoA [91•]. The strain accumulated 404.2 µg of curcumin per plate at a yield of 0.53 mol (mol feruloyl-*N*-acetylcysteamine)$^{-1}$. As no liquid cultures were performed, it was difficult to evaluate the achieved performance, but the general possibility of using fungi for production is interesting.

Additionally, heterologous synthesis of curcumin was recently established in baker's yeast through episomal expression of *dcs* and *curs1* from turmeric and *ferA*, which encodes feruloyl-CoA synthase enzyme (*ferA*), from *Pseudomonas paucimobilis* [61•]. Importantly, the native gene *fdc*, which encodes ferulate decarboxylase, was inactivated to prevent decarboxylation of ferulate to 4-vinylguaiacol. The mutant formed 3 mg l^{-1} of curcumin in a biotransformation setup from ferulate.

Conclusions

Recent efforts have led to microbial strains that produce the plant polyketide curcumin at the milligram scale through streamlined heterologous pathways. Enabled by progress in recent years, a promising era in microbial curcumin production is near. However, much process optimization is needed to reach a viable curcumin fermentation industry. Several challenges must be addressed to unlock the full potential of microbial curcumin production and harness the benefits of curcumin for industry, health, and the environment. To improve economic factors, microbial strains and fermentation processes must be further optimized to attain higher curcumin yields and greater purity while minimizing byproducts and waste. In addition, downstream processing of curcumin, which is conventionally extracted from total cell broth using polar solvents, might be of interest for optimization. These achievements may be crucial for attaining scalable curcumin production with attractive carbon footprints and resource consumption levels. The inhibitory effects of curcumin reported thus far vary greatly. For *E. coli*, the minimum inhibitory concentration (MIC) ranges between 160 and 2000 µg ml^{-1}, depending on the strain and test conditions used [12,102,103]. For yeast, the MIC can even differ by more than 100-fold [12,103]. At this point, forming a clear conclusion about the extent to which strain limitations have been reached is difficult. More work is needed to clarify this topic. Notably, scientific and technical developments should accompany regulatory and safety considerations to ensure the quality and safety of microbial curcumin for consumer use. Therefore, regions that rely heavily on curcumin production for their livelihoods should receive equitable access to microbial curcumin technology.

CRediT authorship contribution statement

S. Beganovic: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **C. Wittmann:** Conceptualization, Funding acquisition, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Data Availability

No data was used for the research described in the article.

Declaration of Competing Interest

Selma Beganovic and Christoph Wittmann declare that they have no competing interest.

Acknowledgements

The authors acknowledge financial support from the Leibniz ScienceCampus Living Therapeutic Materials (Saarbrücken, Germany).

Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.copbio.2024.103112.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Akaberi M, Sahebkar A, Emami SA: **Turmeric and curcumin: from traditional to modern medicine**. In *Studies on Biomarkers and New Targets in Aging Research in Iran: Focus on Turmeric and Curcumin*. Edited by Guest PC. Springer International Publishing; 2021:15-39.

2. Sharifi-Rad J, Rayess YE, Rizk AA, Sadaka C, Zgheib R, Zam W, Sestito S, Rapposelli S, Neffe-Skocińska K, Zielińska D, *et al*.: **Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications**. *Front Pharmacol* 2020, **11**:01021.

3. • El-Saadony MT, Yang T, Korma SA, Sitohy M, Abd El-Mageed TA, Selim S, Al Jaouni SK, Salem HM, Mahmmod Y, Soliman SM, *et al*.: **Impacts of turmeric and its principal bioactive curcumin on human health: pharmaceutical, medicinal, and food applications: a comprehensive review**. *Front Nutr* 2023, **9**:1040259.

Valuable, well-organized survey on curcumin properties and appplications.

4. Zare M, Norouzi Sarkati M, Tashakkorian H, Partovi R, Rahaiee S: **Dextran-immobilized curcumin: an efficient agent against food pathogens and cancer cells**. *J Bioact Compat Polym* 2019, **34**:309-320.

5. Roy S, Priyadarshi R, Ezati P, Rhim J-W: **Curcumin and its uses in active and smart food packaging applications — a comprehensive review**. *Food Chem* 2022, **375**:131885.

6. Chen H-z, Zhang M, Bhandari B, Yang C-h: **Novel pH-sensitive films containing curcumin and anthocyanins to monitor fish freshness**. *Food Hydrocoll* 2020, **100**:105438.

