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Review Article

Synthetic Biology Approaches for the Production of Flavonoids

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Abstract

Flavonoids are phenolic carbon compounds, a class of plant secondary metabolites known for their unique properties such as antioxidant, anti-inflammatory, coloring agent, and many more. Due to their diverse properties, they are being utilized in the industrial sector at a very high pace. The conventional methods are limited to low amounts of biomass from the source. Synthetic biology offers a promising approach for enhancing the bioproduction of flavonoids due to its tremendous advantages over conventional extraction methods. This review focuses on recent developments in applying synthetic biology techniques specifically towards microbial synthesis of flavonoids. It provides a comprehensive overview of the recent advancements in synthetic biology approaches to optimize microbial systems for efficient flavonoid production. Emphasizing genetic engineering, metabolic engineering, and systematic design principles, these techniques represent an immense shift in bioproduction strategies. The insights presented contribute significantly to our understanding of the effectiveness of these methodologies in the production of flavonoids.

Keywords: Pathway Engineering, Metabolic Engineering, Biological Engineering, Chassis, Biosynthesis

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Introduction

The biosynthesis of flavonoids involves a series of enzymatic reactions that convert simple precursor molecules into complex flavonoid compounds. By understanding the biosynthetic pathways of flavonoids, researchers can manipulate these pathways using synthetic biology tools to enhance the production of specific flavonoids with desired properties. This intersection between flavonoid biosynthesis and synthetic biology holds great promise for the development of novel plant-based products with improved nutritional, medicinal, and industrial applications.[1] Flavonoids are not only essential for plants but also play a significant role in human health. They have a variety of beneficial properties, such as anti-cancer, anti-inflammatory, anti-fungal, anti-bacterial, and anti-mutagenic effects, as well as coloring, flavoring, and preservative abilities. Due to these advantageous characteristics, flavonoids are widely used in various industries, including food and beverage, nutraceuticals, cosmetics, pharmaceuticals, and agriculture. They help protect food from light-induced quality deterioration.[2]

Flavonoids are used as natural insecticides, growth regulators, and biopesticides to provide crop protection, apparently improving crop yield and quality. The anti-inflammatory and antioxidant properties of flavonoids have made them highly desirable in the field of cosmetics.[3,4] They are commonly integrated into skincare products to protect the skin from environmental stresses and preserve its vitality. Furthermore, the pharmaceutical industry has harnessed the potential medicinal properties of flavonoids. Additionally, consuming flavonoid-rich foods presents a promising nutraceutical approach to combating diseases associated with a shortened lifespan[5] as they exhibit neuroprotective, anti-tumorous, and cardiovascular protective effects[6] (Figure 1).

Structure of Flavonoid

Flavonoids possess a C3-C6-C3 structural arrangement characterized by two phenyl aromatic rings; A and B, connected by an oxygen-containing heterocyclic pyrene C ring. These compounds are synthesized through two primary biosynthetic pathways: the phenylpropanoid and the polyketide pathway. The phenylpropanoid pathway is responsible for generating the C6-C3 phenylpropanoid skeleton. Based on the degree of saturation in their central (C) ring, flavonoids have been categorized into two groups: Saturated and Unsaturated. Examples of the former are flavanones, dihydroxyflavonols, and flavan-3-ols, while the latter encompasses anthocyanidins, flavones, flavonols, and isoflavones.[7] Furthermore, flavonoids are subcategorized into distinct classes, including flavones, flavonols, isoflavones, anthocyanins, chalcone, aurones, neoflavanoids, and their dihydrogen derivatives. Common sugars, such as Flavonoids 6,7 D-glucose, D-galactose, L-rhamnose, D-xylose, D-glucuronic

