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Review Article

Recent Advancement of Microbial Production of Curcuminoids and Its Industrial Applications: A Review

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Abstract: Turmeric (from *Curcuma longa*) is a yellow colored spice commonly used in daily diet. It has been extensively used in traditional medicine since ancient times to treat various nervous and disorder disease. A little was known about the biosynthesis curcuminoid in turmeric and identified the enzymes involved in the curcuminoid biosynthetic pathway and confirmed the involvement of the phenylpropanoid pathway in the production of these compounds in plants. Traditionally known curcumin has emerged as a modern biological regulator curcuminoids, agroclimatic and soil environmental variation are also influencing the curcumin synthase gene expression, which is correlated with curcumin yield in turmeric cultivars. Microbial production of curcuminoids is very promising and production yield can be improved by using synthetic biology approaches and metabolic engineering tools, to make heterologous production competitive with the current process of curcuminoid's extraction from plants. Type III polyketide synthases are responsible for the production of curcuminoids. Among those DCS and CURS enzymes have been used to synthesize curcuminoids. Synthetic biology and metabolite engineering approaches have generated microbial cell factories that can allow the for the mass production of pharmaceutically and nutraceuticals important microbial metabolites in an environmentally friendly and efficient way. Considering a wide pharmaceutical, industrial and health beneficial applications of curcuminoids, this review focused on microbial production of curcuminoids and substrate recognizing and regulatory mechanism of curcuminoid synthase and obtain the mutant enzymes using mutagenesis study and synthetic biology approaches.

Keywords: Curcuminoids, *Curcuma Longa*, Polyketides, Turmeric

1. Introduction

Turmeric is a yellow coloured spice commonly used in daily food in Asia. Turmeric has been a subject of extensive research for many years and its therapeutic potential against several diseases including cancer, cardio vascular disease, lung and liver diseases etc. has been studied. It is shown to have some preventive as well as therapeutic effect in diseases without causing any toxicity [1]. Curcumin is the major bioactive component present in turmeric plant.

Curcuminoids are polyphenolic compounds, more specifically (C6-C7-C6), isolated from the rhizome of turmeric (*Curcuma longa Linn*.). The rhizome of turmeric contains a mixture of curcuminoids in which curcumin, also known as diferuloyl-methane, is the main active chemical constituent [2]. Curcuminoids are abundantly present in *Curcuma longa* but also occur in other Curcuma species. Other curcuminoids present in rhizome of turmeric include demethoxycurcumin and bisdemethoxycurcumin. However, since different Curcuma species are difficult to distinguish due to their similar morphologies and their different names given in Latin and by Japanese, Chinese, and Indian cultures, pharmacological studies are very scarce [3]. Therefore, the word curcumin is used to represent all the three curcuminoids

found in the turmeric extract.

Type III polyketide synthases (PKS) are responsible for the production of curcuminoids in plants and consist of two phenylpropanoid units chemically derived from the amino acid phenylalanine and connected by a central carbon unit derived from malonyl-coenzyme A (malonyl-CoA). In the last decade, several advances have been made in curcuminoid biosynthesis as a result of the identification of DCS and multiple CURSs from *C. longa* and *O. sativa*. However, DCS and CURS enzymes have been used to synthesize curcuminoids [4].

Very little was known about curcuminoid biosynthesis in turmeric and identified the enzymes in the curcuminoid biosynthetic pathway and confirmed the involvement of the phenylpropanoid pathway in the production of these compounds in plants. The first enzyme identified was phenylalanine ammonia lyase (PAL), which is involved in amino acid metabolism, but also in the biosynthesis of plant natural products, as the branch point between primary and secondary metabolism [5].

Among the type III Polyketide synthase, diketide-CoA synthase (DCS), which catalyses the formation of feruloyl-diketide-CoA from feruloyl-CoA and malonyl-CoA. Curcumin synthase 1 (CURS1), catalyses two reactions. First, it catalyzes the hydrolysis of feruloyl-diketide-CoA in a β-keto acid. Second, using the β-keto acid and other molecule of feruloyl-CoA, it catalyzes the formation of curcumin. β-Keto acid is not detected in vitro because it is not released from the enzyme [6].

