Microbial Production of Curcumin

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Abstract

Curcumin, a polyphenol produced by turmeric (*Curcuma longa*), has attracted increased attention due to its potential as a novel cancer-fighting drug. However, to satisfy the required curcumin demand for health-related studies, high purity curcumin preparations are required, which are difficult to obtain and are very expensive. Curcumin and other curcuminoids are usually obtained through plant extraction. However, these polyphenols accumulate in low amounts over long

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periods in the plant and their extraction process is costly and not environmentally friendly. In addition, curcumin chemical synthesis is complex. All these reasons limit the advances in studies related to the in vitro and in vivo curcumin biological activities. The microbial production of curcumin appears as a solution to overcome the limitations associated with the currently used methods. Curcumin biosynthesis begins with the conversion of the aromatic amino acids, phenylalanine and tyrosine, into phenylpropanoids, the curcuminoid precursors. The phenylpropanoids are then activated through condensation with a CoA molecule. Afterwards, curcuminoids are synthesized by the action of type III polyketide synthases (PKS) that combine two activated phenylpropanoids and a malonyl-CoA molecule. To engineer microbes to produce curcumin, the curcuminoid biosynthetic genes must be introduced as microorganisms lack the enzymatic reactions responsible to synthesize curcuminoids. In this chapter, the advances regarding the microbial production of curcumin are exposed. The heterologous production of curcumin has been mainly achieved in the bacteria *Escherichia coli*. However, other microorganisms have already been explored. Besides the introduction of curcumin biosynthetic genes, the optimization of the microbial chassis must also be considered to maximize the production yields. The strategies employed for this purpose are also herein presented. The maximum titer of curcumin produced by a genetically engineered *E. coli* was 563.4 mg/L with a substrate conversion yield of 100% from supplemented ferulic acid. Moreover, the de novo production of curcumin was accomplished in *E. coli* reaching 3.8 mg/L of curcumin. Overall, the recent developments on curcumin heterologous production are very encouraging.

Keywords

Curcumin biosynthesis · Heterologous production · Synthetic biology · Genetic engineering · *E. coli* · Biosynthetic pathways

Introduction

The polyphenol curcumin produced in turmeric rhizomes has been extensively explored as a natural therapeutic agent. Several studies have demonstrated curcumin biological activities including its promising anticarcinogenic activity. Hereupon, new methodologies for curcumin production have been explored in the last decades. The development of microbial cell factories through genetic engineering of microorganisms is a common alternative for high-level production of scarce natural compounds. The scope of this chapter is to give an overview of the current developments regarding the microbial production of curcumin. Firstly, the curcumin chemistry, value, and extraction will be reviewed followed by its biological activities, relevance, and safety. Next, the curcumin biosynthetic pathway in the plant will be elucidated. Finally, the studies regarding curcumin microbial production will be explored offering a complete description of the works available on the field.

Curcumin

Curcuminoids are polyphenolic compounds found in turmeric (*Curcuma longa*) rhizomes and represent the main active compounds of the plant, approximately 3% of its total chemical composition (Niranjan and Prakash 2008). Since antiquity, rhizomes of this perennial herb have been used in culinary as spice and as natural yellow coloring agent for textiles and cosmetics. In addition, the curcuminoid-rich rhizome extract has been used in Asian traditional medicine, for example, for wound healing or in the treatment of respiratory or digestive disorders. Moreover, it is known as a powerful antioxidant being also used as a preservative (Hewlings and Kalman 2017). There are three major types of curcuminoids found in *C. longa* rhizomes: curcumin (~77%), demethoxycurcumin (~17%), and bisdemethoxycurcumin (~3%) (Amalraj et al. 2017). The term curcumin is commonly used to designate a turmeric extract containing a curcuminoid mixture.

The Chemistry

Curcuminoids consist in two phenolic groups connected by two α,β-unsaturated carbonyl groups, and these compounds are chemically classified as diarylheptanoids (Fig. 1). The chemical structures of the three curcuminoids differ in the number of methoxy groups at ortho-positions of the phenol rings (Priyadarsini 2014). Curcumin has two methoxy groups while demethoxycurcumin has one and bisdemethoxycurcumin none. The molecular formula of the main curcuminoid, curcumin, is $C_{21}H_{20}O_6$, and it has a molecular weight of 368.37 g/mol. Crystallized curcumin [IUPAC name (1E, 6E)-1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadien-3,5-dione; CAS number: 458-37-7] is an orange-yellow crystalline powder water insoluble. Curcumin solubility increases with pH; however, it quickly degrades in alkaline solution. Curcumin is soluble in organic solvents such as methanol, ethanol, or acetone and has two tautomeric forms, −keto and -enol. The enol tautomer is exclusively observed in alkaline conditions, while the keto form is predominant both at acid and neutral pH. Curcumin has three labile protons (at neutral pH), one enolic and the others phenolic corresponding to pKa1, pKa2, and pKa3 values of 7.8, 8.5, and 9.0, respectively. Additionally, curcumin holds an

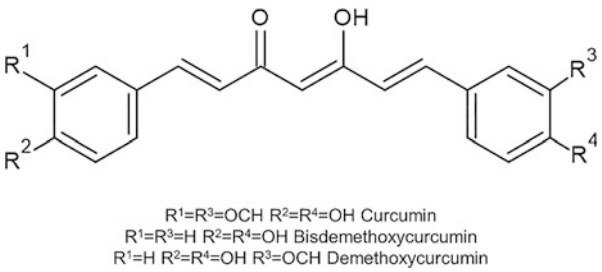

Fig. 1 Structures of curcumin, bisdemethoxycurcumin, and demethoxycurcumin

excellent metal chelating capacity due to its central unsaturated diketone portion (Priyadarsini 2014).

Curcumin Value and Market

Curcumin was first isolated in 1815, but it was only after more than a hundred years that the first study of its effects against human disease was published (in 1937). Later, in 1949, its antibacterial activity was reported (Gupta et al. 2012). In the last decades, several studies have reported and confirmed curcumin biological activities. Curcumin has over 18,000 citations in PUBMED and over 200 clinical trials ongoing or closed (clinicaltrials.gov). Hereupon, curcumin appears as one of the most studied natural therapeutic products. The reported effects for human health include anti-inflammatory, antioxidant, anticancer, wound healing properties, antiviral, among others (Hewlings and Kalman 2017). In addition, other reports showed that curcumin is able to attenuate debilitating effects of conventional therapeutics due to its hemoprotective, chemosensitive, radioprotective, and radiosensitive properties (Goel and Aggarwal 2010). Due to all these potential therapeutic effects, curcumin market size reached 58.2 million dollars in 2020 and is expected to reach 191.9 million dollars in 2028 (GrandViewResearch 2021). Currently, there are several curcumin formulations available in the market, namely Theracurmin® (Integrative Therapeutics) (Kanai et al. 2013), a highly bioavailable form of curcumin produced by surface-controlled colloidal dispersion; Meriva® (Thorne Research) (Belcaro et al. 2010), curcumin encapsulated with hydrophilic carriers; or C3 complex® (Sabinsa Corporation), that combines curcumin with bioperine (Ranjan et al. 2012). All these formulations contain a mixture of the three curcuminoids extracted from the plants and increase their bioavailability that is usually very low as described in the following sections.

Curcumin Extraction, Purification, and Chemical Synthesis

The curcuminoid content of *C. longa* rhizomes varies depending on factors such as the geographical origin, the soil condition, or the weather (Poudel et al. 2019). Conventional curcumin extraction from plants and purification involves a solvent extraction followed by purification with column chromatography. The organic solvents employed include methanol, ethyl acetate, ethanol, hexane, or acetone (Priyadarsini 2014). However, curcuminoids are susceptible to degradation via heat or light and their extraction must rely on methods that can be easily controlled (Zielińska et al. 2020). Recently, more innovative methods have been employed for curcuminoid extraction. For example, carbon dioxide as supercritical antisolvent (Nagavekar and Singhal 2019), microextraction based on eutectic solvents (Aydin et al. 2018), microwave-assisted extraction (Mandal et al. 2008), and supramolecular solvent-based extraction (Menghwar et al. 2018). Some reports also concluded that the use of an enzyme-assisted pretreatment of turmeric before the extraction

increased the curcumin yields (Sahne et al. 2017). However, this strategy makes the process not profitable due to the high costs associated with purified enzymes. Pure curcumin can also be obtained from a curcuminoid mixture through column chromatography. The mixture is usually adsorbed in a silica gel using mixtures of solvents, for instance, dichloromethane/acetic acid or methanol/chloroform, and three fractions containing the individual curcuminoids are obtained (Gupta et al. 1999; Revathy et al. 2011). However, the extraction of curcumin from turmeric rhizomes exhibits several drawbacks. First, the curcumin production is limited by the plant seasonality. Secondly, the geographical location can affect the curcuminoid content of the rhizome extracts, as previously stated (Poudel et al. 2019). In addition, plant crops require a lot of resources to be maintained. Moreover, as secondary metabolite, curcumin accumulates in low amounts making its extraction and purification difficult and expensive (Priyadarsini 2014). Overall, the production of curcumin by means of farming is unprofitable and consequently not appealing.

Pure curcumin can also be obtained by chemical synthesis. The synthesis method involves several steps and reagents and starts with acetylacetone and boron trioxide (B_2O_3). Chemical synthesis could be an alternative to overcome the limitations related to plant extraction, but it relies on the use of harsh and expensive chemicals, extreme conditions, and it is easily contaminated by undesired by-products (Priyadarsini 2014). To reduce the associated costs, the replacement of B_2O_3 by the less costly borontrifluoride has been proposed (Venkata Rao and Sudheer 2011). However, the large-scale chemical synthesis of curcumin remains a challenge due to its complexity (Yixuan et al. 2021). Consequently, more simple, efficient, and environmentally friendly methods are necessary.

Curcumin Biological Activities

The Anticancer Properties

The anticancer activity is one of the most reported biological effects on curcumin. The anticancer effects of this compound are diverse, targeting various levels of regulation during the various stages of carcinogenesis from DNA mutation to the metastasis and apoptosis (Tomeh et al. 2019). The vast elucidation of curcumin anticarcinogenic effects has already led to studies aiming to synthetically modify the functional groups of the curcumin molecule (aromatic side chains, di-keto moiety, methylene site, and carbon linker chain) in order to find structural analogues with relatively stronger pharmacological efficacy for cancer treatment (Rodrigues et al. 2021).

