Jun. 25, 2023, 39(6): 2313-2333

·微生物细胞合成·

杨晓兵 西北农林科技大学葡萄酒学院副教授。研究工作主要为采用合成生物学和代谢工程策略改造圆红冬孢酵母，赋予其合成高价值化合物萜烯类、苯丙烷类的能力，从而高值化利用葡萄园副产物，提高葡萄产业链条的经济性。主持和参与省部级研究课题 6 项，其中陕西省科技厅一般面上项目 1 项；在 *Biotechnology Advances*、*Biotechnology for Biofuels* (*and Bioproducts*)、*Bioresource Technology* 等期刊发表科技论文近 30 篇；申请发明专利 6 项，获得授权发明专利 2 项；受邀参加编写外文图书 1 部(Springer 出版社)。

圆红冬孢酵母基因编辑及天然产物合成的研究进展

高琦豆，董亚琦，黄颖，刘懿娟，杨晓兵*

西北农林科技大学葡萄酒学院，陕西 杨凌 712100

高琦豆，董亚琦，黄颖，刘懿娟，杨晓兵. 圆红冬孢酵母基因编辑及天然产物合成的研究进展[J]. 生物工程学报，2023，39(6): 2313-2333.
GAO Qidou, DONG Yaqi, HUANG Ying, LIU Yijuan, YANG Xiaobing. Advances in gene editing and natural product synthesis of *Rhodotorula toruloides*[J]. Chinese Journal of Biotechnology, 2023, 39(6): 2313-2333.

摘 要：圆红冬孢酵母(*Rhodotorula toruloides*)是一种能够天然合成多种类胡萝卜素和油脂的非模式酵母。该菌能够利用各种廉价原料，耐受甚至同化利用多种有毒木质纤维素水解副产物。目前，该酵母被广泛用于微生物油脂、萜烯类化合物、各种高价值酶、糖醇和聚酮化合物的生产研究。鉴于其广阔的工业应用前景，研究人员对其开展了多维度的理论和技术的探索，包括基因组、转录组、蛋白组、遗传操作平台等。本文着重阐述近年来圆红冬孢酵母的代谢工程和天然产物合成的研究进展，并展望其细胞工厂构建中面临的挑战和可能的应对决策。

关键词：圆红冬孢酵母；非模式酵母；基因工程；天然产物合成；代谢工程

资助项目：中央高校基本科研业务费专项资金(2452018314)；陕西省自然科学基础研究计划(2020JM-177)
This work was supported by the Chinese Universities Scientific Fund (2452018314) and the Natural Science Basic Research Program of Shaanxi Province (2020JM-177).
*Corresponding author. E-mail: yangxb@nwafu.edu.cn
Received: 2022-12-15; Accepted: 2023-02-15; Published online: 2023-02-17

Advances in gene editing and natural product synthesis of *Rhodotorula toruloides*

GAO Qidou, DONG Yaqi, HUANG Ying, LIU Yijuan, YANG Xiaobing*

College of Enology, Northwest A&F University, Yangling 712100, Shaanxi, China

Abstract: *Rhodotorula toruloides* is a non-conventional red yeast that can synthesize various carotenoids and lipids. It can utilize a variety of cost-effective raw materials, tolerate and assimilate toxic inhibitors in lignocellulosic hydrolysate. At present, it is widely investigated for the production of microbial lipids, terpenes, high-value enzymes, sugar alcohols and polyketides. Given its broad industrial application prospects, researchers have carried out multi-dimensional theoretical and technological exploration, including research on genomics, transcriptomics, proteomics and genetic operation platform. Here we review the recent progress in metabolic engineering and natural product synthesis of *R. toruloides*, and prospect the challenges and possible solutions in the construction of *R. toruloides* cell factory.

Keywords: *Rhodotorula toruloides*; non-conventional yeast; genetic engineering; natural product synthesis; metabolic engineering

绿色生物制造是生物经济时代的核心，是解决当前石化资源安全、能源安全、环境安全、粮食安全，实现“双碳”目标的必由之路。基于合成生物学技术改造微生物平台，实现生物炼制木质纤维素生产各类天然产物具有广阔前景。木质纤维素生物质是世界上最为丰富的可再生资源，其主要组分为纤维素、半纤维素和木质素。其中，纤维素和半纤维素部分能够被分解为可发酵单糖如葡萄糖、木糖和甘露糖等被微生物转化利用。脂类、萜类、黄酮类、糖醇等天然产物广泛应用于保健、食品、医药、香料、化妆品等行业[1]。通过合成生物学和代谢工程策略赋能木质纤维素的生物转化，有望实现各种高值化学品的低成本、可持续高效生产[2]。相比植物和动物，微生物具有种类繁多、增殖迅速、底物转化效率高等特点，以工程化微生物为平台，能够实现各类可再生化合物连续化、规模化生产，且不受季节、气候等客观因素影响[3]。当前，微生物催化转化已应用于生物质绿色转化、化学品绿色合成、未来食品生物制造等诸多研究领域[3-5]。寻找与培育高效的微生物平台菌株，对有效转化利用可再生原料，降低绿色生物制造的生产成本、促进菌株工业化应用具有重要意义。

圆红冬孢酵母(*Rhodotorula toruloides*)是一种异宗配合、双极性的担子菌纲(Basidiomycetes)的非模式酵母，该菌 1922 年首次从中国大连分离获得，自 20 世纪 50 年代以来被当作一种潜在的生物技术微生物[6]。该菌具有强大的碳源、氮源利用能力，能够天然合成类胡萝卜素、油脂、苯丙氨酸解氨酶和 D-氨基酸氧化酶等重要化合物[1,7-9]。该菌株具有高密度发酵的特性，能够耐受较低 pH 值、高渗透胁迫、氧化胁迫等，特别是该酵母能够高效利用各种木质纤维素水解液作碳源，对水解液副产物具有很强的耐受和代谢能力，甚至能够利用 5-(羟甲基)糠

醛[10-13]。综上所述，圆红冬孢酵母具有搭建木质纤维素原料生物炼制平台的巨大潜力[2,14-16]。

系统地改造圆红冬孢酵母需要坚实的理论和技术支撑。鉴于圆红冬孢酵母的潜在工业应用价值，研究人员对其进行了多线程的底层理论和技术研究。Zhu 等[17]依据基因组结合转录组数据绘制了圆红冬孢酵母 NP11 的基因组草图(基因组大小为 20.2 Mb，GC 含量 61.9%)，注释了 8 171 个蛋白质编码基因，发掘了圆红冬孢酵母 NP11 中新型脂肪酸合酶系统；利用转录组学和蛋白质组学阐释了氮限制条件下脂质积累的机制，明晰了影响含氮化合物再循环、大分子代谢和自噬诱导等方面的机理。此外，功能基因组学和比较基因组学技术的结合为研究圆红冬孢酵母的染色体结构、蛋白质编码基因及功能 RNA 提供有力支撑[18]。Tiukova 等[19]建立了首个圆红冬孢酵母 NP11 基因组规模的代谢模型，并研究了木质纤维素中木糖代谢到脂质合成的蛋白组学。通过比较葡萄糖和木糖作为碳源对菌株生长动力学、脂质组成、脂肪酸图谱和蛋白质组等方面的影响，发现木糖培养具有较低的生物量和耗糖速率，发酵中能够高效表达参与糖转运、木糖同化的初始步骤和烟酰胺腺嘌呤二核苷酸磷酸(nicotinamide adenine dinucleotide phosphate, NADPH)再生的蛋白质，且含有更高水平的参与过氧化物酶体 β-氧化和氧化应激反应的酶[19]。随后，菌株 IFO0880 的基因组规模代谢模型也相继发表，能够有效预测关于利用葡萄糖、木糖和甘油培养菌株的生长情况，锚定提高亚麻酸、三酰基甘油(triacylglycerol, TAG)和类胡萝卜素等产量的某些关键基因[20-21]。Kim 等[22]通过多组学分析和代谢网络重建，确定了圆红冬孢酵母独特的戊糖和芳香化合物的代谢途径。Dinh 等[21]通过基因组尺度的代谢模型及多组学分析圆红冬孢酵母利用木质纤维素的机制，扩大了先前代谢模型研究覆盖的广度，研究结果将助力圆红冬孢酵母转化木质纤维素生产高值化合物。在基础理论研究不断深入的同时，圆红冬孢酵母基因编辑技术如 CRISPR-Cas9、Cre/Loxp、Flp/FRT 和 RNA 干扰(RNA interference, RNAi)等也取得了诸多进展，编辑技术手段的进步将为该酵母系统理性地改造提供技术支撑[23-25]。

简言之，圆红冬孢酵母的全基因组测序和多组学研究为深度改造代谢路径、调控通路机制及跨越基因编辑壁垒给予了理论保障。合成生物学和代谢工程技术的不断发展，为理性工程改造圆红冬孢酵母天然代谢网络，实现微生物油脂等大宗产品和类胡萝卜素等高价值化合物合成提供了坚实的技术支撑。本文将重点阐述近年来圆红冬孢酵母基因元件挖掘和基因编辑技术开发的进展和挑战，同时总结了圆红冬孢酵母合成天然产物的发展近况，并对其未来发展趋势进行了展望。

1 圆红冬孢酵母基因工程使能工具的研究进展

传统育种工程如诱变育种和适应性实验室进化在提升圆红冬孢酵母的鲁棒性、强化生产效率等方面具有重要价值[26-27]。然而，利用诱变和适应性实验室进化难以实现圆红冬孢酵母菌株的理性改造。因此，研究人员对其基因元件、基因编辑技术开展了一系列的研究工作，该部分主要阐述圆红冬孢酵母基因工具的研究进展。

1.1 基因的表达元件的挖掘

非模式酵母中可靠高效、正交性和鲁棒性强的基因元件的挖掘和改进是合成生物学的重要内容[28-29]。为了不断完善圆红冬孢酵母的代谢工程，当务之急是开发高效遗传编辑功能工

具包。本部分将阐述圆红冬孢酵母启动子、终止子、筛选标记等方面的研究进展以及存在的问题。

1.1.1 启动子与终止子

丰富的启动子文库是理性调控目的基因表达、设计合理代谢路径和维持细胞代谢平衡的基础；而各具特色的启动子适合于不同的应用场景。最初，圆红冬孢酵母中报道了一些可以调节异源基因表达的代谢响应启动子[30-33]。虽然代谢响应启动子便于代谢路径的调控，但要探索菌株潜在的路径工程，仍然需要一套特征良好的组成型启动子文库。Nora 等[34]验证了一组内源组成型启动子，其中有 8 个能够双向启动转录；他们还发掘了一些能够在发酵晚期启动基因表达的启动子，这项工作显著地丰富了圆红冬孢酵母代谢工程可用工具。为了理性分配代谢流及控制基因的表达强度，需要对启动子强弱进行表征。Wang 等[32]通过定量聚合酶链式反应(polymerase chain reaction, PCR)表征了圆红冬孢酵母内源的 5 个启动子强弱，分别为葡萄糖 6-磷酸异构酶启动子(P_{Pgl})>磷酸甘油酸激酶启动子(P_{Pgk})>果糖 1,6-二磷酸醛缩酶启动子(P_{Fba})>磷酸三糖异构酶启动子(P_{Tpi})>甘油醛 3-磷酸脱氢酶启动子(P_{Gpd})。

区别于组成型启动子，使用诱导型启动子能够根据研究的需求改变诱导条件，进而调控蛋白质和 RNA 的表达。一项研究比较几种假定的担子菌 D-氨基酸氧化酶基因(*dao1*)同源物的上游 DNA 序列，确定了圆红冬孢酵母 ATCC 10657 *dao1* 上游保守的 DNA 序列。验证发现包含内含子的 *dao1* 启动子与一个 *dao1* 缺失突变体相结合，在圆红冬孢酵母属和红酵母属中形成了高效且紧密的 D-氨基酸诱导基因表达系统[35]。启动子 P_{Dao1} 具有良好的调控基因表达的潜力，低至 1 mmol/L 的 D-丙氨酸也能够有效诱导 P_{Dao1} 启动子。研究还发现，通过调控 D-丙氨酸诱导剂浓度，能够将 P_{Dao1} 启动子的强度变化范围控制在 10 倍以内。此外，磷酸盐饥饿诱导型、半乳糖诱导型启动子也依次被表征，如内源的 Na^+/Pi 协同转运蛋白启动子 P_{Pho89}、乙醇脱氢酶启动子 P_{Adh2} 和半乳糖激酶启动子 P_{Gal1} 受到磷酸盐和葡萄糖的严格调控。其中，P_{Pho89} 在磷酸盐浓度为$(1.19\times10^{-3}–1.19\times10^{-2})$ mmol/L 时被高效诱导；P_{Adh2} 和 P_{Gal1} 在以半乳糖为唯一碳源的培养基中能够快速诱导基因表达[36]。硝酸还原酶启动子 P_{Nar1}，柠檬酸裂解酶 1 启动子 P_{Icl1}，高亲和性铜转运体启动子 P_{Ctr3} 和磷酸腺苷硫酸还原酶启动子 P_{Met16} 具有独立的诱导和抑制条件(具体详见[36]中表 2)，尽管诱导水平和效率不同，在圆红冬孢酵母中同样能够实现基因的可控表达[30]。作为最小诱导启动子，P_{Nar1} 的调控区域仅 200 bp，在含有硫酸铵的抑制条件下，显示出非常严格的调控。此外，表达小 RNA (sgRNA)的 RNA 聚合酶III启动子已成功用于圆红冬孢酵母 CRISPR-Cas9 编辑系统的开发[23,37]。