Both DCS and CURS1 accept p-coumaroyl-CoA, but at low efficiency, and are capable of synthesizing bisdemethoxycurcumin. The asymmetric curcuminoid desmethoxycurcumin can also be produced from p-coumaroyldiketide-CoA and feruloyl-CoA or from feruloyl-diketide-CoA and p-coumaroyl CoA. DCS and CURS1 share 62% amino acid sequence identity and a conserved Cys-His-Asn catalytic triad [7].

Plants accumulate low quantities of curcuminoids over very long growth periods and are difficult and expensive to isolate and hard to synthesize chemically. These reasons, in combination with the wide benefits of curcuminoids and their application potential, have led to an increased interest in the last 10years, and attempts to implement the heterologous biosynthesis of curcuminoids have been reported [8].

Heterologous production of curcuminoids in microorganisms is highly advantageous, since they can grow on a very cheap and inexpensive substrates compared with plants, easier to manipulate and have very rapid production cycles, allowing curcuminoids to be produced faster and maybe in larger amounts [9].

Microbial production of curcuminoids is very promising and curcuminoid production yield can be improved by using synthetic biology approaches and metabolic engineering tools, to make heterologous production competitive with the current process of curcuminoid's extraction from plants. Considering industrial and health applications of curcuminoids. In this review focus on the microbial production of curcuminoids.

2. Turmeric (*Curcuma longa L*) and Curcuminoids

Curcuma longa L. (Turmeric), a rhizomatous herbaceous perennial plant belonging to the *Zingiberaceae* family, is a native to Southeast Asia and is now widely cultivated in the tropical and subtropical regions of the world [10].

During the last few decades, turmeric has gained global recognition for its medicinal importance after many studies that were conducted to understand its medicinal properties, yielded exciting results [22]. Turmeric has been used for centuries as edible dye and in traditional medicines to treat numerous diseases. The most important biologically active constituent and the principal curcuminoid of turmeric is the polyphenol curcumin.

Polyketides, such as curcuminoids, flavonoids, phloroglucinol, stilbenes, pyrones, etc., represent an important class of structurally diverse and biologically important secondary metabolites in nature [11]. Curcuminoids are natural phenylpropanoids from the plant *Curcuma longa Linn*. Its rhizome contains a mixture of curcuminoids, with curcumin, demethoxycurcumin and bisdemethoxycurcumin present in higher amounts. These compounds present in turmeric have long been used in traditional food and medicine [12]. Despite their numerous benefits to human health, curcuminoids have poor bioavailability and their natural abundance is low, thus making their heterologous biosynthetic production very interesting. Recent evidence suggests that many of its beneficial effects are attributed to the presence of compounds such as curcumin, curcuminoid related compounds and various diarylheptanoids [13].

2.1. Curcuminoid Biosynthesis from Curcuma Longa

At present, traditionally known curcumin has emerged as a modern biological regulator curcuminoids. Agroclimatic and soil environmental variation are also influencing the curcumin synthase gene expression, which is correlated with curcumin yield in turmeric cultivars [14].

The gene expression levels of the multiple curcumin synthases are capable of curcuminoid synthesis which might influence the composition of curcuminoids in different cultivars of turmeric. Genomic and data mining-based studies in turmeric have identified major multiple curcumin synthase enzyme genes involved in curcuminoid biosynthesis pathway viz diketide-CoA synthase (DCS), curcumin synthase 1 (CURS1), curcumin synthase 2 (CURS2) and curcumin synthase 3 (CURS3) [7]. Despite the tremendous worldwide importance of turmeric baring few reports on variation in curcumin content at different cultivated areas not much has been reported yet on the expression profile of CURS gene across different genotypes and during different harvesting periods [14].

Polyketides are structurally and functionally diverse secondary metabolites that are biosynthesized by polyketide synthases (PKSs) using acyl-CoA precursors. Polyketides are synthesized by a family of multifunctional enzymes known as

polyketide synthases (PKSs). Based on the structures of the polyketide products, as well as biochemical features of the PKSs, PKSs are currently classified into types I, II, and III subgroups. Type I PKSs are mega synthases in which catalytic domains are typically found in a single polypeptide [15]. Type II PKSs known as bacterial aromatic polyketide synthase are composed of mostly dissociated, monofunctional enzymes that function repeatedly in the synthesis of a poly-β-ketone backbone [16]. Type II PKSs are involved in the synthesis of aromatic polyketides, such as the aglycons of actinorhodin [17].