The action mechanism of curcumin is still a matter of study since its targets are diverse. A vast number of works showed that curcumin can effectively modulate several molecular targets in various cancer-associated signaling cascades and pathways (Kunnumakkara et al. 2017). The anticancer effects include the down-regulation of transcription factors of proliferative pathways such as the nuclear factor kappa B (NF-κB) (highly activated in carcinogenic cells). The NF-κB

inactivation downregulates the expression of carcinogenic-related genes including cyclin D1, cyclooxygenase-2 (COX-2), and B-cell lymphoma 2 (Bcl-2) (Marín et al. 2007; Marquardt et al. 2015; Shanmugam et al. 2015; Singh and Aggarwal 1995). Curcumin also showed to inhibit signal transducer and activator of transcription 3 (STAT3) phosphorylation that regulates the expression of several genes involved in cell proliferation and migration (Hahn et al. 2018). Moreover, curcumin decreases the expression levels of the downstream effectors in signaling pathways such as Wnt/β-catenin at the mRNA level (Yen et al. 2019). Curcumin can modulate multiple pathways that eventually may lead to apoptosis induction (Patiño-Morales et al. 2020; Talib et al. 2018) through the suppression of p53 pathway (Amin et al. 2015). Curcumin has also been reported as a potential hypomethylation agent preventing the DNA methylation and thus tumor formation (Hassan et al. 2019).

The Antioxidant and Anti-Inflammatory Effects

Curcumin chemically possesses antioxidant properties due to its two Michael acceptors and two phenolic hydroxyl group (Priyadarsini 2014). Hereupon, curcumin is a potent direct antioxidant being capable of scavenging hazardous oxidants rapidly. Furthermore, curcumin may also induce cytoprotective enzymes to ensure long-term physiological protection against the oxidative stress acting as an indirect antioxidant (Lin et al. 2019). This compound showed capacity to increase the levels of some oxide protective enzymes, for instance, heme oxygenase-1, superoxide dismutase, or glutathione peroxidase (Guo et al. 2011).

The inflammation process is associated with several human illnesses including neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, or multiple sclerosis, cardiovascular diseases, respiratory diseases (asthma and bronchitis), or arthritis, among others (Amor et al. 2014). The inflammatory response is strictly correlated with oxidative stress since inflamed cells liberate reactive oxygen species (ROS) leading to oxidative stress (Chapple 1997). On the other hand, ROS can activate intracellular signaling cascades that increase the expression of pro-inflammatory genes (Biswas 2016). The principal mediator of inflammation is the tumor necrosis factor α (TNF-α) (Parameswaran and Patial 2010). Curcumin can inhibit the production of TNF-α, as well as the production of other inflammatory cytokines such as interleukin family. In addition, curcumin is capable of downregulating COX-2, lipoxygenase, and inducible nitric oxide synthase, all enzymes involved in inflammatory response. The downregulation of these enzymes is accomplished via suppression of the NF-κB activation (Hewlings and Kalman 2017).

The Antibacterial, Antifungal, and Antiviral Properties

The curcumin antibacterial activity has already been demonstrated against several pathogenic bacteria (Zorofchian Moghadamtousi et al. 2014). The action mechanism acts through blocking the prokaryotic cell division (Kaur et al. 2010). Nowadays,

due to the emergence of multiresistant bacteria, there is intensive research focused on the discovery of novel antibacterial agents. The antimicrobial effect of curcumin has already been studied in combination with antibiotics such as ampicillin, oxacillin, and norfloxacin. The administrated cocktails led to a synergistic effect with increased antibacterial activity (Mun et al. 2013). The development of curcumin cocktails may be very advantageous because it may result in a wider spectrum of activity and reduce side effects compared to the administration of conventional antibiotics.

Curcumin also demonstrated antifungal properties through the reduction of ergosterol synthesis and consequent ergosterol precursor accumulation which leads to cell death via generation of ROS (Sharma et al. 2010). The appearance of multiresistant *Candida* species has become a problem mainly in hospital environments. Curcumin was found to be a potent fungicide against several *Candida* species (Phuna et al. 2020). Moreover, the synergistic effect with existing fungicides also resulted in a relative significant increase of antifungal activity (Gupta et al. 2018).

Nowadays and more than ever, finding new effective antiviral compounds has become a main concern. The existing antiviral therapies are not always well-tolerated or quite effective and have high associated costs (Zorofchian Moghadamtousi et al. 2014). Numerous studies demonstrated a wide variety of antiviral effects attributed to curcumin. This phytochemical can act at several stages of viral infection from preventing viral entry, viral attachment, replication, protein expression, or acting as a viricide (Mathew and Hsu 2018). Curcumin showed antiviral properties against several types of viruses including RNA virus (e.g., human immunodeficiency viruses, Zika virus, dengue, influenza A, norovirus, viral hemorrhagic septicemia virus) and DNA virus (e.g., herpesvirus and human adenovirus) (Jennings and Parks 2020). However, currently, there are no reports demonstrating curcumin antiviral activity against the novel SARS-Cov-2 virus. Nevertheless, some reviews highlight the potential effects of curcumin to prevent the novel coronavirus infections by blocking direct viral infection and also for the treatment of the coronavirus disease 2019 (COVID-19) associated symptoms (Rattis et al. 2021; Thimmulappa et al. 2021).

Safety and Bioavailability

As a food agent, curcumin is recognized as "generally safe" by the Food and Drug Administration (FDA) (Phipps et al. 2020) with an adequate daily intake value recommended of up to 3 mg per kg of body weight by Joint FAO-WHO Expert Committee Report on Food Additives (JEFCA) (Kocaadam and Şanlier 2017) and European Food Safety Authority (EFSA) (European Food Safety Authority 2014). Other investigations reported that this compound is safe for humans up to 12 g/day without any side effects (Lao et al. 2006).

Although curcumin presents a wide range of therapeutic applications, its bioavailability is low due to its lack of water solubility, a relatively low absorption in intestine, rapid metabolism in liver, and fast elimination through the gallbladder if

administered orally (Siviero et al. 2015). It is only significantly detected in plasma or in tissues after administration of high oral doses (Yang et al. 2007). A study performed with healthy individuals revealed that curcumin is converted into glucuronide and sulfate conjugates and excreted without being properly absorbed (Vareed et al. 2008). For instance, the ingestion of 12 g of curcumin resulted in only 29.7 ng/mL detected in blood after 1 hour of ingestion. This value is far below the effective concentrations reported in the in vitro studies (Lao et al. 2006).

To overcome the poor bioavailability, several synthetic curcumin derivatives have been developed. It was demonstrated that the glycosylation of the aromatic ring improves curcumin solubility and thus its kinetic stability leading to a better therapeutic response (Ferrari et al. 2009). The curcumin kinetic stability can be enhanced by masking the 4-OH groups attached to the phenyl rings, thus avoiding conjugation reactions in the human body (Cao et al. 2014). Other strategies employed include curcumin formulations with oils, polymers, or with natural products (Tomeh et al. 2019). In addition, to increase the targeting and bioavailability of curcumin, different types of delivery systems have been developed based on nanoformulations including polymeric nanoparticles, liposomes, nanogels, peptide formulations, and cyclodextrin complexes (Zielińska et al. 2020).

Curcumin Biosynthesis in *Curcuma longa*

Curcuminoids are secondary metabolites produced by *C. longa*. The biosynthesis of these compounds starts with the phenylpropanoid pathway which channels the carbon fluxes from primary metabolism (amino acid metabolism) to different branches of the secondary metabolism including the synthesis of curcuminoids (Rodrigues et al. 2015a, b). Curcuminoids consist of two phenylpropanoid units derived from phenylalanine connected by a central carbon derived from malonyl-CoA (Katsuyama et al. 2009a). Curcuminoid synthesis reactions are catalyzed by an enzyme cluster called type III polyketide synthases (PKS) (Ramirez-Ahumada et al. 2006).

The Phenylpropanoid Pathway

The enzyme phenylalanine ammonia lyase (PAL) is the first enzyme of this pathway and catalyzes the deamination of phenylalanine to form cinnamic acid (Fig. 2). Cinnamic acid is then hydroxylated into *p*-coumaric acid by cinnamate-4-hydroxylase (C4H). At this point, there are two different ways for *p*-coumaric acid metabolization. First, it may be converted in other phenylpropanoid analogues namely ferulic acid by 4-coumarate 3-hydroxylase (C3H) and caffeic acid *O*-methyltransferase (COMT) having caffeic acid as intermediate, or it can be activated by condensation with coenzyme A (CoA) to form the corresponding hydroxycinnamoyl-CoA thioester. This last reaction is catalyzed by 4-coumarate-CoA ligase (4CL). 4CL can act in multiple substrates, also activating the *p*-coumaric

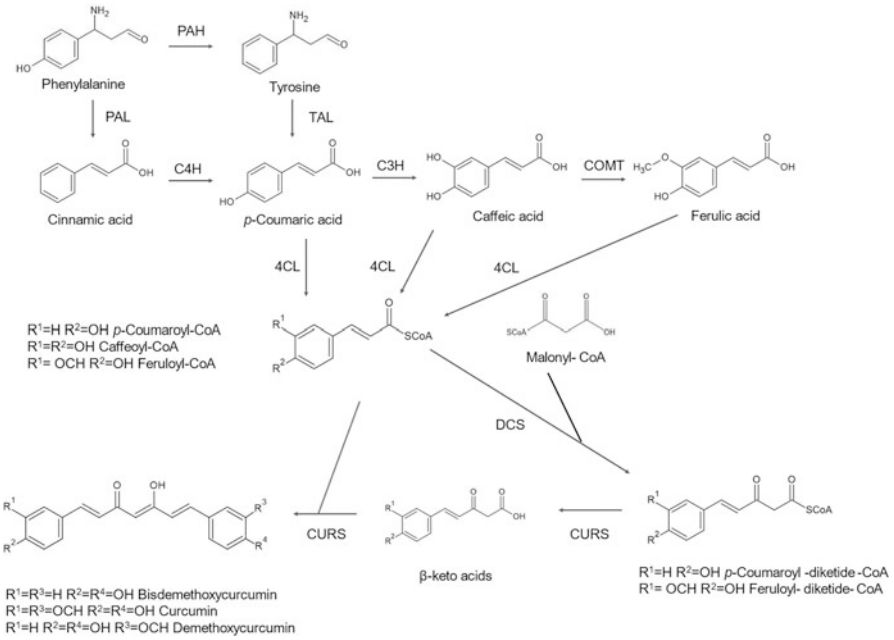

Fig. 2 Curcuminoid biosynthetic pathway in *Curcuma longa. 4CL* 4-coumarate-CoA ligase, *C3H* 4-coumarate 3-hydroxylase, *C4H* cinnamate-4-hydroxylase, *COMT* caffeic acid *O*-methyltransferase, *CURS* curcumin synthase, *DCS* diketide-CoA synthase, *PAH* phenylalanine hydroxylase, *PAL* phenylalanine ammonia lyase, *TAL* tyrosine ammonia lyase

acid analogues (ferulic or caffeic acid) (Ehlting et al. 1999). Activated *p*-coumaric acid, *p*-coumaroyl-CoA, can as well be converted to the other activated analogues, caffeoyl-CoA and then feruloyl-CoA by a set of enzymes which include *p*-coumaroyl shikimate transferase (CST) and caffeoyl-CoA *O*-methyltransferase (CCoAOMT) (Ramirez-Ahumada et al. 2006).