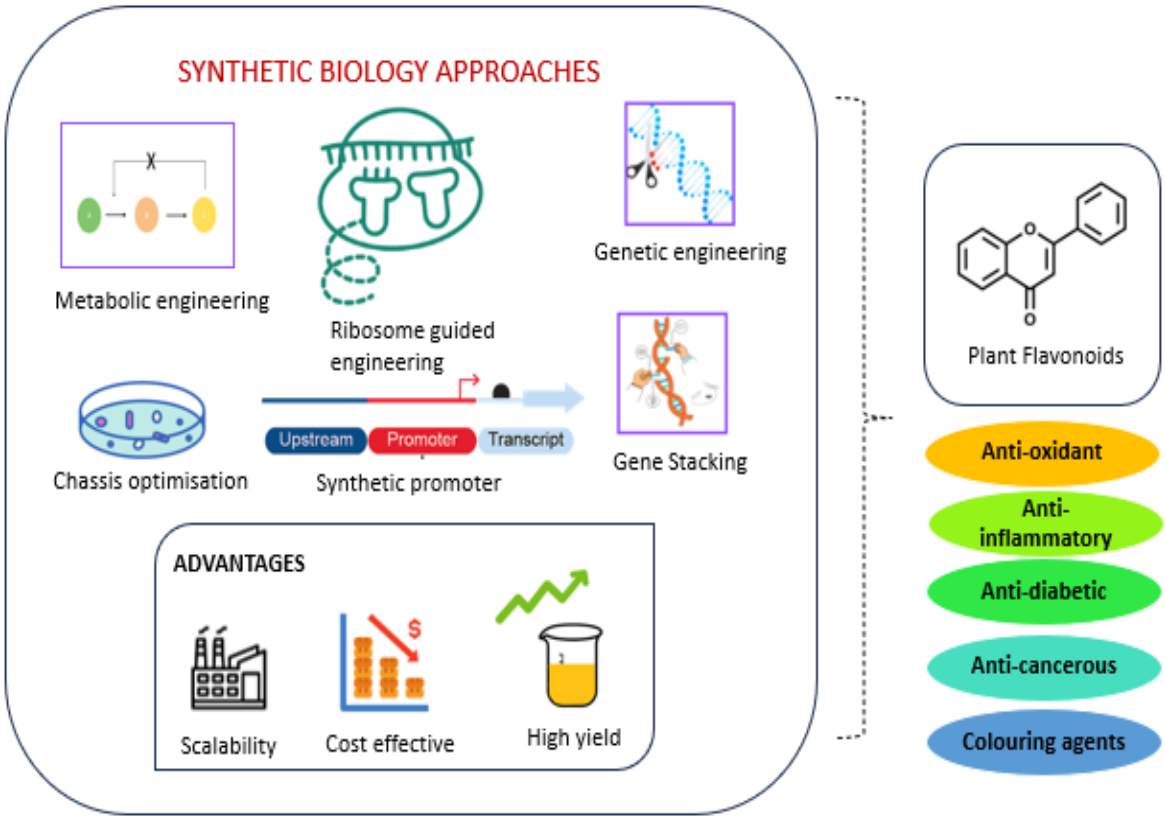

Figure 1. Schematic Representation of Synthetic Biology Approaches for the Production of Flavonoids.

acid, sophorose, rutinose, tangerine peel sugar, and gentianose, can be attached to flavonoids. The diversity in flavonoids achieved through the introduction of various functional group modifications like methylation, methoxylation, glycosylation, acylation, malonylation, and alteration of phenolic hydroxyl group positions[8,9] (Figure 2).

Flavonoids are synthesized via the phenylpropanoid pathway, a complex metabolic route that involves numerous enzymatic steps. This pathway initiates with the conversion of phenylalanine into cinnamic acid, catalyzed by the enzyme phenylalanine ammonia-lyase (PAL). Subsequently, cinnamic acid is transformed into p-coumaric acid through the action of cinnamate 4-hydroxylase (C4H). Further conversion of p-coumaric acid into caffeic acid is facilitated by the enzyme coumaroyl-CoA ligase (4CL). Caffeic acid, in turn, undergoes conversion to p-coumaroyl-CoA under the influence of hydroxycinnamoyltransferase (HCT), and finally, p-coumaroyl-CoA is transformed into naringenin chalcone through the enzymatic action of chalcone synthase (CHS).[10,11] Naringenin chalcone plays a pivotal role as the precursor for a large group of flavonoids, encompassing flavones, flavonols, and flavanones. The conversion of naringenin chalcone into distinct flavonoid types is orchestrated by specific enzymes with dedicated functions. For example, the production of flavones is mediated by flavone synthase (FNS), flavonol synthase (FLS) is responsible for generating flavonols, and chalcone isomerase (CHI) facilitates the production of flavanones.[12]