终止子是构建基因高效表达系统的重要元件。目前，已有多种终止子应用于圆红冬孢酵母表达系统的构建，如内源的终止子 T_{hsp}、T_{gpd}[38-39]。一些富含 T 的序列也能起到终止基因转录的作用，在圆红冬孢酵母 CRISPR-Cas9 系统的构建中，连续的 T 序列能够终止 sgRNA 的转录[40]。此外，研究人员还挖掘了一些异源终止子用于圆红冬孢酵母表达载体的构建，如花椰菜花叶病毒(cauliflower mosaic virus)来源的 T_{35s}、根癌农杆菌(*Agrobacterium tumefaciens*)来源的 T_{nos}[26]。启动子与终止子是调节生物合成途径和生产重组蛋白的重要元件，其文库的不断挖掘为圆红冬孢酵母遗传操作平台的构建奠定基础。

1.1.2 筛选标记和报告基因

筛选标记和报告基因是快速筛选转化子的基础工具，具有指示、表征遗传选择的功能，辅助阳性转化子的快速分选。筛选标记通常分为抗性筛选标记和营养缺陷型标记两大类。就抗性筛选标记而言，诺尔丝菌素、博来霉菌素、潮霉素和遗传霉素在圆红冬孢酵母工程菌的筛选中最为常用。研究人员对常用的营养缺陷选择标记如亮氨酸缺陷 Leu2、尿嘧啶缺陷 Ura3 等在圆红冬孢酵母中的可用性也进行了探索。研究结果表明，Ura3 具有较高的筛选可行性，其敲除菌株能够耐受 1 g/L 5-氟乳清酸[30,41-44]。圆红冬孢酵母的深度代谢工程化改造通常涉及诸多基因，现有的筛选标记仍不能满足科研需求。为了改善筛选标记不足的现状，研究人员构建了基于 Cre/loxP 和 Flp/FRT 的位点特异性重组系统，以实现抗性筛选标记的循环使用(图 1)[24,43]。然而，Cre/loxP 和 Flp/FRT 系统的引入需要在抗性标记两端重复引入特异性序列，经过迭代整合易造成染色体的非特异性切割，导致菌株不稳定。

报告基因主要分为荧光酶素和荧光蛋白两大类，常用于启动子、终止子等基因元件的功能表征及菌株快速筛选。目前，荧光酶素和荧光蛋白均在圆红冬孢酵母中成功应用[26,30,34]。其中，绿色荧光蛋白和红色荧光蛋白常被用于启动子的筛选[34]。报告基因的成功应用，为构建圆红冬孢酵母基因元件文库提供了可靠工具。

1.1.3 2A 肽介导多基因表达

代谢路径的构建与调控通常涉及多个基因协同操作。然而，真核生物中基因通常以单顺反子形式发挥功能，这在很大程度上限制了基因操作的效率。借助病毒来源 2A 肽的“stop-carry on”的转录编码机制，能够在圆红冬孢酵母中实现多基因共表达[38]。即核糖体能够跳过 2A 元件 C-末端的甘氨酸和脯氨酸的肽键合成，致使 2A 序列末端和下游蛋白分离[45]。研究人员已经利用源于猪捷申病毒(porcine teschovirus)的 P2A 和源于明脉扁刺蛾病毒(thoseaasigna virus)的 T2A 序列成功构建了类胡萝卜素、柠檬烯等异源合成路径的表达[38,46]。

1.2 基因表达载体

在基因工程领域，标准化和模块化的表达载体是快速构建优质工程菌株的必要条件[28]。表达载体通常以质粒形式存在，分为游离型和整合型。其中，整合型质粒能够使外源基因整合至基因组而实现目标基因稳定表达。

对圆红冬孢酵母而言，由于缺乏已知的自主复制序列和着丝粒序列，无法构建游离的表达载体，需要将基因整合到基因组中表达[44]。整合型质粒 pEX2、pZPK 等通过根癌农杆菌介导的转化(*Agrobacterium tumefaciens* mediated transformation, ATMT)整合进入圆红冬孢酵母基因组，构建多种代谢路径的稳定表达[43,47-48]。目前，基因元件库及表达载体的挖掘和应用取得了一定的发展，为了基因表达模块的快速搭建，研究人员开发了 Gibson 组装、Golden Gate 等载体构建技术[49-52]。Bonturi 等[53]构建了圆红冬孢酵母模块化、多功能、高效的 Golden Gate 组装系统，其中包含启动子、基因、终止子、多插入位点和抗性基因等标准化工具。圆红冬孢酵母 Golden Gate 工具包完成了复杂代谢途径类胡萝卜素的四表达盒质粒的搭建，使其产量增加 41%，达到 14.0 mg/L。这项尝试填补了圆红冬孢酵母基因组工具包的空白，实现代谢路径表达载体的快速设计和组装。未来，构建更加标准化的元件和正交设计的模块化载体仍然是基因工程不断努力的目标。

1.3 圆红冬孢酵母遗传转化与基因编辑

目前，圆红冬孢酵母的遗传编辑和代谢工程改造工具尚不完善。研究人员为高效代谢调

控该酵母，对其遗传转化和基因编辑技术进行了大量的探索。本部分将主要阐述圆红冬孢酵母的遗传转化方式、基因编辑的进展与挑战。

1.3.1 圆红冬孢酵母遗传转化

高效便捷的转化方法是实施圆红冬孢酵母基因工程改造的前提保障。当前已经成功应用于圆红冬孢酵母的转化方法有：(1) 聚乙二醇(polyethylene glycol, PEG)介导的原生质体转化；(2) 醋酸锂/PEG 介导的化学转化；(3) 根癌农杆菌介导的转化(ATMT)；(4) 电击穿孔法。其中，ATMT 是目前圆红冬孢酵母最常用和可靠的转化方法。

PEG 介导的圆红冬孢酵母原生质体转化操作繁琐，效率低(10^3/μg DNA)且不稳定，现在应用较少[41]。醋酸锂/PEG 介导的转化方法在圆红冬孢酵母中能够实现 10^2/μg DNA 的转化效率[54]。ATMT 最常用，转化效率较高，操作简单，但转化周期长，基因整合位点不可控，其能够理性设计和操作的基因数受限于可用筛选标记的数量[42]。然而，研究人员通过充分利用 ATMT 的基因整合位点的不确定性，迭代转化并筛选转化子文库，同样能够获得感兴趣的菌株[55]。依托转化子文库，结合染色体步移技术能够鉴定 T-DNA 的插入位点，解析酵母的表达与生产特性[56]。此外，通过电穿孔转化法快速转化线性 DNA 片段改造圆红冬孢酵母，转化效率达到 2 000 个转化子(1 μg DNA)[57]。但该方法受限于筛选标记的数量和圆红冬孢酵母极低的同源重组效率。

在圆红冬孢酵母中敲除 Ku70/80 蛋白能够提高同源重组效率，但会降低修复能力和转化效率，损害细胞鲁棒性[43,57]。为了提高同源重组效率，未来可以通过表达异源高效的同源重组系统相关蛋白如 Rad51、Rad52 等来提升同源重组效率[58-59]。Zhang 等[60]通过 Cas 蛋白融合核酸外切酶以提升双链断裂后的末端切除效率，发现在毕赤酵母中融合内源 Mre11 且在 Rad52 过表达时，实现了无缝删除双基因 76.7%–86.7%、三基因 10.8%–16.7%的效率。以上研究为圆红冬孢酵母基因编辑系统优化提供了新方向。

1.3.2 CRISPR 介导的基因组编辑

为了实现圆红冬孢酵母的理性高效基因编辑，研究人员已经在圆红冬孢酵母中构建了 Cre/loxP、Flp/FRT 和 CRISPR-Cas9 基因编辑系统(图 1)[24,43]。如前所述，重复使用 Cre/loxP、Flp/FRT 系统易造成不可控的非理性基因编辑。本部分主要阐述 CRISPR-Cas9 系统的开发应用。目前，多个团队已独立完成了圆红冬孢酵母 CRISPR-Cas9 系统的构建，实现了靶向遗传操作[23,37,61]。

当前，在圆红冬孢酵母中报道的 Cas9 蛋白有两种。一是化脓链球菌(*Streptococcus pyogenes*)的 *Sp*Cas9 核酸酶，该酶使用 5′-NGG-3′作为原间隔相邻基序(PAM)。由于该酶 PAM 序列较短，在圆红冬胞酵母(基因组 GC 含量高)，脱靶率较高。相较而言，来源于金黄色葡萄球菌(*Staphylococcus aureus*)的分子量较小的 *Sa*Cas9 的 PAM 序列(5′-NNGRRT-3′)较长，特异性更强，脱靶率更低[23]。为了高效表达 Cas 蛋白与 sgRNA，研究人员筛选了 RNA 聚合酶II、III类启动子，得到最适配的表达元件组合[37,62]。目前，单基因敲除效率中 *Sa*Cas9 达到 60%以上，*Sp*Cas9 在 50%–95%，而 *Sp*Cas9 对多基因敲除效率达到 78%。Carl 等[54]通过 CRISPR-Cas9 分别敲除了圆红冬孢酵母 IFO0880 的二酰基甘油酰基转移酶(diacylglycerol acyltransferase, Dga1)和卵磷脂胆固醇酰基转移酶(lecithin cholesterol acyltransferase, Lro1)，使脂肪醇产量分别提升了 2.3 倍和 4.4 倍。研究还发现 Dga1 和 Lro1 同时敲除对圆红冬孢酵母是致死的，原因可能是双

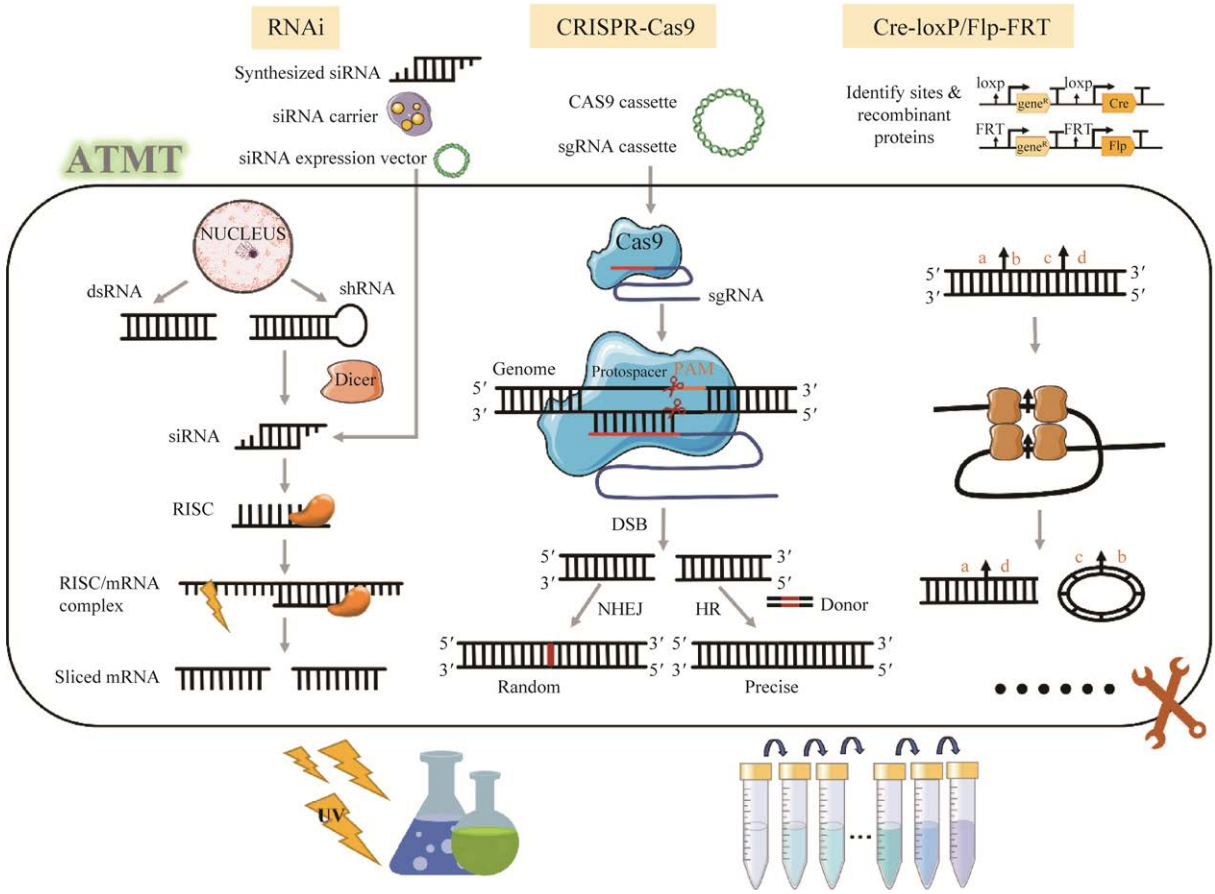

图 1　圆红冬孢酵母的基因工程[24,43]　从左至右依次为 RNA 干扰技术、CRISP-Cas9 技术、Cre/loxP 和 Flp/FRT 位点特异性重组技术. RISC：RNA 诱导的沉默复合物；DSB：双键断裂；NHEJ：非同源末端链接；HR：同源重组；ATMT：根癌农杆菌介导转化