The Type III Polyketide Synthases Reactions

The biosynthesis of curcuminoids is accomplished by the action of two type III PKS. The type of curcuminoid synthesized depends on the activated phenylpropanoids used as substrates. For instance, to synthesize curcumin, diketide-CoA synthase (DCS) (EC number: 2.3.1.218) catalyzes the formation of feruloyl-diketide-CoA from feruloyl-CoA and malonyl-CoA. Afterwards, curcumin synthase (CURS) catalyzes the hydrolysis of feruloyl-diketide-CoA in a β-keto acid and condensates it with another molecule of feruloyl-CoA forming curcumin. On the other hand, a combination of two *p*-coumaroyl-CoA molecules originates bisdemethoxycurcumin, and the combination of *p*-coumaroyl-CoA with feruloyl-CoA originates demethoxycurcumin (Katsuyama et al. 2007b). Three different isoforms of curcumin

Table 1 Identity and kinetic parameters of curcumin synthase (CURS) 1, 2, and 3 from *Curcuma longa*

Enzyme	EC number	Substrate	K_{cat}/K_M (s^{-1} M^{-1})	Molecular weight (kDa)	Isoelectric point	References
CURS1	2.3.1.217	Feruloyl-CoA	1001	21.1	4.93	Katsuyama et al. (2009a) Santhoshkumar and Yusuf (2020)
		Coumaroyl-CoA	75			
CURS2	2.3.1.219	Feruloyl-CoA	1622	20.3	5.28	Katsuyama et al. (2009b) Santhoshkumar and Yusuf (2020)
		Coumaroyl-CoA	176			
CURS3	2.3.1.219	Feruloyl-CoA	2017	20.6	4.96	
		Coumaroyl-CoA	1742			

synthase have been identified in *C. longa*, CURS1, 2, and 3. These enzymes have different substrate specificities (Table 1), which could be the reason to explain the distribution of the three curcuminoids in turmeric (Katsuyama et al. 2009b). CURS1 prefers feruloyl-CoA but also uses *p*-coumaroyl-CoA with low efficiency. CURS2 also uses preferentially feruloyl-CoA while CURS3 uses both substrates almost equally. The substrate specificities of CURS suggest that CURS1 and CURS2 synthesize curcumin preferentially, while CURS3 synthesizes curcumin, demethoxycurcumin, and bisdemethoxycurcumin almost equally (Katsuyama et al. 2009b). CURSs and DCS share a conserved Cys-His-Asn catalytic triad and more than 60% identity. Bioinformatic analysis revealed that CURS's secondary structure is mainly composed of α-helix and random coils. In addition, it was also predicted that CURSs are phosphorylated as a posttranslational modification. The predicted isoelectric points suggest a moderately acidic nature of the proteins (Santhoshkumar and Yusuf 2020).

Curcumin Production in Microorganisms

Attributable to its pharmacological potential, curcumin production and purification has been raising the attention of the scientific community. New extraction and purification methodologies have been evaluated aiming to obtain pure curcuminoids or pure curcumin extracts. However, as mentioned before, plant extraction and the chemical synthesis of curcumin is not ideal due to the associated costs and issues regarding the process sustainability. The microbial production appears as the third alternative to produce curcuminoids (Rodrigues et al. 2015b). Microorganisms can grow in inexpensive substrates and have rapid production cycles surpassing the drawbacks of plant extraction. In addition, the downstream processes are easier to

implement in large-scale fermentations comparatively to rhizome extraction since microbes do not produce other compounds with similar structures that make the curcumin difficult to isolate. The metabolic engineering of microorganisms to produce curcumin was mainly implemented in *Escherichia coli*, a role model host for genetic engineering purposes. However, other studies have reported the production of curcuminoids in *Saccharomyces cerevisiae* and in less conventional hosts such as the bacteria *Pseudomonas putida*, or the eukaryotic fungi *Aspergillus oryzae* and the yeast *Yarrowia lipolytica* (Table 2).

Metabolic Engineering of *E. coli* to Produce Curcuminoids

Advances on metabolic engineering have allowed researchers to develop genetically modified microorganisms able to produce plant-derived compounds such as flavonoids, stilbenes, or curcuminoids (Rodrigues et al. 2015b; Rainha et al. 2020; Gomes et al. 2021). *E. coli* is the most notorious biological chassis for this kind of application, because it is easy to manipulate and to handle. The biosynthesis of curcumin has been accomplished in this organism from multiple substrates including the phenylpropanoid precursors or the aromatic amino acids. To design an artificial curcumin biosynthetic pathway in *E. coli*, several enzymatic steps are required since *E. coli* naturally lacks the curcuminoid precursors. Moreover, the de novo biosynthesis of curcumin in *E. coli* to make the process simpler and more profitable has been reported (Fang et al. 2018). The following sections highlight the advances on *E. coli* metabolic engineering to produce curcuminoids starting with the construction of microorganisms able of synthesizing curcumin from fed precursors. Next, advances on the curcuminoid de novo biosynthesis will be reviewed. Finally, the endogenous chassis optimizations performed to improve curcuminoid biosynthesis will be presented.

Curcuminoid Production from a Fed Precursor in *E. coli*

The first proof-of-concept on the artificial curcuminoid biosynthesis by a microorganism was reported by Katsuyama et al. (2008). To achieve that, a plasmid containing PAL gene from *Rhodotorula rubra* (a multi-substrate enzyme that accepts phenylalanine and tyrosine to produce cinnamic acid and *p*-coumaric acid, respectively) and 4CL gene from *Lithospermum erythrorhizon* was constructed. Another plasmid containing curcuminoid synthase (CUS) gene from *Oryza sativa* and a third one containing acetyl-CoA carboxylase (ACC) from *Corynebacterium glutamicum* were also constructed. The three plasmids were then transformed into *E. coli* BLR DE3 strain. All the genes were placed under control of strong IPTG-inducible T7 promoters that lead to a robust protein expression. ACC was expressed in order to increase the intracellular pool of the curcuminoid extender substrate malonyl-CoA as this previously proved to be important for the synthesis of other plant polyketides (Katsuyama et al. 2007a). The enzyme CUS from *O. sativa* (rice) (NCBI accession number AK109558) used in this work is also a type III PKS, like DCS and CURS, and was identified by the same research group (Katsuyama et al. 2007b) before the

Table 2 Production of curcumin in microorganisms. The host organism, substrate precursor, genes expressed, strategies applied, fermentation conditions, and titers obtained are presented

Host organism	Substrate precursor	Curcuminoid	Genes expressed[a,b]	Strategies applied[c]	Fermentation conditions[d]	Titer (mg/L)	Reference
E. coli BLR (DE3)	*p*-Coumaric acid (1 mM)	Bisdemethoxycurcumin	*Le*4CL *Os*CUS *Cg*ACC	Heterologous genes expression controlled by IPTG inducible promoter Increase MCoA pool by the expression of *Cg*ACC	Flask fermentation LB + M9 100 mL 60 h	91	Katsuyama et al. (2008)
	Ferulic acid (1 mM)	Curcumin				113	
	Cinnamic acid (1 mM)	Dicinnamoylmethane				84	
E. coli BL21 (DE3)	Phenylalanine (3 mM)	Dicinnamoylmethane	*Tf*PAL1 *At*4CL1 *Os*CUS	Heterologous genes expression controlled by IPTG inducible promoter	Flask fermentation LB 50 mL 72 h	360	Wang et al. (2013)
E. coli K-12	Ferulic acid (2 mM)	Curcumin	*At*4CL1 *Cl*DCS *Cl*CURS1	Heterologous genes expression controlled by IPTG/aTc inducible promoters	Flask fermentation LB 50 mL 63 h	70	Rodrigues et al. (2015a)
	Tyrosine (3 mM)	Curcumin	*Rg*TAL *Se*C3H *Ms*CCoAOMT *At*4CL1 *Cl*DCS *Cl*CURS1			0.2	

E. coli BL21 (DE3)	Ferulic acid (2 mM)	Curcumin	*At*4CL1 *Cl*DCS *Cl*CURS1	Heterologous genes expression controlled by IPTG inducible promoter Selection of the best *E. coli* strain to improve production Optimization of induction parameters, carbon source, and culture media	Flask fermentation LB + M9 50 mL 63 h	353.4	Couto et al. (2017)
					Flask fermentation TB 50 mL 63 h	301.2	
E. coli BL21 (DE3)	Ferulic acid (3 mM)	Curcumin	*At*4CL1 *Cl*DCS *Cl*CURS1	Reduction of the number of plasmids	Flask fermentation LB + M9 50 mL 63 h	563	Rodrigues et al. (2020)
	Tyrosine (3 mM)	Curcumin	*Rg*TAL *Se*C3H *At*COMT *At*4CL1 *Cl*DCS	Coculture strategy was applied to reduce metabolic burden Reduction of the number of plasmids used in each module		15.8	
E. coli BL21 star™ (DE3)	Glucose (10 g/L + 100 g)	Bisdemethoxycurcumin	*Rg*TAL *At*4CL *Os*CUS *Rl*MatB *Rl*MatC	Use of a tyrosine overproducing strain Coculture strategy to reduce metabolic burden MatB and MatC overexpression to increase MCoA availability	Bioreactor fermentation AMM 3 L 22 h	6.28	Fang et al. (2018).
E. coli BL21 (DE3)	Glucose (10 g/L)	Dicinnamoylmethane	*At*PAL *Os*4CL *Os*CUS	Use of tyrosine/phenylalanine overproducing strain	Flask fermentation LB + M9 24 h	6.95	Kim et al. (2017)
		Bisdemethoxycurcumin	*Se*TAL *Os*4CL *Os*CUS			4.63	

(continued)

Table 2 (continued)

