Review Article

L-Tyrosine Metabolic Pathway in Microorganisms and Its Application in the Biosynthesis of Plant-Derived Natural Products

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Abstract

L-tyrosine, an aromatic amino acid, is an important upstream precursor for the synthesis of a series of valuable natural products such as flavonoids and phenolic acids. In recent years, regulation of the L-tyrosine metabolic pathway has been devoted to enhancing the production of L-tyrosine and the derived bioactive compounds in microorganisms, usually by increasing the supply of precursors, blocking competitive routes, and modulating the transport system. Here, we reviewed the strategies to promote L-tyrosine production in microbial hosts and the common strategies to produce bioactive compounds in engineered *Escherichia coli* and *Saccharomyces cerevisiae* to better understand and utilize the L-tyrosine metabolic pathway for microbial overproduction of diverse valuable aromatic compounds in the future.

Keywords: Biosynthesis, *Escherichia coli*, L-tyrosine, *Saccharomyces cerevisiae*

Introduction

L-tyrosine, (2S)-2-amino-3-(4-hydroxyphenyl) propionic acid, and the intermediates of L-tyrosine metabolism are important precursors for the synthesis of various value-added secondary metabolites such as phenolic acids, benzylisoquinoline alkaloids, and flavonoids.[1-4] L-tyrosine is also an important raw material in the food, health protection, and chemical industries; therefore, it is of great significance to understand the biosynthesis of L-tyrosine and L-tyrosine-derived downstream pathways.

L-tyrosine is synthesized from chorismate, the end product of the shikimate pathway and common precursor of L-phenylalanine, L-tyrosine, and L-tryptophan.[3] Among the three aromatic amino acids, the yield of L-tyrosine is the lowest.[5] At present, there are four main methods for producing L-tyrosine: protein hydrolysis, chemical synthesis, enzymatic conversion, and microbial fermentation. The chemical synthesis of L-tyrosine is mainly through the hydroxylation of L-phenylalanine or through the condensation of alkaline hydrolysis and ammonia, followed by transformation and other steps, which involve multistep reactions, and the production efficiency is relatively low. In addition, chemical synthesis leaves environmental footprints because of its typically toxic by-products. The enzymatic method has the practical value of a short cycle, high selectivity, simple separation, and purification steps and is environmentally friendly. Still, it is limited due to low enzyme activity and poor stability.[6,7]

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In recent decades, technologies have revolutionized metabolic engineering to provide a sustainable, environmentally friendly, and economically feasible platform for producing natural products originally derived from plants by microbial cell factories. Nonetheless, microbial overproduction of L-tyrosine and its derivatives still remains as challenges owing to the complex gene regulations involved in the metabolic pathways.

Engineered microbial strains with high L-tyrosine productivity must overcome the biosynthetic pathway's control mechanism, as microbial cells have a feedback-inhibition regulatory mechanism. L-tyrosine can only accumulate to a certain level for cell growth,[8] which is far below industrial production. Recently, various metabolic engineering strategies such as "in," "through," "cut off," "block," and "out" methods have been used to optimize the L-tyrosine biosynthetic pathway to acquire a higher yield of L-tyrosine in microorganisms.[9] At the same time, under the guidance of systems biology and synthetic biology, the synthesis pathways of diverse aromatic compounds were designed and constructed, and heterologous genes were introduced into the chassis cells. Thus, the biosynthesis of various plant-derived natural compounds can be realized.[10,11] There have been reviews on microbial hosts' production of natural products *via* manipulation of the L-tyrosine metabolic pathway.[8,12] However, a summary of the strategies for engineering the L-tyrosine metabolic pathway is lacking. This review summarizes the current strategies and biotechnological approaches for high-yield L-tyrosine-producing strains. Significantly, the strategies for the microbial biosynthesis of L-tyrosine-derived valuable compounds in engineered *Escherichia coli (E. coli)* and *Saccharomyces cerevisiae* (*S. cerevisiae)* are highlighted.

