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Recent Advances in the Biosynthesis of Representative Plant-Derived Natural Products in Pichia Pastoris

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Abstract. Pichia pastoris, isolated from California oak trees, is a methylotrophic yeast that utilizes methanol, glucose, etc. It has fast growth, suits high-density fermentation, low cost, and high biosafety, acting as an ideal chassis strain for high-value natural products in biosynthesis for decades. Widely used in microbial synthesis of plant-derived compounds (terpenoids, flavonoids, etc.), it provides green routes for pharmaceuticals, food, chemicals. Researchers have optimized gene expression elements (promoters, terminators) and developed genetic modification technologies like CRISPR/Cas9. This paper focuses on elaborating the key progress of Pichia pastoris in the synthesis of plant-derived natural products in recent years, including the optimization strategies of gene expression elements, breakthroughs in strain modification technologies, and the synthetic pathways and yield improvement achievements of representative products, and prospects the challenges faced in the construction of its cell factory (e.g., modification relying on the trial-and-error method, insufficient gene editing tools and genetic elements) and possible countermeasures (e.g., using multi-omics and computational biology to locate metabolic bottlenecks, and strengthening tool development and element exploration). This paper finds that Pichia pastoris still has broad room for improvement in the synthesis of natural products.

Keywords: Pichia pastoris, Biosynthesis, Natural Products

1. Introduction

Terpenoids, flavonoids, and other plant-derived natural products have garnered significant interest due to their diverse pharmacological activities and great potential as high-value-added chemicals [1]. However, traditional extraction methods are often limited by scarce resources, environmental pressures, and complex extraction and purification processes. Microbial synthetic biology provides a revolutionary approach for the sustainable and large-scale production of these high-value natural products, with advantages including short production cycles, controllable processes, environmental friendliness, and ease of engineering modification.

Pichia pastoris was isolated from oak trees in California and named by Herman Phaff [2]. It is a methylotrophic yeast that can utilize substances such as methanol and glucose as carbon sources. Owing to its advantages of a fast growth rate and suitability for high-density fermentation, it is widely used in production.

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This review centers on the latest advancements in the synthesis of plant-derived natural products using Pichia pastoris. It systematically organizes and analyzes existing studies, with emphasis on elaborating the key technologies and research status in the construction of synthetic pathways. The aim is to provide references and insights for the synthesis of natural products using the Pichia pastoris platform

2. Gene expression elements

2.1. Promoters

Promoters are key elements that regulate gene transcription levels and affect gene expression. The AOX1 promoter is a classic representative of inducible promoters in Pichia pastoris. Its traditional methanol-induced mode exhibits extremely high transcriptional activity when methanol is the sole carbon source [3], but the toxicity of methanol and transcriptional inhibition under glucose/glycerol conditions limit its application. To address this issue, the ethanol-induced transcriptional signal amplification device (ESAD) system developed by Jiangnan University breaks through this bottleneck through a dual-module design: it uses the ethanol-activated ICL1 promoter to drive the LacI-Mit1AD fusion protein, and combines it with the AOX1 core promoter containing multiple copies of the lacO sequence. This design increases the target protein yield by 38% and balances synthesis and growth in the ethanol system [4]. In addition, the FLD1 promoter, another inducible promoter, can achieve expression levels comparable to AOX1 under methanol/methylamine conditions, with lower dependence on methanol, making it suitable for the regulation of complex metabolic networks [5].

Constitutive promoters possess the characteristic of continuous expression without the need for inducers. The glyceraldehyde-3-phosphate dehydrogenase promoter (GAP promoter) is a widely used constitutive promoter in Pichia pastoris. Compared with the AOX1 promoter, it does not require methanol as a carbon source and takes less time for expression, so it is considered an excellent alternative to the AOX1 promoter [6]. In 2023, Jie Lai et al. constructed a hybrid promoter PHY47, which achieved a GFP expression level 2.93 times higher than that of the GAP promoter, demonstrating the great optimization potential of constitutive promoters [7]. During the logarithmic growth phase, the activity of the FDH promoter (PF_DH800) reaches 119% of that of the GAP promoter and 69% of that of the PAOX1 promoter. The expression system constructed based on it exhibits characteristics such as precise biphasic expression, easy construction, minimal impact on normal cellular metabolism, and high intensity [8].