Host organism	Substrate precursor	Curcuminoid	Genes expressed[a,b]	Strategies applied[c]	Fermentation conditions[d]	Titer (mg/L)	Reference
E. coli BL21 (DE3)	Cinnamic acid (0.1 mM)	Diccinamoylmethane	*Pc*4CL *Os*CUS *Nf*ACC	Use of CRISPRi to silence genes of TCA cycle and fatty acid biosynthesis to increase MCoA availability Increase MCoA pool by the expression of *Nf*ACC	Bioreactor fermentation LB 3 L 48 h	11.6	Chu et al. (2020)
E. coli C41 (DE3)	Glucose (15 g/L)	Curcumin	*Se*TAL *Se*C3H *At*COMT *Nt*4CL2 *Cl*DCS *Cl*CURS2	Use of a tyrosine overproducing strain Optimization of the expression ratios of curcumin biosynthetic genes by random mutagenesis	Tube fermentation LB 3 mL 45 h	3.8	Kang et al. (2018)
E. coli BW25113	Ferulic acid (4 mM)	Curcumin	*At*4CL (mutant) *Os*CUS (mutant) *Al*mgs *Ec*ACS	Inactivation of fatty acid biosynthesis genes and ACS overexpression to increase pool of MCoA pool Deletion of the native *curA* to prevent curcumin degradation Use of medium copy plasmids and operon re-arrangement Construction of a mutant CUS by random mutagenesis Expression of *Al*mgs to optimize cell membrane morphology	Flask fermentation YM9 100 mL 48 h Oleic acid was added to media to improve production	537.8	Wu et al. (2020)
A. oryzae RIB40 RkuptrP2-1	Feruloyl-NAC (Feruloyl-CoA analogue) (1.1 mg)	Curcumin	*Os*CUS *Ao*ACC	Increase MCoA pool by deletion of genes involved in the regulation of ergosterol biosynthesis and overexpression of native ACC	Mutants cultivated in agar plates PD 72 h	404.2 µg/plate	Kan et al. (2019)

P. putida KT2440	*p*-Coumaric acid (5 mM)	Bisdemethoxycurcumin	*Os*CUS	*Ech* deletion to increase MCoA pool	Microplate fermentation MOPS 500 µL 48 h	2.15	Incha et al. (2020)
Y. lipolytica PO1f	*p*-Coumaric acid (2 mM)	Bisdemethoxycurcumin	*Nt*4CL *Os*CUS *Yl*Pex10p *Yl*ACC	Overexpression of Pex10p And ACC to increase MCoA pool	Flask fermentation YNB 50 mL 168 h	0.17	Palmer et al. (2020)
S. cerevisiae BY4741	Ferulic acid (0.08 mM)	Curcumin	*Pp*FerA *Cl*DCS *Cl*CURS1	Deletion of *fdc1* to prevent substrate consumption	Flask fermentation YNB 50 mL 72 h	2.7	Rainha et al. (2021a)

[a]*Ag* Aspergillus oryzae, *Al* Acholeplasma laidlawii, *At* Arabidopsis thaliana, *Cg* Corynebacterium glutamicum, *Cl* Curcuma longa, *Ec* Escherichia coli, *Le* Lithospermum erythrorhizon, *Ms* Medicago sativa, *Nf* Nocardia farcinica, *Nt* Nicotiana tabacum, *Os* Oryza sativa, *Pc* Petroselinum crispum, *Pp* Pseudomonas paucimobilis, *Rg* Rhodotorula glutinis, *Rl* Rhizobium leguminosarum, *Se* Saccharothrix espanaensis, *Tf* Trifolium pratense, *Yl* Yarrowia lipolytica

[b]*4CL* 4-coumarate-CoA ligase, *ACC* acetyl-CoA carboxylase, *C3H* 4-coumarate 3-hydroxylase, *C4H* cinnamate-4-hydroxylase, *CCoAOMT* caffeoyl-CoA *O*-methyltransferase, *COMT* caffeic acid *O*-methyltransferase, *CURS* curcumin synthase, *CUS* curcuminoid synthase, *DCS* diketide-CoA synthase, *FerA* feruloyl-CoA synthetase, *MatB* malonate synthetase, *MatC* malonate carrier protein, *mgs* monoglucosyldiacylglycerol synthase, *PAH* phenylalanine hydroxylase, *PAL* phenylalanine ammonia lyase, *Pex10p* peroxisome biogenesis factor, *TAL* tyrosine ammonia lyase

[c]*aTc* anhydrotetracycline, *curA* curcumin reductase gene, *CRISPRi* clustered regularly interspaced short palindromic repeats interference, *Ech* enoyl-CoA hydratase lyase gene, *fdc1* ferulic acid decarboxylase gene, *IPTG* isopropyl β-d-1-thiogalactopyranoside, *MCoA* malonyl-CoA, *TCA* tricarboxylic acid cycle

[d]*AMM* Andrew's magic media, *LB* lysogeny broth, *M9* M9 minimal media, *MOPS* morpholinepropanesulfonic acid minimal media, *PD* polypeptone-dextrin media, *TB* optimized terrific broth, *YM9* yeast extract M9 media, *YNB* yeast nitrogen base media

discovery and characterization of DCS and CURS enzymes in *C. longa* (Katsuyama et al. 2009a). This CUS enzyme is capable of synthesizing curcuminoids from two molecules of activated phenylpropanoids and one extender malonyl-CoA molecule in a single step catalyzing the same reactions carried by DCS and CURS in *C. longa*. Contrary to DCS, CUS also accepts cinnamoyl-CoA acid as substrate to produce the curcuminoid dicinnamoylmethane, however it prefers *p*-coumaroyl-CoA over feruloyl-CoA and cinnamoyl-CoA (Katsuyama et al. 2007b; 2009a). Morita et al. (2010) studied the architecture of CUS active site and concluded that it contains a single downward-expanding active site capable of accommodating two activated *p*-coumaric acid molecules (*p*-coumaroyl-CoA), a malonyl-CoA and a putative H_2O molecule that forms hydrogen bond networks at the active centre. In the following year, Miyazono et al. (2011) annotated the CUS crystal structure. It was found that CUS disobeys the traditional model of head-to-tail polyketide assembling catalyzing only condensation reactions without performing cyclization. The use of "one-pot" CUS for curcuminoid biosynthesis represents a simpler system than the double enzyme DCS/CURS system since it is only required the expression of one enzyme thus reducing the metabolic charge for the cells. The engineered strain constructed by Katsuyama et al. (2008) was grown in the presence of 3 mM of tyrosine or phenylalanine. The production of the correspondent curcuminoid was detected in both assays yielding 53.4 mg/L of bisdemethoxycurcumin (from tyrosine) and 107 mg/L of dicinnamoylmethane (from phenylalanine). When both phenylalanine and tyrosine (3 mM of each) were added to the media, dicinnamoylmethane was produced in higher amounts over bisdemethoxycurcumin, although CUS prefers *p*-coumaroyl-CoA over cinnamoyl-CoA. The authors hypothesized that these differences may be due to the distinct incorporation rates of tyrosine and phenylalanine or *p*-coumaric and cinnamic acids by the multi-substrate enzymes PAL and 4CL. In addition, despite being reported as cytotoxic agents, the produced curcuminoids did not cause inhibitory effects on cellular growth at the reported concentrations. Next, to improve the curcuminoids titers, the hydroxycinnamic acids were directly supplied to the culture media. For that purpose, the PAL step was removed, and *E. coli* mutant harbored only the 4CL, CUS, and ACC genes. Precursors were fed to the culture media at a lower concentration of 1 mM. The engineered *E. coli* strain produced 91 mg/L of bisdemethoxycurcumin from *p*-coumaric acid, 84 mg/L of dicinnamoylmethane from cinnamic acid and 113 mg/L of curcumin from ferulic acid. Additionally, 57 mg/L of curcumin were produced from rice bran pitch, a ferulic acid-rich industrial waste resulting from rice bran. In another study using the same pathway (4CL, CUS, and ACC), Katsuyama et al. (2010) produced 15 asymmetric curcuminoids, 9 of these unnatural, by adding *p*-coumaric acid analogues to the medium. This was only possible due to the wide range of substrate specificities of the enzymes involved, an interesting feature to the discovery of novel drug candidates.

In the subsequent years, a curcuminoid producing engineered *E. coli* reporter strain was developed for the screening of novel PAL enzyme efficiency (Wang et al. 2013). The pathway was composed of different PALs, 4CL1 from *Arabidopsis thaliana*, and CUS from *O. sativa*. Three PALs from *Trifolium pratense* were

screened (PAL1, PAL3, and PAL4). The best results were obtained using PAL1. The curcuminoids production reached 360 mg/L dicinnamoylmethane from 3 mM of phenylalanine, 3.4 times more than in Katsuyama's work (Katsuyama et al. 2008). In this case, ACC was not overexpressed, demonstrating that the natural endogenous malonyl-CoA concentration was enough to ensure the production of these levels of curcuminoids.

The first report addressing the use of the DCS/CURS system from *C. longa* to heterologous produce curcumin in *E. coli* was published by Rodrigues et al. (2015a). At first, Rodrigues et al. (2015a) constructed two different biosynthetic pathways: (1) composed by 4CL1 from *A. thaliana* and *O. sativa* CUS and (2) harboring the same 4CL but in combination with DCS and CURS1 from *C. longa*. CURS1 was selected over the other CURS isoenzymes since it has the highest turnover rate towards feruloyl-CoA. All genes were expressed under the control of T7 promoter. The *E. coli* K-12 strains expressing DCS and CURS enzymes produced 28-fold more curcumin (70 mg/L) than the ones expressing CUS (2.5 mg/L) when 2 mM of ferulic acid were fed to the culture media. Therefore, the two-enzyme system pathway (2) was found to be more efficient than CUS pathway (1) to synthesize curcumin. Interestingly, when *p*-coumaric acid was supplemented, the amount of bisdemethoxycurcumin was very low compared to curcumin for the two pathways. However, in vitro experiments reported that *A. thaliana* 4CL1 and CUS have preference for *p*-coumaric acid and *p*-coumaroyl-CoA, respectively. In another assay, the two carboxylic acids (*p*-coumaric acid and ferulic acid) were both directly supplemented to the culture media, which resulted in the production of the three curcuminoids bisdemethoxycurcumin, demethoxycurcumin, and curcumin. The curcumin titer was higher, as expected, due to CURS1 preference for feruloyl-CoA. Moreover, in the same study, to produce curcumin from tyrosine, a new pathway was designed that synthesized caffeic acid as intermediate. The enzymes TAL (conversion of tyrosine to *p*-coumaric acid) from *Rhodotorula glutinis*, C3H (*p*-coumaric acid to caffeic acid) from *Saccharothrix espanaensis* and CCoAOMT (caffeoyl-CoA to feruloyl-CoA) from *Medicago sativa* were expressed together with 4CL1, DCS, and CURS (Fig. 3). The curcumin production was tested using *p*-coumaric acid or tyrosine as precursors. As expected, the production was higher when *p*-coumaric acid was used since the number of intermediates is lower, resulting in a lower loss of product over the pathway reactions. Interestingly, bisdemethoxycurcumin was the curcuminoid produced in higher amounts when *p*-coumaric acid was supplemented to the media besides CURS1 preference for feruloyl-CoA possible due to high accumulation of *p*-coumaroyl-CoA. Using tyrosine as substrate, the *p*-coumaric acid was present in the media in higher concentrations than caffeic acid. Nevertheless, curcumin was produced in higher amounts than bisdemethoxycurcumin. The curcumin yield reached 0.2 mg/L from 3 mM of tyrosine. Although the titer was low, this work by Rodrigues et al. (2015a) comprises the first report on the curcumin production from an amino acid.

