微生物学通报
Microbiology China
http://journals.im.ac.cn/wswxtbcn

Sep. 20, 2024, 51(9): 3268−3302
DOI: 10.13344/j.microbiol.china.231039
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专论与综述

# 解脂耶氏酵母合成萜类化合物的研究进展

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徐龙兴, 袁丽杰, 刘顺成. 解脂耶氏酵母合成萜类化合物的研究进展[J]. 微生物学通报, 2024, 51(9): 3268-3302.
XU Longxing, YUAN Lijie, LIU Shuncheng. Research progress in the synthesis of terpenoids from *Yarrowia lipolytica*[J]. Microbiology China, 2024, 51(9): 3268-3302.

**摘　要：**解脂耶氏酵母(*Yarrowia lipolytica*)是一种遗传背景清晰、基因编辑工具完备的非常规油脂酵母。萜类化合物是以异戊二烯为基本结构单元，广泛存在自然界的天然次级代谢产物。近年来随着合成生物学的高速发展，代谢工程改造解脂耶氏酵母合成萜类化合物越来越受到人们的关注。本文从增强前体供应、亚细胞器合成和碳源利用等方面对代谢工程改造解脂耶氏酵母合成萜类化合物进行综述，并对未来可能的研究方向进行展望，为后续研究工作提供参考。
**关键词：**解脂耶氏酵母；萜类化合物；合成生物学；代谢工程

# Research progress in the synthesis of terpenoids from *Yarrowia lipolytica*

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**Abstract:** *Yarrowia lipolytica* is a species of unconventional lipid-producing yeast with a well-defined genetic background and a comprehensive suite of gene editing tools. Terpenoids are natural secondary metabolites with isoprene as the basic structural unit and are ubiquitous in nature. In recent years, with the rapid development of synthetic biology, the synthesis of terpenoids by metabolic engineering has attracted increasing attention. In this paper, we reviewed the metabolic engineering in *Y. lipolytica* for the synthesis of terpenoids from

资助项目：河北省自然科学基金(H2021209027, B2022209030)；河北省重点研发计划(23372505D)
This work was supported by the Natural Science Foundation of Hebei Province (H2021209027, B2022209030), and the Key Research and Development Program of Hebei Province (23372505D).
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Received: 2023-12-08; Accepted: 2024-01-30; Published online: 2024-04-08

enhanced precursor supply, subcellular organelle synthesis, and carbon source utilization. In addition, this paper envisions the potential research directions, with a view to providing guidance for the following research.

**Keywords:** *Yarrowia lipolytica*; terpenoids; synthetic biology; metabolic engineering

萜类化合物也称异戊二烯类化合物，是一类由异戊二烯($C_5H_8$)为基本结构单元组成的化合物。根据所含 C5 单元的数量，萜类可分为半萜($C_5$)、单萜($C_{10}$)、倍半萜($C_{15}$)、二萜($C_{20}$)、三萜($C_{30}$)和四萜类($C_{40}$)等。萜类化合物主要在植物信号转导、适应环境、防御、繁殖等方面发挥作用[1-3]，是植物生存过程中不可或缺的物质。同时，因其具有抗炎、抗细胞凋亡、抑制肿瘤增殖和转移等生物活性受到医药领域的广泛关注[4-5]。目前，萜类化合物的获取很大程度上依赖于植物提取和化学合成。然而，植物提取受到气候环境和生长周期的限制，并且提纯程序复杂，产物获取率低；而化学合成耗费高且对环境不友好[6]。

随着合成生物学、分子生物学技术的快速发展和天然产物生物合成路径解析的不断深入，以代谢工程思路构建微生物细胞工厂生产高附加值化合物成为生物化工领域的研究热点[7-8]。自然界中存在两种萜类合成途径，分别是甲羟戊酸(mevalonate, MVA)途径和甲基-D-赤藓糖醇-4-磷酸(methyl-D-erythritol-4-phosphate, MEP)途径[9-10]。其中真核生物主要通过 MVA 途径进行萜类的合成，原核生物则主要为 MEP 途径[11](图 1)。