2.2. Terminators

In the Pichia pastoris expression system, terminators are not only key elements for terminating transcription but also significantly affect the expression level of exogenous genes by regulating mRNA stability. Endogenous terminators (such as AOX1tt) are the most commonly used, but recent studies have found that terminators derived from Saccharomyces cerevisiae(such as ScCYC1tt) are also effective in Pichia pastoris, and the establishment of synthetic terminator libraries has further expanded the scope of regulation. Studies have shown that different terminators can achieve an adjustable range of expression levels up to 17 times by altering the secondary structure stability of the mRNA 3'-UTR [9]

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3. Modification technologies of Pichia pastoris

3.1. Homologous recombination based on linearized plasmid vectors

Homologous recombination based on linearized plasmid vectors is an early commonly used technology. It achieves gene insertion or replacement by inserting the exogenous gene expression cassette into the Pichia pastoris genome via a linearized vector. Common vectors such as pPIC9K and pAO815 can realize single-copy or multi-copy integration. This technology relies on the homologous recombination mechanism, and the transformation efficiency is affected by the method, vector dosage, and homologous recombination efficiency. Electroporation is the most convenient and efficient transformation method, and pretreatment can further improve its efficiency [10].

3.2. Gene editing based on CRISPR/Cas9

The CRISPR/Cas9 system is a highly effective gene-editing tool, and its core function relies on the ribonucleoprotein complex formed by the Cas9 protein and single-guide RNA (sgRNA). This complex specifically recognizes and binds to the target DNA sequence, inducing the generation of DNA double-strand breaks (DSBs). The cell then activates endogenous repair mechanisms, mainly including the homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. Notably, the repair efficiency of the NHEJ pathway is usually higher in Pichia pastoris [11]. The expression efficiency of the Cas9 protein is significantly affected by the codon usage preference of the host; therefore, the HsCas9 optimized with human codons has a significantly higher editing efficiency in Pichia pastoris than the PpCas9 optimized with Pichia pastoris-preferred codons [11]. The targeted recognition function of Cas9 depends on the PAM sequence. To expand the range of target sites, Cas9 variants that recognize different PAM sequences (such as StCas9) can be used [12]. In addition, the nuclease-inactivated Cas protein mutant dCas9, as a functional platform, has been developed into CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) technologies, which are used to reversibly inhibit or activate the expression of specific genes (e.g., regulating the PAOX1 promoter).

3.3. Gene editing based on CRISPR/Cas12a

Cas12a is a type V-A Cas protein containing RuvC and NUC domains, which cleaves to generate 5' overhangs [13]. Cas12a can target regions with high AT content, recognize specific PAM sequences, and can also recognize suboptimal PAM sequences (with reduced cleavage activity) [14]. After optimization, it can realize the rapid introduction of multiple genes and large-fragment deletion. The editing efficiency of single genes reaches 99% ± 0.8%, that of double genes ranges from 65% ± 2.5% to 80% ± 3%, and that of triple genes is 30% ± 2.5% [15].

4. Synthesis of representative plant-derived natural products in Pichia pastoris

4.1. Synthesis of terpenoids

Terpenoids are a very important class of secondary metabolites in plants. They are widely distributed in nature, with large quantities and diverse structures, and are the most abundant class of compounds in natural products. They have anti-tumor, anti-inflammatory, anti-bacterial, anti-viral, anti-malarial and other effects, and are widely used in food, medicine and other fields [16]. At present, Pichia pastoris has been widely used in the synthesis of terpenoids.

