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Review 综述

智能化微生物合成水杨酸的研究进展

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摘　要：水杨酸(salicylic acid, SA)是一种重要的酚类化合物，在植物防御反应中发挥核心作用，并因其具有显著的抗炎、抗菌等生物活性而被广泛应用于医药、化妆品及日用化学品领域。目前，水杨酸的生产主要依赖植物提取和化学合成法，存在过程繁琐、环境污染严重以及对石化原料依赖性高等问题。随着合成生物学、代谢工程和人工智能技术的快速发展，利用机器学习算法辅助设计、自动化平台驱动迭代的智能化微生物细胞工厂实现水杨酸的绿色合成已成为替代传统生产方式的重要研究方向。本文系统综述了水杨酸的微生物合成路径，以“智能化”设计为主线，重点总结了在人工智能与合成生物学工具的驱动下天然产水杨酸微生物资源的发掘与利用、在模式微生物中理性重构与优化水杨酸合成途径的智能代谢工程策略、提升产量的关键智能化技术及其面临的挑战，并对该领域的未来发展趋势进行了展望。

关键词：水杨酸；合成生物学；人工智能；机器学习；微生物合成；莽草酸途径；代谢工程

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Research progress in intelligent driving of salicylic acid biosynthesis

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Abstract: Salicylic acid (SA) is an important phenolic compound that plays a key role in plant defenses and is widely used in pharmaceuticals, cosmetics, and personal care products due to its significant anti-inflammatory and antimicrobial activities. Currently, the production of SA mainly relies on plant extraction and chemical synthesis, which suffers from complex processes, severe environmental pollution, and high dependence on petrochemical resources. With the rapid development of synthetic biology, metabolic engineering, and artificial intelligence (AI) technologies, the green synthesis of SA through intelligently designed microbial cell factories, empowered by machine learning algorithms and automated platforms, has become an important research direction to replace conventional production methods. This review systematically summarizes the microbial biosynthetic pathways of SA. With a focus on the intelligent design theme, this paper highlights the application of AI and synthetic biology tools in the discovery and utilization of natural SA-producing microbial resources and the rational reconstruction and optimization of the SA biosynthetic pathway in model microorganisms *via* intelligent metabolic engineering strategies. Furthermore, it introduces the key intelligent technologies for enhancing yields and the challenges faced. Finally, it discusses the future trends in this field.

Keywords: salicylic acid; synthetic biology; artificial intelligence; machine learning; microbial synthesis; shikimate pathway; metabolic engineering

水杨酸(salicylic acid, SA)，化学名称为 2-羟基苯甲酸，是一种分子式为 $C_7H_6O_3$、分子量为 138.12 g/mol 的单环芳香化合物，具有广泛的生物活性与应用价值[1]。水杨酸的多领域应用如图 1 所示。水杨酸的价值链条正从传统的原料和中间体，向创新药物、高端化妆品、绿色农业投入品以及生物基材料平台等高附加值方向快速延伸。在医药领域，水杨酸是合成乙酰水杨酸(阿司匹林)的关键前体，全球阿司匹林年消费量超过 4 万 t[2]。此外，它也是抗艾滋病药物拉米夫定以及其他多种非甾体抗炎药的重要中间体[3]。在化妆品和个人护理品领域，中高端产品的开发前景不仅在于其基础功能的深化应用，更在于与其他活性成分的复配创新以及新型递送技术的结合。水杨酸凭借其角质溶解、抗菌和抗炎特性被广泛用于痤疮治疗、去角质及抗衰老产品[4]。例如，将水杨酸与透明质酸、烟酰胺或特定植物提取物协同使用，旨在实现“多效修护”[5]；利用微囊包裹、脂质体或聚合物胶束等技术可以实现水杨酸的控释、缓释，减少刺激性，提升其在敏感肌肤护理和高端功能性护肤品中的应用价值[6]。在植物生理方面，利用水

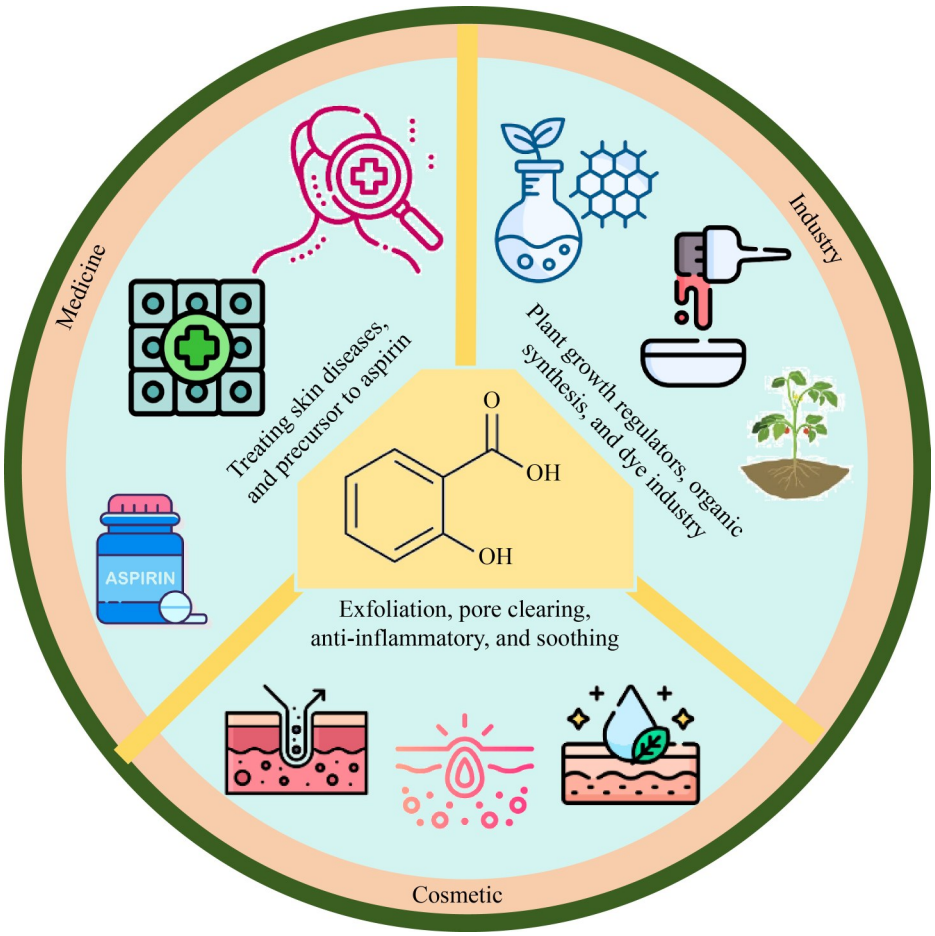

图1　水杨酸的应用

Figure 1　Application of salicylic acid.

杨酸或其类似物作为外源激发子，可预先激活植物的防御系统，减少对化学农药的依赖，符合农业绿色发展理念。目前，基于水杨酸的生物刺激剂和植物免疫诱抗剂正在被开发和商业化，用于提高作物对真菌、细菌及病毒病的抗性。水杨酸作为一种关键激素，参与调节植物生长发育，并在系统获得性抗性(systemic acquired resistance, SAR)中发挥核心作用[7-8]。对某些微生物而言，水杨酸还是铁载体生物合成的前体，协助病原菌如假单胞菌(*Pseudomonas*)、小肠结肠炎耶尔森氏菌(*Yersinia enterocolitica*)和分枝杆菌(*Mycobacterium*)等在铁限制条件下摄取铁元素，与其致病性密切相关。

目前，水杨酸的工业生产主要依赖植物提取和化学合成 2 种方式。植物提取法通常从柳树皮、白珠树等植物组织中获取，虽产品纯度较高，但存在提取效率低、成本高、受季节与地域限制以及潜在生态环境破坏等问题[9]。化学合成则以 Kolbe-Schmitt 反应为主，以苯酚和二氧化碳为原料，在高温高压及强碱性条件下合成水杨酸钠，再经酸化得到水杨酸[10]。尽管该方法具备一定规模优势，但仍面临诸多挑战，包括对石化原料苯酚的依赖、反应条件苛刻、能耗高、生产过程中产生大量废水与废渣造成环境污染，以及产物中常伴有邻甲酚、对羟基苯甲酸等杂质，需经复杂纯化步骤处理[11]。