Type III PKSs, such as chalcone synthase are homodimeric PKSs that synthesize smaller aromatic compounds in bacteria, fungi, and plants. Products synthesized by the various types of PKSs can undergo different sets of post-PKS modifications by decorative enzymes encoded in the biosynthetic pathways, such as cyclases, oxygenases, glycosyltransferases, etc., to afford the structurally diverse natural products.

Polyketides are always responsible for important secondary metabolites biosynthesis involved in plant defense and signal transduction [18]. Studies have shown that the expression of PKSs was regulated by biotic and abiotic elicitors including UV and light stresses, mechanical wounding and plant hormones. Investigations on type III PKSs selectively help plant to adapt to the changing environmental conditions and understand the mechanism of secondary metabolites biosynthesis [19].

Advancements in the polyketide field led to the consensus that polyketides are typically biosynthesized through successive decarboxylative condensations of coenzyme A (CoA)-derived units, into a complex polycyclic multi-carbon compound containing keto or hydroxyl groups [20].

The tremendous importance of turmeric worldwide as a major spice food and as a drug, the molecular and functional analyses of its medicinal value are hampered by lack of tools such as ESTs and ordered genomic contigs. However, things have been changed recently due to the development of turmeric EST database by David Gang's group (ArREST), which has provided a platform for elucidating the curcuminoid biosynthetic pathway in *C. longa* [7], and points toward the role of two Type III Polyketide synthases (PKSs) in the biosynthesis of curcumin. The discovery of Type III PKS has opened up the field of investigation of various pathways leading to the biosynthesis of aromatic polyketides.

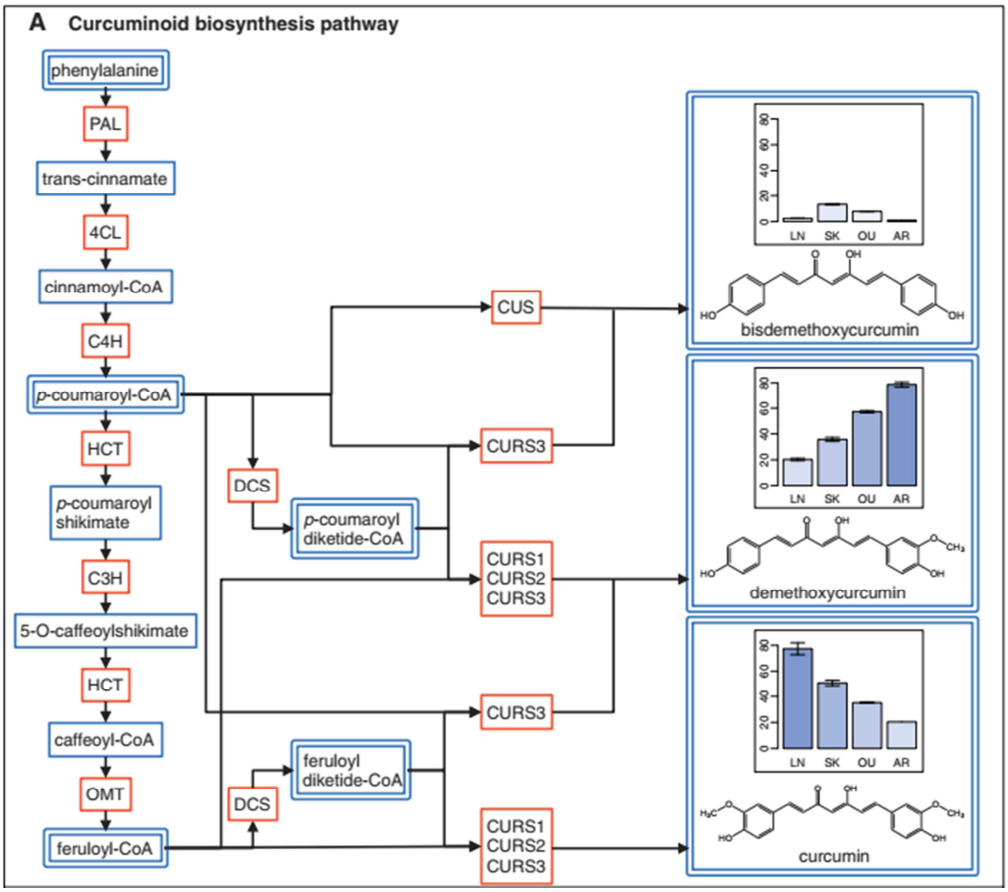

Figure 1. *Curcuminoid biosynthesis pathway from phenylalanine to curcuminoids [21].*