Conventional Techniques of Flavonoid Extraction

Conventional techniques for extracting flavonoids from plants include decoction, maceration, infusion, heat reflux, and percolation. The Soxhlet method,[13] with modern adaptations, has also been widely utilized. The conventional approaches typically employ solvents like water and ethanol, and in some cases, environmentally hazardous solvents such as methanol, acetone, acetonitrile, ethyl acetate, dichloromethane, hexane, and petroleum ether. These methods often operate at high temperatures, exceeding 100 °C, in open containers, leading to the evaporation of solvents and most of them are time-consuming, with extraction periods extending up to 15 days.[14] A variety of unconventional extraction methods, including mechanical, electromagnetic, electric forces, or enzymatic methods, have been introduced to overcome the limitations of traditional approaches. However, these alternative methods are challenging as they are often labor-intensive, requiring a series of pre-treatment steps[15] and residue separation steps,[16,17] along with prolonged extraction times.[18] Furthermore, these methods are unreliable for commercial production since they can only be used for the production of limited quantities. These constraints underscore the need for more efficient, sustainable, scalable, and cost-

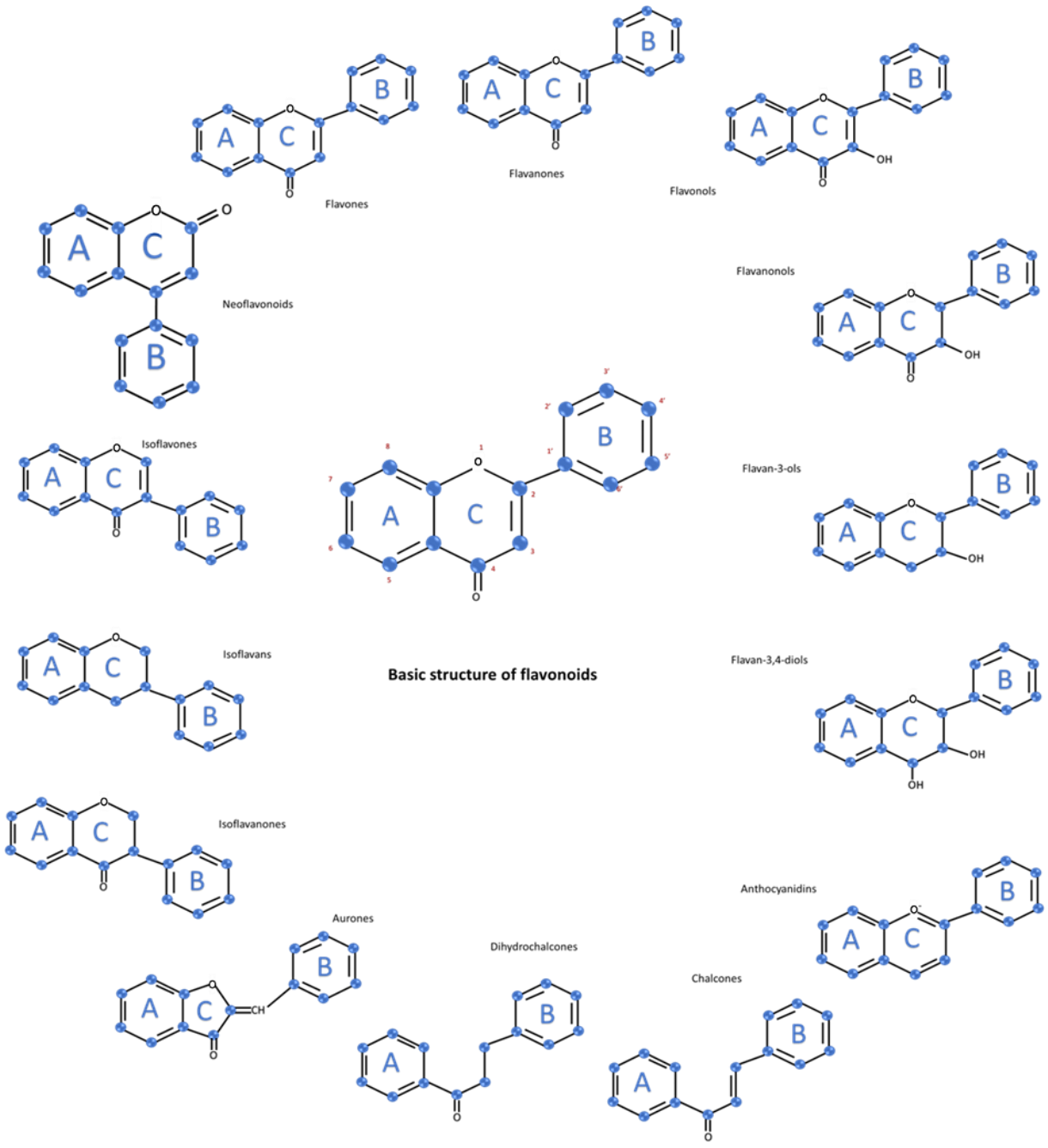

Figure 2. The Figure Depicts the Structure of the C3-C6-C3 Backbone of Flavonoids along with the Structures of Various Classes of Flavonoids.