The use of heat shock promoters to produce curcuminoids was also evaluated by the same research group (Rodrigues et al. 2017a). These promoters may comprise in the future good alternatives to replace T7 promoters frequently used in the expression of heterologous pathways, such as the one of curcuminoids. The expression using T7 promoters is induced through the supplementation of the chemical inducer

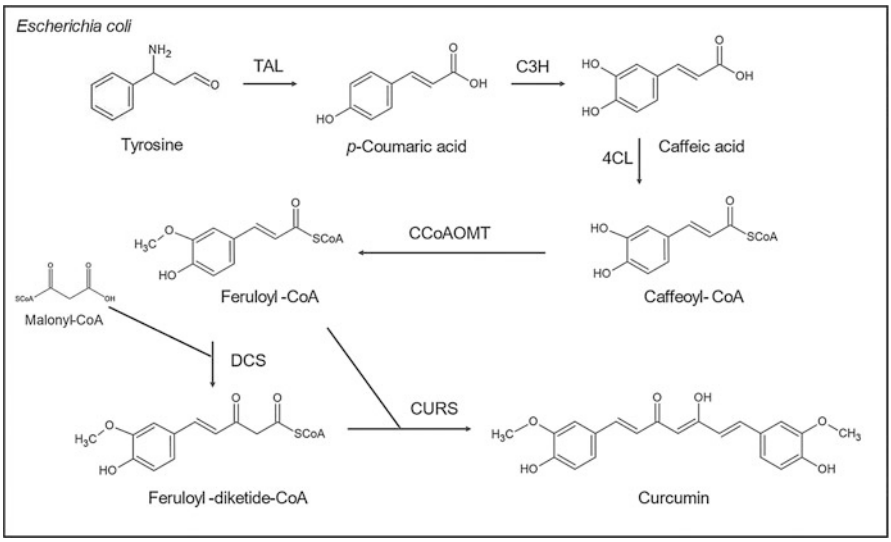

Fig. 3 Strategy developed by Rodrigues et al. (2015a) to produce curcumin in *Escherichia coli*. *4CL* 4-coumarate-CoA ligase, *C3H* 4-coumarate 3-hydroxylase, *CCoAOMT* caffeoyl-CoA *O*-methyltransferase, *CURS* curcumin synthase, *DCS* diketide-CoA synthase, *TAL* tyrosine ammonia lyase

isopropyl β-D-1-tiogalactopiranoside (IPTG) that is potentially toxic and expensive. Although the use of these promoters is feasible on the laboratory scale, on a large scale, their use should be avoided when possible (Rodrigues and Rodrigues 2018). However, heat shock promoters have the disadvantage of not being as strong as the T7 promoters. Nevertheless, this may not always be a disadvantage as it is important to fine-tune the expression of the enzymes of the heterologous pathway. For example, the use of weaker promoters for the expression of highly efficient enzymes to avoid the accumulation of several intermediary compounds can be beneficial. Bearing this in mind, several *E. coli* heat shock promoters were characterized (Rodrigues et al. 2014), and *dnaK* promoter was further used in the construction of curcumin biosynthetic pathway using ferulic acid as substrate. This heat shock promoter was used for DCS and CURS1 expression while 4CL was under the expression of a constitutive lac promoter. Heat shock was applied for 5 min at 48 °C to induce the expression. The curcumin production obtained was very low (0.4 mg/L) and it was verified using ribosome binding site (RBS) calculator tool (https://salislab.net/software/) that the translation initiation rates (TIR) where low when compared to the ones related to T7 promoters. Therefore, RBS calculator tool was used to optimize the RBSs and curcuminoids production increased significantly to 6.3 mg/L. Although the production obtained was very low when compared to the ones obtained using T7 promoters, it was demonstrated that heat shock promoters may be used in specific cases to control the fine-tuning of the pathway.

Afterwards, Couto et al. (2017) using the pathway containing DCS and CURS (and T7 promoters) optimized the fermentation conditions to maximize curcumin

production from ferulic acid. The parameters analyzed included the *E. coli* host strain, the pathway expression induction time and inducer concentration, the culture media, and the carbon source concentration. The greatest performance was obtained using BL21 strain (a strain known to efficiently convert acetate to acetyl-CoA, the precursor of malonyl-CoA) (Castaño-Cerezo et al. 2015). Regarding the induction parameters, an induction at an optical density at 600 nm of 0.9 with the supplementation of IPTG at a concentration of 0.1 mM was found to maximize the curcumin production. Overall, the curcumin production increased to 959.3 μM (353.4 mg/L) in 63 h. This production was obtained using a two-step fermentation process involving a first growth phase in lysogeny broth to increase the heterologous protein production and a curcumin production phase in M9 minimal media. As this two-step production process is laborious and not desirable whenever scale up is considered, other culture media were studied. Optimized terrific broth (TB) demonstrated to be a good option as culture media as it allowed to produce up to 817.7 μM (301.2 mg/L) of curcumin in 63 h. Although the production in TB was lower at 63 h, at 20 h, the production in this medium was significantly higher, which can be advantageous. Overall, the optimization of fermentation conditions in this study (Couto et al. 2017) resulted in almost five-fold improvement on curcumin production relatively to the previous report from Rodrigues et al. (2015a) and to a 3.1-fold increase in relation to Katsuyama's study (Katsuyama et al. 2008).

More recently, the same research group optimized the curcumin production at genetic and fermentative level (Rodrigues et al. 2020). They started to optimize ferulic acid biosynthesis from tyrosine. Since the researchers (Rodrigues et al. 2015a) had previously shown that the conversions of tyrosine to caffeic acid and ferulic acid to curcumin were very efficient, it was hypothesized that the combination of 4CL and CCoAOMT was the main bottleneck. In this sense, instead of using CCoAOMT (conversion of caffeoyl-CoA to feruloyl-CoA), this enzyme was replaced by COMT (conversion of caffeic acid to ferulic acid) from *A. thaliana*. First, to test COMT efficiency, an engineered *E. coli* BL21 expressing COMT was evaluated, and it was found to be capable of completely converting 1 mM of caffeic acid into ferulic acid. Next, the expression of TAL, C3H, and COMT, corresponding to the first part of the pathway, (module 1) led to the synthesis of ferulic acid (Fig. 4). These genes were individually expressed in different plasmid backbones and several plasmid/gene combinations were tested to optimize ferulic acid synthesis. The highest ferulic acid accumulation was achieved by expressing TAL in pRSFDuet-1, C3H in pCDFDuet-1, and COMT in pACYDuet-1. However, accumulation of *p*-coumaric acid was also observed as in other previously constructed pathways. This limitation is related to the low conversion rate of *p*-coumaric acid into caffeic acid because of the C3H low efficiency. Nevertheless, 172.6 mg/L of ferulic acid were synthesized from 3 mM of tyrosine. These researchers also studied the optimization of the second part of the curcumin biosynthesis pathway that corresponds to the conversion of ferulic acid into curcumin. To optimize this second module, the best plasmid backbone for 4CL expression was addressed. It was observed that the combination of pCDFDuet_DCS, pRSFDuet_CURS, and pACYDuet_4CL yielded the highest curcumin titer. The resulting strain could produce 446.7 mg/L of

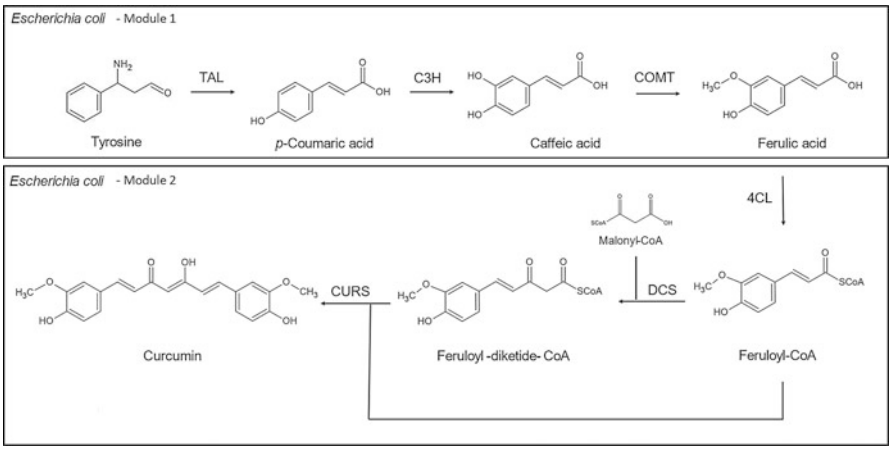

Fig. 4 Strategy developed by Rodrigues et al. (2020) to produce curcumin *Escherichia coli*. *4CL* 4-coumarate-CoA ligase, *C3H* 4-coumarate 3-hydroxylase, *COMT* caffeic acid *O*-methyltransferase, *CURS* curcumin synthase, *DCS* diketide-CoA synthase, *TAL* tyrosine ammonia lyase