Figure 1　Genetic engineering of *Rhodotorula toruloides*[24,43]. From left to right: RNA interference technique, CRISP-Cas9 technique, Cre/loxP and Flp/FRT site specific recombination technique. RISC: RNA induced silencing complex; DSB: Double strand break; NHEJ: Non-homologous end joining; HR: Homologous recombination; ATMT: *Agrobacterium tumefaciens* mediated transformation.

基因敲除导致圆红冬孢酵母无法合成生存必需的 TAG。Jiao 等[40]利用 CRISPR-Cas9 系统敲除了圆红冬孢酵母 NP11 的脂滴结构蛋白 Ldp1 和 Cals，使菌株脂质含量下降 40%以上；研究还表明 Ldp1 缺失菌的脂滴尺寸显著减小。CRISPR-Cas9 技术的发展与应用将为该红酵母的理性编辑提供科学参考。

1.3.3　RNA 干扰技术

直接敲除代谢通路和调控网络中的必需基因会影响菌株的生长活性，甚至致死[63]。RNAi 是一种转录后水平的基因调控技术，能够实现必需基因的弱化调控，并广泛应用于多种真核生物[64-67]。Liu 等[25]首次在圆红冬孢酵母 NP11 中采用 RNAi 技术下调了自噬相关基因 8 (*atg8*)和脂肪酸合成酶基因(*fas1* 和 *fas2*)的表达水平，使基因沉默效率达到 11%–92%。最近，RNAi 技术成功应用于圆红冬孢酵母合成二酰基甘油(diacyl glycerol, DAG)和脂肪酸的代谢途径中。通过该技术下调了合成 TAG 的 3 个基因，使碳代谢流转向目标产物，产量分别提升 2 倍

和 3 倍[68]。

RNAi 下调靶基因的效率不同，可能存在多种影响因素。(1) 整合位点效应引起的“位点特异性”，导致双链 RNA (double-stranded RNA, dsRNA)抑制效率存在差异；(2) 靶基因太长，无法确定 dsRNA 作用的有效区域；(3) RNAi 技术相关元件的设计非理性等[25]。

当前，圆红冬孢酵母的遗传工具、转化方法和基因编辑技术还有待继续丰富，其他酵母中具有功能的元件和技术在红酵母属也逐渐成功应用[30,69]。将来，遗传工具集的不断挖掘、表征、标准化和交互使用将促进圆红冬孢酵母基因编辑技术的多层次发展。

2 圆红冬孢酵母天然产物合成研究进展

基于其天然代谢特性，研究人员利用圆红冬孢酵母开展了油脂、油脂衍生物、类胡萝卜素、柠檬烯、红没药烯等产物的合成工作(图 2)。为促进木质纤维素的生物炼制，研究人员利用代谢工程策略强化了圆红冬孢酵母的木质纤维素水解副产物的耐受性。

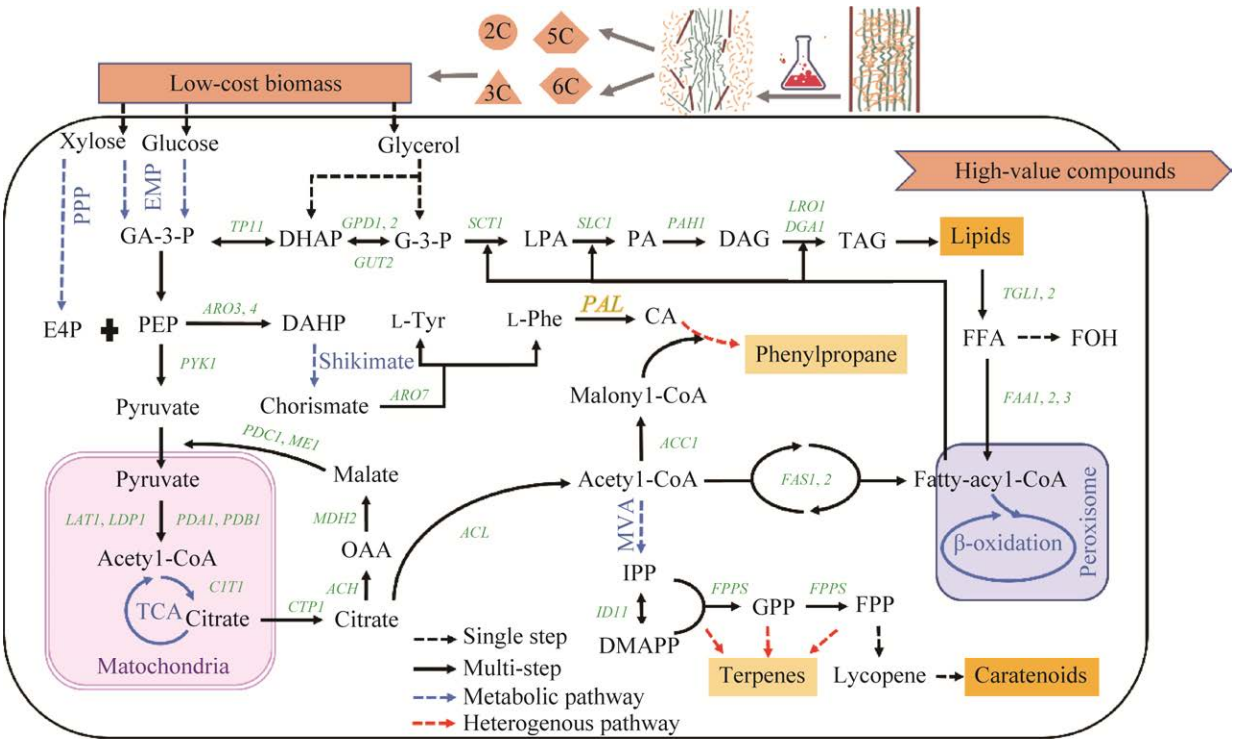

图 2 圆红冬孢酵母生产高值化合物 关键天然产物用不同颜色高亮显示. GA-3-P：3-磷酸甘油醛；DHAP：磷酸二羟基丙酮；G-3-P：甘油-3-磷酸；LPA：溶血磷脂酸；PA：磷脂酸；DAG：二酰甘油；TAG：三酰甘油；FFA：游离脂肪酸；FOH：脂肪醇；OAA：草酰乙酸盐；IPP：异戊烯基二磷酸；DMAPP：二甲基烯丙基二磷酸；PEP：磷酸烯醇式丙酮酸；E4P：赤藓-4-磷酸；DAHP：3-脱氧-D-阿拉伯庚酮糖-7-磷酸

Figure 2 High value compounds produced by *Rhodotorula toruloides*. Key natural products are highlighted with different color. GA-3-P: Glyceraldehyde 3-phosphate; DHAP: Dihydroxyacetone phosphate; G-3-P: Glycerol-3-phosphate; LPA: Lysophosphatidic acid; PA: Phosphatidic acid; DAG: Diacylglycerol; TAG: Triacylglycerol; FFA: Free fatty acid; FOH: Fatty alcohol; OAA: Oxaloacetate; IPP: Isopentenyl diphosphate; DMAPP: Dimethylallyl diphosphate; PEP: Phosphoenolpyruvic acid; E4P: Erythrin 4-phosphate; DAHP: 3-deoxy-D-arabinogeptulose 7-phosphate.

2.1 油脂及其衍生物的合成

油脂是一种可再生燃料，能够作为传统化石燃料的替代品[70]。相比于从植物中提取油脂产品，微生物产油脂具有生产周期短、底物范围广、不占用耕地、易于规模化生产等优势，但同时面临生产成本较高的困境[71-72]。圆红冬孢酵母在营养限制条件下能够积累超过自身干重 70%的油脂[40]。目前，提升圆红冬孢酵母油脂合成的策略集中在优化发酵条件及代谢工程调控方面。发酵体系优化强化油脂发酵的研究已经在相关的文献中进行了系统评述[73]，本部分将重点介绍代谢工程策略强化圆红冬孢酵母合成油脂及其衍生物研究进展。

脂质的合成需要充足的乙酰辅酶 A 和 NADPH 供应。在圆红冬孢酵母 NP11 中引入枯草芽孢杆菌(*Bacillus subtilis*)磷酸转乙酰酶(phosphotransacetylase, Pta)，使细胞量、脂质产量和产率分别提高 8.5%、15.0%和 64.0%[74]。通过表达内源的苹果酸脱氢酶(malic enzyme, Me)改善 NADPH 供应，工程改造的圆红冬孢酵母在磷、氮限制下脂质含量提升 24%，达到 10.8 g/L[7,75]。DAG 到 TAG 的高效转化也是油脂高效合成的关键。过表达内源乙酰辅酶 A 羧化酶(acetyl-CoA carboxylase, Acc1)和 Dga1 使得圆红冬孢酵母 IFO0880 中能够从 70 g/L 葡萄糖和木糖中分别产生 16.4 g/L 和 9.5 g/L 脂质[76]。类似地，过表达内源甾体-Δ9-去饱和酶基因 *scd1* 和 *dga1*，使圆红冬孢酵母 CECT 13085 脂质产量提升 13%，经木质纤维素水解液发酵生产了 39 g/L 的脂质[26]。

不饱和脂肪酸是油脂的重要组成部分，在保护生命健康方面具有重要的价值。Liu 等[77]探究了圆红冬孢酵母的内源脂肪酸去饱和酶(fatty acid desaturase, Fad)基因的分子特征，对于 4 种 Fad 基因的转录调控、Fad 的进化及其功能等方面进行了详细阐述，证明了该酵母作为多不饱和脂肪酸生产平台的潜力。其中，Δ9-Fad 能够合成油酸和棕榈油酸，双功能Δ12/Δ15-Fad2)能够将油酸转化为亚油酸和 α-亚麻酸。特别的是，Fad4 是一种三功能酶(Δ9/Δ12/Δ15-Fad)，在菌株生长、脂质合成及抗应激中起重要作用。基于此，Liu 等[77]在圆红冬孢酵母中高水平生产油酸和一种新型脂肪酸 γ-亚麻酸，使其最大产量分别达到 3.5 g/L 和 2.6 g/L。Wu 等[78]通过过表达内源Δ12-脂肪酸去饱和酶基因 *Rtfad2*，使圆红冬孢酵母亚油酸的含量显著提升到 1.7 mg/L，其他脂肪酸产量达 4.2 g/L，其中棕榈酸(C16:0)、棕榈油酸(C16:1)、硬脂酸(C18:0)、油酸(C18:1)和 α-亚麻酸的含量分别占 15.9%、1.3%、2.4%、48.1%和 3.9%。当 *Rtfad2* 与Δ9-脂肪酸去饱和酶基因 *Rtfad1* 共表达时，*Rtfad1* 的表达被下调，亚油酸可能是圆红冬孢酵母中 *Rtfad1* 表达的关键调控因子，从而控制 C18 脂肪酸的合成。