目前，在大肠杆菌(*Escherichia coli*)、酿酒酵母(*Saccharomyces cerevisiae*)等模式生物中已经成功实现功能性萜类及萜类衍生物的生物合成[12-13]。但是大肠杆菌缺乏完整的内膜系统与转录后修饰，其对真核生物来源基因的表达效率低下[14]。此外，大肠杆菌细胞工厂存在生物安全风险等问题。相较而言，酵母等真核微生物在合成复杂蛋白质及生物活性物质生产方面表现出优良性状。随着快速途径组装工具和代谢网络动态调节系统的开发应用，在酵母细胞中异源合成天然化合物得以实现[15]。

# 1 解脂耶氏酵母及其遗传改造

作为一种产油酵母，解脂耶氏酵母(*Yarrowia lipolytica*)具有耐盐、耐低温并能在低 pH 值的环境生长等特点。在代谢方面，其具有能利用葡萄糖、废弃的食用油、乙醇、甘油等多种底物进行生长的优势，是合成天然产物的优质底盘细胞[16-17]。其次，其具有高通量的三羧酸(tricarboxylic acid cycle, TCA)循环，为萜类化合物的合成提供了丰富的乙酰辅酶 A 前体；其胞质中丰富的脂滴及亚细胞结构也为疏水性产物的储存提供场所[18]。此外，解脂耶氏酵母还具有较高的磷酸戊糖途径(pentose phosphate pathway, PPP)代谢通量，能够为产物合成提供所需辅因子[19]。解脂耶氏酵母的上述优势使其成为一种优秀的代谢工程底盘菌株。

除了代谢方面的优势，作为一种成熟的代谢工程底盘菌株，解脂耶氏酵母还具有完善的基因编辑工具和清晰的遗传背景。近年来随着科学研究的深入，科研人员鉴定并构建了一系列解脂耶氏酵母中的基因表达元件，并通过多种方式实现了基因的高效表达。