2.2. Microbial Curcuminoid Synthase for Curcumin Production

Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* possesses diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities [22]. In addition to its wide variety of biological and pharmacological activities, curcumin, the natural pigment that gives the spice turmeric its yellow color, has long been used as

a traditional Asian medicine and a food additive. Curcumin, bisdemethoxycurcumin and dicinnamoylmethane are chemically bis-α, β-unsaturated β-diketones and called curcuminoids. The intriguing structure of curcuminoids raised the question of whether a single type III PKS catalyzes all the steps in curcuminoid synthesis or only some of them, such as β-keto acid synthesis. Development of synthetic biology and metabolic engineering techniques provides a great opportunity to synthesize curcumin in microorganisms. Some progresses have been made in engineered biosynthesis of plant natural products such as coumarins [23], in microorganisms.

The biosynthesis of curcumin has also been studied. Two type III polyketide synthases (PKSs), diketide-CoA synthase (DCS) and curcumin synthase (CURS), are involved in the biosynthesis of curcuminoids in the herb *Curcuma longa* (Katsuyama et al. 2010). Compared to the collaborative actions of two type III PKSs in *C. longa*, one single type III PKS from *Oryza sativa*, curcuminoid synthase (CUS), was found to synthesize curcuminoids. With supplementation of different carboxylate precursors, curcumin and several analogs were synthesized in *E. coli* [24].

The biosynthesis of curcuminoids using radioactive tracers suggested that they were derived from the phenylpropanoid pathway. Phenylalanine ammonia lyase (PAL) is the first enzyme in the biosynthesis sequence that is involved in amino acid metabolism and the transition point between primary and secondary metabolism. Two type III polyketide synthase (PKSs) enzymes were isolated from the herb *C. longa*, curcumin synthase (CURS) and diketide-CoA synthase (DCS) [7]. Diketide-CoA synthase (DCS) catalyzes the reaction of feruloyl-CoA with malonyl-CoA to form feruloyl-diketide-CoA.

Unlike plant type III PKSs, bacterial type III PKSs usually share less than 50% identity with each other and with plant type III PKSs. Bacterial type III PKSs were first characterized from the Gram-positive *Streptomyces griseus*. RppA showed 30% identity to plant chalcone synthase, and was able to utilize malonyl-CoA as a starter and catalyze the decarboxylative condensation of another four units of malonyl-CoA to form a pentaketide, which undergoes dual cyclization reactions and decarboxylation to generate1,3,6,8-tetrahydroxynaphthalene (THN) (Funa et al. 1999). Recently, a heptaketide pyrone synthase from the soil bacterium *Rhizobium etli* (RePKS) was characterized. Unlike the *R. palmatum aloesone* synthase (ALS), RePKS undergoes dual cyclization reactions and generates a heptaketide pyrone 43 from one unit of acetyl-CoA and six units of malonyl-CoA [25].

The first fungal type III PKS was characterized from the filamentous fungus, *N. crassa*, and showed approximately 26% identity to other plant and bacterial PKSs [26]. Although the iterative type I PKSs synthesizes most fungal polyketides such as lovastatin, fungal type III PKS genes were identified recently in the past decade. Using the alfalfa CHS amino acid sequence as a reference, four putative type III PKSs, namely chalcone synthase-like A, chalcone synthase-like B, chalcone synthase-like C, and chalcone synthase-like D (CsyA, CsyB, CsyC, and CsyD), were found in the filamentous fungus, *Aspergillus oryzae*. Similar CHS-like genes were also identified in *Fusarium graminearum* and *Neurospora crassa* (one homolog each), *Magnaporthe grisea* and *Podospora anserina* (two homologs each), *Phanerochaete chrysosporium (*three homologs each) [27].

Curcumin can be produced by *E. coli* using the biosynthetic pathway. *E. coli* has been the host of choice for the expression of recombinant proteins given its ability to produce high quantities at low costs. However, in large-scale productions, chemical inducers, such as isopropyl β-D-1-thiogalactopyranoside (IPTG), can be expensive and toxic and their presence in waste effluents or in the final product must be eliminated, especially in the production of pharmaceutical-grade proteins and other products intended for human use [28].