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For triterpene synthesis, Liu et al. established a synthetic pathway for dammarenediol-II (a ginsenoside precursor) in Pichia pastoris through the introduction of the dammarenediol-II synthase gene (PgDDS) derived from ginseng [17]. To increase the yield, they adopted the strategy of "increasing precursor supply and reducing competitive consumption". They overexpressed the squalene epoxidase gene ERG1 to boost the synthesis of 2,3-oxidosqualene (a key precursor), which increased the yield by 6.7 times. Then, they replaced the promoter of the lanosterol synthase gene ERG7 with the thiamine-repressible promoter P_{thi11} through the Cre/loxP system to reduce its competitive consumption of 2,3-oxidosqualene, which further increased the yield by 3.6 times. Finally, by adding 0.5 g/L squalene, the yield reached 1.073 mg/g DCW, which was 37.5 times higher than that of the initial strain. This study confirmed the feasibility of producing triterpenes using the branch of the endogenous sterol synthesis pathway in Pichia pastoris.

For sesquiterpene production, numerous studies have focused on the optimization and flux distribution of the mevalonate (MVA) pathway. Xu et al. (2023) constructed a high-yield α-farnesene strain XF22 by modularly regulating the MVA pathway, farnesyl pyrophosphate (FPP) branch, and acetyl-CoA supply. In fed-batch fermentation, this strain achieved a yield of 17.92 g/L with a productivity of 0.107 g/(L · h). Its core strategies include: enhancing the flux of the MVA pathway through promoter strength matching (e.g., regulating key enzymes with P_GAP), replacing the promoter of ERG9 (squalene synthase) with the weak promoter P_{ser1} to reduce FPP shunting, and introducing the Escherichia coli pyruvate dehydrogenase complex (EcPDHs) and acetyl-CoA synthase (EcACS) to increase acetyl-CoA supply [18]. Similarly, Liu et al. introduced the isopentenol utilization pathway (IUP) into peroxisomes to enhance IPP/DMAPP supply through dual regulation of the cytoplasm and peroxisomes. Combined with the co-feeding of sorbitol and oleic acid, the yield of α-farnesene reached 2.56 g/L, which demonstrated the advantage of subcellular compartmentalization in reducing metabolic interference [19].

For more complex oxidized terpenoids, Wriessnegger et al. (2014) achieved efficient conversion from farnesene to (+)-nootkatone by co-expressing the P450 oxidase (HPO) from Hyoscyamus muticus, the P450 reductase (CPR) from Arabidopsis thaliana, and the endogenous alcohol dehydrogenase (ADH-C3). The yield reached 208 mg/L in a bioreactor [20]. Niu et al. used methanol as the sole carbon source, and for the first time realized the de novo synthesis of the sesquiterpene zealexin A1 by optimizing the MVA pathway, enhancing NADPH supply, and improving methanol assimilation efficiency. The yield in a 5-L fermenter reached 102.5 mg/L, which demonstrated the potential of Pichia pastoris to produce high-value terpenoids using one-carbon substrates [21].

4.2. Synthesis of flavonoids

Flavonoids are a class of secondary metabolites widely present in plants. They have various biological activities such as anti-oxidation, anti-inflammation, and anti-virus, and are widely used in medicine, food and other fields. Pichia pastoris, relying on its efficient carbon source utilization ability and powerful heterologous protein expression system, has become an important chassis for the microbial synthesis of flavonoids. Relevant studies have realized the efficient production of a variety of flavonoids and their derivatives.

Naringenin is a natural dietary flavonoid, which is abundant in fruits such as oranges, bergamots, and grapefruits. It has anti-oxidant, anti-inflammatory, anti-infective and other effects [22]. For the synthesis of naringenin, the supply of the precursor L-tyrosine is a key bottleneck. Hasunuma et al. overexpressed the feedback-inhibition-insensitive enzymes from Saccharomyces cerevisiae—3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (ARO4K229L) and chorismate mutase

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(ARO7G141S). This relieved the feedback inhibition of the shikimate pathway in Pichia pastoris and significantly increased the metabolic flux of L-tyrosine. This "L-tyrosine chassis" strategy increased the naringenin yield by 244%, with the yield reaching 1067 mg/L in fed-batch fermentation. At the same time, the study found that the engineered strain could use crude glycerol, a by-product of biodiesel, as a carbon source, and the naringenin yield reached 338 mg/L, which provided the possibility for low-cost production [23].