随着合成生物学、系统代谢工程以及人工智能(artificial intelligence, AI)和机器学习(machine

learning, ML)技术的快速发展，芳香族化合物的生物合成已从传统的“试错式”工程进入基于机器学习和大数据分析的“智能理性设计”时代。利用微生物作为细胞工厂合成水杨酸已成为具有潜力的替代路径[12-13]。微生物合成法以葡萄糖、甘油等可再生碳源为原料，在温和条件下进行反应，符合绿色化学与可持续发展理念[14]。酶催化过程具有高度的区域与立体选择性，有助于提高产物纯度并简化下游分离流程[15]。此外，微生物发酵还具有生长周期短、可实现连续化生产、副产物少、环境污染小等优势[16-17]。然而，该技术目前仍面临产量较低、生产成本缺乏竞争力以及水杨酸对宿主细胞具有一定毒性等问题[18]。通过智能化基因工程手段可精准、高效地调控代谢网络，优化产物合成效率，甚至拓展至水杨酸衍生物的合成[19]。

莽草酸途径作为芳香族化合物合成的核心模块，其高效智能构建与精准动态调控是实现下游高值产物工业化生产的前提[20]。在菌株构建层面，研究者通过对大肠埃希氏菌(*Escherichia coli*)、谷氨酸棒杆菌(*Corynebacterium glutamicum*)等模式微生物的代谢网络进行系统重构，结合基于机器学习的全局转录组分析与智能化代谢流平衡计算，识别出莽草酸合成的关键限速步骤。通过多拷贝质粒或染色体整合策略过表达关键酶基因(*aroB*、*aroE*、*aroL*)，采用规律成簇的间隔短回文重复序列系统及相关蛋白(clustered regularly interspaced short palindromic repeats, CRISPR-associated, CRISPR-Cas9)技术精准阻断竞争性分支途径，并优化莽草酸转运系统；在此技术平台基础上，进一步拓展至芳香族氨基酸等下游产物，对关键酶进行了人工智能辅助的定向进化与功能改造[21-22]，并初步验证了其在医药中间体合成中的应用价值。

为系统总结该领域的研究进展，本文首先阐述水杨酸的微生物合成途径，包括异分支酸途径与植物样合成路径；随后介绍天然产水杨酸微生物的发现及相关研究进展；重点以“智能化”为主线，讨论在模式微生物中理性设计与优化水杨酸合成途径的智能代谢工程策略，涵盖异源途径的智能引入与优化、前体供应的智能强化、产物毒性的智能缓解与动态调控等方面；分析当前面临的关键挑战，并对未来研究方向进行展望。最后对全文进行总结，以期为构建高效、智能的水杨酸微生物合成体系提供理论参考。

1 水杨酸的微生物合成途径

微生物中水杨酸的合成主要通过莽草酸途径产生[23]，主要依赖于 2 条在酶学机制与进化起源上具有显著差异的核心生化途径：异分支酸-丙酮酸裂解酶途径与植物样苯丙烷途径[24]，水杨酸经由莽草酸途径的具体生化反应流程及关键酶如图 2 所示。其中，异分支酸途径因其步骤简洁、催化效率高且易于在微生物宿主中重构，已成为当前研究的重点。在天然菌株中水杨酸常作为铁载体的合成前体，例如在铜绿假单胞菌(*Pseudomonas aeruginosa*)中水杨酸是铁载体绿脓杆菌素(pyochelin)的结构单元，在铁限制条件下协助菌体摄取 Fe^{3+}，对其在宿主体内的生存与致病性至关重要[25]。类似地，小肠结肠炎耶尔森氏菌(*Y. enterocolitica*)合成的耶尔森菌素(yersiniabactin)与结核分枝杆菌(*Mycobacterium tuberculosis*)所产生的分枝杆菌素(mycobactin)也均含有水杨酸结构单元[25]。

1.1 异分支酸-丙酮酸裂解酶途径

异分支酸途径是微生物中合成水杨酸的主要路径，最早在铜绿假单胞菌(*P. aeruginosa*)中被系统阐明[26-27]。1995 年，Serino 等[28]首次克隆并鉴定了铜绿假单胞菌(*P. aeruginosa*) PAO1 中负责水杨酸合成的关键基因 *pchB* 与 *pchA*，这也是微生物中最早被报道的水杨酸生物合成基因。

该途径的生化过程起始于中心碳代谢。磷酸烯醇式丙酮酸(phosphoenolpyruvate, PEP)与赤

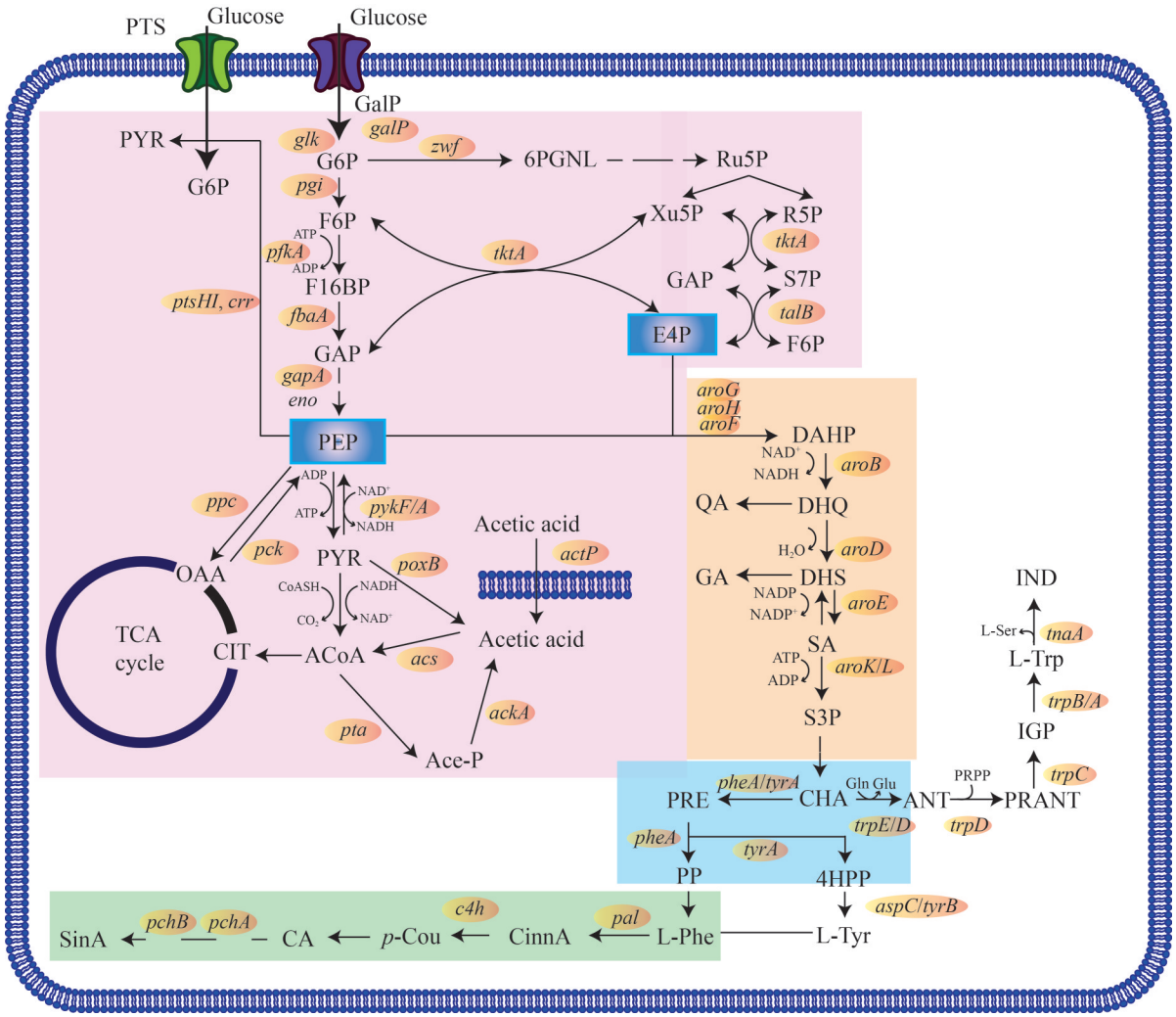

图2　水杨酸的微生物合成途径(以异分支酸途径为主)