## 1.1 基因表达元件

以解脂耶氏酵母为底盘细胞进行代谢工程改造往往需要构建基因表达载体，其中包括启动子、终止子及筛选标记等元件。

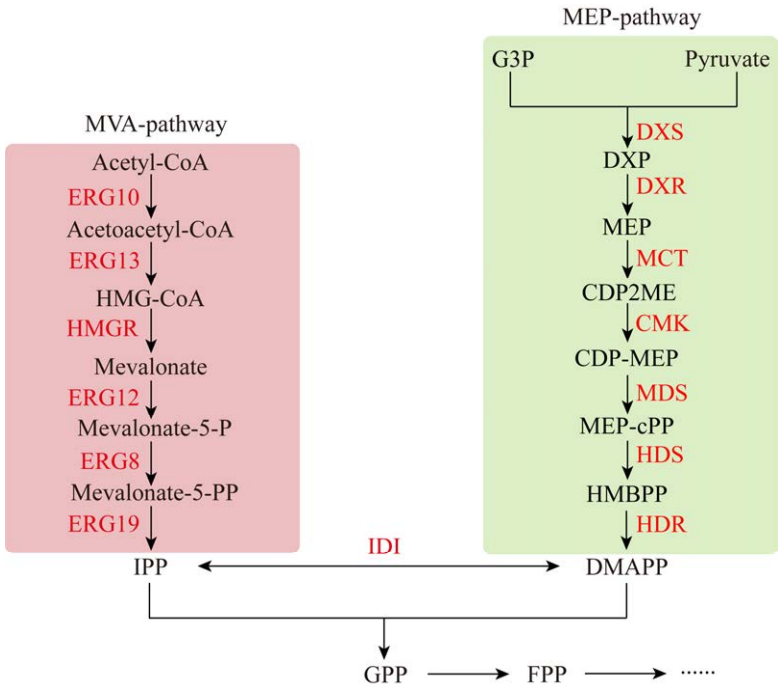



**图 1 微生物中的两种萜类合成途径** DXS：1-脱氧-D-木酮糖-5-磷酸合成酶；DXR：1-脱氧-D-木酮糖-5-磷酸合还原异构酶；MCT：2-C-甲基-D-赤藓糖醇 4-磷酸胞苷酰转移酶；CMK：4-(胞苷 5′-二磷酸)-2-C-甲基-D-赤藓糖醇激酶；MDS：2-C-甲基-D-赤藓糖醇 2,4-环二磷酸合成酶；HDS：1-羟基-2-甲基-2-丁烯基 4-二磷酸合成酶；HDR：1-羟基-2-甲基-2-丁烯基 4-二磷酸还原酶；ERG10：乙酰辅酶 A 乙酰基转移酶；ERG13：羟甲基戊二酰辅酶 A 合酶；HMGR：羟甲基戊二酰辅酶 A 还原酶；ERG12：甲羟戊酸激酶；ERG8：磷酸甲羟戊酸激酶；ERG19：甲羟戊酸二磷酸脱羧酶；IDI：异戊烯基二磷酸异构酶；G3P：3-磷酸甘油醛；DXP：1-脱氧-D-木酮糖-5-磷酸；MEP：2-C-甲基-D-赤藓糖醇 4-磷酸；CDP2ME：4-(胞苷 5′-二磷酸)-2-C-甲基-D-赤藓糖醇；CDP-MEP：2-磷酸-4-(胞苷 5′-二磷酸)-2-C-甲基-D-赤藓糖醇；ME-cPP：2-C-甲基-D-赤藓糖醇 2,4-环二磷酸；HMBPP：1-羟基-2-甲基-2-丁烯基 4-二磷酸；HMG-CoA：3-羟基-3-甲基戊二酰辅酶 A；Mevalonate-5-P：甲羟戊酸-5-磷酸；Mevalonate-5-PP：甲羟戊酸-5-二磷酸；IPP：异戊烯基焦磷酸；DMAPP：二甲基烯丙基焦磷酸；FPP：法尼基焦磷酸；GPP：香叶基焦磷酸

Figure 1 Terpenoid biosynthesis pathway in microorganisms. DXS: 1-deoxy-D-xylulose 5-phosphate synthase; DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT: 2-C-methyl-D-erythritol 4-phosphate cytidine transferase; CMK: 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; MDS: 2-C-methyl-D-erythritol 2,4-dichlorodiphosphate synthase; HDS: 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase; HDR: 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase; ERG10: Acetyl-CoA acetyltransferase; ERG13: Hydroxymethylglutaryl-CoA synthase; HMGR: Hydroxymethylglutaryl-CoA reductase; ERG12: Mevalonate kinase; ERG8: Phosphomevalonate kinase; ERG19: Mevalonate pyrophosphate decarboxylase; IDI: Isopentenyl-diphosphate; G3P: 3-phosphoglyceraldehyde; DXP: 1-deoxy-D-xylulose 5-phosphate; MEP: 2-C-methyl-D-erythritol 4-phosphate; CDP2ME: 4-(cytidine-5′-diphospho)-2-C-methyl-D-erythritol; CDP-MEP: 2-phospho-4-(cytidine-5′-diphospho)-2-C-methyl-D-erythritol; ME-cPP: 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP: 1-hydroxy-2-methyl-2-butenyl 4-diphosphate; HMG-CoA: 3-hydroxy-3-methylglutaryl CoA; Mevalonate-5-P: Mevalonate-5-phosphate; Mevalonate-5-PP: Mevalonate-5-diphosphate; IPP: Isopentenyl pyrophosphate; DMAPP: Dimethylallyl pyrophosphate; FPP: Farnesyl pyrophosphate; GPP: Geranyl pyrophosphate.