Baicalin is derived from the dicotyledonous plant Scutellaria baicalensis. It has various pharmacological effects such as anti-inflammation, anti-virus, anti-bacteria, liver protection, lipid-lowering, and neuroprotection [24]. Its synthesis in Pichia pastoris involves the heterologous expression of glycosyltransferases and pathway coordination. Kang et al. constructed a site-specific glycosylation chassis by introducing glycosyltransferases from 6 sources (such as SbUBGAT and TcC8GT), which could synthesize 18 kinds of 4'-deoxyflavone glycosides. By enhancing the synthesis of UDP-sugar precursors (overexpressing the YPUU gene cluster) and blocking glycoside hydrolysis (knocking out EXG1 and SPR1), the baicalin yield was increased by 18.6 times. Using a co-cultivation strategy and combining with carbon source switching (glucose-ethanol) to regulate module balance, the baicalin yield was finally achieved to 1290.0 mg/L in a bioreactor [25].

For the synthesis of 4'-deoxyflavones (such as baicalein and oroxylin A), researchers adopted pathway modularization and toxicity mitigation strategies. The synthetic pathway was divided into modules such as phenylalanine metabolism, chalcone synthesis, and flavone conversion. By constructing ethanol-inducible (ESADs) and constitutive (CSADs) transcription amplification devices to dynamically balance enzyme expression, the accumulation of toxic intermediates (such as cinnamic acid) was reduced. In addition, by culturing at near-neutral pH (pH 7.1) and using ethanol as the carbon source, the inhibition of cell growth by cinnamic acid was reduced. Finally, the de novo synthesis of baicalein (401.9 mg/L) and oroxylin A (339.5 mg/L) was realized, among which the microbial synthesis of oroxylin A was reported for the first time [26].

4.3. Synthesis of other compounds

In addition, Pichia pastoris has also successfully synthesized several other compounds.

Erythritol is widely present in various plants and fungi. It is a new type of natural sweetener, and due to its unique physical and chemical properties and physiological functions, it is widely used in food, medical treatment, cosmetics, pharmaceuticals and other fields [27]. Researchers realized the efficient synthesis of erythritol by pruning and enhancing the carbon rearrangement network (CRN), and introducing the bacterial ribulose monophosphate (RuMP) pathway and the endogenous xylulose monophosphate (XuMP) pathway to construct a hybrid network, not only was the conversion efficiency of methanol to erythrose-4-phosphate (E4P) improved, but also the production of pentitol by-products was reduced. Finally, the erythritol yield reached 31.5 g/L in a fermenter [28].

Malic acid is an organic acid mainly present in plants and has been widely used in the chemical industry, pharmaceutical industry, food industry, agriculture and other industries [29]. Zhang et al. produced 0.74 g/L of malic acid from glucose by overexpressing a single endogenous mdh1 gene in Pichia pastoris [30]. The study by Feng et al. optimized the malic acid synthesis module (e.g., introducing pyruvate carboxylase and malate dehydrogenase from Rhizopus oryzae), knocked out the by-product (succinic acid, ethanol) synthesis genes using the FLP-FRT system, relieved the metabolic flux limitation of the XuMP cycle by knocking out glucose-6-phosphate isomerase, and combined with nitrogen source optimization. As a result, the malic acid yield reached 2.79 g/L when

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methanol was the sole carbon source, which also verified the effectiveness of remodeling the methanol assimilation pathway for the synthesis of carboxylic acids [31].