Figure 2　Biosynthesis pathway of salicylic acid (predominantly *via* the isochorismate pathway). GLK: Glucokinase; PGI: Phosphoglucose isomerase; PFKA: 6-phosphofructokinase; FBAA: Fructose-1,6-bisphosphate aldolase; GAPA: Glyceraldehyde-3-phosphate dehydrogenase; ENO: Enolase; PTSH/PTSI/CRR: Phosphotransferase system proteins; PPC: Phosphoenolpyruvate carboxylase; PCK: Phosphoenolpyruvate carboxykinase; PYKA/PYKF: Pyruvate kinase; POXB: Pyruvate oxidase; ACS: Acetyl-CoA synthetase; ACKA: Acetate kinase; PTA: Phosphotransacetylase; ACTP: Acetate permease; RPSA: Ribosomal protein S1; TKTA: Transketolase; TALB: Transaldolase; AROF/G/H: 3-deoxy-D-arabinoheptulose-7-phosphate synthase; AROD: 3-dehydrogenase quinate dehydratase; AroE: Shikimate dehydrogenase; AROK/L: Kinase; AROA: 5-enolpyruvylshikimate-3-phosphate synthase; AROC: Chorismate synthase; ASDD: Aspartate semialdehyde dehydrogenase; ASUB: Aspartate-β-semialdehyde dehydrogenase; PHEA/TYRA: Chorismate mutase/prephenate dehydrogenase; TRPE/D: Anthranilate synthase; TRPD: Anthranilate phosphoribosyltransferase; TRPC: Indole-3-glycerolphosphate synthase; TRPB/A: Tryptophan synthase; Pal: Phenylalanine ammonia lyase; ASPC/TYRB: Aspartate aminotransferase/aromatic amino acid aminotransferase; PCHB/PCHA: Isochorismate synthase; MAA: L-valine decarboxylase.

薛糖-4-磷酸(erythrose 4-phosphate, E4P)分别在糖酵解与磷酸戊糖途径(pentose phosphate pathway, PPP)中生成，随后在 3-脱氧-D-阿拉伯庚酮糖酸-7-磷酸合成酶(DAHP synthase)催化下缩合为 2, 4-二氨基-6-羟基嘧啶(2, 4-diamino-6-hydroxypyrimidine, DAHP)，并进一步经由莽草酸途径中包括 AroB、AroD、AroE、AroK/L、AroA 与 AroC 等酶参与的多个酶促步骤，最终生成分支酸(chorismate)[29-30]。分支酸在异分支酸合成酶(isochorismate synthase, ICS) (如 PchA 或 EntC)催化下发生烯丙基侧链迁移，生成异分支酸(isochorismate)[30]。在途径的最后一步，异分支酸在异分支酸-丙酮酸裂解酶(isochorismate pyruvate lyase, IPL)催化下发生 C−C 键裂解，释放丙酮酸并生成水杨酸[31]。

PchA 属于多硫化物硫转移酶(MST)家族，需 Mg^{2+}作为辅因子，其最适 pH 为 7.5−8.0，最适温度为 37 ℃[32-33]。酶催化效率的智能化评估常基于 Michaelis-Menten 动力学模型，其中 v 为反应速率，V_{max} 为最大反应速率，K_m 为底物亲和力常数，如公式(1)所示。通过机器学习拟合实验数据获取 V_{max} 与 K_m 参数，可量化评估 PchA、PchB 等关键酶的催化性能，并指导理性设计高活性突变体，实现途径通量的智能优化。动力学研究表明，铜绿假单胞菌(*P. aeruginosa*)来源的 PchA 对分支酸的 K_m 约为 12 μmol/L，k_{cat} 为 0.6 s^{-1}，催化效率(k_{cat}/K_m)达 5×10^4 mol/(L·s)[30]。值得注意的是，该反应的催化速率接近扩散极限，提示底物结合可能为限速步骤。PchB 为同源二聚体，每个单体约 11 kDa，其活性中心由 2 个单体共同构成，具有“互锁”结构；位于活性中心入口的 Lys42 残基在催化过程中起关键作用[34-35]。该酶对异分支酸的 K_m 值为 12.5 μmol/L，k_{cat} 为 106 min^{-1}，最适 pH 为 6.8[30]。此外，PchB 还表现出较弱的分支酸变位酶活性，可能在一定程度上与主反应竞争底物分支酸[36]。

$$v=\frac{V_{max}[S]}{K_m+[S]} \quad (1)$$

在铜绿假单胞菌(*P. aeruginosa*)中 *pchA* 与 *pchB* 基因构成 *pchBA* 操纵子，其表达受到铁浓度的严格调控。在铁限制条件下，铁摄取调节蛋白的抑制作用被解除，同时 PchR 转录激活因子结合至启动子区域，激活 *pchBA* 的转录[37]。此外，*pchB* 的终止密码子与 *pchA* 的起始密码子存在重叠，提示二者可能通过翻译耦合机制实现协同表达，从而保证 2 种酶以适当化学计量比合成[38]。

1.2 植物样苯丙烷途径

在植物中水杨酸还可通过苯丙烷途径合成，该途径以苯丙氨酸为起始物，依次经苯丙氨酸解氨酶(phenylalanine ammonia lyase, PAL)、肉桂酸-4-羟化酶(cinnamic acid-4-hydroxylase, C4H)、苯甲酸合成酶及苯甲酸-2-羟化酶(benzoate 2-hydroxylase, BA2H)等多步催化最终生成水杨酸[39-41]。最新的研究揭示了一个新的水杨酸生物合成途径，其中苯甲酰辅酶 A 是关键中间体；该研究发现，在杨柳科植物中苯甲酸通过一个三步反应转化为水杨酸；这些步骤包括：苯甲酸与辅酶 A 连接形成苯甲酰辅酶 A；苯甲酰辅酶 A 被羟基化生成 2-羟基苯甲酰辅酶 A；2-羟基苯甲酰辅酶 A 被水解生成水杨酸[42]。然而，在微生物中重构该途径面临诸多挑战：(1) 该途径步骤较长，涉及多个酶促反应，部分酶需依赖复杂的电子传递系统与辅因子 NADPH；(2) 植物来源的酶在微生物宿主中常出现表达活性低、稳定性差等问题，需进行密码子优化与蛋白质工程改造；(3) 整体合成效率普遍较低。尽管有研究尝试在酿酒酵母等真核微生物中引入部分植物苯丙烷代谢酶，但其水杨酸产量仍普遍低于基于异分支酸途径构建的工程菌株[43]。

1.3 两条途径的比较与工程应用选择

从代谢工程角度综合比较，异分支酸途径具有明显优势：该途径从分支酸出发仅需 2 步反应即可生成水杨酸，所涉及的 PchA 与 PchB 酶催化效率高、无需复杂辅酶系统，且其原核来源使其易于在大肠埃希氏菌(*E. coli*)等常用宿主中实现高效异源表达。目前，文献报道中基于该途径构建的工程菌已可实现克级每升的水杨酸产量[44-45]。因此，当前微生物合成水杨酸的研究与菌株开发工作主要集中于对异分支酸途径的进一步优化与应用。

1.4 智能化微生物合成水杨酸的内涵与技术框架

在微生物合成水杨酸的研究与开发中，系统集成人工智能、机器学习、自动化实验平台及先进合成生物学工具，实现从基因设计、途径构建、菌株优化到发酵控制的全程数据驱动与理性设计。其核心技术框架包括：(1) 人工智能/机器学习辅助的蛋白质与途径设计：利用 AlphaFold2、Rosetta 等工具预测与设计高活性/高特异性酶，通过深度学习(deep learning, DL)模型优化代谢途径流量与平衡；(2) 自动化高通量设计-构建-测试-学习(design-build-test-learn, DBTL)循环：借助液体处理机器人、微流控发酵芯片与高通量分析仪器实现菌株的快速构建、筛选与迭代优化；(3) 智能动态调控系统：开发基于生物传感器的反馈控制回路、CRISPRi/a 动态调控开关等，实现产物合成与细胞生长的时空精确解耦；(4) 基因组尺度代谢模型(genome-scale metabolic model, GEM)与机器学习融合：利用 GEM 模拟代谢网络，结合强化学习(reinforcement learning, RL)、元启发式(meta heuristic, MH)方法等优化基因编辑策略，预测非直观工程靶点；(5) 过程数字孪生与智能监控：整合实时多组学数据与过程参数，构建发酵过程数字模型，实现自适应优化与控制。下文将围绕上述智能化技术体系系统综述其在水杨酸微生物合成中的最新研究进展、应用策略与未来方向。

