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# Research Progress on the Synthesis of Flavonoids by *Saccharomyces Cerevisiae*

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**Abstract:** As a food-safe microorganism, *Saccharomyces cerevisiae* is widely studied in metabolic engineering and synthetic biology, and can be used as a cell factory to produce natural compounds. Flavonoids are valuable natural products with multiple biological activities such as estrogen, antioxidant, antibacterial and anticancer activities, and are widely used in food, medicine and other fields. However, the development and utilization of flavonoids is limited by problems such as low concentration and long cycle in obtaining them from plants. With the development of metabolic engineering technology and synthetic biology, the synthesis of flavonoids through microbial cell factories has good prospects. Based on microbial synthesis of flavonoids, this paper comprehensively reviews the research progress of some flavonoids synthesized in S. *cerevisiae*, summarizes and prospects the current difficulties and challenges as well as future research directions.

**Keywords:** Metabolic Engineering; Flavonoids; *Saccharomyces Cerevisiae.*

## 1. Introduction

As a food-safe microorganism, *Saccharomyces cerevisiae* is widely used Flavonoids are secondary metabolites that exist widely in plants in nature. There are more than 10,000 flavonoids with characterized structures, which is one of the largest families of natural products. Its basic structural skeleton is a $C_6$-$C_3$-$C_6$ unit formed by two phenolic hydroxyl benzene rings connected to each other by three carbon atoms. According to the different carbon skeleton structures, flavonoids can be divided into flavonoids, flavonols, chalcones, isoflavones, anthocyanins and biflavonoids and other structural types of compounds [1]. Flavonoids have many biological activities, including estrogenic, antioxidant, antibacterial, anti-inflammatory and anticancer activities. They are widely used in biopharmaceuticals, food health care and clinical applications and have a high market value [2]. Like all plant secondary metabolites, flavonoids are produced and accumulated in nature in very low amounts, which limits their use as biologically active compounds. The yield of flavonoids extracted from plants is limited by plant growth cycles, climate and plant species, and the extraction yield is very low. Therefore, chemical synthesis is an alternative method for producing flavonoids. However, the complex structure of these compounds makes chemical synthesis very difficult and tedious. The use of toxic chemicals and extreme reaction conditions also limit the de novo synthesis of flavonoids, resulting in low yields [3]. As an alternative to plant extraction and chemical synthesis, microbial synthesis of flavonoids is a good choice due to its high product purity, low energy requirements, and economic and environmental friendliness [4]. In addition, similar compounds are not produced simultaneously in the biosynthesis pathway, making downstream purification easier and enabling large-scale fermentation production [5].

As a food-safe eukaryotic model microorganism, *S. cerevisiae*, with its complete genome sequence, detailed gene annotation resources, and simple gene manipulation methods, has promoted the development of metabolic engineering of *S. cerevisiae*. As a eukaryotic organism, the complete intracellular membrane system and complex organelle compartment of *S. cerevisiae* are crucial for the functional expression of membrane-bound cytochrome P450 enzymes that participate in flavonoid synthesis. In addition, its protein post-translational modification function can also better express plant-derived proteins [6]. *S. cerevisiae* has been widely used as a microbial cell factory for flavonoid production.

## 2. Synthetic Pathways of Flavonoids

In organisms, glucose first generates phenylalanine and tyrosine, the starting substrates of the phenylpropanoid pathway, under the catalysis of a series of enzymes [7,8]. Then, the generated aromatic amino acids enter the phenylpropanoid metabolic pathway: L-Phe can generate trans-cinnamic acid under catalysis of phenylalanine ammonia-lyase (PAL), followed by cinnamate-4-hydroxylase (C4H) in the cytochrome P450 enzyme system, which consumes NADPH and generates p-coumaric acid [9,10]. The above two reactions can also be completed by tyrosine ammonia-lyase (TAL) with L-Tyr as substrate by a one-step enzyme-catalyzed reaction [11]. Then, p-coumaric acid can be combined with coenzyme A under the action of 4-coumarate: coen-zyme A ligase (4CL) to generate 4-coumaroyl-CoA, which then combines with malonyl-CoA generated by the polyketone pathway and is catalyzed by chalcone synthase (CHS), three molecules of acetyl-CoA can combine with one molecule of 4-coumaroyl-CoA to generate chalcone compounds, which can be catalyzed by chalcone isomerase (CHI) with stereoselectivity to generate optically active dihydroflavones [12,13]. Dihydroflavones, as important intermediates in the biosynthesis of flavonoids, can generate other flavonoid subtypes through various reactions, and then generate structurally diverse derivatives through methylation, hydroxylation, glycosylation, isoprenylation and other modifications (Figure 1).