2.3. Pharmaceutical Significance of Curcumin

Turmeric powder obtained from rhizomes of *C. longa* is extensively used as a spice, food preservative, natural dye in food industry and in cosmetics and drugs [29]. The rise in demand for high-curcumin, turmeric varieties in the food, pharmaceutical and cosmetic industries is presently due to its medicinal values. However, curcumin accumulation often exhibiting spatio-temporal and environmental variation [14], limits its export potential.

Chinese medicines recommend turmeric in treatment for a large number of disorders and diseases. Curcuminoid, a phenylpropanoid derivative, is a mixture of curcumin (50–60% of the curcuminoids), demethoxycurcumin and bisdemethoxycurcumin that imparts yellow colour to turmeric.

The medicinal properties of curcuminoids such as anti-inflammatory, anti-oxidant, antimutagenic, anti-diabetic, anti-bacterial, hepatoprotective and expectorant are reported extensively. It is also well known in treating conditions ranging from arthritis and inflammation to Alzheimer's disease and cancer [30].

Curcumin has been shown to inhibit a number of different molecules involved in inflammation including phospholipase, lipooxygenase, COX-2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, MCP-1, interferon-inducible protein, tumour necrosis factor, and interleukin-12 [31].

Curcumin, the most common antioxidant constituent of *Curcuma longa* rhizome extract, was reported to enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver. The ethanolic extract of *Curcuma Longa* rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg and 500 mg/kg, and the protective effect was dose dependent. The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification. The volatile oils and curcumin of Curcuma longa exhibit potent anti-inflammatory effects [32].

Over the past few years various research has shown that curcumin targets multiple signalling pathways and regulates the expression of several transcription factors, inflammatory cytokines, enzymes associated with inflammation and cancer, growth factors, receptors, adhesion molecules, antiapoptotic proteins, and cell cycle proteins [33].

Studies have proven bisdemethylcurcumin (BDC) is more potent as an anti-inflammatory agent as indicated by suppression of TNF induced NF-κB activation, more potent as an anti-proliferative agent, and more potent in inducing reactive oxygen species (ROS). Hispolon analogues, which lacks one aromatic unit in relation to curcumin, also exhibited enhanced anti-inflammatory and anti-proliferative activities. The beneficial effect of curcumin (anti-inflammatory compound) in sepsis appears to be mediated by the upregulation of PPAR-γ, leading to the suppression of pro inflammatory cytokine, TNF-α expression and release [34].

Curcumin has been found to possess anticancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis. Curcumin has shown anti-proliferative effect in multiple cancers, and is an inhibitor of the transcription factor NF-B and downstream gene products (including c-myc, Bcl-2, COX- 2, NOS, Cyclin D1, TNF-a, interleukins and MMP-9). In addition, curcumin affects a variety of growth factor receptors and cell adhesion molecules involved in tumour growth, angiogenesis and metastasis [35].

Turmeric rhizome powder is very useful in treatment of diabetes mellitus. The ingestion of 6g *Curcuma longa* increased postprandial serum insulin levels, but did not seem to affect plasma glucose levels or GI, in healthy subjects. The results indicate that *Curcuma longa* may have an effect on insulin secretion [36]. The active principles in the rhizome of Turmeric plant viz; curcuminoids lower lipid peroxidation by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase at higher levels. Antioxidant properties of *curcuma longa* are due to curcumin and its three derivatives (dimethoxy curcumin, bisdemethoxycurcumin and diacetyl curcumin) [37].

Curcuma longa is known to contain curcuminoids, glycosides, terpenoids, and flavonoids. Maximal inhibition of the enzyme Human Pancreatic Amylase (HPA) was obtained with Curcuma longa isopropanol extract and acetone extract. This inhibitory action on HPA causes reduction in starch hydrolysis leading to lowered glucose levels [38].

2.4. Advanced Techniques for Improving Curcumin Biosynthesis

Combinatorial biosynthesis is a new tool in the generation of novel natural products and for production of rare and expensive natural products. The concept of combinatorial biosynthesis involves genetically engineered enzymes that are used to make novel, complex compounds as drug development candidates. Two types of enzyme systems operate like an assembly line, produce a diverse group of natural products, and have proven to be useful for generation of compound libraries. These systems are composed of multiple modules, in which an individual module consists of either a polyketide synthase (PKS) or a non-ribosomal peptide synthetase (NRPS). Each module has a specific set of catalytic domains that determine the structure of the metabolic product [39].