2 天然产水杨酸微生物及其研究

2.1 铜绿假单胞菌

铜绿假单胞菌(*P. aeruginosa*)是研究最为深入的天然水杨酸生产者和模式菌株[46]。该菌是一种革兰氏阴性、机会性病原菌，广泛分布于土壤、水体和医院环境中。在铜绿假单胞菌(*P. aeruginosa*) *PAO1* 基因组中，水杨酸合成相关基因位于 *pch* 生物合成基因簇，该基因簇包含编码转录激活因子的 *pchR*、编码绿脓杆菌素后续合成酶 NRPS 的 *pchD*、*pchC*、*pchE* 和 *pchF*，编码异分支酸合成酶的 *pchB* 和 *pchA*，以及编码转运和调控蛋白的 *pchG*、*pchH*[47]。在铁限制环境下，*pch* 基因簇被激活，合成的水杨酸进一步转化为绿脓杆菌素[48]。绿脓杆菌素能够螯合环境中的 Fe^{3+}，形成的铁-绿脓杆菌素复合物通过外膜受体 FptA 被识别并转运入细胞[49]。研究表明，*pchA* 或 *pchB* 缺失突变株在铁限制条件下生长受到严重抑制，且在小鼠感染模型中毒力显著降低，证明了水杨酸-绿脓杆菌素系统在致病性中的重要作用[50]。铜绿假单胞菌(*P. aeruginosa*) *pchBA* 基因为后续的异源表达和代谢工程提供了关键的基因资源。Serino 等[51]在 1997 年首次证明，将 *pchBA* 基因导入大肠埃希氏菌 *entC* 突变株(缺失异分支酸合成酶)可恢复水杨酸的合成，这一发现奠定了在模式微生物中重构水杨酸合成途径的基础。

2.2 其他假单胞菌属

除铜绿假单胞菌(*P. aeruginosa*)外，多种假单胞菌也能合成水杨酸或其衍生物。荧光假单胞菌(*Pseudomonas fluorescens*) WCS374 菌株含有 *pmsBAC* 基因簇，其中 *pmsC* 和 *pmsB* 分别编码异分支酸合成酶和异分支酸裂解酶，与 *pchA*

和 *pchB* 同源[52]。该菌株合成的铁载体假单胞菌素也包含水杨酸结构，研究表明 *pmsC* 和 *pmsB* 可在大肠埃希氏菌中异源表达并产生水杨酸[51]。恶臭假单胞菌(*Pseudomonas putida*)的某些菌株含有类似的水杨酸合成基因，且具有较强的有机溶剂耐受性，是构建非天然产物细胞工厂的良好宿主。某些恶臭假单胞菌(*P. putida*)菌株具有水杨酸合成基因，这些菌株的水杨酸合成基因或相关通路可被进一步开发，用于实现特定非天然化合物的合成[1,53]。

2.3 其他细菌

小肠结肠炎耶尔森氏菌(*Y. enterocolitica*)的高致病性岛(high-pathogenicity island, HPI)上的 *irp9* 基因编码一种双功能酶，能够直接从分支酸生成水杨酸，无需经过异分支酸中间体，这一发现拓展了微生物合成水杨酸的催化机制多样性[54]。结核分枝杆菌(*M. tuberculosis*)合成的铁载体分枝杆菌素含有水杨酸结构，*MbtI* 基因编码的水杨酸合成酶(salicylate synthase)是药物开发的重要靶点[55-56]。某些链霉菌属(*Streptomyces* spp.)在次级代谢产物合成中也涉及水杨酸或其衍生物的生成，但相关研究相对较少[57-58]。

2.4 植物内生菌

一些植物内生菌也被发现能够合成水杨酸，如芽孢杆菌属(*Bacillus* spp.)、固氮螺菌属(*Azospirillum* spp.)和无色杆菌属(*Achromobacter* spp.)等，这可能在植物-微生物互作和诱导植物系统抗性中发挥作用[58]。然而，Ye 等[59]提出，细菌在体外培养中分泌的“游离”水杨酸可能是一种人工现象，在自然环境中水杨酸通常会被迅速掺入铁载体或其他代谢产物中，因此游离水杨酸分泌诱导植物抗性的机制尚不明确。

2.5 天然菌株的局限性

尽管天然菌株为水杨酸合成提供了宝贵的遗传资源，但直接用于工业生产存在诸多限制：首先，存在致病性问题，铜绿假单胞菌(*P. aeruginosa*)、小肠结肠炎耶尔森氏菌(*Y. enterocolitica*)等是人类病原菌，无法在开放环境中大规模培养，存在生物安全风险[60]；其次，某些菌株生长条件苛刻，对培养基成分、温度、pH 等有特殊要求，增加了生产成本；再次，天然菌株的水杨酸代谢网络复杂，作为铁载体前体，水杨酸合成受到多重调控，且产物会被迅速转化，难以大量积累，远低于工业需求；最后，部分菌株缺乏完善的遗传操作工具，从而限制了代谢改造。

表 1 系统归纳并直观比较了天然产水杨酸微生物的主要菌种、合成途径、产物关联、调控机制及应用潜力等信息。

3 智能化驱动的代谢工程策略构建水杨酸高产菌株

构建高效的水杨酸生产细胞工厂是当前研究的核心内容。大肠埃希氏菌(*E. coli*)和酿酒酵母(*Saccharomyces cerevisiae*)因其遗传背景清晰、操作工具完善而成为首选宿主[61]。随着 AI 与自动化技术的融合，代谢工程策略正从传统的经验性、局部性改造，向基于数据驱动和全局优化的“智能化”设计范式转变。

3.1 异源途径的智能引入与优化

异源途径的成功引入是构建水杨酸生产菌株的基础。在基因来源选择方面，异分支酸合成酶和异分支酸裂解酶的组合选择至关重要。研究表明，源自铜绿假单胞菌(*P. aeruginosa*)的 PchA 与 PchB 组合在大肠埃希氏菌(*E. coli*)中的表达效果最佳，成为构建工程菌的首选[29-31,44]。其他来源的酶如荧光假单胞菌(*P. fluorescens*)的 PmsC-PmsB 组合、大肠埃希氏菌(*E. coli*)自身的 EntC[29]以及甲基单胞菌属(*Methylomonas*)的双功能酶 AmS[62]也被研究，但其效率均不及 PchA-PchB 系统。

表1　代表性天然产水杨酸微生物及其特性

Table 1　Characteristics of representative natural salicylic acid-producing microorganisms

Microbial name	Classification/ Characteristics	Key genes/ Enzymes	Main products/Functions	Value and limitations	References
Pseudomonas aeruginosa	Gram-negative, opportunistic pathogen; widely present in the environment; the most thoroughly studied type strain	*pchBA* manipulator	Salicylic acid, as a precursor to the siderophore *P. aeruginosa*, assists in iron uptake under iron-restricted conditions and is closely related to pathogenicity	Value: It provides the first elucidated microbial pathway and key genes (*pchA*/*B*) for salicylic acid synthesis, which are core gene resources for subsequent heterologous expression Limitations: Human pathogens pose biosafety risks and cannot be used for open industrial fermentation; the products are rapidly converted as precursors and are difficult to accumulate	[46,48-53]
Pseudomonas fluorescens WCS374	Gram-negative, commonly found in soil and plant rhizosphere; some strains have biocontrol potential	The *pmsBAC* gene cluster is homologous to *pchA*/*B*	Salicylic acid is the structural unit of siderophore pseudomonasin	Value: This study demonstrates the conservation of the pathway in different *Pseudomonas* species; *pmsC*/*B* can be used for heterologous expression Limitations: Its research and application depth is not as advanced as that of *P. aeruginosa*	[53-54]
Pseudomonas putida	Gram-negative; known for its excellent tolerance to organic solvents and aromatic compounds; commonly used in environmental engineering	Some strains contain similar salicylic acid synthesis gene clusters	It may be related to secondary metabolism or environmental adaptation	Value: Due to its strong tolerance, it is a highly promising non-pathogenic host for building cell factories of aromatic compounds, especially suitable for two-phase extraction fermentation Limitations: Genetic manipulation toolkits and the depth of basic research are generally not as advanced as those for model bacteria such as *E. coli*	[55]
Yersinia enterocolitica	Gram-negative, enteric pathogens	*irp9* gene	Salicylic acid is a component of the siderophore *Yersinia*	Value: Its Irp9 can directly generate salicylic acid from branched acid without the need for heterobranched acid intermediates, representing a unique catalytic mechanism and expanding the diversity of synthetic pathways	[56]

(待续)

(续表1)