### 1.1.1 启动子

启动子是在基因的转录水平上调节和影响基因表达的关键元件。目前，在解脂耶氏酵母中已经表征了多个启动子，如组成型启动子 $P_{hp4d}$ 和诱导型启动子 $P_{XPR2}$、$P_{TEF}$ 启动子等；其中 $P_{hp4d}$ 被证明比 $P_{TEF}$ 具有更强的启动子活性[20]。Juretzek 等[21]基于解脂耶氏酵母积累脂质的特性，鉴定了一系列解脂耶氏酵母内源性启动子；并分析了 $P_{G3P}$、$P_{ICL1}$、$P_{POT1}$、$P_{POX1}$、$P_{POX2}$ 及 $P_{POX5}$ 等在不同碳源条件下的强度和诱导作用。Wong 等[22]表征了包括 $P_{TEF}$ 和 11 个与脂肪代谢相关的启动子在内的 12 个内源启动子，并比较了启动子强度：$P_{TEF}$>$P_{GAP}$>$P_{ACL2}$>$P_{ICL}$>$P_{IDH2}$>$P_{FAS1}$>$P_{DGA1}$>$P_{FAS2}$>$P_{ZWF1}$>$P_{POX4}$>$P_{ACC}$>$P_{IDP2}$；随后，其利用这些启动子设计了一组模块化克隆载体。Blazeck 等[23]通过改造核心启动子序列和上游激活序列(upstream activation sequence, UAS)来调节启动子的强度；将 $P_{TEF}$ 序列截短为多个区域并与 UAS 连接，发现将 UAS 的串联数量控制在 8–16 个可以显著提高组成型启动子的活性。此外，在启动子中插入特定的内含子序列也有助于提高基因的表达水平[24]。Hong 等[25]在 $P_{FBA1}$ 启动子中添加部分内含子序列，构建了 $P_{FBA1in}$ 启动子，该启动子活性较 $P_{FBA}$ 提高了 5 倍。Tai 等[20]构建的 $P_{FBA1in}$ 启动子表达水平比无内含子的 $P_{TEF}$ 提高了 17 倍。Trassaert 等[26]分离并表征了编码赤藓糖激酶的基因 *EYK1* 的启动子，$P_{EYK1}$ 在赤藓糖醇和赤藓糖存在的条件下表达水平增强；通过鉴定上游激活序列 $UAS1_{EYK1}$ 并构建包含串联重复序列的 $UAS1_{XPR2}$ 和 $UAS1_{EYK1}$ 杂合启动子诱导基因高效表达。Park 等[27-28]在赤藓糖醇分解代谢相关基因 *EYD1* 和 *EYK1* 的启动子区域内鉴定到了 4 个保守的顺式调控模块，并发现模块上游激活序列参与了赤藓糖醇依赖的启动子诱导表达；在此基础上构建了组成型启动子 $P_{EYK1\text{-}3AB}$ 或 $P_{HU8EYK}$，其表达强度分别为内源性强启动子 $P_{TEF}$ 的 2.8 倍和 2.5 倍。Xiong 等[29]从解脂耶氏酵母基因组分离了 6 个铜离子($Cu^{2+}$)诱导型启动子和 5 个抑制型启动子，抑制型启动子在无 $Cu^{2+}$ 条件下表现出相对较高的活性，但在加入 $Cu^{2+}$ 后活性几乎被抑制；通过串联 UAS 改造诱导型启动子，成功提高了其动态调控范围。相关研究运用上述 $Cu^{2+}$ 抑制型启动子抑制角鲨烯生成，显著增加了萜类化合物合成[30]。

Wang 等[31]在解脂耶氏酵母中筛选了 81 个主要参与碳代谢和氮代谢的内源性启动子，经过比较，获得了 15 个强启动子、41 个中等强度启动子和 25 个弱启动子；并用鉴定的启动子文库表达 UDP 糖基转移酶，成功实现了红景天苷的合成。Georgiadis 等[32]在甘油的培养条件下，选择高表达的 3 个基因 *H3*、*ACBP* 和 *TMAL*，分别将它们的启动子元件与报告基因整合并判断其强度，发现 $P_{H3}$ 启动子强度超过强启动子($P_{FBA1in}$、$P_{EXP1}$ 和 $P_{TEF1in}$)；将上游激活序列 1B (UAS1B8)分别与 H3 (260)和 TMAL (250)的最小启动子序列相连，并与 UAS1B8-TEF1 (136)进行比较，发现新的杂交启动子表现出更高的转录活性，这也为后续研究增加了启动子库。

### 1.1.2 终止子

终止子是转录过程中必不可少的调控元件，其通过调节转录机制完成转录过程，进而影响 mRNA 的稳定性[33]。在解脂耶氏酵母中通常使用 *CYC1t*、*XPR2t* 和 *LIP2t* 等几种终止子，相较于启动子，终止子的研究仍然较少[34-35]。Curran 等[36]在酿酒酵母中表征了多种终止子，发现不同的终止子对基因转录水平的影响十分明显；后续通过 *CYC1t* 突变，发现基因的表达水平与终止子序列也有关系。Curran 等[37]构建的人工终止子在酿酒酵母和解脂耶氏酵母中都具有很强的功能，较天然终止子序列更短、效