The Biosynthesis of L-tyrosine

L-tyrosine originates from the shikimate pathway, the main pathway for the formation of common aromatic compounds, by providing precursors to the L-tyrosine biosynthetic branch pathway, the L-phenylalanine biosynthetic branch pathway, and the L-tryptophan biosynthetic branch pathway.[13] The biosynthesis of L-tyrosine is costly because it consists of multiple enzymatic steps that consume many metabolic precursors, including phosphoenolpyruvate (PEP), erythrose-4-phosphate (E4P), L-glutamate, as well as ATP, and NADPH. The L-tyrosine biosynthetic pathway in *E. coli* and yeast is shown in Figure 1. The L-tyrosine synthesis begins with the condensation of E4P and PEP, which is catalyzed by 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase (Aro3/4, AroG/F/H) to form DAHP. DAHP is dephosphorylated by 3-dehydroquinate (DHQ) synthase (Aro1, AroB) to form DHQ through an intramolecular aldol condensation; DHQ dehydratase (Aro1, AroD) removes one molecule H_2O of DHQ to generate 3-dehydroshikimate (DHS). The resultant DHS is then converted to shikimate (SHIK) by shikimate dehydrogenase (Aro1, AroE);[14] shikimate kinase (Aro1, AroK/AroL) phosphorylates shikimate to obtain shikimate-3-phosphate (S3P). Subsequently, S3P is catalyzed by 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Aro1, AroA) to generate EPSP; chorismate (CHA) synthase (Aro2, AroC) eliminates phosphoric acid from EPSP to form CHA. In *E. coli*, chorismate mutase/prephenate dehydrogenase (tyrA) is a bifunctional enzyme that catalyzes the conversion of CHA to prephenate and prephenate to 4-hydroxyphenylpyruvate (4-HPP), and then, aromatic-amino-acid transaminase (tyrB) catalyzes the transamination reaction from 4-HPP to L-tyrosine.[15] In microorganisms, a high concentration of L-tyrosine inhibits DAHP synthase activity. In *S. cerevisiae*, DAHP synthase (Aro3 and Aro4) is sensitive to L-tyrosine-induced feedback inhibition.[4,16] In addition, in *E. coli*, the expression of *aroF* and *tyrA* is negatively regulated by L-tyrosine.[17]

Engineering of L-tyrosine Biosynthetic Pathway to Improve L-tyrosine Production

The L-tyrosine biosynthetic pathway, together with its downstream pathways, has been extensively utilized to produce a variety of valuable products. Therefore, there is an increasing demand to improve the yield of L-tyrosine. To achieve this, optimization of the L-tyrosine metabolic pathway mainly focuses on reducing negative regulation, blocking competitive pathways, optimizing the shikimate metabolism pathway, and manipulating the L-tyrosine transport system. Table 1 lists the practical biosynthesis strategies used to achieve a high yield of L-tyrosine in *E. coli* and *S. cerevisiae* in recent years.

Reducing negative regulation

In L-tyrosine synthesis, the accumulation of L-tyrosine induces feedback inhibition of the expression of pathway genes, and the global regulator *TyrR* inhibits the transcription of shikimate pathway genes (*aroF*, *aroG*, and *aroL*) and transporters (*aroP* and *tyrP*). Overexpressing the feedback resistant (fbr) version of *aroG* and *tyrA* could eliminate the feedback inhibition induced by aromatic amino acids and promote L-tyrosine yield in wild-type *E. coli* K12.[18] To develop a superior L-tyrosine-producing strain, four candidate genes (*tyrR*, *csrA*, *pgi*, and *ppc*) were knocked out in 14 different strains in combination with synthetic sRNAs.[19] Finally, the optimally engineered *E. coli* S17-1 harboring anti-*tyrR* and anti-*csrA* produced the highest tyrosine titer (2 g/L).[19]

Blocking the competitive pathway

Blocking the competitive pathway is also a promising approach for improving L-tyrosine production. The L-tyrosine metabolic pathway competes with the L-tryptophan and L-phenylalanine pathways for precursor substances [Figure 1]. Blocking these two competitive pathways saves carbon sources, directs the metabolic flux toward the synthesis of L-tyrosine, and releases L-phenylalanine and L-tryptophan-induced feedback inhibition of DAHP synthase, which is conducive to the accumulation of L-tyrosine. Since both L-phenylalanine and L-tyrosine are converted from prephenate, the L-phenylalanine pathway is the main competitive pathway for L-tyrosine synthesis.