Microbial name	Classification/ Characteristics	Key genes/ Enzymes	Main products/Functions	Value and limitations	References
				Limitations: Human pathogens pose a biosafety risk	
Mycobacterium tuberculosis	Gram-positive, obligate aerobic, cause tuberculosis	*mbtI* gene	Salicylic acid is a key structural unit of siderophore mycotoxins	Value: MbtI is an important target for developing novel anti-tuberculosis drugs; studying its synthetic pathway helps to understand the iron acquisition mechanism of pathogens Limitations: Pathogenic bacteria, slow growth, operations must be carried out in a biosafety level 3 laboratory, high research threshold	[57-58]
Bacillus spp., *Azospirillum* spp., *Achromobacter* spp., etc.	They live within plant tissues and do not cause obvious diseases; most of them can promote plant growth	The specific synthetic genes are usually not fully elucidated and may differ from known pathways	The secretion of free salicylic acid may play a signaling role in inducing systemic resistance in plants and in plant-microbe interactions	Value: Provides resources for understanding microbe-plant interactions and developing biostimulants; environmentally friendly and highly safe Limitations: The synthesis mechanisms are mostly unclear; the authenticity and mechanism of action of secreted “free” salicylic acid in the natural environment are still controversial; the yield is extremely low	[59-60]

表达系统的智能化设计对途径效率有显著影响。传统策略依赖经验选择启动子与载体。强启动子如T7虽然表达强度高，但可能导致蛋白错误折叠或形成包涵体。在载体选择方面，高拷贝质粒能提供高基因剂量，但代谢负担大、遗传不稳定[63]；中拷贝质粒能平衡表达水平和稳定性；染色体整合遗传稳定性最佳，适合长期连续发酵，但基因剂量较低，可通过多位点整合提高表达量[64]。智能化方法则利用机器学习模型，基于启动子序列特征、核糖体结合位点强度、质粒拷贝数与宿主生长关联等大数据，预测最优的表达单元组合[65-66]。例如，基于神经网络(neural network, NN)或随机森林(random forest, RF)算法的启动子强度预测工具可快速从库中筛选出适合PchA/PchB协同表达的启动子对，避免表达失衡。对于诱导型系统(如LacI/Ptrc)，强化学习算法可用于优化诱导时机与IPTG浓度，在动态发酵过程中实现生长与生产的最优平衡。CRISPR-Cas辅助的多位点基因组整合技术结合自动化菌落挑选与验证平台已能高效、精准地将途径基因整合至基因组特定位点，大幅提升菌株构建的“智能化”与通量。

Lin等[44]使用质粒携带*pchA*和*pchB*基因，在以甘油为碳源的培养条件下获得了约1.2 g/L的水杨酸产量。Noda等[45]采用染色体整合策略将大肠埃希氏菌(*E. coli*)自身的异分支酸合成酶(由*menF*编码)和*pchB*整合到a*roK*/*aroL*缺失菌株的基因组中，在优化培养条件下达到了

11.5 g/L 的水杨酸产量，葡萄糖摩尔转化率超过40%，代表了水杨酸微生物合成领域的重要里程碑；该研究中虽未明确使用人工智能工具，但其系统性的多重工程策略本身正是后续智能化全局优化算法(如 OptKnock)所要学习和模拟的对象。

密码子优化是提高异源蛋白表达水平的关键策略[67-68]。来自铜绿假单胞菌(*P. aeruginosa*)的 *pchA* 和 *pchB* 基因在大肠埃希氏菌(*E. coli*)中的密码子使用偏好性与宿主存在差异，可能导致翻译效率低、蛋白表达量不足或错误折叠。传统的密码子优化主要基于密码子适应指数(codon adaptation index, CAI)[69]。智能化密码子优化则更进一步，利用深度学习模型分析宿主全基因组翻译动力学数据，在优化密码子使用的同时，预测并避免形成抑制翻译的 mRNA 二级结构或隐蔽的剪切位点，从而更精准地提升蛋白表达水平与可溶性[70]。

3.2 中心碳代谢与莽草酸途径的智能强化

提高水杨酸产量的核心在于增加前体磷酸烯醇式丙酮酸(PEP)、赤藓糖-4-磷酸(erythrose-4-phosphate, E4P)、分支酸、异分支酸的供应，将碳流导向目标产物。传统的强化策略依赖于对已知限速步骤的逐一修改，而智能化方法旨在通过系统建模与数据挖掘识别非直观的瓶颈并进行全局优化[71]。基因组尺度代谢模型(GEM)结合通量平衡分析(flux balance analysis, FBA)或 ^{13}C 代谢通量分析(metabolic flux analysis, MFA)数据可定量评估不同工程策略对 PEP 和 E4P 供应以及整体代谢网络的影响，从而理性选择最优的改造组合，避免因单点改造引发的代谢失衡[72]。具体而言，在智能代谢工程中代谢通量分析通过求解约束优化问题，量化细胞内各反应的代谢流分布，如公式(2)所示。

$$\begin{aligned} &min \sum_i (v_i - v_{i,exp})^2 \\ &s.t. S \cdot v = 0,\ v_{min} \le v \le v_{max} \end{aligned} \tag{2}$$

其数学模型以代谢物平衡方程“$S \cdot v=0$”为核心约束，通过最小化实验测得通量 $v_{i,exp}$ 与模型预测值之间的差异，识别代谢网络中的瓶颈反应与冗余路径。该优化框架为人工智能算法提供了可计算的代谢状态表示，是智能预测与动态调控的基础。

增强前体 PEP 和 E4P 的供应是关键策略之一。PEP 是莽草酸途径的关键前体，但在大肠埃希氏菌(*E. coli*)中 PEP 主要通过磷酸转移酶系统(phosphotransferase system, PTS)用于葡萄糖摄取，同时被丙酮酸激酶(PYKF 和 PYKA)迅速转化为丙酮酸，导致用于莽草酸途径的 PEP 有限。代谢工程策略包括替换 PTS 系统为 *galP-glk* 系统，直接以葡萄糖激酶磷酸化葡萄糖，保留 PEP[73]；敲除 *pykF* 和 *pykA* 基因，阻断 PEP 到丙酮酸的转化[74]；过表达编码 PEP 合成酶的 *ppsA*，催化丙酮酸逆向生成 PEP[75]。E4P 来自磷酸戊糖途径，通过过表达编码转酮醇酶的 *tktA* 和编码转醛醇酶的 *talB*，并重定向碳流，削弱糖酵解，增强 PPP，可为莽草酸途径提供更多 E4P[76-77]。

解除反馈抑制是提高途径通量的重要手段。人工智能辅助的蛋白质工程技术已深刻改变了反馈抑制解除突变体的发现过程。利用 AlphaFold2 等蛋白质结构预测工具可快速获得 DAHP 合成酶的三维结构模型。结合分子动力学模拟与机器学习分类器可预测关键氨基酸位点突变对底物结合口袋构象及反馈抑制物结合能力的影响，从而理性设计出反馈抑制完全解除且酶活保持甚至增强的新型突变体，替代传统的随机诱变筛选[78]。莽草酸途径的首个酶，DAHP 合成酶受到严格的反馈抑制调控，是整个途径的限速步骤[79]。大肠埃希氏菌(*E. coli*)的 DAHP 合成酶同工酶 AroF、AroG、AroH 分别被酪氨酸、苯丙氨酸和色氨酸反馈抑制[80]。最常用的策略是使用 $aroG^{fbr}$ 变体使酶对苯丙氨酸不敏感[81]，并通过强启动子和高拷贝载体大量表达突变酶，显著增加 DAHP 合成通量[82]。

在酿酒酵母中，ARO4^{K229L}突变体对酪氨酸不敏感，已被广泛用于芳香族化合物生产菌株的构建[41]。

过表达莽草酸途径限速酶可进一步提高通量。转录组学与蛋白质组学的多组学数据整合分析，结合机器学习方法可以更准确地识别在特定工程背景下的新限速步骤，实现动态、精准的途径强化，而非简单的全部过表达[83-84]。除 DAHP 合成酶外，3-脱氢奎尼酸合成酶(3-dehydroquinate synthase, AroB)[85]、莽草酸脱氢酶(shikimate dehydrogenase, AroE)[86]和分支酸合成酶(chorismate synthase, AroC)[87]均是途径中的关键限速酶。典型策略是将 *aroB*、*aroD*、*aroE*、*aroC* 等基因构建成一个操纵子或分别过表达，实现途径整体强化[88]。