effective techniques for the production of flavonoids.[19]

Flavonoid Synthetic Biology

Synthetic biology involves the intentional engineering of biological systems to introduce novel functionalities that do not occur naturally.[20] This process entails using standardized components, such as genes and proteins, and employing engineering principles to systematically design and construct these systems.[21] This engineering approach extends to flavonoid synthetic biology, where synthetic biology techniques are applied to enhance the biosynthesis of flavonoids within microbial systems. The advancement of these methodologies has significantly facilitated the identification and character ization of key enzymes involved in flavonoid biosynthesis, enabling the optimization of metabolic pathways and leading to enhanced efficiency in flavonoid production.[22]

Various microbial systems have been utilized as microbial cell factories for the production of flavonoids, including *Escherichia coli*,[23] *Saccharomyces cerevisiae*,[24] *Pichia pastoris*,[25] *Yarrowia lipolytica*,[26] and *Aspergillus niger*.[27] The selection of the appropriate microbial host depends on multiple considerations, such as the complexity of the biosynthetic pathway, the toxicity of intermediate compounds, and the availability of genetic manipulation tools.[28]

Synthetic Biology Approaches for the Production of Flavonoids

The field of synthetic biology has helped in the production of flavonoids within microbial systems through various strategies. Some of these approaches include pathway engineering, gene dosage tuning, and metabolic flux analysis. Key techniques include chassis optimization, optimization of gene expression levels, and the modification or deletion of non-essential genes to achieve optimal flavonoid production.

Chassis Optimization

Chassis optimization is a technique, in which the selection, design, and genetic engineering of host organisms are necessary to serve as a robust and reliable platform for constructing synthetic biological systems, including flavonoid production.[29] The key objective is to construct a standardized and reusable biological platform that possesses the ability to achieve predictable and controlled outcomes in terms of growth, yield, and stress tolerance under specific environmental conditions.[30] Furthermore, it aims to enhance the performance and reliability of the microbes being used as the chassis. *E. coli* has been well explored as a chassis for the production of flavonoids, with biosynthetic pathways in *E. coli* leading to the de novo synthesis of flavanones such as naringenin, pinocembrin, eriodictyol, and homoeriodictyol.[31] Furthermore, yeast has been engineered to function as a microbial chassis for the *de novo* synthesis of various flavonoids, including naringenin, liquiritigenin, kaempferol, resokaempferol, quercetin, and fisetin.[32]

Pathway Enzyme Engineering

Synthetic biology also involves pathway enzyme engineering as a commonly employed strategy to enhance flavonoid production. Its objective is to rewire metabolism processes within the cell to promote the synthesis of target compounds, thereby facilitating the development of more efficient and sustainable production processes.[33] It employs the application of directed evolution and rational design techniques to develop enzymes with improved activity or modified substrate specificity.[11,34] For example, the existence of crystal structures for PALs from *Rhodobacter sphaeroides*, *Petroselinum crispum*, and *Rhodosporidium toruloides*, alongside histidine ammonia-lyase derived from the bacterium *Pseudomonas putida*, facilitate the potential use of homology modeling and rational design manipulating the structural insights. This approach facilitates crafting new TALs that exhibit improved tyrosine specificity and higher enzymatic activity.[35,36] It involves subsequent techniques, these include:

i) Codon Optimization

Codon optimization involves scientific methods intended to improve the codon composition of a recombinant gene, considering various factors without altering the amino acid sequence.[37] Codon optimization is usually carried out at the start of microbial engineering for flavonoid production. For example, the expression of codon-optimized genes encoding chalcone synthase (CHS) and chalcone isomerase (CHI) in the *Corynebacterium glutamicum* strain has led to increased production of naringenin and eriodictyol.[38]

ii) Fusion Expression

Fusion expression is a beneficial tool for optimizing flavonoid production. It involves the formation of fusion proteins by linking the sequences of two or more genes, creating a hybrid protein with combined functional properties. Enzyme activity can be efficiently improved by the translational fusion of several enzymes.[39] An Alfalfa flavanone 3-hydroxylase promoter-gus fusion has been introduced in *Nicotiana benthamiana* for the controlled regulation of flavonoid production.[40]