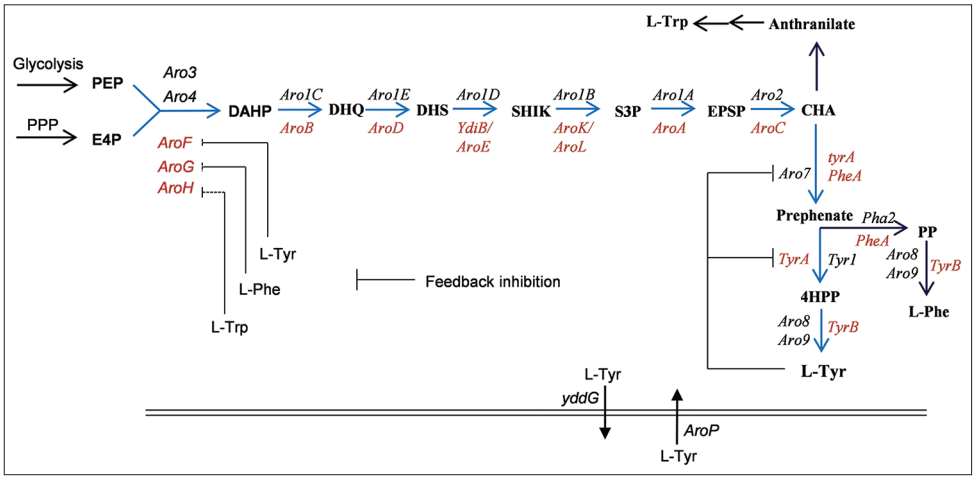

Figure 1: Biosynthetic pathway of L-tyrosine in *Escherichia coli* and *Saccharomyces cerevisiae.* Red: *Escherichia coli* genes, black: *Saccharomyces cerevisiae* genes, DAHP: 3-deoxy-D-arabino-heptulosonate-7-phosphate, DHQ: 3-dehydroquinate, SHIK: Shikimic acid, S3P: shikimate-3-phosphate, EPSP: 5-enolpyruvylshikimate-3-phosphate, CHA: Chorismate, PP: Phenylpyruvate, 4HPP: 4-hydroxyphenylpyruvate, L-Phe: L-phenylalanine, L-Tyr: L-tyrosine, L-Trp: L-tryptophan, *AroF*/*AroG*/*AroH:* 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase genes, *AroB*: 3-dehydroquinate synthase gene, *AroD*: 3-dehydroquinate dehydratase gene, *AroE*: Dehydroshikimate reductase gene, *AroK*/*AroL*: shikimate kinase genes, *AroA*: EPSP synthase gene, *AroC:* Chorismate synthase gene, pheA/tyrA: Chorismate mutase/prephenate dehydrogenase gene, *tyrB*: Aromatic-amino-acid transaminase gene, *AroP:* Aromatic amino acid H(+) symporter gene, *yddG*: Aromatic amino acid exporter gene, *Aro3*/*Aro4:* 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase genes, *Aro2*: Chorismate synthase, *Aro7*: Chorismate mutase gene, Tyr1: Prephenate dehydrogenase gene, *Aro8*/*Aro9:* Aromatic acid aminotransferase gene, *Pha2:* Prephenate dehydratase gene

Table 1: Optimization of metabolic engineering for microbial production of L-tyrosine

Host	Metabolic engineering strategies	Titer (g/L)	References
E. coli WSH-Z06	*tyrA^{fbr}, aroG^{fbr}, aroP*	44.5	[6]
E. coli MG1655	*aroE, tyrB, aroC, aroA, aroL, aroD, aroB, ppsA, tktA, tyrA^{fbr}, aroG^{fbr}*	2.169	[5]
E. coli K12	*tyrA^{fbr}, aroG^{fbr}, ydeO, evgA, relA, purF, hisH*	13.8	[18]
E. coli S17-1	*ppsA, tktA, aroF, aroK, tyrC28, aroG^{fbr}, tyrA, anti-tyrR, anti-csrA, sRNA*	21.9	[19]
E. coli MG1655	*aroE, aroD, aroB, aroG^{fbr}, ppsA, tktA, tyrB, tyrA^{fbr}, aroC, aroA, aroL*	0.686	[20]
E. coli XL1-Blue	*ΔtyrP, aroG^{fbr}, aroL, tyrA^{fbr}, aroG^{fbr}, aroL, tyrC*	43.14	[21]
E. coli HG	*ΔpheA, ΔtyrR, aroG^{fbr}, tyrA^{fbr}*	55.54	[22]

E. coli: Escherichia coli, fbr: Feedback resistant

Optimizing the shikimate metabolism pathway

Since a large amount of metabolic flux passes through the shikimate pathway, it is meaningful to optimize the shikimate pathway and direct the carbon flux into the L-tyrosine biosynthesis. The shikimate pathway consists of seven enzymatic steps that convert PEP and E4P into their end-product chorismate.[14] The shikimate pathway is usually optimized by overcoming the rate-limiting steps in the pathway to promote the conversion between substances. Two main bottlenecks need to be resolved to increase the supply of L-tyrosine precursors in the shikimate metabolism pathway. One major bottleneck was quinate/shikimate dehydrogenase (YdiB), which induces the overproduction of intermediates and by-products, and an overexpression of *AroE* in the place of *YdiB* increased the shikimate supply by approximately 5-fold.[5] Another bottleneck was due to the low expression of *AroB,* which was reduced by codon optimization.[5] In addition, the availability of PEP and E4P is also an important approach for optimizing the shikimate metabolic pathway. This approach, mainly by increasing the supply of precursor substances to boost the production of L-tyrosine, is a straightforward and highly efficient strategy for improving the overall bioproduction performance.