削弱竞争途径是引导代谢流向水杨酸合成的有效方法。基于 GEM 的 OptKnock、OptForce 等计算算法可系统性地计算为最大化代谢产物合成通量所需敲除的基因组合，这些组合可能包括非直观的、与目标产物合成无直接关联的基因，从而实现碳流的全局最优重定向[89]。这类算法是代谢工程“智能化”的重要体现。分支酸是莽草酸途径的核心分支点，可进入多条下游途径合成芳香族氨基酸(Phe、Tyr、Trp)和其他化合物，与水杨酸合成竞争底物。通过敲除芳香族氨基酸合成途径的关键基因 *pheA*、*tyrA*、*trpE* 或 *trpD*[90-92]可大幅提高流向水杨酸的碳通量。定量分析表明，在 *pheA*/*tyrA* 双敲除菌株中流向水杨酸的碳通量可增加 40%−60%[93]。需要注意的是，这些氨基酸是细胞必需的，敲除相应基因会导致菌株营养缺陷，因此需要在培养基中补充相应的氨基酸[94]。

辅因子工程对维持途径高通量至关重要。代谢控制分析(metabolic control analysis, MCA)与辅因子供需平衡的数学模型结合实时监测技术可用于定量评估辅因子限制程度，并指导动态辅因子工程策略的设计，例如在生长后期诱导表达辅因子再生酶以匹配产物合成需求[95]。莽草酸途径的多个反应需要辅因子。增强 NADPH 供应的策略包括过表达磷酸葡萄糖脱氢酶(由 *zwf* 编码)和 6-磷酸葡萄糖酸脱氢酶(由 *gnd* 编码)，增强 PPP 氧化支路[96]；此外，引入外源的 NADH 激酶，如来自粪肠球菌(*Enterococcus faecalis*)的 *ppnK*，催化 NADH 转化为 NADPH[97]；使用 NAD(P)H 转氢酶系统平衡 NADH 和 NADPH 比例[98]。ATP 供应优化则需要确保充分供氧，维持高 ATP/ADP 比值，并敲除 ATP 消耗型竞争途径[99]。

3.3 智能动态调控与产物外排

水杨酸在胞内积累会对微生物生长产生多方面抑制作用，包括膜损伤[100]、蛋白变性[101]、DNA 损伤[102]和代谢紊乱[103]。研究表明，当胞内水杨酸浓度超过 1 g/L 时大肠埃希氏菌(*E. coli*)的生长显著受抑，OD_{600} 降低 50% 以上[19]。静态的基因改造难以平衡生长与生产的矛盾，智能化动态调控系统提供了更优解决方案。

动态调控策略可有效解决毒性问题。动态代谢调控系统的智能设计常借助常微分方程组描述产物合成、酶表达及外排的时序变化。产物 P 浓度变化受合成速率 k_{syn}、降解与外排速率共同影响；酶量 E 则受诱导信号 $Inducer\,(t)$ 动态调控，如公式(3)所示。

$$\begin{aligned}\frac{d[P]}{dt} &= k_{syn} - k_{deg}[P] - k_{export}[P] \\ \frac{d[E]}{dt} &= \alpha \cdot Inducer\,(t) - \beta \cdot [E]\end{aligned} \tag{3}$$

该模型可用于仿真不同调控策略下产物积累轨迹，指导生物传感器与反馈回路的设计。“生长-生产两阶段”策略在生长期抑制水杨酸合成，在稳定期开启合成[104]。基于生物传感器的动态调控包括营养感应型调控[105-106]、群体感应型调控[107]和产物响应型调控[108-109]。近年来，人工智能算法被用于优化生物传感器的设计。通过机器学习分析大量启动子-调控因子互作数据可以预测或设计出具有所需动力学参数，如灵敏度、动态范围、响应阈值的新型生物传感

器元件，从而实现对水杨酸浓度的更精确感知与调控[110-112]。Gao 等[113]开发了一种可逆热调控系统，通过温度变化控制基因表达，实现了生长与生产的精确分离。基于 CRISPR 的动态调控使用 CRISPRi 技术在转录水平动态调控竞争途径或合成途径的基因表达强度，提供了更精细的调控手段[114]。

产物外排工程是代谢工程中的一项关键策略，通过将目标化合物从胞内主动转运至胞外能够有效减轻产物对细胞生长的毒性作用，并弱化反馈抑制，从而显著提高最终产量。机器学习模型可用于从转运蛋白序列数据库中预测具有潜在水杨酸转运活性的候选蛋白，缩小实验筛选范围[115]。此外，适应性进化与全基因组测序结合机器学习分析可快速鉴定出在高水杨酸压力下发生富集的关键膜蛋白突变，为外排泵工程提供直接靶点[116]。对于水杨酸而言，促进其胞外分泌主要可通过 2 类转运系统实现：一是过表达天然特异性转运蛋白，二是利用广谱性的多药外排泵。工程策略包括过表达候选转运蛋白，筛选能提高水杨酸分泌效率的转运体；对转运蛋白进行定向进化，提高对水杨酸的特异性和转运效率；平衡外排速率与合成速率，避免因过度外排导致胞内中间体不足。此外，水杨酸的转运过程受多种因素调控，包括宿主微生物的种类、培养环境以及其他细胞调控机制。需要指出的是，在植物体系中水杨酸作为一种关键信号分子，其积累与调控与胁迫响应及生长发育密切相关。近年研究表明，水杨酸信号传导不仅涉及多种蛋白，脂质信号也在其应答过程中扮演重要角色[117]。

宿主耐受性工程是提升水杨酸生产效率的关键策略之一[118]。通过适应性进化方法，在逐步提高水杨酸浓度的选择压力下进行连续传代培养可获得耐受性显著提高的突变菌株。这些耐受性突变通常涉及多个细胞层面，包括膜蛋白组成改变、应激响应系统调整以及中心代谢途径的重编程。全基因组重测序与转录组学结合的全基因组关联分析(genome-wide association study, GWAS)与机器学习方法可系统性解析耐受性突变的关键基因与通路，为理性的耐受性工程改造提供蓝图[119]。全局转录因子工程通过过表达或定向改造全局调控因子可增强细胞对多种环境胁迫的整体耐受能力。同时，膜工程策略通过调整细胞膜脂肪酸组成以增加膜流动性或稳定性，或过表达膜稳定蛋白(如分子伴侣)也能有效增强细胞对水杨酸胁迫的适应能力。

3.4 水杨酸高产菌株的智能构建与案例分析

系统代谢工程策略的应用已显著提升了水杨酸的微生物合成效率。大肠埃希氏菌(*E. coli*)高产菌株的构建已取得显著进展。Zhang 等[120]通过对大肠埃希氏菌(*E. coli*)进行代谢工程改造，实现了以廉价的可再生碳源为原料高效生产水杨苷；他们首先研究了从分支酸合成水杨酸的酶，随后表达来自杨树的水杨苷合成酶基因，构建了从分支酸到水杨苷的合成途径，并通过优化培养条件水杨苷的产量得到了显著提高。Chen 等[121]的研究将水杨酸合酶 Irp9 引入到产苯丙氨酸的大肠埃希氏菌(*E. coli*)中，构建了最短的水杨酸生物合成途径；后续的蛋白质工程改造使 Irp9 的催化效率提高了 33.5%，并报道了摇瓶中水杨酸的最高浓度为 3.72 g/L。Lin 等[44]以大肠埃希氏菌(*E. coli*)野生型菌株为起点，通过导入携带 *pchA* 和 *pchB* 的质粒，并优化碳源，在摇瓶中获得约 1.2 g/L 的水杨酸。Noda 等[45]的工作代表了重要突破，他们以大肠埃希氏菌(*E. coli*)苯丙氨酸过量生产菌 ATCC 31882 为起始菌株，通过敲除 *pykF* 和 *pykA*，替换 PTS 系统为 *galP-glk* 系统，过表达 $aroG^{fbr}$、*aroB*、*aroD*、*aroE*，并染色体整合 *menF* 和 *pchB*，在优化培养条件下实现了 11.5 g/L 的水杨酸产量，葡萄糖摩尔转化率超过 40%。Sun 等[122]利用水杨酸作为顺,顺-黏康酸合成的前体，通过引入异

分支酸合成酶、异分支酸丙酮酸裂解酶、水杨酸1-单加氧酶和儿茶酚1,2-双加氧酶实现了2.4 g/L水杨酸和最终产物黏康酸的高效积累，展示了以水杨酸为平台分子进一步合成其他高价值芳香族化合物的潜力。