curcumin from 3 mM of supplemented ferulic acid (2 mM in the beginning of the fermentation and 1 mM after 15 h), a 20.9% improvement on productivity. After optimizing both pathway modules separately, the curcuminoid biosynthesis was evaluated from caffeic acid in a strain with COMT, 4CL, DCS, and CURS1 genes, and from tyrosine in a strain harboring the complete pathway (TAL, C3H, 4CL, DCS, and CURS1) expressed in four plasmids. As expected, when caffeic was supplemented, the curcumin titer (46.6 mg/L) and yield decreased significantly when compared with that obtained when ferulic acid was directly supplemented, since in this case ferulic acid was produced in low amounts. Using tyrosine as substrate, the curcumin production was even lower (0.82 mg/L). Nevertheless, it increased significantly in relation to the pathway that contained CCoAOMT instead of COMT, demonstrating that COMT is a better option. Next, to increase the production efficiency, the high metabolic charge caused by the expression of several plasmids was distributed using a coculture strategy. Two strains, each one carrying a different module for the curcumin synthesis, were constructed (Fig. 4). The coculture cultivation of the two strains leads to the production of 5.5 mg/L of curcumin, 6.8 times more than the one achieved with the monoculture system. The authors predicted that the metabolic burden caused by the expression of the complete pathway reduced the titers and yields in the monoculture system. In order to reduce this metabolic burden, the number of plasmids carrying the heterologous enzymes was reduced in all pathways tested. The reduction of the number of plasmids of the *E. coli* strain harboring the complete pathway (from TAL to CURS) to three resulted in the production of 6.1 mg/L of curcumin from 3 mM of tyrosine, 7.5 times higher than the one achieved with four plasmids. Afterwards, the plasmid number of each module was also reduced to optimize production. By reducing the number of plasmids from three to two, it was possible to produce 563.4 mg/L of curcumin

from ferulic acid (module 2) which represents a percent yield of 100%. Currently, this value represents the highest curcumin titer produced from ferulic acid by a microorganism. The same strategy was also applied to the first module of the pathway reaching an improvement of 49% (257.3 mg/L) on produced ferulic acid from tyrosine. Then, the optimized modules were then applied to a coculture system which resulted in the production of 15.9 mg/L of curcumin, representing a 6.8-fold improvement relatively to the optimized monoculture. This highest titer was obtained using a coculture ratio of 2:1, where the strain carrying the first module of the pathway was inoculated in a higher concentration. This report represents the highest amount of curcumin obtained from tyrosine in a genetically modified microorganism. During the monoculture and coculture experiments, the other curcuminoids were also produced. The proportion of curcuminoids produced was highly dependent on the coculture ratio evaluated. The highest total curcuminoids titer obtained was 41.5 mg/L and was achieved when the strain carrying the second part of the pathway was inoculated in a higher amount which was not in concordance with what was verified to achieve high concentrations of curcumin. This demonstrated that the increased expression of the first module is more important for the curcumin production as its production is more dependent on the expression of C3H and COMT while the production of, for example, bisdemethoxycurcumin is only dependent on TAL step that is highly efficient and fast. On top of that, during the experiments it was demonstrated that the strain carrying the first part of the module was in a clear disadvantage, because even when it was inoculated in higher amounts, the strain carrying the second module prevailed during the fermentation. Therefore, in the future, coculture engineering can be more explored to favor the production of one specific curcuminoid.

De Novo Curcuminoid Production in *E. coli*

From the economic point of view, the development of engineered microbes capable of producing natural added-value compounds such as curcuminoids from inexpensive substrates is very advantageous at industrial level since it makes the process more profitable. Furthermore, the use of organic wastes instead of purified compounds will contribute to a circular process valuing industrial by-products and wastes that until then were worthless. The unique report addressing the use of wastes to produce curcumin was the one already mentioned by Katsuyama et al. (2008) which used ferulic-rich rice bran pitch as substrate to produce curcumin in a genetically modified *E. coli*. However, the production of curcumin from simple sugars is still a more profitable process than the ones requiring the supplementation of expensive phenylpropanoid acids or the aromatic amino acids directly to the media. To achieve that, the metabolic fluxes of the chassis may be adjusted to improve the native biosynthesis of aromatic amino acids since it is strictly regulated.

The production of curcuminoids from sugars was first reported by Fang et al. (2018). For that purpose, a pathway composed by TAL gene from *R. glutinis* was expressed in a tyrosine overproducing *E. coli* strain (*E. coli* rpoA14) and 4CL1 from *A. thaliana* and CUS from *O. sativa* were expressed in an *E. coli* BL21 wild-type strain. To develop the tyrosine-overproducing strain, the genes encoding tyrosine

repressor (TyrR) and bifunctional chorismate mutase/prephenate dehydratase (PheA) were deleted to direct the pathway to tyrosine production. Also, feedback-insensitive versions of phospho-2-dehydro-3-deoxyheptonate aldolase ($AroG^{fbr}$) and chorismate mutase-prephenate dehydrogenase ($TyrA^{fbr}$) were overexpressed to eliminate tyrosine- and phenylalanine-mediated repression (Santos et al. 2012). A coculture strategy was used (ratio 10:1 -*E. coli* BL21: *E. coli* rpoA14) and optimized using both genetically engineered strains. The obtained titer of bisdemethoxycurcumin was 6.28 mg/L in a lab-scale bioreactor starting with 10 g/L of glucose and then supplementing 10 g/h of glucose until 100 g.

Kim et al. (2017) described for the first time the de novo production of curcuminoids by a single *E. coli* strain. At first, the production of bisdemethoxycurcumin was tested by two different constructs: the first one where *O. sativa* 4CL and CUS expression were controlled by an independent T7 promoter in the same plasmid and the second one where 4CL and CUS were organized in an operon (same promoter). Both constructs were transformed in an *E. coli* BL21 wild-type strain along with a plasmid carrying *S. espanaensis* TAL. The operon-type construct resulted in higher bisdemethoxycurcumin production. Next, the same pathway was transformed in a tyrosine overproducing *E. coli* strain (TyrR and PheA deletion mutant). The bisdemethoxycurcumin production increased almost 15 times in this strain relatively to the wild-type strain. Afterwards, to maximize the tyrosine pool, AroG and 3-phosphoshikimate 1-carboxyvinyltransferase (AroA) were overexpressed resulting in 6.02 mg/L of bisdemethoxycurcumin which represented a 1.3-fold increment on production. In another experiment, the production of dicinnamoylmethane was addressed. For that purpose, *A. thaliana* PAL and the 4CL/CUS operon were expressed in an *E. coli* strain with TyrA and TyrR deleted to increase the supply of phenylalanine. The production level reached 6.95 mg/L. Finally, the authors developed an engineered strain for de novo bisdemethoxycurcumin and dicinnamoylmethane production co-expressing both TAL and PAL with 4CL/CUS operon in a tyrosine/phenylalanine overproducing strain (ΔTyrR, AroG, TyrA). The resulting strain presented production values of 0.16 mg/L for dicinnamoylmethane, 6.02 mg/L for bisdemethoxycurcumin, and 1.11 mg/l for cinnamoyl *p*-coumaroylmethane (asymmetric curcuminoid synthesized from *p*-coumaroyl-CoA and cinnamoyl-CoA). All the curcuminoids produced were synthesized from 10 g/L of glucose.

As previously mentioned, to develop an *E. coli* strain increasingly adapted to the production of curcumin, several heterologous enzymatic steps are required. The number of reactions increases when simpler substrates are used. Moreover, the endogenous synthesis of the aromatic amino acids is strictly regulated being required to construct tyrosine/phenylalanine overproducing strains, as reported above, to ensure the de novo biosynthesis of curcumin in appropriate amounts. As mentioned, for large-scale applications, the utilization of simpler substrates is advantageous because it makes the process more profitable. The implemented overexpression systems usually rely on the use of T7 promoters to control the pathway expression. This often results in an excessive metabolic burden for the cells. Therefore, strategies to overcome that charge have been explored, namely the use of coculture systems (Rodrigues et al. 2020; Fang et al. 2018). However, the biosynthetic metabolic flux

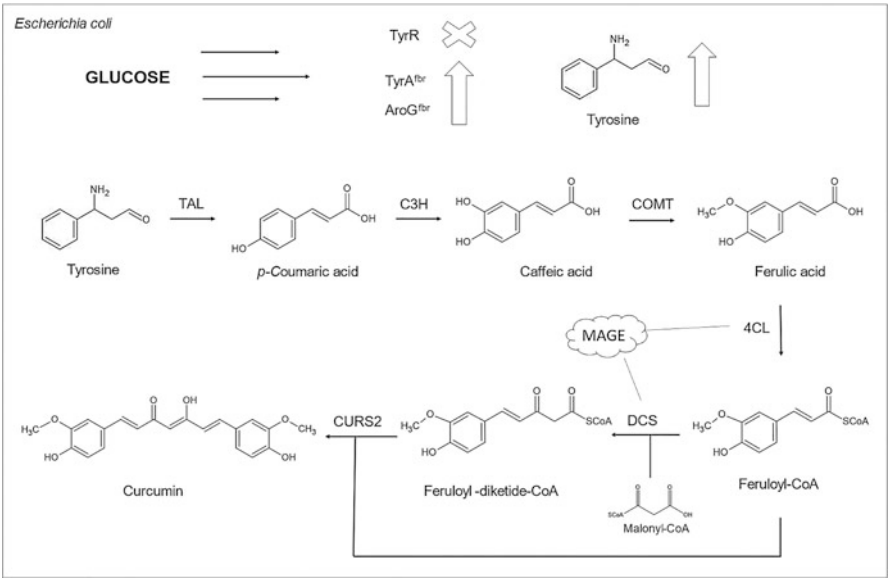

Fig. 5 Strategy developed by Kang et al. (2018) to produce curcuminoids in *Escherichia coli. 4CL* 4-coumarate-CoA ligase, *AroG*^{fbr} phospho-2-dehydro-3-deoxyheptonate aldolase (feedback inhibition-resistant), *C3H* 4-coumarate 3-hydroxylase, *COMT* caffeic acid *O*-methyltransferase, *CURS2* curcumin synthase 2, *DCS* diketide-CoA synthase, *MAGE* multiplex automatic genome engineering, *TAL* tyrosine ammonia lyase, *TyrA*^{fbr} chorismate mutase-prephenate dehydrogenase (feedback inhibition-resistant), *TyrR* tyrosine repressor

may be optimal when the enzymes are expressed in a proper ratio than at maximal. Kang et al. (2018) applied the multiplex automatic genome engineering (MAGE) tool to optimize the expression ratios of curcumin biosynthetic genes. MAGE was used to randomly modify the 5′ untranslated region (5′-UTR) of the genes used in a de novo artificial curcumin biosynthetic pathway. The pathway was composed by TAL and C3H from *S. espanaensis*, COMT from *Nicotiana tabacum,* 4CL2 from *A. thaliana*, and DCS and CURS2 from *C. longa* (Fig. 5). The pathway was divided into two modules: the first one, for de novo ferulic acid production and the second one, for curcumin production from ferulic acid. Each module was integrated in a specific region of *E. coli* genome. The *E. coli* strain used was previously constructed by the same research group for overproduction of tyrosine (ΔTyrR, TyrA^{fbr}, AroG^{fbr}) (Kang et al. 2015). The engineered strain could produce the three main curcuminoids from 15 g/L of glucose, however, in very low amounts (0.01 mg/L of curcumin and 0.03 mg/L of total curcuminoids). The developed strain was the first engineered strain to successfully synthesize curcuminoids by inserting the full biosynthetic pathway into *E. coli* chromosome. Afterwards, the MAGE tool was applied to this strain to fine-tune the expression of curcumin biosynthetic genes. In total, 25 mutant strains were obtained with an increased curcuminoid production relative to the parent strain, but only 8 of the 25 exhibited more than fourfold increment on curcumin production. The strain with lower expression levels of the enzymes 4CL

and DCS resulted in a 38.2-fold improvement in the curcumin titer (3.8 mg/L) and 12.6-fold improvement in the total of curcuminoids (3.9 mg/L). Therefore, the genetic expression fine-tune of curcumin pathway genes may lead to higher titers by reducing the host metabolic charge resultant of the expression of the biosynthetic pathway. This is extremely useful for the design of complex pathways with several reactions involved such as the de novo biosynthesis of curcumin.