Catharanthine is an alkaloid compound extracted from Catharanthus roseus and is a precursor of the anti-cancer drug vinblastine. Researchers divided the catharanthine biosynthesis pathway into three functional modules (NPT module: carbon source to nerolidol; STR module: nerolidol to loganin; CAN module: loganin to catharanthine) and carried out modular optimization. The optimization strategies included: selecting high-activity/specificity enzymes (e.g., replacing CrISY with NmISY2 to reduce by-products), improving the solubility and activity of the key enzyme PAS through MBP fusion expression, amplifying the rate-limiting enzyme genes (e.g., CrSGD, CrGS, etc.), knocking out the competitive pathway (OYE3A/B), enhancing the function of cytochrome P450 enzymes (overexpressing RAD52, CYB5, etc.), and optimizing the carbon source (adding trehalose or mannitol). Finally, the engineered Pichia pastoris strain achieved a catharanthine yield of 2.57 mg/L in a fermenter [32].

5. Conclusion

Pichia pastoris has been used in biosynthesis for decades. Its biosynthetic capacity, fermentation production cost, and biosafety have been widely recognized by the industry, and it has been used as a chassis strain for the synthesis of various natural products. In recent years, researchers have continuously optimized the metabolic pathway of Pichia pastoris to improve the yield and production efficiency of natural products. Through technical means such as pathway modularization, enzyme engineering modification, and rational configuration of carbon sources, researchers have successfully realized the effective synthesis of a variety of bioactive natural products.

However, Pichia pastoris still has broad room for improvement in the synthesis of natural products. Firstly, most of the current modification strategies still rely on the trial-and-error method. In the future, it is necessary to use multi-omics technologies (genomics, transcriptomics, proteomics, metabolomics) and computational biology models to systematically analyze the interaction between heterologous pathways and the endogenous metabolic network of Pichia pastoris, and accurately locate rate-limiting nodes and metabolic bottlenecks. This will promote the transformation of engineering modification from semi-empirical to highly rational predictive design. Secondly, compared with model microorganisms such as Saccharomyces cerevisiae, there are relatively few gene editing tools for Pichia pastoris, and the available genetic elements are not sufficient. In the future, it is necessary to strengthen the development of gene editing tools and the exploration of genetic elements.

References

[1] D.J. Newman, G.M. Cragg, Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019, Journal of Natural Products 83(3) (2020) 770-803.

[2] H.J. Phaff, M.W. Miller, M. Shifrine, The taxonomy of yeasts isolated from Drosophila in the Yosemite region of California, Antonie van Leeuwenhoek 22(2) (1956) 145-61.

[3] C.S. Dharmarathna, S. Kumar, Y.I.N.S. Gunawardena, R.S. Dassanayake, C. Hettiarachchi, AOX1 promoter-driven expression of yeast-enhanced green fluorescent protein in Pichia pastoris, The Microbe 1 (2023) 100012.

[4] X. Wang, X. Zhang, Y. Mao, Y. Wu, X. Lv, L. Liu, W. Han, S. Yin, R. Wu, J. Chen, Y. Liu, Ethanol-Inducible Bioproduction of Human α-Lactalbumin in Komagataella phaffii, Journal of agricultural and food chemistry 73(15) (2025) 9246-9260.

[5] D. Resina, M. Maurer, O. Cos, C. Arnau, M. Carnicer, H. Marx, B. Gasser, F. Valero, D. Mattanovich, P. Ferrer, Engineering of bottlenecks in Rhizopus oryzae lipase production in Pichia pastoris using the nitrogen source-regulated FLD1 promoter, New Biotechnology 25(6) (2009) 396-403.