Metabolic Engineering

The process by which the metabolic processes of an organism can be altered to facilitate the production of biofuels, pharmaceuticals, biochemical products, and other high-value compounds, including secondary metabolites, is known as Metabolic Engineering.[41] This can be achieved through the manipulation of metabolic pathways and the modification of genes, enzymes, and other cellular components of an organism. The incorporation of native biosynthetic pathways of flavonoids from plants into microbial hosts offers a feasible method for the production of flavonoids.[42] An example of this involves the biosynthesis of catechin, a flavonol found in tea and cocoa. Zhao et al. (2015) have engineered *E. coli* to produce catechin by introducing the genes encoding for the enzymes flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and leucoantho cyanidin reductase (LAR).[43] Several strategies are employed in metabolic engineering, which include:

i) Overexpression of Biosynthetic Genes:

Gene overexpression involves enhancing the expression level of specific genes within the cell genome responsible for flavonoid production. The overexpression of flavonoid structural genes and transcription factors has been found to regulate flavonoid production, leading to differences in yield and types of flavonoids.[44] One such example is, that overexpressing CtAC01 results in the increased accumulation of quercetin and its glycosylated derivatives, such as quercetin 3-β-D-glucoside and rutin, in safflower (*Carthamus tinctorius* L.).[45] Similarly, the production of chrysin, wogonin, and baicalein also increased significantly after the elicitation of hairy roots of *Scutellaria* with MeJA+Chi which resulted in the upregulation of MYB7 and FNSII2 genes in *Scutellaria bornmuelleri*.[46]

ii) Feedback Inhibition

Feedback inhibition is a regulatory mechanism where the end product of a biosynthetic pathway inhibits an enzyme, thereby controlling metabolite production. Without such regulation, there is a risk of over-accumulation of flavonoids within microbial cells, which can lead to feedback inhibition.[47] To counteract this issue, specific transporters can be employed to transport the final product into the extracellular space, reducing the intracellular concentration of flavonoids and mitigating feedback inhibition.[48]. Moreover, the construction of logic gates with higher-order processing capabilities has been explored in the context of managing flavonoid production effectively. This approach involves interconnecting logic gates into higher-order computation, leveraging inter-cellular communication enabled by quorum-sensing systems.[49] For example, the dependence of phenylalanine ammonia-lyase (PAL) feedback inhibition on flavonols was confirmed through chemical implementation. Specifically, the use of naringenin effectively restored PAL repression in the tt4 ugt78d1 ugt78d2 system.[50] This intricate approach showcases how manipulation of pathway regulation can be achieved to modulate flavonoid production efficiently.

iii) Transcriptional Regulation

Transcriptional regulation plays a vital role in optimizing flavonoid production. The mechanism involves governing gene expression through controlled binding of transcription factors to specific DNA sequences, thereby influencing the transcription initiation rate.[51] This process is crucial in microbial flavonoid production as it directly affects the expression of genes involved in flavonoid biosynthesis. The regulation of flavonoid biosynthesis involves transcription factors such as MYB and bHLH, which directly control the expression of genes encoding enzymes responsible for flavonoid production.[52] Additionally, introducing plant-derived genes encoding modification enzymes into microbes enables the microbial production of complex flavonoids.[53] Since transcriptional regulation actively governs the expression of essential genes associated with biosynthesis, it plays a significant role in microbial flavonoid production by altering both the yield and the type of flavonoid.[54] Moreover, optimizing microbial flavonoid production underscores the importance of transcriptional regulation, as seen in the proposal of transcription factor-based biosensors for microbial flavonoid production.[55]

In yeast, the regulation of the shikimate pathway occurs at multiple levels, involving feedback genes and transcriptional regulators. This regulation is utilized to decrease the flux of the shikimate pathway, resulting in the overproduction of tyrosine, a precursor for (2S)-naringenin in flavonoid biosynthesis.[56] Additionally, specific transcription factors involved in flavonoid biosynthesis, such as AaYABBY5, have been identified in *Arabidopsis annua*. These transcription factors play a crucial role in regulating the expression of genes related to flavonoid production.[57] Overexpression of transcription factors from the bHLH and MYB family, such as MY2 in *A. annua*, leads to increased levels of anthocyanin[58] and artemisinin.[59]

iv) Optimization of Culture Conditions

Optimizing culture conditions involves adjusting various environmental and nutritional parameters within biological systems to maximize the yield of flavonoids. This includes adjustments in growth factors such as light, temperature, pH, nutrient availability, and the composition of nutrient media.[47] An example of this is the supplementation of *Phellinus igniarius* with citric acid, resulting in a significant increase in flavonoid accumulation. The flavonoid content markedly increased when treated with 2.77 mM citric acid over 69.74 hours.[60]