7. Cvek M, Paul UC, Zia J, Mancini G, Sedlarik V, Athanassiou A: **Biodegradable films of PLA/PPC and curcumin as packaging materials and smart indicators of food spoilage**. *ACS Appl Mater Interfaces* 2022, **14**:14654-14667.

8. Jakubczyk K, Drużga A, Katarzyna J, Skonieczna-Żydecka K: **Antioxidant potential of curcumin — a meta-analysis of randomized clinical trials**. *Antioxidants* 2020, **9**:1092.

9. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, Hu C, Xu R: **Anti-inflammatory effects of curcumin in the inflammatory diseases: status, limitations and countermeasures**. *Drug Des Dev Ther* 2021, **15**:4503-4525.

10. Tomeh MA, Hadianamrei R, Zhao X: **A review of curcumin and its derivatives as anticancer agents**. *Int J Mol Sci* 2019, **20**:1033.

11. Huang L, Chen C, Zhang X, Li X, Chen Z, Yang C, Liang X, Zhu G, Xu Z: **Neuroprotective effect of curcumin against cerebral ischemia-reperfusion via mediating autophagy and inflammation**. *J Mol Neurosci* 2018, **64**:129-139.

12. Adamczak A, Ożarowski M, Karpiński TM: **Curcumin, a natural antimicrobial agent with strain-specific activity**. *Pharmaceuticals* 2020, **13**:153.

13. Himesh S, Sharan PS, Mishra K, Govind N, Singhai AK: **Qualitative and quantitative profile of curcumin from ethanolic extract of *Curcuma longa***. *Int Res J Pharm* 2011, **2**:180-184.

14. Neves MIL, Strieder MM, Vardanega R, Silva EK, Meireles MAA: **Biorefinery of turmeric (*Curcuma longa* L.) using non-thermal and clean emerging technologies: an update on the curcumin recovery step**. *RSC Adv* 2020, **10**:112-121.

15. Harish BS, Umesha K, Venugopalan R, Maruthi Prasad BN: **Photo-selective nets influence physiology, growth, yield and quality of turmeric (*Curcuma longa* L.)**. *Ind Crops Prod* 2022, **186**:115202.

16. Sellaperumal C, Eapen SJ, Manimaran B, Berliner J, Senthamizh SB, Mahendar B, Giridhar K, Sivakumar V: **Plant-parasitic nematodes, a looming threat to turmeric cultivation in India: results of recent surveys**. *Nematology* 2023, **1**:1-9.

17. Chenniappan C, Daniel GM, Ponnusamy P, Narayanasamy M: **Association of multiple fungal pathogens to rhizome rot disease of turmeric**. *Arch Phytopathol Plant Prot* 2020, **53**:440-458.

18. Theerawitaya C, Kyaw MMM, Samphumphuang T, Pinruan U, Wiriyathanawudhiwong N, Cha-um S: **Shoot meristem culture eliminates bacterial and fungal infections from elite varieties of turmeric (*Curcuma longa* L.)**. *Vitr Cell Dev Biol Plant* 2022, **58**:146-154.

19. Mangang IB, Minj R, Manickam L, Rangarajan J, Shanmugasundaram S: **Turmeric — a super food — prevention of post-harvest losses using radio frequency disinfestation**. *Arch Phytopathol Plant Prot* 2022, **55**:1450-1463.

20. Yixuan L, Qaria MA, Sivasamy S, Jianzhong S, Daochen Z: **Curcumin production and bioavailability: a comprehensive review of curcumin extraction, synthesis, biotransformation and delivery systems**. *Ind Crops Prod* 2021, **172**:114050.

21. Becker J, Wittmann C: **Advanced biotechnology: metabolically engineered cells for the bio-based production of chemicals and fuels, materials, and health-care products**. *Angew Chem* 2015, **54**:3328-3350.

22. Becker J, Wittmann C: **Systems metabolic engineering of *Escherichia coli* for the heterologous production of high value molecules — a veteran at new shores**. *Curr Opin Biotechnol* 2016, **42**:178-188.

23. Becker J, Wittmann C: **From systems biology to metabolically engineered cells — an omics perspective on the development of industrial microbes**. *Curr Opin Microbiol* 2018, **45**:180-188.