DOI: 10.54254/2753-8818/2026.PJ30537

[6] H.R. Waterham, M.E. Digan, P.J. Koutz, S.V. Lair, J.M. Cregg, Isolation of the Pichia pastoris glyceraldehyde-3-phosphate dehydrogenase gene and regulation and use of its promoter, Gene 186(1) (1997) 37-44.
[7] J. Lai, L. Song, Y. Zhou, H. Zong, B. Zhuge, X. Lu, Fine-Tuned Gene Expression Elements from Hybrid Promoter Libraries in Pichia pastoris, ACS Synthetic Biology 13(1) (2024) 310-318
[8] Q. Shen, J. Cui, Y. Wang, Z.-C. Hu, Y.-P. Xue, Y.-G. Zheng, Identification of a novel growth-associated promoter for biphasic expression of heterogenous proteins in Pichia pastoris, Applied and Environmental Microbiology 90(2) (2024) e01740-23
[9] Y. Ito, G. Terai, M. Ishigami, N. Hashiba, Y. Nakamura, T. Bamba, R. Kumokita, T. Hasunuma, K. Asai, J. Ishii, A. Kondo, Exchange of endogenous and heterogeneous yeast terminators in Pichia pastoris to tune mRNA stability and gene expression, Nucleic acids research 48(22) (2020) 13000
[10] X. Yang, Y.M. Wang, P. Zhao, X.Y. Yu, Study on Strategy Improvement of Yeast Transformation Experiment Based on ANP Method, Applied Mechanics and Materials 217-219 (2012) 931-936
[11] A. Weninger, A.M. Hatzl, C. Schmid, T. Vogl, A. Glieder, Combinatorial optimization of CRISPR/Cas9 expression enables precision genome engineering in the methylotrophic yeast Pichia pastoris, Journal of biotechnology 235 (2016) 139-49.
[12] Y. Sun, N. Yan, L. Mu, B. Sun, J. Deng, Y. Fang, S. Shao, Q. Yan, F. Han, Z. Zhang, K. Xu, sgRNA-shRNA Structure Mediated SNP Site Editing on Porcine IGF2 Gene by CRISPR/StCas9, Frontiers in genetics 10 (2019) 347.
[13] D.C. Swarts, M. Jinek, Cas9 versus Cas12a/Cpf1: Structure-function comparisons and implications for genome editing, Wiley interdisciplinary reviews. RNA 9(5) (2018) e1481.
[14] B. Zetsche, O.O. Abudayyeh, J.S. Gootenberg, D.A. Scott, F. Zhang, A Survey of Genome Editing Activity for 16 Cas12a Orthologs, The Keio journal of medicine 69(3) (2020) 59-65.
[15] X. Zhang, S. Gu, X. Zheng, S. Peng, Y. Li, Y. Lin, S. Liang, A Novel and Efficient Genome Editing Tool Assisted by CRISPR-Cas12a/Cpf1 for Pichia pastoris, ACS Synthetic Biology 10(11) (2021) 2927-2937
[16] K.W. George, J. Alonso-Gutierrez, J.D. Keasling, T.S. Lee, Isoprenoid drugs, biofuels, and chemicals--artemisinin, farnesene, and beyond, Advances in biochemical engineering/biotechnology 148 (2015) 355-89.
[17] X.-B. Liu, M. Liu, X.-Y. Tao, Z.-X. Zhang, F.-Q. Wang, D.-Z. Wei, Metabolic engineering of Pichia pastoris for the production of dammarenediol-II, Journal of biotechnology 216 (2015) 47-55.
[18] J.-Z. Xu, S.-L. Chen, S.-Q. Niu, Systematic modulating carbon metabolism to improve α-farnesene production in Pichia pastoris, Fuel Processing Technology 247 (2023) 107757.
[19] H. Liu, S.L. Chen, J.Z. Xu, W.G. Zhang, Dual Regulation of Cytoplasm and Peroxisomes for Improved A-Farnesene Production in Recombinant Pichia pastoris, ACS Synth Biol 10(6) (2021) 1563-1573.