v) Introduction of Heterologous Pathways

The successful introduction of heterologous pathways involves incorporating genes responsible for flavonoid biosynthesis from one organism into a different host organism, enabling the production of compounds not naturally synthesized by its genome.[61] The most frequently used heterologous hosts include various eukaryotic microorganisms such as yeast *Saccharomyces cerevisiae, Pichia pastoris, Candida boidinii, Hansenula polymorpha, Pichia methanolica,* and *Yarrowia lipolytica as well as other filamentous fungi like* Aspergilli.[62] For example, maize transcription factor genes *LC* and *C1* were introduced into tomatoes to enable heterologous expression, resulting in the production of flavonoids in tomato flesh. Previously, anthocyanins were only found in the leaves of tomatoes, but the introduction of LC/C1 transcription factors upregulated the expression of genes necessary for flavonoid production.[63] Additionally, a four-step heterologous pathway was constructed within engineered L-tyrosine Escherichia coli, incorporating enzymes such as tyrosine ammonia lyase (TAL), 4-coumarate: CoA ligase (4CL), chalcone synthase (CHS), and chalcone isomerase (CHI). This enabled the direct production of naringenin from glucose.[64]

vi) Metabolic Flux Optimization

Metabolic flux optimization is a strategy that involves manipulating the metabolic pathways of a cell or organism to enhance the production of a specific metabolite or molecule. The objective of this approach is to maximize the flow of precursors through the targeted pathway while minimizing the competing pathways. This is achieved through the regulation of gene expression within the flavonoid biosynthesis pathway and ensuring the availability of necessary precursors and cofactors for efficient flavonoid production.[65] Computational methods such as Flux Balance

Analysis (FBA) are employed to maximize the growth rate of microorganisms per unit of carbon consumption. FBA is a valuable tool used to optimize various aspects of metabolic networks, including maximizing biomass and ATP production or minimizing metabolic adjustments.[66]

Gene Stacking

Gene stacking is a procedure that involves combining multiple bioparts, including the gene of interest, into the genome of the host plant. This technique is utilized in synthetic biology to enhance the production of flavonoids. It involves the design and construction of new biological components, devices, and systems to engineer plants for increased production of flavonoids or specific types of flavonoids with desired characteristics.[67] An example that illustrates this approach is the gene stacking in *Arabidopsis thaliana* plants to boost the production of proanthocyanidins, a specific type of flavonoid known for its role in plant defense against pathogens and diseases. In this case, genes CsF3'5'H and CsANR2 from the tea plant were introduced into *Arabidopsis*, resulting in elevated proanthocyanidin production in the new plant germplasm.[68]

RNA Interference (RNAi) or Post-Translational Gene Silencing

RNA interference (RNAi) is a valuable tool in synthetic biology for manipulating the expression of protein-coding genes and controlling cellular processes through sequence-specific gene silencing. The RNAi process involves two key steps; the first step involves the introduction of double-stranded RNA (dsRNA) into the cell and the subsequent degradation of the target mRNA. The introduction of dsRNA can be achieved through methods such as direct insertion into the cell via bombardment, virus-mediated transfer, and infiltration. The second step involves the transformation of cells with vectors that facilitate the production of dsRNA within the cell.[69] An effective application of RNAi is the simultaneous silencing of GmFNSII-1 and GmFNSII-2 in the hairy roots of soybean, which resulted in a reduction in apigenin and luteolin production but a significant increase in genistein, a flavonoid compound.[70]

Riboswitch-Guided Engineering

Riboswitch-guided engineering involves using riboswitches to regulate gene expression by altering their conformation in response to the binding of specific effector molecules.[71] Some RNA biosensors act through a cis-regulatory mechanism, resulting in faster response times. These cis-regulating RNA biosensors are referred to as riboswitches.[72] High-throughput screening, based on synthetic naringenin riboswitches, has yielded an optimized strain with a three-fold increase in naringenin production compared to the parental strain. This optimized strain achieved the highest reported concentration of 260.3 mg/l naringenin when glycerol and p-coumaric acid were used as substrates. These strategies contribute significantly to the improvement of flavonoid production in synthetic biology applications.[73]

Genetic Circuits

Genetic circuits are complex networks that consist of genes and regulatory components such as transcription factors and proteins that enable cells to control gene expression and metabolic processes.[74] This concept of genes functioning as biological circuits traces its origins to pioneering work by Jacob and Monod, notably their investigation of the lac operon.[75] A genetic circuit comprises three vital elements: a sensor that detects signals, internal logic circuits that process these signals, and an actuator for converting the processed signals into desired outputs.[76] These genetic circuits can be engineered to regulate the expression of genes involved in flavonoid biosynthesis, resulting in enhanced production of flavonoids.