Manipulating L-tyrosine transport system

In addition to the strategies mentioned above, L-tyrosine production can be promoted by manipulating the L-tyrosine transport system by blocking the cellular uptake of L-tyrosine or by increasing L-tyrosine efflux.[6] The results of a fed-batch fermentation experiment on a 3 L fermentor showed that the L-tyrosine production of the *aroP* and *tyrP* gene knockout mutants was increased to 44.5 and 35.1 g/L, respectively.[6] To enhance the L-tyrosine yield by manipulating the L-tyrosine transport system, single (Δ*tyrP*/Δ*aroP*) and double genes (Δ*tyrP* Δ *aroP*) were knocked out in the wild-type strain and the *tyrR* knockout mutant, respectively, and the six strains were transformed with pTY13 (*aroG^{fbr}-aroL-tyrC* overexpressed). The wild-type and *tyrR* knockout mutants transformed with pTY13 were used as controls, and the

fermentation results showed that *tyrP* was knocked out in the three strains with the highest L-tyrosine production, which indicated that *tyrP* knockout seemed to be more effective than *tyrR* in promoting L-tyrosine production in microorganisms.[21] In summary, the ability of microorganisms to effectively utilize amino acids is vital for the regulation of endogenous biosynthesis.

Other ways

For most high-yield L-tyrosine-producing engineered *E. coli* strains, the expression of critical enzymes is induced by isopropyl-β-D-thiogalactoside (IPTG). However, IPTG is unsuitable for large-scale industrial production owing to its relatively high cost and environmental pollution. Temperature-inducible expression plasmids are more suitable for industrial production, as their expression can simply be induced by changing the culture temperature. Therefore, the engineered *E. coli* can be induced to produce L-tyrosine by the alteration of culture temperature. The heat-inducible plasmid containing the two feedback resistance enzymes ($aroG^{fbr}$ and $tyrA^{fbr}$) was introduced into a phenylalanine-producing strain, *E. coli* HGX, to produce a high yield of L-tyrosine.[22]

L-tyrosine Downstream Pathway

L-tyrosine serves as a good precursor of diverse high-value aromatic compounds, most of which are plant-derived natural products. With a series of modifications and optimizations in the early stage, engineered *E. coli* and *S. cerevisiae* strains with a high yield of L-tyrosine were obtained, and then, the downstream biosynthetic pathways of L-tyrosine-derived bioactive natural products were constructed in the microorganisms. To achieve microbial hyperproduction of plant-derived natural products, researchers should obtain plant-derived key pathway enzymes and screen isoenzymes from various plant sources. In plants, tyrosine is metabolized by tyrosine hydroxylase, transaminase, aldehyde synthase, reductase, and lyase.[3] For example, tyrosine aminotransferase (TAT) catalyzes the reversible reaction between tyrosine and 4-HPP. Wang[23] characterized the functions of TAT1 and TAT2 in tyrosine metabolism and degradation in *Arabidopsis thaliana.* Pathway genes can be obtained from several different species,[24-27] and they can then be combined and screened to obtain the most effective biosynthetic pathway. Figure 2 shows the L-tyrosine downstream pathways involved in the synthesis of bioactive natural products.

Engineering of L-tyrosine Downstream Pathway for the Production of Plant-derived Natural Products

Many researchers have summarized the synthesis of L-tyrosine derivatives in microorganisms. Tan[28] reviewed the recent advances of L-tyrosine derivatives catalyzed by enzymatic biocatalysts. Shen[12] reviewed the latest progress in the biosynthesis of aromatic chemicals and conducted a systematic overview of the biosynthesis of L-tyrosine derivatives. Most related articles describe the biosynthetic process of L-tyrosine derivatives but lack summaries of the strategies in the metabolic pathways. The following section introduces the application strategies utilizing the L-tyrosine metabolic pathway to biosynthesize various bioactive natural products in microorganisms. Table 2 summarizes the synthetic strategies used to optimize the production of L-tyrosine derivatives in *S. cerevisiae* and *E. coli.*