脂肪醇是一种脂肪酸衍生物，在工业、食品、医疗等行业应用广泛。Liu 等[79]通过在培养基中添加 tergitol 等表面活性剂，降低脂肪醇的生物毒性，将产量提升了 4.3 倍，在 2 L 生物反应器上产量达到 1.6 g/L。当在圆红冬孢酵母中仅过表达海洋杆菌(*Marinobacter aquaeolei*)的脂肪酰辅酶 A (fatty acyl-CoA, Far)基因时，以不同碳源在摇瓶上发酵产生 0.8–2.0 g/L 脂肪醇；以蔗糖为碳源，在 7 L 生物反应器中分批补料发酵，可生产超过 8 g/L 的 C16–C18 脂肪醇[80]。最近，一项研究通过在圆红冬孢酵母 IFO0880 中过表达胞质乙酰辅酶 A 和丙二酰辅酶 A 合成相关基因 ATP-柠檬酸裂解酶基因 *acl1* 和 *acc1*，以及敲除酰基转移酶基因 *dga1* 和 *lro1*，使脂肪醇产量提升 1.8–4.4 倍。尤其删除 *lro1* 的组合表达菌株在 250 mL 生物反应器中产量达到 3.7 g/L[54]。

生物柴油和石化柴油具有相似的能量密度和燃烧性能，具有高润滑性和低排气量的优点，且生物柴油与现有燃油基础设施具有高度兼容性，是传统柴油的可再生替代品[81]。目前，可再生柴油的生产主要通过化学酯交换作用产生，需要依赖植物油和动物油作为原料，这种方式会增加土地压力，产生环境问题，因此生物生产更具有综合竞争力[82]。在模式宿主中，脂质前体的供应限制了传统柴油替代品脂肪酸乙酯(fatty acid ethyl ester, FAEEs)的产量，通过外源添加脂肪酸可以将其产量提高至 0.52 g/L[83]。圆红冬孢酵母作为利用多种碳源高密度发酵的高脂质生产宿主，具有高产 FAEEs 的潜力。其在含有 10%体积的乙醇培养基中孵育 84 h，就可将 73%的中性甘油酯转化为 FAEEs[1,39,84]。Zhang 等[81]通过筛选不同来源的蜡脂合酶基因，在圆红冬孢酵母中构建了 FAEEs 生物合成路径，通过对双功能蜡酯合成酶/酰基辅酶 A-二酰基甘油酰基转移酶(Ws/Dga1)基因突变及发酵优化，FAEEs 达到 9.97 g/L (表 1)。

2.2 萜类化合物的合成

类胡萝卜素是一类重要的高价值萜类化合物，广泛应用于食品、化妆品和制药行业。圆红冬孢酵母能够天然合成 β-胡萝卜素、红酵母红素、γ-胡萝卜素等多种类胡萝卜素[6,94]。其中，红酵母红素是比 β-胡萝卜素更强的抗氧化剂，能够保持细胞膜的稳定性、调节免疫系统及提高钛材料的抗菌能力[95]。目前，通过改造圆红冬孢酵母甲羟戊酸途径高产某种类胡萝卜素的报道较少。相应地，通过随机突变和菌株筛选提高类胡萝卜素产量的报道较多。例如，Bao 等[96]通过 ATMT 对筛选的 9 个类胡萝卜素突变菌株做 Box-Behnken 发酵优化，使红色突变菌 A1-15-BRQ 的红酵母红素产量达到 21.3 mg/L，占类胡萝卜素总含量的 94.4%。通过发酵优化，其产量是野生菌的 17 倍，发酵周期减少了 67%。

值得关注的是圆红冬孢酵母中内源的甲羟戊酸(mevaleric acid, MVA)途径已被工程化改造合成多种萜烯化合物(表 1)。通过在圆红冬孢酵母中引入植物、细菌、真菌等不同来源的 16 种萜烯合酶，能够实现 1,8-桉树脑、桧萜、罗勒烯、蒎烯、柠檬烯和萜烯等单萜化合物的生产，其中 1,8-桉树脑的产量最高，达到了 34.6 mg/L[89]。Liu 等[46]引入毛番茄(*Solanum habrochaites*)来源的橙花基焦磷酸合酶(neryl-diphosphate synthase, *Sl*Npps)与甜橙(*Citrus sinensis*)来源的柠檬烯合酶(limonene synthase, *Clt*Ls)构建柠檬烯合成途径，并通过引入粪肠球菌(*Enterococcus faecalis*)的乙酰辅酶 A 乙酰转移酶/羟甲基戊二酰-辅酶 A (hydroxy-methylglutaryl-coA, HMG-CoA)还原酶 *Ef*Mvae 和 HMG-CoA 合酶 *Ef*Mvas 以及来自马氏甲烷八叠球菌(*Methanosarcina mazei*)的甲羟戊酸激酶(mevalonate kinase, *Mm*Mk)强化柠檬烯合成的前体供应，结合蛋白质融合策略(*Sl*Npps::*Clt*Ls)提高催化效率，使柠檬烯产量达到 393.5 mg/L。Yaegashi 等[2]在圆红冬孢酵母中引入甜没药烯合酶(bisabolene synthase, Bis)和青蒿素前体紫穗槐二烯合酶(amorphadiene synthase, Ads)实现了倍半萜甜没药烯和青蒿素前体紫穗槐二烯的异源合成，发酵原料木质纤维素经新型生物相容性离子液胆碱 α-酮戊二酸或碱性预处理后，甜没药烯在工程菌中产量分别达到 261 mg/L (实验室规模)及 680 mg/L (高重力加料生物反应器)，青蒿素前体紫穗槐二烯在 5 mL 试管中以葡萄糖为碳源发酵产量达 36 mg/L。类似地，Geiselman 等[88]以玉米秸秆水解物为原料异源合成二萜贝壳杉烯，通过强启动子表达藤仓赤霉(*Gibberella fujikuroi*)的贝壳杉烯合酶(kaurene synthase, Ks)和来源于家鸡(*Gallus gallus*)的法尼希基焦磷酸合酶突变体，在 2 L

表 1　代谢工程改造圆红冬孢酵母合成天然产物策略汇总

Table 1　Metabolic engineering strategies for the synthesis of natural products by *Rhodotorula toruloides*

Products	Strains	Engineering strategies	Carbon sources	Fermentation	Titer	References
Lipids						
Lipids	NP11	Mnp, Vp	Glucose, 5-HMF	Shake-flask	45%[#*]	[85]
Diacylglycerols/ FFA	NP11	↓Dga1, Lro1, Are1; Tgl5::Ldp1	Glucose	Shake-flask	30%/50%[#*]	[68]
Palmitoleic acid	NP11	*Sc*Ole1, *Rt*Δ9fad	Peptone, glucose	Shake-flask	450.0 mg/L[*]	[86]
OA	NP11	*Sc*Ole1, *Rt*Δ9fad	Peptone, glucose	Shake-flask	2.3 g/L[*]	[86]
Lipid	CGMCC 2.1389	Vhb	Glucose	Fed-batch bioreactor	49.0 g/L	[87]
Linoleic acid	NBRC 8766	*Rt*Fad1, *Rt*Fad2	Glucose	Shake-flask	1.7 g/L	[78]
Fatty acid ethyl esters	IFO0880	Ws/Dga, Ku70Δ	Glucose	Fed-batch cultivation	9.97 g/L	[81]
Fatty alcohol (C16, C18)	IFO0880	Acl1, Acc1; Dga1Δ, Lro1Δ	Glucose	Mini bioreactors	3.7 g/L	[54]
Terpenes						
Carotenoid	NP11	Carb, Carrp, Ggpps	Glucose	Fed-batch bioreactor	2.1 mg/g	[38]
Carotenoid	NP11	Ldp1Δ, CalsΔ	Glucose	Shake-flask	3.2 mg/g	[40]
Ent-kaurene	/	Ks, Ggpp, promoter engineering	Glucose[1]	Scale-up of cultivation	1.4 g/L	[88]
Limonene	NP11	Ls::Npps, Hmgr, *Ef*Mvae, *Ef*Mvas, *Mm*Mk	Glucose	Small system	393.5 mg/L	[46]
1,8-cineole	IFO0880	Hyp3	Glucose, xylose[1]	Fed-batch bioreactor	34.6 mg/L	[89]
1,8-cineole	IFO0880	Gpp, Hmgr, Mk, Pmk	Glucose[1]	Fed-batch bioreactor	1.4 g/L	[90]
α-bisabolene	IFO0880	Bis, Hmgr, Mk, Pmk	Glucose[1]	Fed-batch bioreactor	2.6 g/L	[90]
Others						
Triacetic acid lactone	IFO0880	*Gh*Ps Acl1, Acc1	Glucose	Fed-batch bioreactor	28 g/L	[91]
Resveratrol	NP11	*At*C4h, *At*4cl::*Vl*Sts, *At*Atr2, *Rt*Cyb5, *Rt*Aro4, *Rt*Aro7	Glucose	Shake-flask	125.2 mg/L	[92]
Naringenin	/	4cl, Chs	Galactose	Shake-flask	0.038 mg/L	[93]

[*]: Roughly estimated according to the chart and appendix information. [#]: Proportion of content. [1]: Glucose derived from corn stover hydrolysate. In the engineering strategy column, the listed genes or proteins indicate overexpression, the addition of ↓ before the enzymes indicates down-regulation, the addition of Δ after the enzymes indicates knockout, and :: represents enzyme fusion. /: No data.

生物反应器中合成了 1.4 g/L 的贝壳杉烯。对比其他生产贝壳杉烯的工程菌株，如大肠杆菌(578 mg/L)、构巢曲霉(*Aspergillus nidulans*，未定量)等，圆红冬孢酵母生产具有明显优势[97-98]。

2.3　其他产物

除了上述各种产物，圆红冬孢酵母还能合成许多具有重要应用价值的酶，如苯丙氨酸解氨酶(phenylalanine ammonia lyase, Pal)、D-氨基酸氧化酶(D-amino acid oxidase, Ddo)、头孢菌素酯酶和环氧化物水解酶，以及糖醇类物质、黄酮类物质、聚酮化合物、非核糖体肽等。

苯丙氨酸解氨酶具有很多工业及潜在的医疗用途，如治疗肝脏苯丙氨酸羟化酶活性受损引起代谢紊乱的苯丙酮尿症[45,99]。圆红冬孢酵

母的苯丙氨酸解氨酶是一种双功能酶，能够将L-苯丙氨酸转化为反式肉桂酸，将 L-酪氨酸转化为对香豆酸，继而合成一系列重要的芳香族氨基酸衍生物，包括苯基丙烷类、黄酮类化合物[6,17,100]。圆红冬孢酵母来源的苯丙氨酸解氨酶Pal已在酵母中用于白藜芦醇的合成[101]。近期，Zhang等[92]将来源于拟南芥(*Arabidopsis thaliana*)的香豆酰辅酶 A 连接酶 *At*4cl 和葡萄(*Vitis labrusca*)来源的芪合酶 *Vl*Sts 引入圆红冬孢酵母，利用其内源 Pal 实现了白藜芦醇的合成。然后，引入肉桂酸 4 羟化酶 *At*C4h、融合蛋白 *At*4cl::*Vl*Sts、细胞色素 P450 还原酶 2 (cytochrome P450 reductase 2, *At*Atr2)和过表达内源细胞色素 B5 (cytochrome B5, *Rt*Cyb5)及优化内源莽草酸途径后，白藜芦醇产量达到 125.2 mg/L。Lee等[93]在圆红冬孢酵母中引入 4cl 和查尔酮合酶 Chs，通过外源添加酪氨酸实现柚皮素(0.038 mg/L)和对香豆酸(16.9 mg/L)的合成，该研究进一步证明了利用圆红冬孢酵母内源双功能 Pal 酶异源合成黄酮类物质的可能性。Lee 等[93]还通过气相色谱-质谱分析发现，工程菌株代谢通量偏向糖酵解及三羧酸(tricarboxylic acid, TCA)循环，为圆红冬孢酵母合成黄酮类物质提供了足够的前体丙二酰辅酶 A。上述研究为利用圆红冬孢酵母高效的磷酸戊糖途径，内源充足的乙酰辅酶 A 池和高活性的 Pal 酶构建芳香族氨基酸类衍生物细胞工厂提供了科学参考。