相比大肠埃希氏菌(*E. coli*)，在酿酒酵母(*S. cerevisiae*)中构建水杨酸生产菌株的研究相对较少，主要挑战在于真核细胞的莽草酸途径部分在不同细胞器中进行，这增加了工程复杂性、异源原核酶在酵母中的表达和活性不理想、酵母对芳香族化合物的耐受性可能不如大肠埃希氏菌(*E. coli*)[123]。尽管如此，酵母作为真核生物，更适合表达含P450酶的复杂植物途径，具有发达的内膜系统便于膜结合酶的功能表达，更适合食品和医药应用。Zhang等[124]的研究也指出了酿酒酵母(*S. cerevisiae*) CEN.PK2-1C可能更适合合成以苯丙氨酸为前体的天然产物。未来需要更系统地优化包括密码子优化、添加细胞器定位信号、平衡ARO4/ARO3的表达和反馈抑制解除、强化ERG10等前体供应酶。

从表2可以看出研究进展的几个显著特征。大肠埃希氏菌是目前的主流宿主，所有高产菌株(>5 g/L)均基于大肠埃希氏菌构建，证明了其作为水杨酸生产底盘的优越性。系统代谢工程的重要性在最高产量(11.5 g/L)的菌株中得到充分体现，该菌株整合了多个关键策略：前体供应强化、反馈抑制解除、竞争途径削弱、莽草酸途径限速酶过表达以及高效异源酶导入。

发酵条件优化的价值在同一种菌从摇瓶(6.2 g/L)到发酵罐(11.5 g/L)产量提升近1倍中得到验证[45]，表明过程工程优化对提高产量至关重要。比较不同研究可见，敲除芳香族氨基酸合成途径的菌株产量明显高于未敲除的菌株，验证了将碳流导向水杨酸的策略有效性。

真核宿主的研究仍有待突破，酵母和谷氨酸棒杆菌(*C. glutamate*)中的水杨酸产量远低于大肠埃希氏菌(*E. coli*)，说明在非模式或真核宿主中重构异分支酸途径仍面临挑战，需要更深入

表2 微生物合成水杨酸的代表性研究总结

Table 2 Summary of representative studies on microbial biosynthesis of salicylic acid

Chassis	Strategy	Carbon source	Culture methods	Yield/(g/L)	Key conditions	References
E. coli	Introducing Irp9	Glucose	Shake flask	3.72	Pathway construction based on high-phenylalanine-producing strains	[121]
E. coli	Introducing *pchA* and *pchB*	Glycerin	Shake flask	1.20	Using glycerol instead of glucose as a carbon source, the effects of different precursor supply methods were verified	[44]
E. coli	Δ*pheA*, Δ*tyrA*; overexpress *aroG*^{fbr}, *aroB*, *aroE*; introducing *entC* and *pchB*	Glucose	Shake flask	~2.00	Knock out competing pathways and enhance precursor supply; phenylalanine and tyrosine need to be supplemented in the culture medium	[122]
E. coli	Δ*pykF*, Δ*pykA*; non-PTS; overexpress *aroG*^{fbr}, *aroB*, *aroD*, *aroE*; introducing *menF* and *pchB*	Glucose	Shake flask	6.20	Systems metabolic engineering enhances precursors, pathways, and transport; supplementation of aromatic amino acids is necessary	[45]
E. coli		Glucose	2 L bioreactor	11.50	By scaling up from shake flasks to fermenters and optimizing oxygen supply and process control, yields have been significantly increased	[45]

Δ indicates gene knockout; *fbr* indicates a feedback-resistant mutant.

的宿主适应性改造。结合近年蛋白质智能工程、人工智能驱动的动态调控等策略在其他微生物产物合成中取得的显著进展，并参考当前最高产菌株的基础，通过系统性的智能化迭代，有望在未来将水杨酸的微生物合成产量推向具有工业竞争力的水平。

3.5 系统生物学与人工智能在菌株优化中的深度应用

系统生物学方法结合人工智能技术为水杨酸高产菌株的构建提供了强大的技术支撑。基因组尺度代谢模型结合机器学习算法能够预测不同基因敲除/过表达组合对水杨酸产量的影响[125]。深度学习模型可以从海量实验数据中识别非线性代谢关系，发现非直观的代谢瓶颈，指导代谢工程策略的理性设计[126]。传统的定向进化受限于筛选通量，仅能探索极小区域。基于深度学习的适应度景观模型将蛋白质工程问题转化为序列函数拟合任务。给定一个无标注的蛋白质序列数据集，可通过自监督预训练学习氨基酸序列的概率分布，如公式(4)所示。该目标函数源自变分自编码器，其隐含假设是：天然蛋白质序列在潜空间 z 中形成致密聚类，而距离聚类中心较远的序列往往不稳定或活性较低。

$$f(seq)=E_{z\sim q(z|seq)}[\log p(seq|z)]-\beta\cdot D_{KL}(q(zseq)\|p(z)) \quad (4)$$

此外，使用 OptKnock[127]、OptForce[128] 等算法可计算出最优的基因敲除组合，使水杨酸生产与细胞生长耦合，实现生长选择性筛选高产突变株。基于机器学习的产量预测模型将基因表达、发酵参数等多维特征 x_i 映射为水杨酸产量预测值 $\hat{y}$。线性回归模型通过权重 θ_i 量化各因素贡献，而深度学习模型则通过多层非线性变换捕捉复杂特征交互，如公式(5)所示。此类数据驱动模型可实现高产菌株的智能筛选与发酵工艺的虚拟优化，大幅降低实验试错成本。

$$\hat{y}=\theta_0+\sum_{i=1}^{n}\theta_i x_i+\epsilon \quad (5)$$

组学分析为深入理解工程菌的代谢状态提供了有力工具。转录组学可以比较野生型与工程菌的基因表达谱，识别代谢瓶颈和应激响应基因，发现意外上调或下调的途径，指导进一步优化[129]。代谢组学可以定量分析胞内代谢物池，识别积累的中间体或缺乏的前体，验证代谢工程改造是否按预期改变代谢流[130]。蛋白质组学可以测定关键酶的实际表达水平，评估基因表达是否转化为蛋白水平，发现翻译后修饰或蛋白降解问题[131]。^{13}C-MFA 使用同位素标记底物追踪碳原子在代谢网络中的流动，精确测定各个代谢反应的通量，定量识别限速步骤[132]。通过整合多组学数据可全面理解工程菌的代谢状态，实现更精准的菌株改造。人工智能技术，特别是深度学习和神经网络算法，在多组学数据整合分析中发挥着越来越重要的作用，能够从复杂的生物学数据中挖掘出传统方法难以发现的代谢调控规律。

4 总结与展望

微生物合成水杨酸的研究已取得显著进展，但要实现大规模工业化应用仍面临产量与经济性、途径效率、宿主耐受性、过程集成与放大等多方面挑战。

4.1 当前面临的主要挑战

经济性分析显示，化学法水杨酸成本约为 2−3 USD/kg，而生物法当前估算成本约为 5−8 USD/kg[18]，需将产量提升至 20−30 g/L 以上并优化下游工艺方能在成本上与化学法竞争。宿主耐受性方面，即使采用动态调控与产物外排策略，水杨酸的细胞毒性仍限制最终产量。当培养液中浓度超过 10−15 g/L 时，即使胞内浓度不高，胞外高浓度仍会对细胞造成应激[133]。未来需筛选或构建耐受性更强的菌株，并开发如双相萃取发酵、膜分离等原位产物去除技术[134]。

在从实验室研究迈向工业化生产的过程中，

过程集成与放大面临着诸多严峻的工程挑战。首先，在大型发酵罐中物理尺度的增加导致传质与混合效率显著下降，反应器内会形成明显的溶氧梯度与底物浓度梯度，造成局部区域氧气或营养供给不足，从而严重影响微生物代谢的稳定性与最终产物的合成效率。与此同时，发酵过程中水杨酸本身的积累会持续降低发酵液的 pH 值，这一方面要求精确且持续地添加碱液进行中和，增加了过程控制的复杂性；另一方面又不可避免地导致盐分的积累，可能抑制细胞生长并为后续纯化环节带来额外负担。随着发酵规模的扩大和运行周期的延长，整个系统面临的染菌风险也急剧升高，对设备密封性、无菌操作及过程监控提出了极高要求。放大生产后的下游纯化环节也更为复杂，培养基中的残余组分、细胞代谢产物以及大量的细胞碎片都会对目标产物的分离纯化造成显著干扰；现有的主流纯化工艺，如酸化沉淀与溶剂萃取等，在应对这种复杂性时通常在收率、成本控制及环境影响方面存在局限，亟需进一步优化与创新以实现整体过程的经济可行性[135]。此外，目前多数研究使用葡萄糖或甘油作为碳源，这些“第一代”底物与食品和燃料生产竞争，未来需开发木质纤维素水解物、农业废弃物等廉价可再生底物的生产工艺以进一步提高可持续性[136]。