Eliminating negative-feedback regulation in synthetic pathways

In microorganisms, the biosynthesis of specific metabolites is often controlled by strict negative-feedback regulation, which affects the efficiency and yield of the target product.[53] To obtain a high production, it is necessary to reduce the feedback inhibition in the biosynthetic pathway and direct more metabolic flux toward the precursor substances of the desired products. At the same time, it is also necessary to minimize the impact on the primary metabolism of the strain. In *E. coli*, feedback-insensitive $tyrA^{fbr}$ and $aroG^{fbr}$ were overexpressed to alleviate the tyrosine-induced feedback inhibition and improve the yield of caffeic acid (CA).[29] No significant difference in genkwanin production was observed between strains with *tyrA* and *aroG* overexpression and non-overexpression,[50] whereas, by introducing $tyrA^{fbr}$ and $aroG^{fbr}$ into the engineered *E. coli* genkwanin-producing strain from tyrosine, the yield of genkwanin was 2.3-fold that of the strain overexpressing wild-type *tyrA* and *aroG*; so the key to increasing the yield of genkwanin was to eliminate the tyrosine-induced feedback inhibition.[50] In *S. cerevisiae,* overexpression of the feedback-insensitive versions of DAHP synthase $ScAro4p^{K229L}$ and chorismate mutase $ScAro7p^{G141S}$ increased the production of L-tyrosine. Finally, the yield of resveratrol increased by 78%.[41] The $Aro4^{K229L}$ and $Aro7^{G141S}$ mutations were overexpressed to alleviate the feedback inhibition and redirect carbon flux to improve the titer of tyrosol and salidroside in the engineered *S. cerevisiae*.[46] Coexpression of module 1 ($aroG^{fbr}$-$tyrA^{fbr}$-*aroE*) and module 2 (*ppsA-tktA-glk*), together with knockout of the regulatory genes and alternative genes, appeared to be effective for enhancing the production of L-tyrosine, providing a sufficient L-tyrosine supplement for the production of salvianic acid A (SAA).[34] In the engineered *S. cerevisiae*, Koopman[48] doubled the yield of naringenin in shake flask culture (10.4 μM) by relieving the feedback inhibition of DAHP synthase. Meanwhile, this strategy also promoted the accumulation of phenylethanol. The extracellular concentration of phenylethanol increased by 100 times,[48] suggesting that while increasing the metabolic flux of the aromatic amino acid pathway, we should also pay attention to lessening the intermediates and unwanted by-products.

Enhancing the metabolic flux in the biosynthetic pathway

The main methods to increase metabolic flux are to overexpress pathway genes and block competitive