D-氨基酸氧化酶是一种黄酮酶，能够将头孢菌素 C 脱氨基生成 7-氨基头孢烷酸(半合成头孢菌素的关键中间体)。D-氨基酸氧化酶也是用于治疗慢性尿毒症药品 γ-酮酸和检测 D-氨基酸生物传感器的重要材料[1]。圆红冬孢酵母中的 D-氨基酸氧化酶具有高动力学活性，能够高效催化 D-氨基酸氧化脱氨生成相应的 2-氧酸和氨，同时将分子氧还原为过氧化氢[102]。D-氨基酸氧化酶的高效异源表达是一个重要挑战，因而圆红冬孢酵母内源 D-氨基酸氧化酶的存在为拓展该菌新应用领域提供了新思路[103]。

糖醇如赤藓糖醇、阿拉伯糖醇等是一类存在于水果、蔬菜中的低热量的天然甜味剂，也是众多生物基化学品的原料[104-105]。利用微生物生产各种糖醇比化学方式更为安全和可持续[106-108]。圆红冬孢酵母具有良好的糖醇生产能力。Jagtap 等[109]利用圆红冬孢酵母 IFO0880 转化木糖生产阿拉伯糖醇，转化效率最高达到32%，产生 49 g/L D-阿拉伯糖醇。此外，圆红冬孢酵母还能够利用半乳糖生产半乳糖醇。在氮丰富条件下，圆红冬孢酵母能够利用 40 g/L 半乳糖生产 8.4 g/L 半乳糖醇，转化率达到了21% (质量分数)。

植物来源的聚酮化合物三乙酸内酯是公认的生物精炼行业中很有前景的平台化学品。Cao等[91]将非洲菊(*Gerbera hybrida*)的 2-吡喃酮合酶基因 *Gh*Ps 引入圆红冬孢酵母中，过表达 Acl将三乙酸内酯产量提升 45%，进一步过表达 Acc1 使其产量提升 29%，以葡萄糖为碳源分批补料发酵使三乙酸内酯产量达到 28 g/L。

非核糖体肽构成了多种有价值的次级代谢产物，常用于药物、聚合物和染料等工业生产。微生物生产染料，是解决化学合成负面影响的可替代途径。枯草芽孢杆菌(*B. subtilis*)来源的 4′-磷酸泛酰巯基乙胺基转移酶(4′-phosphopantetheinyl transferase, Sfp)能够激活淡紫灰链霉菌(*Streptomyces lavendulae*)的蓝色素合成酶 A (blue pigment synthetase A, Bps A)，激活的 Bps A 催化 2 个 L-谷氨酰胺转化为靛蓝。将此异源途径引入圆红冬孢酵母中，利用廉价碳源和氮源实现了 2.9 g/L 蓝色颜料靛蓝的生产[110]。这为可持续、异源生产非核糖体肽提供了新选择。

3 总结与展望

圆红冬孢酵母具有独特的发酵性能和天然产物合成潜能，还具有高效转化利用木质纤维素水解液、高密度发酵、强鲁棒性等优势[81,111]。目前，圆红冬孢酵母的多组学基础信息、代谢调控的分子机制、发酵特性和代谢工程改造等方面已取得一系列重要进展。然而，利用圆红冬孢酵母在生产高价值天然产物方面仍有很大提升空间(表 2)。

首先，尽管多种转化方法已经在圆红冬孢酵母中得到应用，但转化时效长、效率低、步骤繁琐、转化子筛选困难仍然是亟待解决的问题。其主要原因在于圆红冬孢酵母内源同源重组效率低，且缺乏能够自主复制的游离型表达质粒。因此，开发精准基因编辑技术和构建高效同源重组策略是提升圆红冬孢酵母可编辑性、构建高价值化合物细胞工厂的关键。

其次，除了遗传操作工具不足限制了可实施策略外，还可能存在菌株内源对异源代谢途径产生强力竞争，代谢流难以高效转入异源路径；异源蛋白在菌株中的活性及稳定性差，酶的转化效率低等问题[92-93,135]。例如，利用圆红冬孢酵母内源苯丙氨酸解氨酶，虽有望开启芳香族氨基酸类衍生物异源合成的新征程。然而，在利用圆红冬孢酵母生产白藜芦醇的案例中，诸如过表达莽草酸途径关键酶、添加浅蓝菌素等策略对于白藜芦醇的生产并无明显改善，提示该酵母中的代谢网络调控可能与常规的酿酒酵母等存在差异[1,136]。因而，深度解构圆红冬孢酵母内源脂质代谢、萜烯类代谢和氨基酸代谢等网络调控机制，将为其理性设计改造，实

表 2 不同菌株合成天然产物产量汇总

Table 2 Summary of production of natural products synthesized by different yeasts

Products	*Rhodotorula toruloides*	*Yarrowia lipolytica*	*Saccharomyces cerevislae*
Lipids			
Lipids	12.7 g/L[87]	7.5 g/L[112]	1.78 g/L[113]
Diacylglycerols	30%#*[68]	/	/
FFA	50%#*[68]	/	129 mg/g DCW[114]
Palmitoleic acid	450.0 mg/L[86]	45.73%#[115]	928 mg/L[116]
OA	2.3 g/L*[86]	4.48 g/L[117]	/
Linoleic acid	1.7 g/L[78]	0.9 g/L[118]	/
Fatty acid ethyl esters	1.02 g/L[81]	13.5 g/L[119]	35.05 mg/L[120]
Fatty alcohol	2 g/L[80]	1.19 g/L[121]	252 mg/L[122]
Terpene			
Carotenoid	3.2 mg/g[40]	1.5 g/L[123]	142 mg/L[124]
Ent-kaurene[a]	1.4 g/L[88]	/	/
Iimonene	393.5 mg/L[46]	58.4 mg/L[125]	1 446.56 mg/L[126]
1,8-cineole[a]	1.4 g/L[90]	/	/
α-bisabolene[a]	2.6 g/L[90]	1 058.1 mg/L[127]	Unquantified[128]
Others			
Triacetic acid lactone[a]	28 g/L[91]	35.9 g/L[129]	10 g/L[130]
Resveratrol	125.2 mg/L[92]	819.1 g/L[131]	210 mg/L[132]
Naringenin	0.038 mg/L[93]	124.1 mg/L[133]	212.6 mg/L[134]

*: Roughly estimated according to the chart and appendix information. #: Proportion of content. [a]: Bioreactor fermentation, and unlabeled products are shake-flask fermentation. /: No data.

现油脂化学品、异戊二烯类衍生物和氨基酸衍生物的合成提供新选择[137]。阐明圆红冬孢酵母的代谢网络及其调控机制，并结合异源酶筛选与适配、酶融合、蛋白支架以及亚细胞结构区室化等策略，为提升相关产物的产量提供理论和技术支撑[92,138-139]。

最后，随机诱变和适应性实验室进化同样是获取目标菌株的重要策略，是理性基因编辑技术的有效补充。如通过适应性实验室进化能够显著提高酵母菌株对木质纤维素水解液中有毒副产物的耐受性[140]。虽然随机诱变和实验室进化周期长、效率低、高通量筛选困难，但通过该策略获得菌株将为理性编辑提供合适的实验材料。总之，合成生物技术、代谢工程策略和自动化高通量筛选的迅速发展将为圆红冬孢酵母理性工程化改造提供关键技术支撑。相信在不久的将来，圆红冬孢酵母天然的代谢特性将被充分开发利用，从而赋能绿色生物制造。

REFERENCES

[1] PARK YK, NICAUD JM, LEDESMA-AMARO R. The engineering potential of *Rhodosporidium toruloides* as a workhorse for biotechnological applications[J]. Trends in Biotechnology, 2018, 36(3): 304-317.

[2] YAEGASHI J, KIRBY J, ITO M, SUN J, DUTTA T, MIRSIAGHI M, SUNDSTROM ER, RODRIGUEZ A, BAIDOO E, TANJORE D, PRAY T, SALE K, SINGH S, KEASLING JD, SIMMONS BA, SINGER SW, MAGNUSON JK, ARKIN AP, SKERKER JM, GLADDEN JM. *Rhodosporidium toruloides*: a new platform organism for conversion of lignocellulose into terpene biofuels and bioproducts[J]. Biotechnology for Biofuels, 2017, 10(1): 1-13.

[3] JONES AD, BOUNDY-MILLS KL, BARLA GF, KUMAR S, UBANWA B, BALAN V. Microbial Lipid Alternatives to Plant Lipids[M]. New York: Springer New York, 2019: 1-32.

[4] SILLMAN J, NYGREN L, KAHILUOTO H, RUUSKANEN V, TAMMINEN A, BAJAMUNDI C, NAPPA M, WUOKKO M, LINDH T, VAINIKKA P, PITKÄNEN JP, AHOLA J. Bacterial protein for food and feed generated *via* renewable energy and direct air capture of CO_2: can it reduce land and water use?[J]. Global Food Security, 2019, 22: 25-32.

[5] STEPHENS E, ROSS IL, KING Z, MUSSGNUG JH, KRUSE O, POSTEN C, BOROWITZKA MA, HANKAMER B. An economic and technical evaluation of microalgal biofuels[J]. Nature Biotechnology, 2010, 28(2): 126-128.

[6] BUZZINI P, INNOCENTI M, TURCHETTI B, LIBKIND D, van BROOCK M, MULINACCI N. Carotenoid profiles of yeasts belonging to the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and *Sporidiobolus*[J]. Canadian Journal of Microbiology, 2007, 53(8): 1024-1031.

[7] WANG YN, ZHANG SF, ZHU ZW, SHEN HW, LIN XP, JIN X, JIAO X, ZHAO ZK. Systems analysis of phosphate-limitation-induced lipid accumulation by the oleaginous yeast *Rhodosporidium toruloides*[J]. Biotechnology for Biofuels, 2018, 11(1): 1-15.

[8] WU SG, ZHAO X, SHEN HW, WANG Q, ZHAO ZK. Microbial lipid production by *Rhodosporidium toruloides* under sulfate-limited conditions[J]. Bioresource Technology, 2011, 102(2): 1803-1807.

[9] LI Q, KAMAL R, WANG Q, YU X, ZHAO ZK. Lipid production from amino acid wastes by the oleaginous yeast *Rhodosporidium toruloides*[J]. Energies, 2020, 13(7): 1576.

[10] SÀNCHEZ I NOGUÉ V, BLACK BA, KRUGER JS, SINGER CA, RAMIREZ KJ, REED ML, CLEVELAND NS, SINGER ER, YI XN, YEAP RY, LINGER JG, BECKHAM GT. Integrated diesel production from lignocellulosic sugarsviaoleaginous yeast[J]. Green Chemistry, 2018, 20(18): 4349-4365.

[11] LI YH, ZHAO ZB, BAI FW. High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture[J]. Enzyme and Microbial Technology, 2007, 41(3): 312-317.

[12] XU JY, LIU DH. Exploitation of genus *Rhodosporidium* for microbial lipid production[J]. World Journal of Microbiology and Biotechnology, 2017, 33(3): 1-13.

[13] ANDRé A, CHATZIFRAGKOU A, DIAMANTOPOULOU P, SARRIS D, PHILIPPOUSSIS A, GALIOTOU-PANAYOTOU M, KOMAITIS M, PAPANIKOLAOU S. Biotechnological conversions of bio-diesel-derived crude glycerol by *Yarrowia lipolytica* strains[J]. Engineering in Life Sciences, 2009, 9(6): 468-478.

[14] SINGH G, JAWED A, PAUL D, BANDYOPADHYAY

KK, KUMARI A, HAQUE S. Concomitant production of lipids and carotenoids in *Rhodosporidium toruloides* under osmotic stress using response surface methodology[J]. Frontiers in Microbiology, 2016, 7: 1686.

[15] WU CC, TSAI YY, OHASHI T, MISAKI R, LIMTONG S, FUJIYAMA K. Isolation of a thermotolerant *Rhodosp*oridium *toruloides* DMKU3-TK16 mutant and its fatty acid profile at high temperature[J]. FEMS Microbiology Letters, 2018, 365(21): fny203.

[16] WU CC, OHASHI T, MISAKI R, LIMTONG S, FUJIYAMA K. Ethanol and H_2O_2 stresses enhance lipid production in an oleaginous *Rhodotorula toruloides* thermotolerant mutant L1-1[J]. FEMS Yeast Research, 2020, 20(4): foaa030.

[17] ZHU ZW, ZHANG SF, LIU HW, SHEN HW, LIN XP, YANG F, ZHOU YJ, JIN GJ, YE ML, ZOU HF, ZHAO ZK. A multi-omic map of the lipid-producing yeast *Rhodosporidium toruloides*[J]. Nature Communications, 2012, 3(1): 1112.