24. Panzarini E, Mariano S, Tacconi S, Carata E, Tata AM, Dini L: **Novel therapeutic delivery of nanocurcumin in central nervous system related disorders**. *Nanomaterials* 2021, **11**:2.

25. Novais C, Molina AK, Abreu RMV, Santo-Buelga C, Ferreira ICFR, Pereira C, Barros L: **Natural food colorants and preservatives: a review, a demand, and a challenge**. *J Agric Food Chem* 2022, **70**:2789-2805.

26. Serpa Guerra AM, Gómez Hoyos C, Velásquez-Cock JA, Vélez Acosta L, Gañán Rojo P, Velásquez Giraldo AM, Zuluaga Gallego R: **The nanotech potential of turmeric (*Curcuma longa* L.) in food technology: a review**. *Crit Rev Food Sci Nutr* 2020, **60**:1842-1854.

27. Etxabide A, Kilmartin PA, Maté JI: **Color stability and pH-indicator ability of curcumin, anthocyanin and betanin containing colorants under different storage conditions for intelligent packaging development**. *Food Control* 2021, **121**:107645.

28. Oliveira Filho JGd, Bertolo MRV, Rodrigues MÁV, Marangon CA, Silva GdC, Odoni FCA, Egea MB: **Curcumin: a multifunctional molecule for the development of smart and active biodegradable polymer-based films**. *Trends Food Sci Technol* 2021, **118**:840-849.

29. Seidi Damyeh M, Mereddy R, Netzel ME, Sultanbawa Y: **An insight into curcumin-based photosensitization as a promising and green food preservation technology**. *Compr Rev Food Sci Food Saf* 2020, **19**:1727-1759.

30. Yu X, Zou Y, Zhang Z, Wei T, Ye Z, Yuk H-G, Zheng Q: **Recent advances in antimicrobial applications of curcumin-mediated photodynamic inactivation in foods**. *Food Control* 2022, **138**:108986.

31. Munir Z, Banche G, Cavallo L, Mandras N, Roana J, Pertusio R, Ficiarà E, Cavalli R, Guiot C: **Exploitation of the antibacterial properties of photoactivated curcumin as 'Green' tool for food preservation**. *Int J Mol Sci* 2022, **23**:2600.

32. Aurum FS, Nguyen LT: **Efficacy of photoactivated curcumin to decontaminate food surfaces under blue light emitting diode**. *J Food Process Eng* 2019, **42**:e12988.

33. Ma T, Chen Y, Zhi X, Du B: **Cellulose laurate films containing curcumin as photoinduced antibacterial agent for meat preservation**. *Int J Biol Macromol* 2021, **193**:1986-1995.

34. Gao J, Matthews KR: **Effects of the photosensitizer curcumin in inactivating foodborne pathogens on chicken skin**. *Food Control* 2020, **109**:106959.

35. de Oliveira EF, Tosati JV, Tikekar RV, Monteiro AR, Nitin N: **Antimicrobial activity of curcumin in combination with light against *Escherichia coli* O157:H7 and *Listeria innocua*: applications for fresh produce sanitation**. *Postharvest Biol Technol* 2018, **137**:86-94.

36. Chen L, Song Z, Zhi X, Du B: **Photoinduced antimicrobial activity of curcumin-containing coatings: molecular interaction, stability and potential application in food decontamination**. *ACS Omega* 2020, **5**:31044-31054.

37. Temba BA, Fletcher MT, Fox GP, Harvey J, Okoth SA, Sultanbawa Y: **Curcumin-based photosensitization inactivates *Aspergillus flavus* and reduces aflatoxin B1 in maize kernels**. *Food Microbiol* 2019, **82**:82-88.

38. Song L, Zhang F, Yu J, Wei C, Han Q, Meng X: **Antifungal effect and possible mechanism of curcumin mediated photodynamic technology against *Penicillium expansum***. *Postharvest Biol Technol* 2020, **167**:111234.

39. Wei C, Zhang F, Song L, Chen X, Meng X: **Photosensitization effect of curcumin for controlling plant pathogen *Botrytis cinerea* in postharvest apple**. *Food Control* 2021, **123**:107683.

40. Tao R, Zhang F, Tang Q-j, Xu C-s, Ni Z-J, Meng X-h: **Effects of curcumin-based photodynamic treatment on the storage quality of fresh-cut apples**. *Food Chem* 2019, **274**:415-421.