Combinatorial Biosynthesis

Combinatorial biosynthesis and synthetic biology are interconnected fields that utilize genetic and metabolic engineering to design and construct novel biosynthetic pathways for the production of desired compounds. Com binatorial biosynthesis is primarily focused on using different enzymes and substrates to generate a diverse range of both natural and unnatural compounds. Synthetic biology, on the other hand, attempts to create and engineer novel biological systems to produce specific products.[77] An example of this synergy is the development of a single plasmid multigene expression system, termed pMGE-T7, which has been created to facilitate the heterologous production of naringenin in E. coli via combinatorial biosynthesis.[78]

Synthetic Promoters

Synthetic promoters play a crucial role in the context of flavonoid production as they enable precise regulation of gene expression to meet specific metabolic pathway requirements (Table 1).[79] These promoters are designed to control gene expression predictably and can be customized to suit the specific requirements of the metabolic pathway under construction. By utilizing synthetic promoters, it is feasible to manipulate the expression of genes involved in flavonoid biosynthesis, maximizing flavonoid production while minimizing any adverse side effects.[10] Notably, hybrid promoters have been developed in *Y. lipolytica* for heterologous gene expression. These hybrid promoters encompass elements derived from nitrogen (UAS1B) and the XPR2 promoter, which can be controlled by pH. Additionally, UASTEF is another synthetic promoter derived from the translation elongation factor 1-α (TEF1-α) gene, which has been specifically designed for use in *Y. lipolytica*.[80]

Table 1. Different Types of Flavonoids have been Produced Using Synthetic Biology Approaches

Flavonoid	Class	Substrate used	Microbe	Technique used	Ref.
Apigenin	Flavones	p-coumaric acid	*E. coli* API and *E. coli* GPI (co-culture)	Biotransformation	81
luteolin	Flavones	Naringenin as supply from culture	*Streptomyces albus*	Culture optimization	82
baicalein	Flavones	Tyrosine	*E. coli*	Enzyme self-assembly strategy	83
liquiritigenin	Flavanones	phenylalanine	*S. cerevisiae*	Gene overexpression	84
naringenin	Flavanones	p-ceramic acid	*S. cerevisiae*	Metabolic flux optimization	85
		D-xylose	*E. coli, S. cerevisiae*	Codon optimization and metabolic flux amplification	86
		Acetate	*E. coli*	Optimum flux rerouting	87
		Malonate	*C. glutamicum*	Pathway engineering	88
Eriodictyol	Flavanones	naringenin	*C. glutamicum*	Pathway engineering	89
pinocembrin	Flavanones	L. phenylalanine	*E. coli*	Codon optimization, Use of promoter	90
Kaempferol	flavanol	p-coumaric acid	*S. cerevisiae*	Gene overexpression	91
di-hydroquerecitin	Flavanols	Eriodictyol	*S. cerevisiae*	Gene expression	92
myricetin-3-O-α-L-rhamnoside	Flavonols	Myrecitin as supply from culture	*E. coli*	Culture optimization	93
Triacetin	Flavonol	Glycerol	*E. coli*	Metabolic engineering	94
Scutellarin	Flavonol	L-phenylalanine	*Y. lipolytica*	Metabolic engineering	95
Catechin	Flavanonol	Eriodictyol and dihydrokaempferol	*E. coli*	Combinatorial metabolic engineering	96

Ethical, Safety, and Regulatory Considerations

Ethical, safety, and regulatory considerations have become important in flavonoid synthetic biology. The production of flavonoids using synthetic biology approaches raises several ethical concerns, including the potential to produce novel compounds with unknown ecological and health impacts. Safety measures are crucial to ensure that engineered microorganisms do not pose any risks to the environment or human health. Regulatory frameworks need to be established to govern the production and use of synthetic flavonoids, addressing concerns about biosafety, biosecurity, and potential misuse of knowledge.[97] Furthermore, using synthetic biology tools to produce flavonoids necessitates careful ethical evaluation, particularly regarding the potential for unintended consequences and blurring of boundaries between living organisms and machines. The societal implications of synthetic biology, including its ethical, safety, and security aspects, must be considered to ensure responsible research and development.[98,99]