Because of the rapid development in molecular biological techniques and the explosive accumulation of the genome information from a variety of organisms [40], combinatorial biosynthesis now has a wider definition. Bioinformatic prediction of the catalytic property of a single gene product and novel chemical entities directed by a gene cluster in the huge genome databases facilitates combining metabolic pathways to generate artificially designed biosynthetic pathways for production of targeted compounds in different organisms. As a consequence, the host organisms provide precursors from their own primary and secondary metabolism that are converted to the expected secondary product through the expression of foreign genes.

Downstream modifying enzymes are then used to obtain flavonoids and other bioactive substances. Recent studies in the engineering and structural characterization of polyketide synthases (PKSs) have facilitated the use of target enzymes as biocatalysts to produce novel functionally optimized polyketides, which can serve as potential drug leads [41].

There is an increasing interest in curcumin and other curcuminoids due to their several recognized beneficial effects, synthetic biology and metabolic engineering constitute good approaches to improve their availability. The discovery of CUS from *O. sativa* allowed the production of curcuminoids in *E. coli* [42].

Recombinant *E. coli* strains have been developed for the production of curcuminoids. The *E. coli* strain was engineered with genes for diketide-CoA synthase (DCS) and curcumin synthase (CURS1), isolated from *C. longa* and the gene for 4-coumaroyl-CoA ligase isolated from *Arabidopsis thaliana* [43], produced curcumin in *E. coli* through heat induction using the ibpA and dnaK heat shock promoters.

3. Major Enzymes Involved in Curcumin Production

Polyketides are widely distributed among prokaryotic and eukaryotic organisms. They are named polyketides because they all arise from polyketo chains. They are biosynthesized through a serious of decarboxylative condensation reactions catalysed by a group of enzymes called polyketide synthases (PKSs), which divide further into three main groups: Type I, Type II and Type III PKSs [44]. Type III PKSs have, compared to the other types, drawn much attention, as they are smaller and simpler but still maintain the catalytic function of polyketide chain elongation and cyclization.

Type III polyketide synthases (PKSs) are distributed in diverse organisms, including plants, fungi, and bacteria, and are responsible for the syntheses of various biologically and pharmaceutically important compounds. The universal

reactions catalysed by type III PKSs are as follows: (i) transfer of acyl-CoA (called the starter substrate) to the catalytic Cys, resulting in an acyl-PKS complex; (ii) decarboxylation of malonyl-CoA (called the extender substrate) to form an active anion; (iii) Claisen condensation of the active anion with the acyl moiety attached to the catalytic Cys to generate an acyl-CoA that has an additional two-carbon unit; (iv) extension of the polyketide chain by repeating reactions i–iii; and (v) cyclization of the resultant polyketide chain and release from the enzyme [45].

The reaction by this enzyme, named CUS (curcuminoid synthase), begins with the thioesterification of the thiol moiety of Cys-174 by the starter molecule, p-coumaroyl-CoA. Decarboxylative condensation of the first extender substrate, malonyl-CoA, onto the thioester of p-coumarate results in the formation of a diketide-CoA intermediate. Subsequent hydrolysis of the intermediate yields a β-keto acid, which is then joined to the Cys-174-bound p-coumarate by decarboxylative condensation to form bisdemethoxycurcumin. The incorporation of an additional phenylpropanoid unit violates the traditional head-to-tail model of polyketide assembly, i.e., a switchover of the role of a diketide intermediate from a growing chain to an extender unit the growing diketide intermediate is hydrolysed to a β-keto acid that subsequently serves as the second extender to form curcuminoids. CUS also accepts other phenylpropanoid-derived CoA esters, such as cinnamoyl-CoA and feruloyl-CoA as a substrate to produce dicinnamoylmethane and curcumin.

Numerous type III PKSs have been identified till date, and the range of reactions and product classes discovered is due to differences in intramolecular cyclizations of the linear polyketide intermediate, the number of polyketide extension steps involved, and the choice of starter and extender CoAs used. The diverse polyketide scaffolds catalysed by type III PKSs can be classified into chalcone, stilbene, pyrone, chromone, stilbene carboxylate, bibenzyl, benzalacetone, curcuminoid, benzophenone, biphenyl, acridone, quinolone, phloroglucinol, resorcinol, and naphthalene families respectively [45]. By characterizing various type III PKSs, we can understand reaction mechanisms better and thus, direct the generation of unnatural polyketides using synthetic enzymology [46].