[18] CORADETTI ST, PINEL D, GEISELMAN GM, ITO M, MONDO SJ, REILLY MC, CHENG YF, BAUER S, GRIGORIEV IV, GLADDEN JM, SIMMONS BA, BREM RB, ARKIN AP, SKERKER JM. Functional genomics of lipid metabolism in the oleaginous yeast *Rhodosporidium toruloides*[J]. eLife, 2018, 7: e32110.

[19] TIUKOVA IA, BRANDENBURG J, BLOMQVIST J, SAMPELS S, MIKKELSEN N, SKAUGEN M, ARNTZEN MØ, NIELSEN J, SANDGREN M, KERKHOVEN EJ. Proteome analysis of xylose metabolism in *Rhodotorula toruloides* during lipid production[J]. Biotechnology for Biofuels, 2019, 12(1): 1-17.

[20] TIUKOVA IA, PRIGENT S, NIELSEN J, SANDGREN M, KERKHOVEN E. Genome-scale model of *Rhodotorula toruloides* metabolism[J]. Biotechnology and Bioengineering, 2019, 116(12): 3396-3408.

[21] DINH HV, SUTHERS PF, CHAN SHJ, SHEN YH, XIAO TX, DEEWAN A, JAGTAP SS, ZHAO HM, RAO CV, RABINOWITZ JD, MARANAS CD. A comprehensive genome-scale model for *Rhodosporidium toruloides* IFO0880 accounting for functional genomics and phenotypic data[J]. Metabolic Engineering Communications, 2019, 9: e00101.

[22] KIM J, CORADETTI ST, KIM YM, GAO YQ, YAEGASHI J, ZUCKER JD, MUNOZ N, ZINK EM, BURNUM-JOHNSON KE, BAKER SE, SIMMONS BA, SKERKER JM, GLADDEN JM, MAGNUSON JK. Multi-omics driven metabolic network reconstruction and analysis of lignocellulosic carbon utilization in *Rhodosporidium toruloides*[J]. Frontiers in Bioengineering and Biotechnology, 2021, 8: 612832.

[23] JIAO X, ZHANG Y, LIU XJ, ZHANG Q, ZHANG SF, ZHAO ZK. Developing a CRISPR/Cas9 system for genome editing in the basidiomycetous yeast *Rhodosporidium toruloides*[J]. Biotechnology Journal, 2019, 14(7): 1900036.

[24] SUN WY, YANG XB, WANG XY, JIAO X, ZHANG SF, LUAN YS, ZHAO ZK. Developing a flippase-mediated maker recycling protocol for the oleaginous yeast *Rhodosporidium toruloides*[J]. Biotechnology Letters, 2018, 40(6): 933-940.

[25] LIU XJ, ZHANG Y, LIU HD, JIAO X, ZHANG Q, ZHANG SF, ZHAO ZK. RNA interference in the oleaginous yeast *Rhodosporidium toruloides*[J]. FEMS Yeast Research, 2019, 19(3): 31-41.

[26] DÍAZ T, FILLET S, CAMPOY S, VÁZQUEZ R, VIÑA J, MURILLO J, ADRIO JL. Combining evolutionary and metabolic engineering in *Rhodosporidium toruloides* for lipid production with non-detoxified wheat straw hydrolysates[J]. Applied Microbiology and Biotechnology, 2018, 102(7): 3287-3300.

[27] PINHEIRO MJ, BONTURI N, BELOUAH I, ALVES MIRANDA E, LAHTVEE PJ. Xylose metabolism and the effect of oxidative stress on lipid and carotenoid production in *Rhodotorula toruloides*: insights for future biorefinery[J]. Frontiers in Bioengineering and Biotechnology, 2020, 8: 1008.

[28] NORA LC, WESTMANN CA, MARTINS-SANTANA L, de FÁTIMA ALVES L, MONTEIRO LMO, GUAZZARONI ME, SILVA-ROCHA R. The art of vector engineering: towards the construction of next-generation genetic tools[J]. Microbial Biotechnology, 2019, 12(1): 125-147.

[29] MARTINS-SANTANA L, NORA LC, SANCHES-MEDEIROS A, LOVATE GL, CASSIANO MHA, SILVA-ROCHA R. Systems and synthetic biology approaches to engineer fungi for fine chemical production[J]. Frontiers in Bioengineering and Biotechnology, 2018, 6: 117.

[30] JOHNS AMB, LOVE J, AVES SJ. Four inducible promoters for controlled gene expression in the oleaginous yeast *Rhodotorula toruloides*[J]. Frontiers in Microbiology, 2016, 7: 1666.

[31] LIU YB, AMY YAP SH, JOHN KOH CM, JI LH.

Developing a set of strong intronic promoters for robust metabolic engineering in oleaginous *Rhodotorula* (*Rhodosporidium*) yeast species[J]. Microbial Cell Factories, 2016, 15: 1-9.

[32] WANG YN, LIN XP, ZHANG SF, SUN WY, MA SJ, ZHAO ZK. Cloning and evaluation of different constitutive promoters in the oleaginous yeast *Rhodosporidium toruloides*[J]. Yeast, 2016, 33(3): 99-106.

[33] LIU Y, KOH CMJ, NGOH S T, JI L. Erratum to: engineering an efficient and tight D-amino acid-inducible gene expression system in *Rhodosporidium*/*Rhodotorula* species[J]. Microbial Cell Factories, 2017, 16(1): 107.

[34] NORA LC, WEHRS M, KIM J, CHENG JF, TARVER A, SIMMONS BA, MAGNUSON J, HARMON-SMITH M, SILVA-ROCHA R, GLADDEN JM, MUKHOPADHYAY A, SKERKER JM, KIRBY J. A toolset of constitutive promoters for metabolic engineering of *Rhodosporidium toruloides*[J]. Microbial Cell Factories, 2019, 18(1): 1-11.

[35] LIU YB, KOH CMJ, TE NGOH S, JI LH. Engineering an efficient and tight D-amino acid-inducible gene expression system in *Rhodosporidium*/*Rhodotorula* species[J]. Microbial Cell Factories, 2015, 14(1): 1-16.

[36] 马斯佳. 圆红冬孢酵母诱导性遗传操作平台的构建[D]. 大连: 大连工业大学硕士学位论文, 2015.
MA SJ. Inducible expression vector set for engineering in the oleaginous yeast *Rhodosporidium toruloides*[D]. Dalian: Master's Thesis of Dalian Polytechnic University, 2015 (in Chinese).

[37] SCHULTZ JC, CAO MF, ZHAO HM. Development of a CRISPR/Cas9 system for high efficiency multiplexed gene deletion in *Rhodosporidium toruloides*[J]. Biotechnology and Bioengineering, 2019, 116(8): 2103-2109.

[38] JIAO X, ZHANG Q, ZHANG SF, YANG XB, WANG Q, ZHAO KZ. Efficient co-expression of multiple enzymes from a single promoter mediated by virus 2A sequence in the oleaginous yeast *Rhodosporidium toruloides*[J]. FEMS Yeast Research, 2018, 18(8): foy086.

[39] WEN Z, ZHANG S, ODOH CK, JIN M, ZHAO ZK. *Rhodosporidium toruloides*-a potential red yeast chassis for lipids and beyond[J]. FEMS Yeast Research, 2020, 20(5): foaa038.

[40] JIAO X, LYU LT, ZHANG Y, HUANG QT, ZHOU RH, WANG SA, WANG S, ZHANG SF, ZHAO ZK. Reduction of lipid-accumulation of oleaginous yeast *Rhodosporidium toruloides* through CRISPR/Cas9-mediated inactivation of lipid droplet structural proteins[J]. FEMS Microbiology Letters, 2021, 368(16): fnab111.

[41] TULLY M, GILBERT HJ. Transformation of *Rhodosporidium toruloides*[J]. Gene, 1985, 36(3): 235-240.

[42] LIN XP, WANG YN, ZHANG SF, ZHU ZW, ZHOU YJ, YANG F, SUN WY, WANG XY, ZHAO ZK. Functional integration of multiple genes into the genome of the oleaginous yeast *Rhodosporidium toruloides*[J]. FEMS Yeast Research, 2014, 14(4): 547-555.

[43] KOH CMJ, LIU YB, MOEHNINSI, DU MG, JI LH. Molecular characterization of KU70 and KU80 homologues and exploitation of a KU70-deficient mutant for improving gene deletion frequency in *Rhodosporidium toruloides*[J]. BMC Microbiology, 2014, 14(1): 1-10.

[44] TSAI YY, OHASHI T, KANAZAWA T, POLBUREE P, MISAKI R, LIMTONG S, FUJIYAMA K. Development of a sufficient and effective procedure for transformation of an oleaginous yeast, *Rhodosporidium toruloides* DMKU3-TK16[J]. Current Genetics, 2017, 63(2): 359-371.

[45] de FELIPE P, LUKE GA, HUGHES LE, GANI D, HALPIN C, RYAN MD. E unum pluribus: multiple proteins from a self-processing polyprotein[J]. Trends in Biotechnology, 2006, 24(2): 68-75.

[46] LIU SS, ZHANG MY, REN YY, JIN GJ, TAO YS, LYU LT, ZHAO ZK, YANG XB. Engineering *Rhodosporidium toruloides* for limonene production[J]. Biotechnology for Biofuels, 2021, 14(1): 1-11.

[47] FATMA Z, SCHULTZ JC, ZHAO HM. Recent advances in domesticating non-model microorganisms[J]. Biotechnology Progress, 2020, 36(5): 30081-300819.

[48] KIRIYA K, TSUYUZAKI H, SATO M. Module-based systematic construction of plasmids for episomal gene expression in fission yeast[J]. Gene, 2017(637): 14-24.

[49] CELIŃSKA E, LEDESMA-AMARO R, LARROUDE M, ROSSIGNOL T, PAUTHENIER C, NICAUD JM. Golden gate assembly system dedicated to complex pathway manipulation in *Yarrowia lipolytica*[J]. Microbial Biotechnology, 2017, 10(2): 450-455.

[50] LARROUDE M, PARK YK, SOUDIER P, KUBIAK M, NICAUD JM, ROSSIGNOL T. A modular golden gate toolkit for *Yarrowia lipolytica* synthetic biology[J]. Microbial biotechnology, 2019, 12(6): 1249-1259.

[51] PRIELHOFER R, BARRERO JJ, STEUER S, GASSLER T, ZAHRL R, BAUMANN K, SAUER M, MATTANOVICH D, GASSER B, MARX H. GoldenPiCS: a golden gate-derived modular cloning system for applied synthetic biology in the yeast *Pichia pastoris*[J]. BMC Systems Biology, 2017, 11(1): 1-14.

[52] RAJKUMAR AS, VARELA JA, JUERGENS H, DARAN JM G, MORRISSEY JP. Biological parts for *Kluyveromyces marxianus* synthetic biology[J]. Frontiers in Bioengineering and Biotechnology, 2019, 7: 97.

[53] BONTURI N, PINHEIRO MJ, de OLIVEIRA PM, RUSADZE E, EICHINGER T, LIUDŽIŪTĖ G, de BIAGGI JS, BRAUER A, REMM M, ALVES MIRANDA E, LEDESMA-AMARO R, LAHTVEE PJ. Development of a dedicated golden gate assembly platform (RtGGA) for *Rhodotorula toruloides*[J]. Metabolic Engineering Communications, 2022, 15: e00200.

[54] CARL SJ, SHEKHAR M, EMILY G, ANDREA M, HOANG D, COSTAS M, ZHAO HM. Metabolic engineering of *Rhodotorula toruloides* IFO0880 improves C16 and C18 fatty alcohol production from synthetic media[J]. Microbial Cell Factories, 2022, 21(1): 1-14.

[55] WANG SX, CHEN HQ, TANG X, ZHANG H, CHEN W, CHEN YQ. Molecular tools for gene manipulation in filamentous fungi[J]. Applied Microbiology and Biotechnology, 2017, 101(22): 8063-8075.

[56] LIN XP, GAO N, LIU SS, ZHANG SF, SONG S, JI CF, DONG XP, SU YC, ZHAO ZK, ZHU BW. Characterization the carotenoid productions and profiles of three *Rhodosporidium toruloides* mutants from *Agrobacterium tumefaciens*-mediated transformation[J]. Yeast, 2017, 34(8): 335-342.

[57] LIU HD, JIAO X, WANG YN, YANG XB, SUN WY, WANG JH, ZHANG SF, ZHAO KZ. Fast and efficient genetic transformation of oleaginous yeast *Rhodosporidium toruloides* by using electroporation[J]. FEMS Yeast Research, 2017, 17(2): fox017.