[20] T. Wriessnegger, P. Augustin, M. Engleder, E. Leitner, M. Müller, I. Kaluzna, M. Schürmann, D. Mink, G. Zellnig, H. Schwab, H. Pichler, Production of the sesquiterpenoid (+)-nootkatone by metabolic engineering of Pichia pastoris, Metabolic Engineering 24 (2014) 18-29.
[21] T. Niu, X. Yan, J. Wang, H. Song, Y. Cui, X. Cai, A.K. Chang, J. Qiao, M. Wen, J. He, Engineering of Pichia pastoris for the De Novo Synthesis of the Sesquiterpene Zealexin A1 from Methanol, ACS Sustainable Chemistry & Engineering 12(34) (2024) 12786-12794.
[22] X. Huang, H. Wu, X. Wu, W. Su, P. Li, Naringenin/naringin therapeutic effects and the role of intestinal microflora in them, Pharmacological Research 219 (2025) 107871.
[23] R. Kumokita, T. Bamba, K. Inokuma, T. Yoshida, Y. Ito, A. Kondo, T. Hasunuma, Construction of an l-Tyrosine Chassis in Pichia pastoris Enhances Aromatic Secondary Metabolite Production from Glycerol, ACS Synthetic Biology 11(6) (2022) 2098-2107.
[24] Y.-Q. Wen, Y.-Z. Wang, C.-X. Zhao, B.-Y. Zhao, J.-G. Wang, Baicalin: an active natural product with potential medicinal values, Journal of Asian Natural Products Research 27(8) (2025) 1087-1111.
[25] Y. Kang, Z. Qian, H. Yu, J. Lu, Q. Zhao, X. Qiao, M. Ye, X. Zhou, M. Cai, Programmable Biosynthesis of Plant-Derived 4'-Deoxyflavone Glycosides by an Unconventional Yeast Consortium, Small methods 8(8) (2024) e2301371.
[26] Z. Qian, J. Yu, X. Chen, Y. Kang, Y. Ren, Q. Liu, J. Lu, Q. Zhao, M. Cai, De Novo Production of Plant 4'-Deoxyflavones Baicalein and Oroxylin A from Ethanol in Crabtree-Negative Yeast, ACS Synth Biol 11(4) (2022) 1600-1612.
[27] M. Li, Z. Ni, Z. Li, Y. Yin, J. Liu, D. Wu, Z. Sun, L. Wang, Research progress on biosynthesis of erythritol and multi-dimensional optimization of production strategies, World Journal of Microbiology and Biotechnology 40(8) (2024) 240.
[28] S. Wang, J. Fang, M. Wang, S. Yu, Y. Xia, G. Liu, Y. Zhang, Y. Li, T. Zhu, Rewiring the methanol assimilation pathway in the methylotrophic yeast Pichia pastoris for high-level production of erythritol, Bioresource Technology

DOI: 10.54254/2753-8818/2026.PJ30537

427 (2025) 132430.
[29] G. Liu, Y. Zhou, H. Luo, X. Cheng, R. Zhang, W. Teng, A comparative evaluation of different types of microbial electrolysis desalination cells for malic acid production, Bioresour Technol 198 (2015) 87-93.
[30] T. Zhang, C. Ge, L. Deng, T. Tan, F. Wang, C4-dicarboxylic acid production by overexpressing the reductive TCA pathway, FEMS microbiology letters 362(9) (2015).
[31] F. Guo, Z. Dai, W. Peng, S. Zhang, J. Zhou, J. Ma, W. Dong, F. Xin, W. Zhang, M. Jiang, Metabolic engineering of Pichia pastoris for malic acid production from methanol, Biotechnology and bioengineering 118(1) (2021) 357-371.
[32] J. Gao, Y. Zuo, F. Xiao, Y. Wang, D. Li, J. Xu, C. Ye, L. Feng, L. Jiang, T. Liu, D. Gao, B. Ma, L. Huang, Z. Xu, J. Lian, Biosynthesis of catharanthine in engineered Pichia pastoris, Nature Synthesis 2(3) (2023) 231-242.