The most important amino acid residues for type III PKSs are Cys-164, His-303, and Asn-336, which create a catalytic triad. Cys-164 in CHS forms a thioester bond with *p*-coumaroyl-CoA (starter substrate) and a polyketide intermediate. His-303 and Asn-336 are responsible for acyl-PKS complex formation and the decarboxylative condensation of malonyl-CoA [11].

Type III PKSs showing novel and interesting catalytic properties in various organisms, a type III PKS from *O. sativa*, which catalyzes the synthesis of curcuminoids from p-coumaroyl-CoA as a starter and malonyl-CoA as an extender [47].

Type III PKSs are homodimeric enzymes and are structurally simpler and mechanistically different from type I and II PKSs. The relative simplicity, versatility, and unusually broad substrate specificity of type III PKSs make them ideal candidates for the engineering of biocatalysts, and in doing so, access to bioactive polyketide compound libraries can be readily made available [48] and previously only found in plants, but in the past 15 years, more bacterial type III PKSs were characterized [49], fungal type III PKSs were only recently discovered [27]. Unlike the type I and II PKSs, type III PKSs generally utilize CoA thioesters as substrates and do not entail the involvement of ACP domains. Furthermore, type III PKSs are able to accomplish an entire series of decarboxylative condensations and cyclization reactions in a single active site [50].

Type III PKSs share a similar three-dimensional αβαβα fold and a conserved Cys-His-Asn catalytic triad, subtle differences in the active site cavities result in diverse substrate specificities and different product profiles. Notably, manipulation of the biosynthetic reaction by the incorporation of unnatural substrates has led to the generation of novel polyketide libraries. For instance, when both the starters and the extenders were substituted with non-physiological substrates, CHS from the herb *Scutellaria baicalensis* was able to catalyze the formation of unnatural polyketides [4].

4. Site Directed Mutagenesis

Crystallographic analyses of the plant type III PKSs (alfalfa CHS [37], *O. sativa* CUS [51], have enabled the understanding of the basis of starter molecule selectivity, chain elongation, and cyclization of the linear polyketide intermediate. These structural studies have provided insights into the mechanistic details of type III PKSs, illustrating how subtle modifications in the active site cavity can expand the biosynthetic repertoire and result in the generation of diverse products. Manipulation of active site residues by random mutagenesis [52], and/or site-directed mutagenesis has proved useful in the understanding of and subsequently, engineering of the substrate specificity and activity of type III PKSs. Mutagenesis studies with the aim of broadening substrate specificity have been carried out in plant-derived type III PKSs [53].

5. Crystallography

Crystallography studies have shown that type III PKSs share a common three-dimensional structure and catalytic machinery which contains a conserved Cys-His-Asn catalytic triad to produce structurally diverse polyphenols with remarkable biological activities [45]. Substitution of non-catalytic residues located at the substrate binding, CoA binding and cyclization pockets can change the preference of substrates, number of condensation as well as cyclization manner and thus have impacts on their product selectivity [54].

6. Conclusion

Bioactive secondary metabolites obtained from turmeric

plants like flavonoids and curcuminoids have shown health benefits. They have been used in nutraceuticals and functional foods. Those secondary metabolites produced by plants in a limited amount and under some specific environmental stress. Metabolic engineering and synthetic biology approaches have generated microbial cell factories that can allow the largescale production of pharmaceutically and nutraceuticals important metabolites in an environmentally friendly and efficient way. One of the advantages of genetically engineered microorganisms is the ability to produce pure bioactive compounds that do not require extensive downstream processing. In addition, they offer the ability to produce novel, non-natural derivatives with potentially better efficiency and activity. Thus, in this review we focused on microbial production of curcuminoids and substrate recognizing and regulatory mechanism of curcuminoid synthase and obtain the mutant using metabolic engineering and synthetic biology approaches.

Author Declarations

I declare that this manuscript is original has not been published before and is not currently being considered for publication elsewhere. I know of no conflict of interest associated with this publication

Abbreviations

CURS: Curcumin synthase
DCS: Diketide-CoA synthase
PAL: Phenylalanine ammonia lyase
PKS: Polyketide synthases
UV: Ultraviolet

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