4.2 未来研究方向与发展策略

水杨酸高效微生物合成可采取多维度优化策略(图 3)。基于人工智能的蛋白质工程将成为提升水杨酸合成途径效率的关键突破口。借助 AlphaFold 等人工智能蛋白质结构预测工具、机器学习辅助的定向进化或基于深度学习的理性设计策略可对 PchB 酶进行系统性改造，旨在提高其催化效率或底物亲和力，并有效消除其分支酸变位酶副反应，从而减少底物损耗。

合成生物学工具的快速发展为水杨酸合成途径的精细优化提供了全新可能。采用人工智能驱动的模块化途径设计理念可利用标准化生物元件构建基因表达调控元件库，或通过设计-构建-测试-学习(DBTL)循环结合机器学习算法快速组装与评估不同途径组合的效率，并通过强化学习优化设计策略[137]。为突破天然途径的代谢瓶颈，可探索设计非天然的水杨酸合成新路线以绕过现有途径中的限速步骤[138]。此外，通过合成蛋白质支架将途径中的多个关键酶有序组织为多酶复合体，可实现“底物通道”效应，减少中间代谢物的扩散损失，从而提高整体途径效率[139]。CRISPR-Cas 系统的广泛应用也将助力多位点基因组编辑与基因表达的精细调控，为代谢网络的动态优化提供强大工具[140]。

在宿主系统选择方面，拓展非模式微生物的应用将有助于发掘更具优势的细胞工厂。恶臭假单胞菌对芳香族化合物具有天然耐受性，且其代谢网络适用于芳环类物质的转化，尤其适用于有机溶剂两相萃取发酵体系[141]。谷氨酸棒杆菌作为工业上成熟的氨基酸生产菌种，不仅具备强健的莽草酸途径，还具有公认安全地位，适合用于医药级产品的生产[142-143]。毕赤酵母(*Pichia pastoris*)以其高密度发酵能力和高效蛋白表达系统著称，适用于复杂酶系的异源表达[144]。此外，嗜热菌因其发酵过程可降低污染风险并减少冷却能耗也展现出独特的应用前景[145]。

过程工程的系统强化是实现水杨酸微生物合成产业化的重要保障。下游分离纯化是实现水杨酸微生物合成产业化的关键瓶颈，其挑战主要源于发酵液体系复杂、产物浓度相对较低，以及传统酸化沉淀与溶剂萃取工艺存在收率低、成本高、环境不友好等问题。未来的发展必须摒弃“先发酵，后处理”的传统割裂思维，转而致力于过程强化与智能集成。为突破此瓶颈，必须发展过程强化与智能集成策略：一方面，通过开发高效的原位产物去除技术，例如双相萃取、树脂吸附、膜分离及电渗析等能够有效降低发酵液中水杨酸的反馈抑制，并简化下游提取流程；另一方面，深度融合在线监测、机

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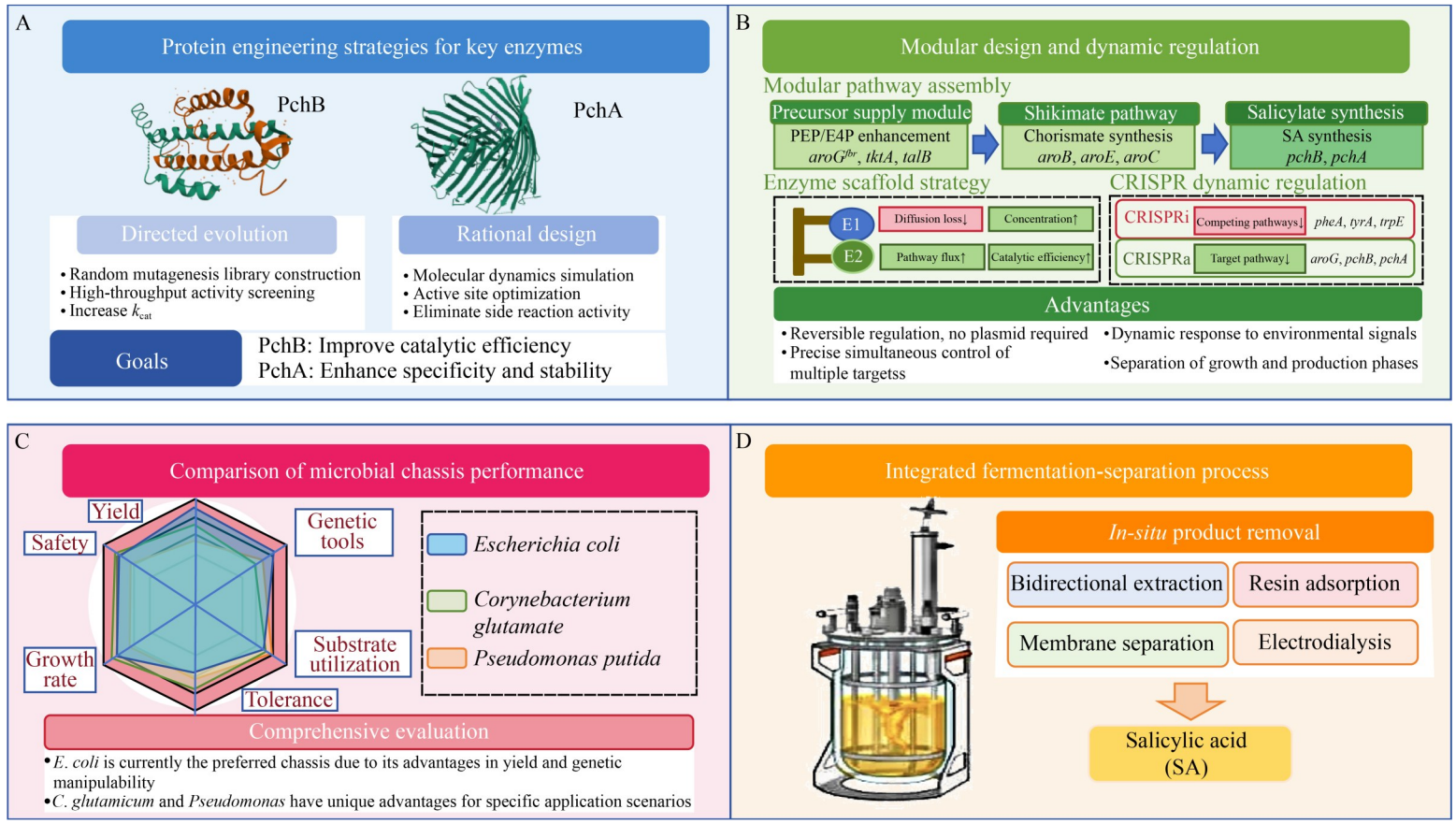

图3　水杨酸高效微生物合成系统的多维度优化策略示意图

Figure 3　Schematic diagram of multi-dimensional optimization strategies for an efficient microbial biosynthesis system of salicylic acid. A: Protein engineering of key enzymes (Key enzymes in synthetic pathways are engineered through directed evolution and rational design to improve their catalytic efficiency, stability, and eliminate side reactions); B: Integrated application of synthetic biology tools (Modular pathway design and enzyme scaffold technology are used to construct multi-enzyme complexes to achieve a substrate channel effect, and the CRISPR system is used for dynamic regulation to achieve precise control and optimization of metabolic flux); C: Development and comparison of diverse microbial chassis (The performance of different hosts such as *Escherichia coli*, *Corynebacterium glutamicum*, and *Pseudomonas putida* is compared in terms of yield, safety, genetic manipulation, and other aspects to expand the range of host selection); D: Enhanced coupling of fermentation and downstream processes (A variety of in situ product removal technologies are demonstrated for integration with fermentation processes to alleviate product inhibition and simplify downstream purification).

器学习优化以及全流程数字孪生等智能化工具，实现从发酵到纯化的精准控制与系统优化，从而推动该技术走向经济可行的绿色制造[146]。通过开发新型、绿色的分离介质与技术，并深度融合在线监测、人工智能与数字孪生等智能化工具，实现从细胞工厂到最终产品的高效、低碳转化，是水杨酸微生物合成技术走向产业化应用的必由之路。

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