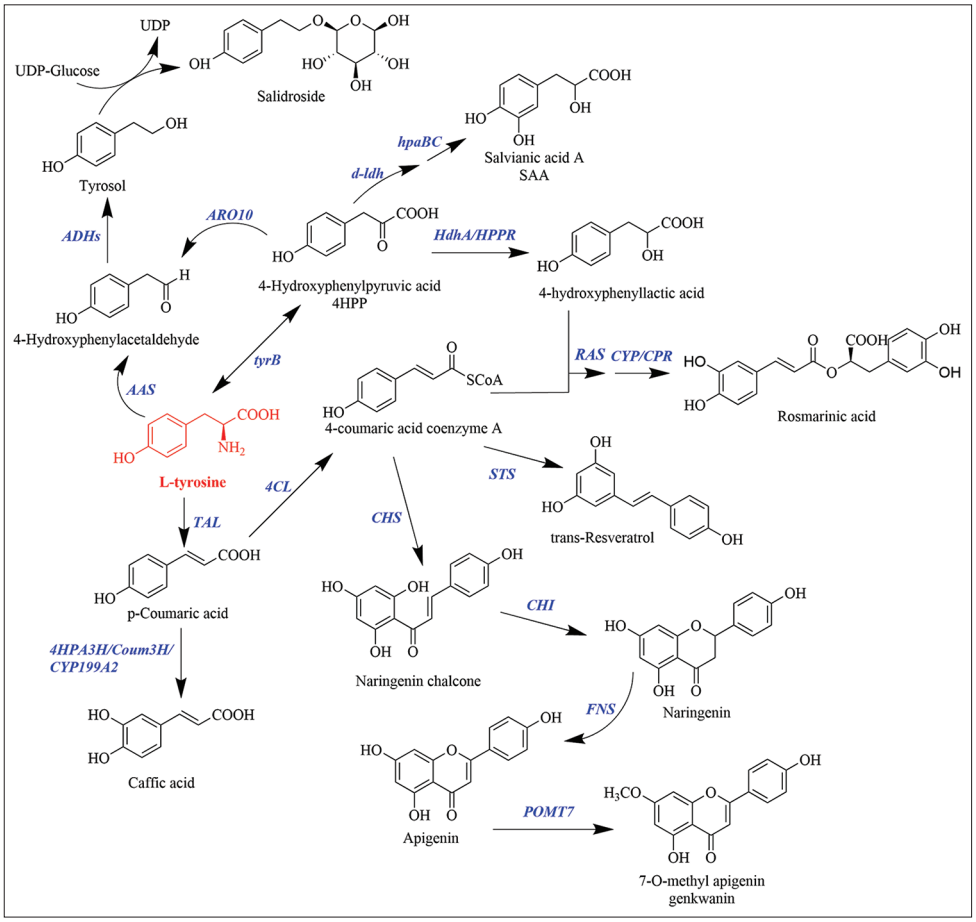

Figure 2: Biosynthesis of L-tyrosine derivatives in microorganisms. Blue: Pathway genes, *ADHs*: Endogenous alcohol dehydrogenases, *AA*: Aromatic aldehyde synthase, *d-ldh*: D-lactate dehydrogenase, *hpaB:* Endogenous hydroxylase complex, *HdhA*: 2-hydroxy acid dehydrogenase, *HPPR*: Hydroxyphenylpyruvate reductase, *TAL*: Tyrosine ammonia lyase, *4HP3H*: 4-hydroxyphenylacetic acid 3-hydroxylase, *Coum3H*: 4-coumaric acid 3-hydroxylase, *CYP*: Cytochrome P450, *CPR*: P450 reductase, *4CL:* 4-coumaric acid coenzyme A ligase, STS: Resveratrol synthase, *RAS*: Rosmarinic acid synthase, *CHS*: Chalcone-flavanone isomerase, *CHI:* Chalcone synthase, *FNS*: Oxidoreductase flavone synthase, *POMT7*: Apigenin 7-O-methyltransferase

pathways. Overexpression of pathway genes is a relatively basic and easy-to-implement method. However, this is not always effective for enhancing the yield of the desired products. For example, the overexpression of some genes affects fundamental cell growth, thus lowering the final yield of the target products. In the biosynthesis of L-tyrosine derivatives, it is usually necessary to block the L-phenylalanine pathway to direct more metabolic flux from chorismic acid to L-tyrosine. CA is a natural phenolic compound derived from L-tyrosine, and overexpression *of* $tyrA^{fbr}$, *ppsA*, *tktA*, and $aroG^{fbr}$ in recombinant *E. coli* that synthesize CA effectively directs more metabolic flux from carbon sources to the L-tyrosine metabolic pathway.[29] L-tyrosine is also an important precursor of resveratrol biosynthesis. Overexpressing the chorismate mutase $ScAro7p^{G141S}$ and feedback-inhibition resistant versions of DAHP synthase $ScAro4p^{K229L}$ improved the supply of L-tyrosine, thus directly increased the production of resveratrol by 78%.[40] In *S. cerevisiae*, most of the carbon flux of PEP flows to pyruvate by pyruvate kinase, and pyruvate decarboxylase (PDC1, PDC5, and PDC6) converts pyruvate into acetaldehyde, which is partially converted to ethanol and partially used for cell growth.[4,44] At the same time, pyruvate decarboxylase also acts on the conversion from phenylpyruvate to phenylethanol *via* the Ehrlich pathway. The aromatic decarboxylase-encoding gene *aro10* shows sequence homology with pyruvate decarboxylase and can catalyze the decarboxylation of phenylpyruvate.[54] Restricting the activity of the Ehrlich pathway by knocking out the three pyruvate decarboxylase genes (*aro10*, *pdc5*, and *pdc6*) increased the metabolic flux toward naringenin, leading to a 22-fold decrease in the concentration of extracellular phenylethanol and a 3-fold increase in extracellular naringenin compared to both ancestor strains.[48] In the engineered tyrosol-producing strain, *pdc1* was knocked out to reduce the carbon flux toward ethanol and cell growth, and the production of tyrosol increased by 32.49%.[44] In engineered *S. cerevisiae*, *pdc1*, *pha2,* and *trp3* were disrupted to inhibit the biosynthetic pathways of ethanol, phenylalanine, and tryptophan, respectively, and