41. Bhavya ML, Shewale SR, Rajoriya D, Hebbar HU: **Impact of blue LED illumination and natural photosensitizer on bacterial pathogens, enzyme activity and quality attributes of fresh-cut pineapple slices**. *Food Bioprocess Technol* 2021, **14**:362-372.

42. Mansouri K, Rasoulpoor S, Daneshkhah A, Abolfathi S, Salari N, Mohammadi M, Rasoulpoor S, Shabani S: **Clinical effects of curcumin in enhancing cancer therapy: a systematic review**. *BMC Cancer* 2020, **20**:791.

43. Abadi AJ, Mirzaei S, Mahabady MK, Hashemi F, Zabolian A, Raee P, Aghamiri S, Ashrafizadeh M, Aref AR, Hamblin MR, *et al.*: **Curcumin and its derivatives in cancer therapy: potentiating antitumor activity of cisplatin and reducing side effects**. *Phytother Res PTR* 2022, **36**:189-213.

44. Fadus MC, Lau C, Bikhchandani J, Lynch HT: **Curcumin: an age-old anti-inflammatory and anti-neoplastic agent**. *J Tradit Complement Med* 2017, **7**:339-346.

45. Trigo-Gutierrez JK, Vega-Chacón Y, Soares AB, Mima EG: **Antimicrobial activity of curcumin in nanoformulations: a comprehensive review**. *Int J Mol Sci* 2021, **22**:7130.

46. Hussain Z, Thu HE, Amjad MW, Hussain F, Ahmed TA, Khan S: **Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: a review of new trends and future perspectives**. *Mater Sci Eng C* 2017, **77**:1316-1326.

47. Alonso-Español A, Bravo E, Ribeiro-Vidal H, Virto L, Herrera D, Alonso B, Sanz M: **The antimicrobial activity of curcumin and xanthohumol on bacterial biofilms developed over dental implant surfaces**. *Int J Mol Sci* 2023, **24**:2335.

48. Akolade JO, Oloyede HOB, Onyenekwe PC: **Encapsulation in chitosan-based polyelectrolyte complexes enhances antidiabetic activity of curcumin**. *J Funct Foods* 2017, **35**:584-594.

49. Mohammadi E, Behnam B, Mohammadinejad R, Guest PC, Simental-Mendía LE, Sahebkar A: **Antidiabetic Properties of curcumin: insights on new mechanisms**. In *Studies on Biomarkers and New Targets in Aging Research in Iran: Focus on Turmeric and Curcumin*. Edited by Guest PC. Springer International Publishing; 2021:151-164.

50. Teter B, Morihara T, Lim GP, Chu T, Jones MR, Zuo X, Paul RM, Frautschy SA, Cole GM: **Curcumin restores innate immune Alzheimer's disease risk gene expression to ameliorate Alzheimer pathogenesis**. *Neurobiol Dis* 2019, **127**:432-448.

51. Zhou X, Venigalla M, Raju R, Münch G: **Pharmacological considerations for treating neuroinflammation with curcumin in Alzheimer's disease**. *J Neural Transm* 2022, **129**:755-771.

52. Jin T, Zhang Y, Botchway BOA, Zhang J, Fan R, Zhang Y, Liu X: **Curcumin can improve Parkinson's disease via activating BDNF/PI3k/Akt signaling pathways**. *Food Chem Toxicol* 2022, **164**:113091.

53. Donadio V, Incensi A, Rizzo G, Fileccia E, Ventruto F, Riva A, Tiso D, Recchia M, Vacchiano V, Infante R: **The effect of curcumin on idiopathic Parkinson disease: a clinical and skin biopsy study**. *J Neuropathol Exp Neurol* 2022, **81**:545-552.

54. Barchitta M, Maugeri A, Favara G, Magnano San Lio R, Evola G, Agodi A, Basile G: **Nutrition and wound healing: an overview focusing on the beneficial effects of curcumin**. *Int J Mol Sci* 2019, **20**:1119.

55. Kumari A, Raina N, Wahi A, Goh KW, Sharma P, Nagpal R, Jain A, Ming LC, Gupta M: **Wound-healing effects of curcumin and its nanoformulations: a comprehensive review**. *Pharmaceutics* 2022, **14**:2288.