In the context of microbial production of flavonoids, synthetic biology approaches have the potential to improve the pharmacokinetics of flavonoids for clinical use, raising ethical considerations related to the development and use of modified compounds.[51] Moreover, the use of synthetic biology pipelines for the rapid and tailored production of antiviral flavonoids requires careful ethical and safety assessments to mitigate potential risks and ensure responsible application.[100] As flavonoid synthetic biology advances, it is important to address these issues to ensure responsible and sustainable development. Environmental risks in synthetic biology are assessed in terms of biosafety and biosecurity because synthetic organisms may be released into the environment, potentially leading to unintended effects on plant physiology and ecological systems.[101] This can have consequences for biodiversity and natural sources of flavonoids. There is also a concern about the misuse of knowledge, which could result in creating novel pathogens harmful to the environment.[102] Safety considerations are pivotal in the development of synthetic biology to ensure that they do not pose health hazards. Rigorous safety evaluations are essential, and producers must adhere to established safety guidelines and protocols for the approval of synthetic products.[103] Regulatory agencies such as the FDA and FSSAI play a crucial role in evaluating safety protocols and ensuring proper monitoring of the safety and suitability of synthetic flavonoids for human consumption.

Future Prospects and Directions

Flavonoid synthetic biology holds promising prospects and directions, driven by recent advancements in synthetic biology and metabolic engineering. These disciplines offer potential solutions to the challenges that have hindered flavonoid drug discovery, such as low production efficiency from plants and chemical synthesis.[104] Researchers are now focusing on producing flavonoids in microorganisms using metabolic engineering and synthetic biology, which opens new avenues for improved pharmacokinetics of flavonoids.[105] Moreover, the repositioning of microbial biotechnology against COVID-19 has highlighted the potential of synthetic biology in the fast and tailored production of valuable antiviral flavonoids, emphasizing the application of division of labor through co-cultivation/microbial community approaches to the Design, Build, Test, Learn (DBTL) principle.[106] The future of flavonoid synthetic biology also involves the development of innovative strategies for improving flavonoid production using synthetic biology approaches, such as genetically encoded biosensors for in vivo metabolite analysis and high-throughput screening methods using fluorescence-activated cell sorting (FACS).[107] Furthermore, the potential convergence between synthetic biology and bioelectronics presents opportunities for developing synthetic biology-based therapies, offering novel therapeutic strategies for future clinical applications.[108] The field of synthetic biology is actively moving forward, with immense

advancements in the synthetic biology of yeast, indicating the beginning of further development and the era of customized synthetic biology and automation.[109] Additionally, modeling is likely to play a larger role in guiding synthetic biology, revealing subtle non-intuitive designs and becoming a significant field in the future.[110] These advancements are expected to address the challenges in flavonoid drug discovery and pave the way for fast and tailored production of valuable flavonoids.[12]

Conclusion

In conclusion, flavonoids, ubiquitous phytopigments, play multifaceted roles in nature and industries. Synthetic biology offers significant potential for sustainable production of high -value products with considerable advantages. Conventional approaches are constrained by reliance on natural sources, which makes it challenging to increase production. The potential of flavonoid synthetic biology holds promise for meeting the increasing demand for these valuable compounds, with innovative approaches that not only enhance production but also maintain ethical and safety standards. As the field continues to evolve, it is poised to contribute significantly to various industries and our understanding of flavonoids, which enables the efficient synthesis of flavonoids in microbial systems, yielding higher purity and productivity. These advanced technologies facilitate their discovery and commercialization, streamlining the efforts of research institutions and industrial agencies. Nevertheless, further investigations are required to overcome these technological barriers and optimize manufacturing methods, especially for flavonoids that are not produced via synthetic biological routes. The development and optimization of additional synthetic biology chassis are necessary for enhanced production, necessitating the amalgamation of the various strategies outlined above. Furthermore, it is essential to address ethical, safety, and regulatory considerations in flavonoid synthetic biology to ensure responsible and sustainable development. Environmental risks, safety assessments, and adherence to regulatory guidelines are crucial aspects that must be carefully managed to ensure the safe and beneficial application of synthetic flavonoids.

Authors' Contributions

KK and A conceived and designed the draft of the manuscript. A wrote the first draft of the manuscript. HC and YS reviewed, edited, and made additions to the data for the manuscript. All authors commented on the subsequent version of the manuscript. All authors have read and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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