Table 2: Engineered microbial strains to produce L-tyrosine derivatives

Classification	Target product	Substrate	Microbial strains	Pathway gene	Yield	References
Phenolic acid compounds	CA	Glucose	BW25113	*4HPA3H, RcTAL*	50.2 mg/L	[29]
		Glucose	DE3	*RgTAL, Pc4CL, Coum3H*	106 mg/L	[30]
		Tyrosine	BY4741	*RtTAL, 4HPA3H (EchpaB, PahpaC)*	289.4 ±4.6 mg/L	[31]
		Glucose	MG1655	*RgTAL, RpCYP199A2*	280 mg/L	[32]
		Glucose	YXWP-113	*ORgTAL, OHpaB, HpaC, OTyrC*	769.3 mg/L	[33]
	SAA	Glucose	BW25113	*d-ldh, hpaBC, tyrA^{fbr}, aroG^{fbr}, aroE, ppsA, tktA, glk*	7.1 g/L	[34]
		Glucose	BW25113	*d-ldh, hpaBC*	5.6 g/L	[35]
	RA	Glucose	BW27784	*EchpaBC, HdhA, RsTAL, At4CL2, CbRAS*	1.8±0.3 μM	[36]
		CA	BMGA	*At4CL, CbRAS, hpaBC, Lpldh*	130 mg/L	[37]
		Glucose, xylose	K12, BL21, P2H and P2I	*RgTAL, Pc4CL, hpaBC, MoRAS, d-ldh^{Y52A}*	172 mg/L	[38]
		Glucose	CEN.PK 113-7D	*FjTAL, CbTAT, CbHPPR, At4CL1, MoRAS, CbCYP-CPR*	5.93±0.06 mg/L	[39]
Other alcohol compounds	Resveratrol	Ethanol	CEN. PK102-5B	*HaTAL, At4CL1, VvSTS1, ScAro4^{K229L}, ScAro7^{G141S}, ScACC1^{S659A}, ^{S1157A}*	531.41 mg/L	[40]
		Glycerin	W3110	*Sc4CL, VvSTS, RgPAL*	22.6 mg/L	[41]
		Glucose	EC1118	*At4CL, VvSTS*	3.4710 mg/L	[42]
	Tyrosol, salidroside	Glucose	BY4742	*PcAAS*	Salidroside 732.5 mg/L, tyrosol 1394.6 mg/L	[43]
		Glucose	BY4741	*PcAAS, EcADH, EcTyrA*	126.74±6.70 mg/g	[44]
		Glucose	HLF-Dα	*PcAAS, EcTyrA M53I/A354V, Xfpk*	Salidroside 1.82 g/L, tyrosol 8.48 g/L	[45]
		Glucose	CEN. PK2-1C	*Aro4 ^{K229L}, Aro3 ^{K222L}, Aro7^{G141S}, RrU8GT33*	Salidroside 26.55±0.43 g/L, tyrosol 9.90±0.06 g/L	[46]
Flavonoids	Baicalin, baicalein	Tyrosine, phenylalanine	BL21	*RtPAL, Pc4CL, PhCHS, MsCHI, PcFNS, SbFNS*	Baicalein 23.6 mg/L, baicalin 106.5 mg/L	[47]
	Naringenin	Glucose	CEN. PK2-1C	*AtPAL, coC4H, coCPR1, AtCHI1, AtCHS3, coCHS3, At4CL3, coTAL1*	414.63 μM	[48]
		Glucose	BL21	*PhCHS, MsCHI, RgTAL, Pc4CL*	41.5 mg/L	[49]
	Apigenin, genkwanin, apigenin-7-O-β-d-glucopyranoside	Glucose	BL21	*Os4CL, PeCHS, MtCHI*	Apigenin 30 mg/L, genkwanin 41 mg/L	[50]
		4-coumaric acid	BL21	*Nt4CL2, PhCHS, MsCHI, PcFNSI, PaGT3*	Apigenin-7-O-β-d-glucopyranoside 16.6 mg/L	[51]
	Sakuranetin	Glucose	BL21-Gold	*MsCHI, PhCHS, MatBC, NoMT*	29.7 mg/L	[52]

CA: Caffeic acid, SAA: Salvianic acid A, RA: Rosmarinic acid, fbr: Feedback resistant

heterologous phosphoketolase (*Xfpk*) was overexpressed to promote the biosynthesis of tyrosol.[45] Furthermore, heterologous expression of *Xfpk* alone increased tyrosol production by approximately 135 times compared to the parent strain.[45]

Microbial coculture

Modular coculture engineering is an effective approach to obtain higher bioproduction efficiency by dividing a complete biosynthetic pathway into two or more separate serial modules. In the coculture biosynthesis of most L-tyrosine derivatives, upstream pathways will first synthesize p-coumaric acid, then further produce the target product. The biosynthesis system of resveratrol was divided into two modules: one strain with *pheA* disrupted and tyrosine ammonia lyase (*TAL)* overexpressed to obtain a high yield of p-coumaric acid, and the other strain was inserted with *4CL* and *STS* to promote resveratrol production. Finally, the yield of resveratrol reached 22.6 mg/L at 30 h.[41] An *E. coli* modular coculture system was designed to produce sakuranetin, using glucose as the sole carbon source.[52] The upstream module was engineered to produce the intermediate p-coumaric acid, whereas the downstream module was responsible for transforming p-coumaric acid to sakuranetin.[52] By optimizing the inoculation ratio of the two strains, a production of 29.7 mg/L sakuranetin was obtained by coculture system in 48 h.[52] A coculture system was established to form the biosynthetic pathway of naringenin. The upstream module was used to synthesize the pathway intermediates tyrosine and p-coumaric acid, and the downstream module was used to convert them into naringenin.[49] Then, the coculture system was optimized by screening the host strains and investigating the effect