56. Kahkhaie KR, Mirhosseini A, Aliabadi A, Mohammadi A, Mousavi MJ, Haftcheshmeh SM, Sathyapalan T, Sahebkar A: **Curcumin: a modulator of inflammatory signaling pathways in the immune system**. *Inflammopharmacology* 2019, **27**:885-900.

57. Makuch S, Więcek K, Woźniak M: **The immunomodulatory and anti-inflammatory effect of curcumin on immune cell populations, cytokines, and in vivo models of rheumatoid arthritis**. *Pharmaceuticals* 2021, **14**:309.

58. Choi YH, Han DH, Kim S-w, Kim M-J, Sung HH, Jeon HG, Jeong BC, Seo SI, Jeon SS, Lee HM, *et al.*: **A randomized, double-blind, placebo-controlled trial to evaluate the role of curcumin in prostate cancer patients with intermittent androgen deprivation**. *Prostate* 2019, **79**:614-621.

59. Lopresti AL, Smith SJ, Jackson-Michel S, Fairchild T: **An investigation into the effects of a curcumin extract (Curcugen®) on osteoarthritis pain of the knee: a randomised, double-blind, placebo-controlled study**. *Nutrients* 2022, **14**:41.

60. Thota RN, Rosato JI, Dias CB, Burrows TL, Martins RN, Garg ML: **Dietary supplementation with curcumin reduce circulating levels of glycogen synthase kinase-3β and islet amyloid polypeptide in adults with high risk of type 2 diabetes and Alzheimer's disease**. *Nutrients* 2020, **12**.

61. • Rainha J, Rodrigues JL, Faria C, Rodrigues LR: **Curcumin biosynthesis from ferulic acid by engineered *Saccharomyces cerevisiae***. *Biotechnol J* 2022, **17**:e2100400.
First demonstration of curcumin production in yeast.

62. Dong NQ, Lin HX: **Contribution of phenylpropanoid metabolism to plant development and plant–environment interactions**. *J Integr Plant Biol* 2021, **63**:180-209.

63. Becker J, Wittmann C: **A field of dreams: lignin valorization into chemicals, materials, fuels, and health-care products**. *Biotechnol Adv* 2019, **37**:107360.

64. Weiland F, Kohlstedt M, Wittmann C: **Guiding stars to the field of dreams: metabolically engineered pathways and microbial platforms for a sustainable lignin-based industry**. *Metab Eng* 2022, **71**:13-41.

65. Yao T, Feng K, Xie M, Barros J, Tschaplinski TJ, Tuskan GA, Muchero W, Chen J-G: **Phylogenetic occurrence of the phenylpropanoid pathway and lignin biosynthesis in plants**. *Front Plant Sci* 2021, **12**:704697.

66. Katsuyama Y, Matsuzawa M, Funa N, Horinouchi S: **In vitro synthesis of curcuminoids by type III polyketide synthase from *Oryza sativa***. *J Biol Chem* 2007, **282**:37702-37709.

67. Katsuyama Y, Matsuzawa M, Funa N, Horinouchi S: **Production of curcuminoids by *Escherichia coli* carrying an artificial biosynthesis pathway**. *Microbiology* 2008, **154**:2620-2628.

68. Katsuyama Y, Kita T, Funa N, Horinouchi S: **Curcuminoid biosynthesis by two type III polyketide synthases in the herb *Curcuma longa***. *J Biol Chem* 2009, **284**:11160-11170.

69. Katsuyama Y, Kita T, Horinouchi S: **Identification and characterization of multiple curcumin synthases from the herb *Curcuma longa***. *FEBS Lett* 2009, **583**:2799-2803.

70. Rodrigues JL, Prather KL, Kluskens LD, Rodrigues LR: **Heterologous production of curcuminoids**. *Microbiol Mol Biol Rev MMBR* 2015, **79**:39-60.

71. •• Rodrigues JL, Gomes D, Rodrigues LR: **A combinatorial approach to optimize the production of curcuminoids from tyrosine in *Escherichia coli***. *Front Bioeng Biotechnol* 2020, **8**:59.
Interesting study that addresses the issue of pathway balancing through a novel co-culture concept.

72. Rainha J, Rodrigues LR, Rodrigues JL: **Microbial production of curcumin**. Microbial Production of Food Bioactive Compounds. Springer; 2022:1-35.