E. coli Chassis Optimizations

To increase the curcumin productivity on a genetically modified *E. coli,* in addition to the introduction of the heterologous genes, the chassis also needs to be optimized to increase the bioavailability of all the precursors needed. Besides the mentioned construction of aromatic amino acids overproducing strains, other intrinsic modifications should be aforethought such as the increase of the endogenous biosynthesis of malonyl-CoA. Some studies speculated that the endogenous supply of the curcuminoid extender unit malonyl-CoA was limiting the curcuminoid production. Malonyl-CoA is synthesized from acetyl-CoA and is involved in fatty acid production that may compete with the curcumin biosynthesis for the use of malonyl-CoA. Katsuyama et al. (2008) suggested that the ACC overexpression may improve curcuminoid synthesis. However, they did not test the curcuminoids production without the ACC expression to support that argument. Other works also speculated that the low availability of this curcuminoid precursor was constraining the production (Fang et al. 2018). Fang and coworkers tested four different approaches to enhance malonyl-CoA availability in its curcuminoid producer *E. coli* BL21 expressing 4CL and CUS. The strategies included: (1) the overexpression of the *E. coli* ACC enzyme cluster to directly improve malonyl-CoA biosynthesis via carboxylation of acetyl-CoA; (2) targeted gene deletions in tricarboxylic acid (TCA) cycle to redirect the flux for the synthesis of phosphoenolpyruvate, the precursor of acetyl-CoA; (3) overexpression of malonate synthetase (MatB) and malonate carrier protein (MatC) from *Rhizobium leguminosarum* and supplementation of malonate to directly synthesize malonyl-CoA; and (4) inhibition of fatty acids biosynthesis with cerulenin to prevent malonyl-CoA deviation. However, only strategy (3) drastically improved the production of bisdemethoxycurcumin. The synthesized bisdemethoxycurcumin increased 25-fold (5 mg/L) using strategy (3) and five-fold using strategy (4). These results demonstrated that the enhancement of malonyl-CoA pool maybe important to increase the synthesis of curcuminoids. Nevertheless, Rodrigues et al. (2020) concluded that the bioavailability of malonyl-CoA was not limiting production as they obtained a percent yield of 100% and higher titers than Katsuyama's and Fang's research group (Katsuyama et al. 2008; Fang et al. 2018). However, Rodrigues et al. (2020) suggested that malonyl-CoA could limit the obtention of higher titers in the future.

Novel synthetic biology techniques have continuously been developed increasing the genetic toolkits available for researchers to perform genetic modifications in microorganisms (Rodrigues et al. 2017b; Rodrigues and Rodrigues 2017). The most notable one is the clustered regularly interspaced short palindromic repeats (CRISPR) that appears as a simple, efficient, cheap, and rapid technology for genetic

engineering purposes. The CRISPR applications created range from ordinary genetic procedures such as genome editing to more complex processes, for instance, to control the genetic expression of native targeted genes (Rainha et al. 2021b). Recently, Chu et al. (2020) applied CRISPR interference (CRISPRi) in a curcuminoid *E. coli* producer expressing 4CL from *Petroselinum crispum* and *O. sativa* CUS. CRISPRi allows to silence multiple genes at transcriptional level, which may be very advantageous for the study of complex pathways. The goal of applying CRISPRi in this study was to increase the intracellular malonyl-CoA by regulating some genes involved in TCA cycle and fatty acid biosynthesis thus preventing acetyl-CoA consumption (malonyl-CoA precursor) and/or malonyl-CoA deviation, respectively. The triple repression of FabF (3-oxoacyl-[acyl-carrier-protein] synthase), FabD ([acyl-carrier-protein] *S*-malonyltransferase) (fatty acid biosynthesis) and malate dehydrogenase (TCA cycle) using CRISPRi resulted in the highest dicinnamoylmethane production (11.6 mg/L) from cinnamic acid, increasing the production titer in 5.76 times relatively to the wild type. The authors also tested if the solo expression of the heterologous ACC cluster from *Nocardia farcinica* could also improve the curcuminoid synthesis. In fact, the dicinnamoylmethane production increased twofold relatively to the wild type, demonstrating that the expression of the heterologous ACC cluster increased the endogenous malonyl-CoA pool and consequently the production titers.

Besides the increment of malonyl-CoA supply, other chassis optimizations may include the inhibition of metabolic pathways to prevent product deviation or even the modification of chassis phenotypic characteristics to accommodate the production. Wu et al. (2020) constructed a curcumin *E. coli* BW25113 producer expressing CUS from *O. sativa* and a 4CL mutant version of *A. thaliana* (Fig. 6). This primary strain

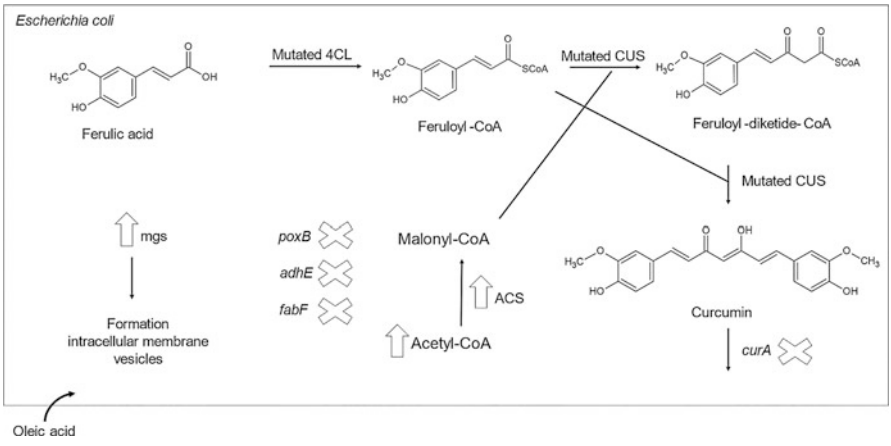

Fig. 6 Strategy developed by Wu et al. (2020) to produce curcumin in *Escherichia coli*. *4CL* 4-coumarate-CoA ligase, *ACS* acetyl-CoA synthetase, *adhE* aldehyde-alcohol dehydrogenase, *curA* curcumin reductase, *CUS* curcuminoid synthase, *fabF* 3-oxoacyl-[acyl-carrier-protein] synthase, *mgs* monoglucosyldiacylglycerol synthase, *poxB* pyruvate oxidase. Oleic acid was added to prevent membrane rigidity

produced 5.5 mg/L of curcumin from ferulic acid. Next, to increase the supply of malonyl-CoA, the genes *poxB* (pyruvate oxidase; TCA cycle), *adhE* (aldehyde-alcohol dehydrogenase; acetyl-CoA degradation), and *fabF* (fatty acids biosynthesis) were inactivated and acetyl-CoA synthetase (ACS) was overexpressed to direct metabolic fluxes through acetyl-CoA synthesis and thus malonyl-CoA. These genetic modifications resulted in a curcumin titer 2.8 times higher relatively to the primary strain. The authors also reported that the deletion of the native *curA* gene, encoding for curcumin reductase, seemingly responsible for curcumin reduction, resulted in a 1.7-fold improvement on the production. Moreover, it was demonstrated that the use of medium copy plasmids to express curcuminoid biosynthetic genes increased curcumin yields over high copy plasmids. Furthermore, 4CL and CUS genes were arranged in an operon, and the operon architecture was optimized. The operon containing CUS gene upstream of 4CL gene improved five times the curcumin titer. Afterwards, the authors to find a CUS with increased expression levels used random mutagenesis to modify the CUS sequence. The use of a mutated CUS with high expression levels improved curcumin levels 3.8 times (95.8 mg/L). In addition, as it is known that hydrophobic compounds in *E. coli*, such as curcumin, accumulate in membrane compartments and only at high concentrations pass to the media, the authors hypothesized that by increasing the hydrophobic compound content of the cell membrane, the curcumin production would also increase. To evaluate that, a monoglucosyldiacylglycerol synthase (mgs) gene from *Acholeplasma laidlawii* was overexpressed. The mgs overexpression in *E. coli* led to the formation of massive intracellular membrane vesicles, which could enhance the storage of hydrophobic compounds (Eriksson et al. 2009). Membrane-engineered cells became bigger in length and smaller in width and curcumin production increased 2.2-fold. Moreover, it was found that curcumin reduced the *E. coli* membrane fluidity. To prevent membrane rigidity caused by curcumin accumulation, unsaturated fatty acids such as oleic acid and palmitoleic acid were added to the culture media which also promoted curcumin biosynthesis. Shake flask of the final strain resulted in 1.46 mM (537.8 mg/L) of curcumin from 4 mM of ferulic acid. Nevertheless, after all these modifications, the titers obtained are in the same range of the titers obtained by Rodrigues et al. (2020) (563.4 mg/L, from 3 mM of ferulic acid) that did not perform chassis modifications and used DCS and CURS enzyme instead of CUS. This demonstrates that DCS and CURS may be a better option than CUS to produce curcuminoids in *E. coli*.