[58] CAI P, DUAN XP, WU XY, GAO LH, YE M, ZHOU YJ. Recombination machinery engineering facilitates metabolic engineering of the industrial yeast *Pichia pastoris*[J]. Nucleic Acids Research, 2021, 49(13): 7791-7805.

[59] GAO JQ, GAO N, ZHAI XX, ZHOU YJ. Recombination machinery engineering for precise genome editing in methylotrophic yeast *Ogataea polymorpha*[J]. iScience, 2021: 102168.

[60] ZHANG K, DUAN XP, CAI P, GAO LH, WU XY, YAO L, ZHOU YJ. Fusing an exonuclease with Cas9 enhances homologous recombination in *Pichia pastoris*[J]. Microbial Cell Factories, 2022, 21(1): 1-9.

[61] OTOUPAL PB, ITO M, ARKIN AP, MAGNUSON JK, GLADDEN JM, SKERKER JM. Multiplexed CRISPR-Cas9-based genome editing of *Rhodosporidium toruloides*[J]. Msphere, 2019, 4(2): e00099-19.

[62] ZHENG XM, ZHENG P, ZHANG K, CAIRNS TC, MEYER V, SUN JB, MA YH. 5S rRNA promoter for guide RNA expression enabled highly efficient CRISPR/Cas9 genome editing in *Aspergillus niger*[J]. ACS Synthetic Biology, 2019, 8(7): 1568-1574.

[63] GAO JQ, LI YX, YU W, ZHOU YJ. Rescuing yeast from cell death enables overproduction of fatty acids from sole methanol[J]. Nature Metabolism, 2022, 4(7): 932-943.

[64] SAURABH S, VIDYARTHI AS, PRASAD D. RNA interference: concept to reality in crop improvement[J]. Planta, 2014, 239(3): 543-564.

[65] SMIALOWSKA A, DJUPEDAL I, WANG JW, KYLSTEN P, SWOBODA P, EKWALL K. RNAi mediates post-transcriptional repression of gene expression in fission yeast *Schizosaccharomyces pombe*[J]. Biochemical and Biophysical Research Communications, 2014, 444(2): 254-259.

[66] HAMEDIRAD M, LIAN JZ, LI HJ, ZHAO HM. RNAi assisted genome evolution unveils yeast mutants with improved xylose utilization[J]. Biotechnology and Bioengineering, 2018, 115(6): 1552-1560.

[67] GORLACH JM, McDADE HC, PERFECT JR, COX GM. Antisense repression in *Cryptococcus neoformans* as a laboratory tool and potential antifungal strategy[J]. Microbiology, 2002, 148(1): 213-219.

[68] ZHANG Y, ZHANG SF, CHU YD, ZHANG Q, ZHOU RH, YU D, WANG S, LYU LT, XU GW, ZHAO ZK. Genetic manipulation of the interconversion between diacylglycerols and triacylglycerols in *Rhodosporidium toruloides*[J]. Frontiers in Bioengineering and Biotechnology, 2022, 10:10349.

[69] WANG YN, ZHANG SF, PÖTTER M, SUN WY, LI L, YANG XB, JIAO X, ZHAO ZK. Overexpression of Δ12-fatty acid desaturase in the oleaginous yeast *Rhodosporidium toruloides* for production of linoleic acid-rich lipids[J]. Applied Biochemistry and Biotechnology, 2016, 180(8): 1497-1507.

[70] 林金涛, 沈宏伟, 张泽会, 胡翠敏, 靳国杰, 谭海东,

赵宗保. 圆红冬孢酵母两阶段培养法生产微生物油脂[J]. 生物工程学报, 2010, 26(7): 997-1002.
LIN JT, SHEN HW, ZHANG ZH, HU CM, JIN GJ, TAN HD, ZHAO ZB. Microbial lipid production by *Rhodosporidium toruloides* in a two-stage culture mode[J]. Chinese Journal of Biotechnology, 2010, 26(7): 997-1002 (in Chinese).

[71] LEONG WH, LIM JW, LAM MK, UEMURA Y , HO YC. Third generation biofuels: a nutritional perspective in enhancing microbial lipid production[J]. Renewable and Sustainable Energy Reviews, 2018, 91(8): 950-961.

[72] YAN Q, PFLEGER BF. Revisiting metabolic engineering strategies for microbial synthesis of oleochemicals[J]. Metabolic Engineering, 2020, 58: 35-46.

[73] XU JY, DU W, ZHAO XB, LIU DH. Renewable microbial lipid production from oleaginous yeast: some surfactants greatly improved lipid production of *Rhodosporidium toruloides*[J]. World Journal of Microbiology and Biotechnology, 2016, 32(7): 1-9.

[74] YANG XB, SUN WY, SHEN HW, ZHANG SF, JIAO X, ZHAO ZK. Expression of phosphotransacetylase in *Rhodosporidium toruloides* leading to improved cell growth and lipid production[J]. RSC Advances, 2018, 8(43): 24673-24678.

[75] ZHANG SY, ITO M, SKERKER JM, ARKIN AP, RAO CV. Metabolic engineering of the oleaginous yeast *Rhodosporidium toruloides* IFO0880 for lipid overproduction during high-density fermentation[J]. Applied Microbiology and Biotechnology, 2016, 100(21): 9393-9405.

[76] ZHANG SY, SKERKER JM, RUTTER CD, MAURER MJ, ARKIN AP, RAO CV. Engineering *Rhodosporidium toruloides* for increased lipid production[J]. Biotechnology and Bioengineering, 2016, 113(5): 1056-1066.

[77] LIU YB, KOH CMJ, YAP SA, CAI L, JI LH. Understanding and exploiting the fatty acid desaturation system in *Rhodotorula toruloides*[J]. Biotechnology for Biofuels, 2021, 14(1): 1-17.

[78] WU CC, OHASHI T, KAJIURA H, SATO Y, MISAKI R, HONDA K, LIMTONG S, FUJIYAMA K. Functional characterization and overexpression of Δ12-desaturase in the oleaginous yeast *Rhodotorula toruloides* for production of linoleic acid-rich lipids[J]. Journal of Bioscience and Bioengineering, 2021, 131(6): 631-639.

[79] LIU D, GEISELMAN GM, CORADETTI S, CHENG YF, KIRBY J, PRAHL JP, JACOBSON O, SUNDSTROM ER, TANJORE D, SKERKER JM, GLADDEN J. Exploiting nonionic surfactants to enhance fatty alcohol production in *Rhodosporidium toruloides*[J]. Biotechnology and Bioengineering, 2020, 117(5): 1418-1425.

[80] FILLET S, GIBERT J, SUÁREZ B, LARA A, RONCHEL C, ADRIO JL. Fatty alcohols production by oleaginous yeast[J]. Journal of Industrial Microbiology and Biotechnology, 2015, 42(11): 1463-1472.

[81] ZHANG Y, PENG J, ZHAO HM, SHI SB. Engineering oleaginous yeast *Rhodotorula toruloides* for overproduction of fatty acid ethyl esters[J]. Biotechnology for Biofuels, 2021, 14(1): 1-11.

[82] SOCCOL CR, DALMAS NETO CJ, SOCCOL VT, SYDNEY EB, Da COSTA ESF, MEDEIROS ABP, de SOUZA VANDENBERGHE LP. Pilot scale biodiesel production from microbial oil of *Rhodosporidium toruloides* DEBB 5533 using sugarcane juice: performance in diesel engine and preliminary economic study[J]. Bioresource Technology, 2017, 223: 259-268.

[83] YU KO, JUNG J, KIM SW, PARK CH, HAN SO. Synthesis of FAEEs from glycerol in engineered *Saccharomyces cerevisiae* using endogenously produced ethanol by heterologous expression of an unspecific bacterial acyltransferase[J]. Biotechnology and Bioengineering, 2012, 109(1): 110-115.

[84] JIN GJ, ZHANG YX, SHEN HW, YANG XB, XIE HB, ZHAO ZK. Fatty acid ethyl esters production in aqueous phase by the oleaginous yeast *Rhodosporidium toruloides*[J]. Bioresource Technology, 2013, 150: 266-270.

[85] LYU LT, CHU YD, ZHANG SF, ZHANG Y, HUANG QT, WANG S, ZHAO ZK. Engineering the oleaginous yeast *Rhodosporidium toruloides* for improved resistance against inhibitors in biomass hydrolysates[J]. Frontiers in Bioengineering and Biotechnology, 2021, 9: 768934.

[86] TSAI YY, OHASHI T, WU CC, BATAA D, MISAKI R, LIMTONG S, FUJIYAMA K. Delta-9 fatty acid desaturase overexpression enhanced lipid production and oleic acid content in *Rhodosporidium toruloides* for preferable yeast lipid production[J]. Journal of Bioscience and Bioengineering, 2019, 127(4): 430-440.

[87] WANG S, KAMAL R, ZHANG Y, ZHOU RH, LV LT, HUANG QT, QIAN S, ZHANG SF, ZHAO ZK.

Expression of VHb improved lipid production in *Rhodosporidium toruloides*[J]. Energies, 2020, 13(17): 4446.

[88] GEISELMAN GM, ZHUANG X, KIRBY J, TRAN-GYAMFI MB, PRAHL JP, SUNDSTROM ER, GAO YQ, MUNOZ MUNOZ N, NICORA CD, CLAY DM, PAPA G, BURNUM-JOHNSON KE, MAGNUSON JK, TANJORE D, SKERKER JM, GLADDEN JM. Production of ent-kaurene from lignocellulosic hydrolysate in *Rhodosporidium toruloides*[J]. Microbial Cell Factories, 2020, 19(1): 1-12.

[89] ZHUANG X, KILIAN O, MONROE E, ITO M, TRAN-GYMFI MB, LIU F, DAVIS RW, MIRSIAGHI M, SUNDSTROM E, PRAY T, SKERKER JM, GEORGE A, GLADDEN JM. Monoterpene production by the carotenogenic yeast *Rhodosporidium toruloides*[J]. Microbial Cell Factories, 2019, 18(1): 1-15.

[90] KIRBY J, GEISELMAN GM, YAEGASHI J, KIM J, ZHUANG X, TRAN-GYAMFI MB, PRAHL JP, SUNDSTROM ER, GAO YQ, MUNOZ N, BURNUM-JOHNSON KE, BENITES VT, BAIDOO EEK, FUHRMANN A, SEIBEL K, WEBB-ROBERTSON BJ M, ZUCKER J, NICORA CD, TANJORE D, MAGNUSON JK, et al. Further engineering of *R. toruloides* for the production of terpenes from lignocellulosic biomass[J]. Biotechnology for Biofuels, 2021, 14(1): 1-16.

[91] CAO MF, TRAN VG, QIN JS, OLSON A, MISHRA S, SCHULTZ JC, HUANG CS, XIE DM, ZHAO HM. Metabolic engineering of oleaginous yeast *Rhodotorula toruloides* for overproduction of triacetic acid lactone[J]. Biotechnology and Bioengineering, 2022, 119(9): 2529-2540.

[92] ZHANG MY, GAO QD, LIU YJ, FANG ZM, GONG ZW, ZHAO ZK, YANG XB. Metabolic engineering of *Rhodotorula toruloides* for resveratrol production[J]. Microbial Cell Factories, 2022, 21(1): 1-9.

[93] LEE JJL, NG KR, LIANG JQ, CUI X, LI A, CHEN WN. Engineering the phenylpropanoid pathway in *Rhodosporidium toruloides* for naringenin production from tyrosine by leveraging on its native *PAL* gene[J]. ACS Food Science & Technology, 2023, 3(1): 92-99.

[94] MEZZOMO N, FERREIRA SRS. Carotenoids functionality, sources, and processing by supercritical technology: a review[J]. Journal of Chemistry, 2016, 2016: 1-16.

[95] DU C, LI YC, GUO YH, HAN M, ZHANG WG, QIAN H. The suppression of torulene and torularhodin treatment on the growth of PC-3 xenograft prostate tumors[J]. Biochemical and Biophysical Research Communications, 2016, 469(4): 1146-1152.

[96] BAO RQ, GAO N, LV J, JI CF, LIANG HP, LI SJ, YU CX, WANG ZY, LIN XP. Enhancement of torularhodin production in *Rhodosporidium toruloides* by *Agrobacterium tumefaciens*-mediated transformation and culture condition optimization[J]. Journal of Agricultural and Food Chemistry, 2019, 67(4): 1156-1164.

[97] BROMANN K, TOIVARI M, VILJANEN K, RUOHONEN L, NAKARI-SETÄLÄ T. Engineering *Aspergillus nidulans* for heterologous ent-kaurene and gamma-terpinene production[J]. Applied Microbiology and Biotechnology, 2016, 100(14): 6345-6359.