73. Zhang L, Gao B, Wang X, Zhang Z, Liu X, Wang J, Mo T, Liu Y, Shi S, Tu P: **Identification of a new curcumin synthase from ginger and construction of a curcuminoid-producing unnatural fusion protein diketide-CoA synthase: curcumin synthase**. *RSC Adv* 2016, **6**:12519-12524.

74. Barros J, Dixon RA: **Plant phenylalanine/tyrosine ammonia-lyases**. *Trends Plant Sci* 2020, **25**:66-79.

75. Rodrigues JL, Araujo RG, Prather KL, Kluskens LD, Rodrigues LR: **Production of curcuminoids from tyrosine by a metabolically engineered *Escherichia coli* using caffeic acid as an intermediate**. *Biotechnol J* 2015, **10**:599-609.

76. Couto MR, Rodrigues JL, Rodrigues LR: **Optimization of fermentation conditions for the production of curcumin by engineered *Escherichia coli***. *J R Soc Interface* 2017, **14**:20170470.

77. Jeong H, Barbe V, Lee CH, Vallenet D, Yu DS, Choi SH, Couloux A, Lee SW, Yoon SH, Cattolico L, *et al.*: **Genome sequences of Escherichia coli B strains REL606 and BL21(DE3)**. *J Mol Biol* 2009, **394**:644-652.

78. Rosano GL, Ceccarelli EA: **Recombinant protein expression in *Escherichia coli*: advances and challenges**. *Front Microbiol* 2014, **5**:172.

79. Yoon SH, Han MJ, Jeong H, Lee CH, Xia XX, Lee DH, Shim JH, Lee SY, Oh TK, Kim JF: **Comparative multi-omics systems analysis of *Escherichia coli* strains B and K-12**. *Genome Biol* 2012, **13**:R37.

80. Phue J-N, Noronha SB, Hattacharyya R, Wolfe AJ, Shiloach J: **Glucose metabolism at high density growth of *E. coli* B and *E. coli* K: differences in metabolic pathways are responsible for efficient glucose utilization in *E. coli* B as determined by microarrays and Northern blot analyses**. *Biotechnol Bioeng* 2005, **90**:805-820.

81. •• Kang S-Y, Heo KT, Hong Y-S: **Optimization of artificial curcumin biosynthesis in *E. coli* by randomized 5′-UTR sequences to control the multienzyme pathway**. *ACS Synth Biol* 2018, **7**:2054-2062.
Demonstration of *de novo* curcumin biosynthesis from glucose, elegantly solving the problem of approprate balancing of the six-enzyme biosnythetic pathway.

82. Giesselmann G, Dietrich D, Jungmann L, Kohlstedt M, Jeon EJ, Yim SS, Sommer F, Zimmer D, Muhlhaus T, Schroda M, *et al.*: **Metabolic engineering of *Corynebacterium glutamicum* for high-level ectoine production: design, combinatorial assembly, and implementation of a transcriptionally balanced heterologous ectoine pathway**. *Biotechnol J* 2019, **14**:e1800417.

83. •• Wu J, Chen W, Zhang Y, Zhang X, Jin J-M, Tang S-Y: **Metabolic engineering for improved curcumin biosynthesis in *Escherichia coli***. *J Agric Food Chem* 2020, **68**:10772-10779.
Comprehensive approach that integrates a high-efficiency cell factory with a detoxifed production environment through improved membrane fluidity.

84. Sun Y, Lee CC, Hung WC, Chen FY, Lee MT, Huang HW: **The bound states of amphipathic drugs in lipid bilayers: study of curcumin**. *Biophys J* 2008, **95**:2318-2324.

85. Kohlstedt M, Sappa PK, Meyer H, Maass S, Zaprasis A, Hoffmann T, Becker J, Steil L, Hecker M, van Dijl JM, *et al.*: **Adaptation of *Bacillus subtilis* carbon core metabolism to simultaneous nutrient limitation and osmotic challenge: a multi-omics perspective**. *Environ Microbiol* 2014, **16**:1898-1917.

86. Kohlstedt M, Weimer A, Weiland F, Stolzenberger J, Selzer M, Sanz M, Kramps L, Wittmann C: **Biobased PET from lignin using an engineered cis, cis-muconate-producing *Pseudomonas putida* strain with superior robustness, energy and redox properties**. *Metab Eng* 2022, **72**:337-352.