Other Organisms

Until now, *E. coli* has been the main biological chassis used for the production of curcuminoids. However, in the last years, other microorganisms have been explored for the same purpose. The filamentous fungi *Aspergillus oryzae* was the first organism to be engineered to produce curcuminoids after *E. coli* (Kan et al. 2019). In a first attempt, the *O. sativa* CUS, 4CL from *L. erythrorhizon* and PAL from the yeast *Rhodotorula rubra*, a multi-substrate PAL capable of producing *p*-coumaric

acid/cinnamic acid from tyrosine/phenylalanine were expressed. However, the engineered strains were not capable of producing any curcuminoids. On the other hand, when feruloyl-N-acetylcysteamine (feruloyl-NAC), a feruloyl-CoA analogue, was added to the agar plates, curcumin was produced. On the other hand, bisdemethoxycurcumin was not produced when *p*-coumaroyl-NAC was supplemented. The researchers postulated that *p*-coumaroyl-NAC might be degraded by endogenous enzymes since its consumption was observed. Therefore, the authors postulated that mutants did not produce curcumin in the initial test due to a failed functional expression of 4CL. Hereupon, they constructed a recombinant *A. oryzae* strain expressing only CUS under control of the *amyB* promoter. The *amyB* promoter is a strong, maltose-inducible promoter usually used to express recombinant genes in *A. oryzae*. The produced curcumin was found to be accumulated in the mycelium. The yield obtained was 64.1 μg/plate, representing a conversion rate from feruloyl-NAC to curcumin of 9%. In order to increase the production, the authors attempted to increase the endogenous supply of the curcuminoid extender molecule malonyl-CoA. For that purpose, the genes for acetyl-CoA hydrolase (ACH) (responsible to convert the acetyl-CoA into acetate), serine protein kinase (snfA) (capable of inhibiting ACC via phosphorylation), and palmitoyl-CoA ligase (faaA) (involved in malonyl-CoA metabolism) were individually disrupted in the recombinant strain. In addition, the native ACC gene was also overexpressed through the replacement of the native promoter by *tef1* promoter which is a strong and constitutive promoter. The deletion of snfA resulted in the highest yield, increasing the production to 151 μg/plate. The other disrupted strains also had increments on curcumin production, while ACC overexpression did not generate significant results. To increase further the curcumin titers, two genes involved on the regulation of ergosterol biosynthesis were deleted (sterol regulatory element-binding protein (SREBP) and SREBP cleavage-activating protein (SCAP)). The downregulation of ergosterol served to prevent the acetyl-CoA deviation to ergosterol biosynthesis. The final engineered strain increased the production of curcumin to 404.2 μg/plate. The authors confirmed that indeed the malonyl-CoA levels increased in the final strain which led to a higher curcumin production. This work also represented the first report of the production of a plant secondary metabolite in the fungi *A. oryzae*.

Some microorganisms have reported capacity to metabolize aromatic compounds such as the hydroxycinnamic acids, the precursors of curcuminoids. The metabolic pathways present in these microorganisms may share common reactions to the synthesis of curcuminoids. Hereupon, instead of developing mutant microorganisms carrying a complete curcumin artificial pathway it may be employed a microbe with some of the desirable reactions already available on it. This can drastically reduce the number enzymes required to be expressed. Moreover, model microbes such as *E. coli* do not naturally have the robustness traits desired for industrial applications such as high solvent and pH toleration (Mukhopadhyay 2015). Therefore, Incha et al. (2020) used the bacteria *Pseudomonas putida* to produce the *p*-coumaric acid-derived curcuminoid, bisdemethoxycurcumin. *P. putida* has known ability to catabolize ferulic and *p*-coumaric acids. The first reaction of this catabolic pathway starts with the condensation of the phenylpropanoids with a CoA molecule, a similar reaction to the one performed by 4CL

in plants, that in *P. putida* is catalyzed by feruloyl/*p*-coumaroyl-CoA synthetase (Fcs). The following reaction is carried by enoyl-CoA hydratase lyase (Ech) to form vanillin. To produce bisdemethoxycurcumin in *P. putida*, the authors first studied the optimal conditions to express a type III PKS addressing the optimal glucose concentration to maximize the synthesis of malonyl-CoA. Next, to prevent *P. putida* from consuming *p*-coumaroyl-CoA, Ech gene was knocked out. The native Fcs gene was then over-expressed using an arabinose-inducible *araBAD* promoter. This engineered strain, in the presence of *p*-coumaric acid, had a relatively high lag time suggesting that accumulation of *p*-coumaroyl-CoA was toxic to the cells. To avoid that, instead of overexpressing Fcs, the Fcs expression was only relied on its native chromosomal expression. To synthesize bisdemethoxycurcumin, CUS from *O. sativa* was expressed in a plasmid also under control of araBAD promoter. The engineered strain was grown during 72 h to induce CUS expression. After 72 h, the cells were harvested and incubated in a media with *p*-coumaric acid for another 72 h. Interestingly, when 10 mM of *p*-coumaric acid were added, the production levels were very low (0.1 mg/L). However, using half of the substrate concentration (5 mM), the production reached 2.15 mg/L, once again suggesting the toxicity of *p*-coumaroyl-CoA, or even *p*-coumaric acid. To extract bisdemethoxycurcumin throughout the fermentation the authors used oleyl alcohol. In the end, the authors concluded that more genetic modifications are still required to avoid the observed intermediary toxicity. Nevertheless, the natural biological characteristics of *P. putida* (high solvent toleration, broad carbon source metabolisms, endogenous phenylpropanoid activation capacity) make it an attractive host to produce curcumin mainly from organic wastes such as lignocellulosic feedstocks (rich in *p*-coumaric/ferulic acids) and contributing to the circular economy.

Due to the reported antimicrobial activity of curcumin (Tyagi et al. 2015), its production in microorganisms seems to be contradictory since high concentrations of this compound could lead to cell death or affect the microbial growth thus limiting production yields. However, some microorganisms have evolved to adapt to extreme conditions. Consequently, the use of a high-tolerant microbe as a host chassis to produce curcumin may lead to high production titers. The oleaginous yeast *Yarrowia lipolytica* has reported high tolerance to phenolic compounds (Konzock et al. 2021). Therefore, Palmer et al. (2020) constructed a recombinant strain for bisdemethoxycurcumin production. For that purpose, a previously engineered *Y. lipolytica* strain overexpressing Pex10p, a peroxisome biogenesis factor, was used as background. Although Pex10p is a structural protein associated with the formation of peroxisomes, being not directly involved with acetyl-CoA biosynthesis, the engineered strain showed high capacity to synthesize acetyl-CoA relatively to the wild type. Furthermore, the native ACC was overexpressed to increase the malonyl-CoA concentration synthesized from acetyl-CoA. To produce curcuminoids, the codon optimized versions of 4CL from *N. tabacum* and *O. sativa* CUS were expressed in this recombinant strain. Flask fermentations supplemented with 2 mM of *p*-coumaric acid led to the production of 0.17 mg/L of bisdemethoxycurcumin.

Recently, Rainha et al. (2021a) reported the production of curcumin from ferulic acid in the model yeast *Saccharomyces cerevisiae*. In this work, two different

pathways were tested: the first one composed by 4CL1 from *A. thaliana* and CUS and the other composed by the same 4CL in combination with DCS and CURS1 from *C. longa*. By supplementing 16 mg/L of ferulic acid, the yeast carrying the first pathway produced 88.8 μg/L of curcumin while the second pathway produced 1067.0 μg/L. This result demonstrates, once again, the higher efficiency of the two-enzyme system to produce curcumin. In addition, it was observed that the higher concentrations of ferulic acid (1 mM) resulted in non-detectable curcumin production, thus indicating a probable toxicity of this compound to the cells. Moreover, the 4CL enzyme was also replaced on the second pathway by feruloyl-CoA synthetase (FerA) from *Pseudomonas paucimobilis*. FerA, like Fcs, is a bacterial enzyme that is capable of catalyzing the same reaction as 4CL, however, with less energy requirements. While 4CL is an AMP forming enzyme, FerA is an ADP-forming enzyme (Masai et al. 2002; Shockey et al. 2003). The replacement of 4CL by FerA resulted in 1413.3 μg/L, a 1.3-fold improvement relatively to 4CL pathway. Moreover, to prevent ferulic acid consumption by *S. cerevisiae*, the gene *fdc1*, encoding ferulic acid decarboxylase was deleted. The curcumin biosynthetic pathway was introduced in this strain yielding the production of 2.7 mg/L of curcumin, a 1.9-fold improvement on curcumin production.

As demonstrated, up to date, the curcumin production in microorganisms was mainly achieved in the model bacteria *E. coli*. More recently, other organisms have started to be explored. This newly explored microorganisms might bring new advantages for the microbial production of curcumin at industrial scale such as the high pH and solvent tolerance. In addition, the use of a eukaryotic chassis such as *S. cerevisiae* might facilitate the correct expression of plant genes due to its ability to perform posttranslational modifications. However, the use of other organisms is still very little explored, and the production yields are far from the ones obtained in *E. coli.*

Conclusion and Future Perspectives

Curcumin has shown over the last years a great potential to be employed as a natural therapeutic agent due to its wide biological activities. The microbial production of curcumin appears as a solution to overcome limitations associated with the plant extraction of this compound. The microbial production allows obtaining curcumin in a rapid, cheaper, and more environmentally friendly way. Remarkable advances have been made regarding the heterologous production of curcumin using *E. coli* as a biological chassis. However, more genetic modifications are still required to maximize the production yields by directing more carbon flux to the biosynthesis of curcumin. Nevertheless, curcumin production in *E. coli* reached interesting titers. Using ferulic acid as substrate the curcumin produced reached 563.4 mg/L with a 100% conversion yield. From tyrosine, 15.9 mg/L of curcumin were produced, and the de novo synthesis reached 3.8 mg/L. In addition, the use of coculture systems proved to be important to increase the curcumin titers in genetically modified *E. coli*. In the future, the engineering of the coculture systems must be evaluated, for

instance, by using a combination of different organisms. The combination of the lessons learned in the different studies appears as one of the next steps to increase production yields in *E. coli*. For example, the most efficient enzymes and pathways must be introduced in efficient tyrosine/phenylalanine *E. coli* overproducing strains for de novo synthesis of curcumin. In addition, the strain engineering to increase the malonyl-CoA pool must also be taken into account. The use of other microorganisms remains very little explored although their utilization as biological chassis might bring advantages over *E. coli*. However, more studies addressing the potential of these microorganisms are still necessary. *S. cerevisiae* has already been extensively studied to produce other polyphenols (e.g., resveratrol) yielding good results. Moreover, the use of renewable feedstocks to produce curcumin is not very much explored. This can be very important to monetize the process and contribute to circular economy.

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