of the medium carbon source, IPTG induction time, and inoculation ratio. The final shake flask fermentation reached the highest naringenin concentration of 41.5 mg/L in 36 h.[49] To synthesize apigetrin, two recombinant engineered *E. coli* strains were designed. The upstream module was introduced with *4CL*, *CHS, CHI,* and *FNSI* to produce apigenin from p-coumaric acid, and the downstream module was designed to improve the expression of UDP-glucose and glycosyltransferase to convert apigenin into apigetrin.[51] By optimizing the coculture inoculation ratio, fermentation temperature, and media components, a production of 16.6 mg/L apigenin was finally obtained.[51] Li[38] used a three-strain coculture engineering strategy to synthesize rosmarinic acid (RA). The two upstream modules were the CA and SAA modules, which produce two parallel precursors of RA, and the downstream module included the condensation of CA and SAA to produce RA.[38] By optimizing the carbon substrates and inoculation ratio of the three strains, the coculture system produced a yield of 172 mg/L RA, which was 38-fold higher than that of the original monoculture strain.[38] In a modular coculture system, modulating the inoculation ratio between coculture strains during the fermentation process is very important. As the coculture technology in *E. coli* is mature and easy to operate, *E. coli* is the most popular host for the coculture engineering of L-tyrosine derivatives. Moreover, L-tyrosine derivatives can be synthesized using a coculture system with two different microbial species.[55] Zhang[56] *et al*. developed a coculture system composed of engineered *E. coli* and *S. cerevisiae* to produce naringenin from D-xylose, and the yield of naringenin was 21.16 ± 0.41 mg/L, which was about eight times that of monoculture yeast. In comparison with monoculture engineering, coculture engineering decreases the excessive metabolic burden on each strain, controls different pathway modules flexibly, reduces the interference between different pathway enzymes, provides diversified and suitable cellular environments for different enzymes, and enables flexible and effective utilization of various substrates.[57,58] Although coculture engineering has distinct advantages and opportunities, several challenges remain, including maintaining the coexistence and balanced growth between coculture systems and transferring key biosynthetic intermediates between different coculture microorganisms. Therefore, continued efforts in delicate coculture design, construction, and regulation are required for superior coculture engineering.[59]

Establishment of dual synthetic pathways

The dual synthesis pathway is also practical for mitigating the impact of an insufficient supply of critical intermediates and promoting bioproduction by assembling two biosynthetic pathways in a single microbial strain performance. For example, the establishment of a novel dual pathway mediated by 4-hydroxyphenylacetate 3-hydroxylase (4HPA3H) and TAL to synthesize CA from L-tyrosine extended the native L-tyrosine metabolic pathway of *E. coli*, reduced the feedback inhibition induced by tyrosine, and directed more metabolic flux toward the tyrosine synthesis.[29] After 48-h cultivation, the yield of CA reached 50.2 mg/L in the shake flask.[29] A dual synthetic pathway was designed and established to synthesize RA from engineered *S. cerevisiae*. RA can be produced by the conversion of 4-coumarolyl-coA and 4-hydroxyphenyllactic acid through RA synthase (RAS) and cytochrome P450 CYP98A14-CPR, or by conjugating caffeoyl-coA and 3,4-dihydroxyphenyllactic acid.[39] By optimizing the enzyme variants, the copy number of pathway genes, and the precursor supply of the metabolic pathway, the production of RA was up to 5.93 ± 0.06 mg/L.[39]

Conclusion and Prospective

L-tyrosine has drawn much attention because of its wide applications in the nutritional, health-protection, and cosmetic industries. In addition, L-tyrosine is used as an essential precursor for a range of different high-value derivatives. Limitations in plant extraction, chemical synthesis, and enzymatic methods for these valuable compounds have triggered interest in microbial biosynthesis. In recent years, going along with the rapid development of systems biology and synthetic biology, microbial cells have been extensively utilized to obtain L-tyrosine and L-tyrosine-derived bioactive natural products. Nevertheless, industrial production of these compounds through microbial cell factories remains a challenge to overcome. First, the yield of most L-tyrosine derivatives produced by microbial biosynthesis is still too low (μg or mg/L) for industrial production. The main reasons for this include the low availability of precursors of the target products, the complex regulations in the biosynthetic process, disruption of competing pathways, and the low activities of the enzymes used in microbial biosynthesis.[60] In addition to overcoming these challenges, the fundamental microbial production limitations could be overcome by improving the utilization of carbon substrates and regulating the supply of cofactors (NADPH/NADH and ATP/ADP) in the pathway. Second, another major bottleneck for the industrial application of these compounds is the high production cost, including high-cost substrates, high-cost antibiotics and inducers, and expensive product purification processes. Finally, the microbial fermentation process should be easy to operate. Most of the biosynthesis of L-tyrosine derivatives uses glucose as a carbon substrate due to its price advantage. However, the fermentation cycle is quite long (especially the fermentation of *S. cerevisiae*), and batch feeding is required. Therefore, the culture conditions need to be optimized to obtain a suitable method for industrial production. Microbial biosynthesis is a promising approach for obtaining large-scale production of L-tyrosine and L-tyrosine downstream value-added chemicals. In future, these challenges must be overcome to improve the biotechnological yields of aromatic products. Some high-yield microbial biosynthesis can gradually replace chemical synthesis and extraction to obtain plant-derived natural products efficiently.

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Conflicts of interest

There are no conflicts of interest.

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