87. Weiland F, Barton N, Kohlstedt M, Becker J, Wittmann C: **Systems metabolic engineering upgrades *Corynebacterium glutamicum* to high-efficiency cis, cis-muconic acid production from lignin-based aromatics**. *Metab Eng* 2023, **75**:153-169.

88. Becker J, Wittmann C: **Systems metabolic engineering of *Escherichia coli* for the heterologous production of high value molecules — a veteran at new shores**. *Curr Opin Biotechnol* 2016, **42**:178-188.

89. Rousta N, Hellwig C, Wainaina S, Lukitawesa L, Agnihotri S, Rousta K, Taherzadeh MJ: **Filamentous fungus *Aspergillus oryzae* for food: from submerged cultivation to fungal burgers and their sensory evaluation — a pilot study**. *Foods* 2021, **10**:2774.

90. Yuan H, Zhang W, Xiao G, Zhan J: **Efficient production of gamma-aminobutyric acid by engineered *Saccharomyces cerevisiae* with glutamate decarboxylases from Streptomyces**. *Biotechnol Appl Biochem* 2020, **67**:240-248.

91. •• Kan E, Katsuyama Y, Maruyama J-i, Tamano K, Koyama Y, Ohnishi Y: **Production of the plant polyketide curcumin in *Aspergillus oryzae*: strengthening malonyl-CoA supply for yield improvement**. *Biosci Biotechnol Biochem* 2019, **83**:1372-1381.
Demonstration of curcumin production in fungi while providing the first genome-based pathway layout.

92. Manasa PSL, Kamble AD, Chilakamarthi U: **Various extraction techniques of curcumin — a comprehensive review**. *ACS Omega* 2023, **8**:34868-34878.

93. Jiang T, Ghosh R, Charcosset C: **Extraction, purification and applications of curcumin from plant materials — a comprehensive review**. *Trends Food Sci Technol* 2021, **112**:419-430.

94. Binello A, Grillo G, Barge A, Allegrini P, Ciceri D, Cravotto G: **A cross-flow ultrasound-assisted extraction of curcuminoids from *Curcuma longa* L.: process design to avoid degradation**. *Foods* 2020, **9**:743.

95. Fernández-Marín R, Fernandes SCM, Andrés MA, Labidi J: **Microwave-assisted extraction of *Curcuma longa* l. Oil: optimization, chemical structure and composition, antioxidant activity and comparison with conventional soxhlet extraction**. *Molecules* 2021, **26**:1516.

96. Sahne F, Mohammadi M, Najafpour GD, Moghadamnia AA: **Enzyme-assisted ionic liquid extraction of bioactive compound from turmeric (*Curcuma longa* L.): isolation, purification and analysis of curcumin**. *Ind Crops Prod* 2017, **95**:686-694.

97. Nagavekar N, Singhal RS: **Supercritical fluid extraction of *Curcuma longa* and *Curcuma amada* oleoresin: optimization of extraction conditions, extract profiling, and comparison of bioactivities**. *Ind Crops Prod* 2019, **134**:134-145.

98. Chao I-C, Wang C-M, Li S-P, Lin L-G, Ye W-C, Zhang Q-W: **Simultaneous quantification of three curcuminoids and three volatile components of *Curcuma longa* using pressurized liquid extraction and high-performance liquid chromatography**. *Molecules* 2018, **23**:1568.

99. Gökdemir B, Baylan N, Çehreli S: **Application of a novel ionic liquid as an alternative green solvent for the extraction of curcumin from turmeric with response surface methodology: determination and optimization study**. *Anal Lett* 2020, **53**:2111-2121.

100. Kotha RR, Luthria DL: **Curcumin: biological, pharmaceutical, nutraceutical, and analytical aspects**. *Molecules* 2019, **24**:2930.

101. Miyazono K-i, Um J, Imai FL, Katsuyama Y, Ohnishi Y, Horinouchi S, Tanokura M: **Crystal structure of curcuminoid synthase CUS from *Oryza sativa***. *Protein Struct Funct Bioinforma* 2011, **79**:669-673.

102. Gunes H, Gulen D, Mutlu R, Gumus A, Tas T, Topkaya AE: **Antibacterial effects of curcumin: an in vitro minimum inhibitory concentration study**. *Toxicol Ind Health* 2013, **32**:246-250.

103. Wang Y, Lu Z, Wu H, Lv F: **Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens**. *Int J Food Microbiol* 2009, **136**:71-74.