[98] KONG MK, KANG HJ, KIM JH, OH SH, LEE PC. Metabolic engineering of the *Stevia rebaudiana* ent-kaurene biosynthetic pathway in recombinant *Escherichia coli*[J]. Journal of Biotechnology, 2015, 214: 95-102.

[99] HOSKINS J, JACK G, PEIRIS RD, STARR DT, WADE H, WRIGHT E, STERN J. Enzymatic control of phenylalanine intake in phenylketonuria[J]. The Lancet, 1980, 315(8165): 392-394.

[100] CUI JD, QIU JQ, FAN XW, JIA XW Tan ZL. Biotechnological production and applications of microbial phenylalanine ammonia lyase: a recent review[J]. Critical Reviews in Biotechnology, 2014, 34(3): 258-268.

[101] SHIN SY, JUNG SM, KIM MD, HAN NS, SEO JH. Production of resveratrol from tyrosine in metabolically engineered *Saccharomyces cerevisiae*[J]. Enzyme and Microbial Technology, 2012, 51(4): 211-216.

[102] PILONE MS, POLLEGIONI L. D-amino acid oxidase as an industrial biocatalyst[J]. Biocatalysis and Biotransformation, 2002, 20(3): 145-159.

[103] POLLEGIONI L, MOLLA G. New biotech applications from evolved D-amino acid oxidases[J]. Trends in Biotechnology, 2011, 29(6): 276-283.

[104] GREMBECKA M. Sugar alcohols—their role in the modern world of sweeteners: a review[J]. European Food Research and Technology, 2015, 241(1): 1-14.

[105] JAGTAP SS, BEDEKAR AA, LIU JJ, JIN YS, RAO CV. Production of galactitol from galactose by the oleaginous yeast *Rhodosporidium toruloides*

IFO0880[J]. Biotechnology for Biofuels, 2019, 12(1): 1-13.

[106] KORDOWSKA-WIATER M. Production of arabitol by yeasts: current status and future prospects[J]. Journal of Applied Microbiology, 2015, 119(2): 303-314.

[107] REGNAT K, MACH RL, MACH-AIGNER AR. Erythritol as sweetener—wherefrom and whereto?[J]. Applied Microbiology and Biotechnology, 2018, 102(2): 587-595.

[108] RZECHONEK DA, DOBROWOLSKI A, RYMOWICZ W, MIROŃCZUK AM. Recent advances in biological production of erythritol[J]. Critical Reviews in Biotechnology, 2018, 38(4): 620-633.

[109] JAGTAP SS, RAO CV. Production of D-arabitol from D-xylose by the oleaginous yeast *Rhodosporidium toruloides* IFO0880[J]. Applied Microbiology and Biotechnology, 2018, 102(1): 143-151.

[110] WEHRS M, GLADDEN JM, LIU YZ, PLATZ L, PRAHL JP, MOON J, PAPA G, SUNDSTROM E, GEISELMAN GM, TANJORE D, KEASLING JD, PRAY TR, SIMMONS BA, MUKHOPADHYAY A. Correction: sustainable bioproduction of the blue pigment indigoidine: expanding the range of heterologous products in *R. toruloides* to include non-ribosomal peptides[J]. Green Chemistry, 2019, 21(21): 6027-6029.

[111] SAINI R, HEGDE K, BRAR SK, VEZINA P. Advanced biofuel production and road to commercialization: an insight into bioconversion potential of *Rhodosporidium* sp.[J]. Biomass and Bioenergy, 2020, 132: 105439.

[112] QIAO KJ, IMAM ABIDI SH, LIU HJ, ZHANG HR, CHAKRABORTY S, WATSON N, KUMARAN AJIKUMAR P, STEPHANOPOULOS G. Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*[J]. Metabolic Engineering, 2015, 29: 56-65.

[113] WATSUNTORN W, CHUENGCHAROENPHANICH N, NILTAYA P, BUTKUMCHOTE C, THEERACHAT M, GLINWONG C, QI W, WANG ZM, CHULALAKSANANUKUL W. A novel oleaginous yeast *Saccharomyces cerevisiae* CU-TPD4 for lipid and biodiesel production[J]. Chemosphere, 2021, 280: 130782.

[114] FERREIRA R, TEIXEIRA PG, SIEWERS V, NIELSEN J. Redirection of lipid flux toward phospholipids in yeast increases fatty acid turnover and secretion[J]. Proceedings of the National Academy of Sciences of the United States of America, 2018, 115(6): 1262-1267.

[115] TEIXEIRA SOUZA KS, LACERDA RAMOS C, SCHWAN RF, DIAS DR. Lipid production by yeasts grown on crude glycerol from biodiesel industry[J]. Preparative Biochemistry & Biotechnology, 2017, 47(4): 357-363.

[116] ZHANG Y, PANG J, LIU S, NIE KL, DENG L, WANG F, LIU JF. Harnessing transcription factor Mga2 and fatty acid elongases to overproduce palmitoleic acid in *Saccharomyces cerevisiae*[J]. Biochemical Engineering Journal, 2022, 181: 108402.

[117] WANG KF, SHI TQ, WANG JP, WEI P, LEDESMA-AMARO R, JI XJ. Engineering the lipid and fatty acid metabolism in *Yarrowia lipolytica* for sustainable production of high oleic oils[J]. ACS Synthetic Biology, 2022, 11(4): 1542-1554.

[118] ZHANG BX, CHEN HQ, LI M, GU ZN, SONG YD, RATLEDGE C, CHEN YQ, ZHANG H, CHEN W. Genetic engineering of *Yarrowia lipolytica* for enhanced production of *trans*-10, *cis*-12 conjugated linoleic acid[J]. Microbial Cell Factories, 2013, 12(1): 1-8.

[119] WEI LJ, MA YY, CHENG BQ, GAO Q, HUA Q. Metabolic engineering *Yarrowia lipolytica* for a dual biocatalytic system to produce fatty acid ethyl esters from renewable feedstock *in situ* and in one pot[J]. Applied Microbiology and Biotechnology, 2021, 105(21): 8561-8573.

[120] SHI WQ, LI J, CHEN YF, CHEN YF, GUO XW, XIAO DG. Enhancement of C6-C10 fatty acid ethyl esters production in *Saccharomyces cerevisiae* CA by metabolic engineering[J]. LWT, 2021, 145: 111496.

[121] CORDOVA LT, BUTLER J, ALPER HS. Direct production of fatty alcohols from glucose using engineered strains of *Yarrowia lipolytica*[J]. Metabolic Engineering Communications, 2020, 10: e00105.

[122] HU YT, ZHU ZW, GRADISCHNIG D, WINKLER M, NIELSEN J, SIEWERS V. Engineering carboxylic acid reductase for selective synthesis of medium-chain fatty alcohols in yeast[J]. Proceedings of the National Academy of Sciences of the United States of America, 2020, 117(37): 22974-22983.

[123] LARROUDE M, CELINSKA E, BACK A, THOMAS S, NICAUD JM, LEDESMA-AMARO R. A synthetic biology approach to transform *Yarrowia lipolytica* into a competitive biotechnological producer of β-carotene[J]. Biotechnology and Bioengineering, 2018, 115(2): 464-472.

[124] BU X, LIN JY, DUAN CQ, KOFFAS MAG, YAN GL. Dual regulation of lipid droplet-triacylglycerol metabolism and ERG9 expression for improved β-carotene production in *Saccharomyces cerevisiae*[J]. Microbial Cell Factories, 2022, 21(1): 1-13.

[125] CHENG BQ, WEI LJ, LV YB, CHEN J, HUA Q. Elevating limonene production in oleaginous yeast *Yarrowia lipolytica via* genetic engineering of limonene biosynthesis pathway and optimization of medium composition[J]. Biotechnology and Bioprocess Engineering, 2019, 24(3): 500-506.

[126] ZHANG X, LIU X, MENG YH, ZHANG LJ, QIAO JJ, ZHAO GR. Combinatorial engineering of *Saccharomyces cerevisiae* for improving limonene production[J]. Biochemical Engineering Journal, 2021, 176: 108155.

[127] ZHU K, ZHAO BX, ZHANG YH, KONG J, RONG LX, LIU SQ, WANG YP, ZHANG CY, XIAO DG, FOO JL, YU AQ. Mitochondrial engineering of *Yarrowia lipolytica* for sustainable production of α-bisabolene from waste cooking oil[J]. ACS Sustainable Chemistry & Engineering, 2022, 10(29): 9644-9653.

[128] ICHINOSE H, UKEBA S, KITAOKA T. Latent potentials of the white-rot basidiomycete *Phanerochaete chrysosporium* responsible for sesquiterpene metabolism: CYP5158A1 and CYP5144C8 decorate (E)-α-bisabolene[J]. Enzyme and Microbial Technology, 2022, 158: 110037.

[129] MARKHAM KA, PALMER CM, CHWATKO M, WAGNER JM, MURRAY C, VAZQUEZ S, SWAMINATHAN A, CHAKRAVARTY I, LYND NA, ALPER HS. Rewiring *Yarrowia lipolytica* toward triacetic acid lactone for materials generation[J]. Proceedings of the National Academy of Sciences of the United States of America, 2018, 115(9): 2096-2101.

[130] VICKERYCR, CARDENAS J, BOWMAN ME, BURKART MD, Da SILVA NA, NOEL JP. A coupled *in vitro*/*in vivo* approach for engineering a heterologous type III PKS to enhance polyketide biosynthesis in *Saccharomyces cerevisiae*[J]. Biotechnology and Bioengineering, 2018, 115(6): 1394-1402.

[131] LIU MS, WANG C, REN XF, GAO S, YU SQ, ZHOU JW. Remodelling metabolism for high-level resveratrol production in *Yarrowia lipolytica*[J]. Bioresource Technology, 2022, 365: 128178.

[132] COSTA CE, ROMANÍ A, TEIXEIRA JA, DOMINGUES L. Resveratrol production for the valorisation of lactose-rich wastes by engineered industrial *Saccharomyces cerevisiae*[J]. Bioresource Technology, 2022, 359: 127463.

[133] PALMER CM, MILLER KK, NGUYEN A, ALPER HS. Engineering 4-coumaroyl-CoA derived polyketide production in *Yarrowia lipolytica* through a β-oxidation mediated strategy[J]. Metabolic Engineering, 2020, 57: 174-181.

[134] LI HB, MA WJ, LYV Y, GAO S, ZHOU JW. Glycosylation modification enhances (2S)-naringenin production in *Saccharomyces cerevisiae*[J]. ACS Synthetic Biology, 2022, 11(7): 2339-2347.

[135] KANG W, MA T, LIU M, QU JL, LIU ZJ, ZHANG HW, SHI B, FU S, MA JC, LAI LTF, HE SC, QU JN, WING-NGOR AU S, HO KANG B, YU LAU WC, DENG ZX, XIA J, LIU TG. Modular enzyme assembly for enhanced cascade biocatalysis and metabolic flux[J]. Nature Communications, 2019, 10: 4248.

[136] OSORIO-GONZÁLEZ CS, HEGDE K, BRAR SK, KERMANSHAHIPOUR A, AVALOS-RAMÍREZ A. Challenges in lipid production from lignocellulosic biomass using *Rhodosporidium* sp.; A look at the role of lignocellulosic inhibitors[J]. Biofuels, Bioproducts and Biorefining, 2019, 13(3): 740-759.

[137] QIAO KJ, WASYLENKO TM, ZHOU K, XU P, STEPHANOPOULOS G. Lipid production in *Yarrowia lipolytica* is maximized by engineering cytosolic redox metabolism[J]. Nature Biotechnology, 2017, 35(2): 173-177.

[138] GREWAL PS, SAMSON JA, BAKER JJ, CHOI B, DUEBER JE. Peroxisome compartmentalization of a toxic enzyme improves alkaloid production[J]. Nature Chemical Biology, 2021, 17(1): 96-103.

[139] DUEBER JE, WU GC, MALMIRCHEGINI GR, MOON TS, PETZOLD CJ, ULLAL AV, PRATHER KLJ, KEASLING JD. Synthetic protein scaffolds provide modular control over metabolic flux[J]. Nature Biotechnology, 2009, 27(8): 753-759.

[140] LIU ZJ, RADI M, MOHAMED ETT, FEIST AM, DRAGONE G, MUSSATTO SI. Adaptive laboratory evolution of *Rhodosporidium toruloides* to inhibitors derived from lignocellulosic biomass and genetic variations behind evolution[J]. Bioresource Technology, 2021, 333: 125171.

(本文责编 陈宏宇)