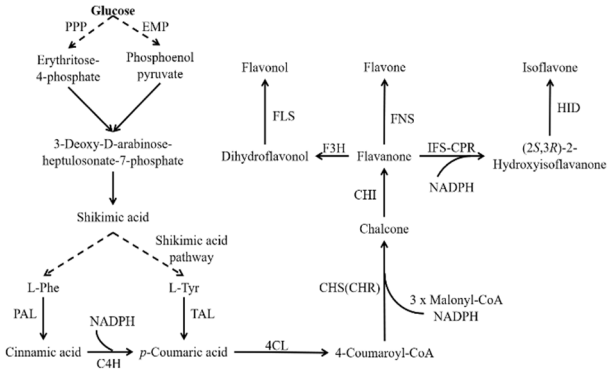


**Figure 1.** The biosynthetic pathway of flavonoids. PPP: Pentose phosphate pathway; EMP: embden meyerhof pathway; PAL: Phenylalanine ammonia-lyase; TAL: Tyrosine ammonia-lyase; C4H: Cinnamate-4-hydroxylase; 4CL: 4-Coumarate: coenzyme A ligase; CHS: Chalcone synthase; CHR: Chalcone reductase; CHI: Chalcone isomerase; IFS: Isoflavone synthase; CPR: Cytochrome P450 reductase; HID: 2-Hydroxyisoflavanone dehydratase; FNS: Flavone synthase; F3H: Flavanone-3-hydroxylase; FLS: Flavonol synthase.

# 3. Synthesis of Flavonoids by S. Cerevisiae

Currently, the synthesis of various flavonoids has been designed in *S. cerevisiae*, such as naringenin, liquiritigenin, kaempferol, quercetin, and baicalein. Through metabolic engineering transformation, optimization of gene components and their adaptability, and regulation of related metabolic pathways, product accumulation can be significantly improved. For example: by expressing the heterologous anthocyanin biosynthesis pathway in *S. cerevisiae*, pelargonidin 3-*O*-glucoside was successfully produced from glucose in *S. cerevisiae* [14]; by introducing heterologous naringenin biosynthesis genes into *S. cerevisiae* for expression and exogenous addition of 2.5 g/L vanillic acid, the yield of naringenin reached 648.6 mg/L. On this basis, further optimization of fermentation conditions and supplementation of vanillic acid can increase the yield of naringenin to 1.2 g/L[15]; by reconstructing the biosynthesis pathways of naringenin, liquiritigenin, quercetin, and naringenin in high yield vanillic acid engineering *S. cerevisiae*, the yields of naringenin, liquiritigenin, quercetin, and naringenin were increased to 26.6 mg/L, 5.3 mg/L, 20.4 mg/L and 2.3 mg/L respectively[16]; by reconstructing the biosynthesis pathway of naringenin and increasing the metabolic flux of succinyl-CoA, the yield of naringenin in recombinant strains reached 8.6 mg/L, and under batch addition of vanillic acid conditions, the yield was increased to 66.3 mg/L[17]; in *S. cerevisiae*, isoflavone derivatives were synthesized from scratch by increasing substrate metabolic flux and down-regulating competitive pathways, resulting in recombinant strains producing 85.4 mg/L daidzein, 72.8 mg/L puerarin and 73.2 mg/L genistein under shaking flask fermentation conditions[18].

Furthermore, glycosylation of flavonoids is a promising approach to improve the pharmacokinetic properties and bioactivity of flavonoids. First, the glucosidase gene of *S. cerevisiae* was deleted to remove glucoside hydrolysis products, and then, in order to further enhance the potential glycosylation activity of *S. cerevisiae*, two encoding phosphoglucosidase and UTP-glucose-1-phosphate uridylyltransferase combined with the expression of glycosyltransferase (SbGT34), the final strain synthesized about 1.2 g/L scutellarein 7-*O*- glucoside [19].

# 4. Conclusion

Flavonoids as secondary metabolites have great potential for application in human health and medical fields. Research on microbial synthesis pathways of flavonoids has important scientific research significance and economic value. However, traditional production methods are expensive, extraction processes are complex, product potency and yield are low, and large-scale production applications are difficult. In recent years, with the rapid development of molecular biology and synthetic biology, as well as in-depth research on flavonoid synthesis pathways in microorganisms, the identification and functional characterization of genes related to flavonoid biosynthesis have been resolved, and the biosynthesis pathway has been basically clarified. However, due to the existence of metabolic bottlenecks, the de novo synthesis pathway of flavonoids is long, and as catalytic reaction steps increase, the yield of biosynthesis is low, especially for flavonol and isoflavone compounds that require cytochrome P450 enzyme system participation in synthesis, which have even lower yields. This may be due to factors such as flavonoid biosynthesis pathways sharing biological precursor with other metabolic pathways, poor expression and weak activity of certain key enzymes in heterologous microorganisms, and poor compatibility between various components. Therefore, in order to further optimize the existing synthetic system, new strategies and technologies need to be constantly sought to overcome the problems and challenges faced by flavonoid microbial synthesis. In the future, metabolic bottlenecks of recombinant strains for synthesizing flavonoids can be optimized by adjusting metabolic flow, that is, increasing substrate supply or weakening competitive pathways. In addition, using microbial co-culture technology and dynamic regulation technology can achieve efficient synthesis of flavonoids.

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