果更好，还可以减少天然终止子重复使用导致的同源重组机率。因此，在代谢工程改造解脂耶氏酵母的过程中，终止子的改造也逐渐进入人们的视野。

#### 1.1.3 筛选标记

工程菌株的筛选通常需要使用遗传筛选标记。解脂耶氏酵母中常用的筛选标记可分为营养缺陷标记和抗生素筛选标记。目前，营养缺陷型筛选标记包括亮氨酸(*LEU2*)、尿嘧啶(*URA3*)等筛选标记[38-39]。当工程菌株培养在含有 5-氟乳清酸(5-fluorooroticacid, 5-FOA)和尿嘧啶的培养基中时，尿嘧啶原养型菌株($URA3^+$)不能存活，而尿嘧啶营养缺陷型菌株($URA3^-$)被保留，从而恢复底盘细胞的营养缺陷，实现尿嘧啶筛选标记在解脂耶氏酵母中的重复使用[40]。此外，通过截短基因片段中 *URA3* 的启动子能够有效地提高菌株整合基因片段的拷贝数，实现目的基因的高水平表达[40]。使用 *LEU2* 筛选标记可能会影响菌株的脂质合成，亮氨酸营养缺陷型菌株在含亮氨酸的培养基中培养时，菌株的脂肪酸产量和生物量在一定程度上与培养基中亮氨酸的含量呈正相关[39]。

目前，解脂耶氏酵母可用的有限筛选标记数量，在一定程度上限制了菌株构建的进程。野生型解脂耶氏酵母对潮霉素 B (hygromycin B)、诺尔斯菌素(nourseothricin)等抗生素敏感，利用抗生素抗性基因作为筛选标记，能够在一定程度上避免营养缺陷标记使用次数的限制[41-42]。Fickers 等[43]基于 *URA3* 和潮霉素 B 两种筛选标记，设计了解脂耶氏酵母中的 Cre-Loxp 重组系统；Cre 重组酶表达后识别筛选标记表达框两端的 Loxp 位点并进行切割和重组，该系统能有效地进行基因组整合表达和标记回收。其中，Cre 酶质粒通过潮霉素筛选标记进行筛选[42]。Liu 等[42]通过利用潮霉素 B 筛选标记进行基因的整合，成功实现了反式橙花叔醇的高效生产。

### 1.2 基因表达方式

特定基因在宿主中的表达是代谢工程研究中的重点。根据基因片段在解脂耶氏酵母中的存在方式，可以将其分为游离质粒表达和基因组整合表达。其中，根据基因片段在基因组中的整合方式，又可以分为同源重组和非同源重组。

#### 1.2.1 基于游离质粒的表达

目前，在解脂耶氏酵母中未发现天然的游离质粒，随着代谢工程的深入研究，应用于解脂耶氏酵母的人工游离质粒得以构建出来[44-45]。对于人工游离质粒而言，染色体自主复制序列(autonomously replicating sequence, ARS)尤为关键(表 1)。解脂耶氏酵母的 ARS 由复制起点(origin, ORI)和着丝粒(centromere, CEN)构成；其中 ARS 主要有 4 种，包括 ARS1、ARS2、ARS18 和 ARS68[23]。ARS1 和 ARS18 又分别包含 ORI1001 和 ORI3018[48]。

Heslot 等[49]使用 ARS 和 CEN 设计游离型表达载体，发现在单细胞中的拷贝数较低。而且，游离质粒在菌株传代过程中遗传稳定性差，不利于异源基因稳定表达[44]。有研究显示，将解脂耶氏酵母着丝粒序列与启动子上游融合，能增强游离质粒的数量和表达水平[25,50]。相关报

**表 1 解脂耶氏酵母中常用质粒的自主复制序列/着丝粒**

Table 1 ARS/CEN of plasmids commonly used in *Yarrowia lipolytica*

| 质粒 Plasmid | 自主复制序列/着丝粒 Autonomously replicating sequence/Centromere | 参考文献 Reference |
|---|---|---|
| JMP113 | ARS68 | [43] |
| P-UAS1B8-TEF | ORI1001, CEN1 | [23] |
| CRISPR-cas9 | ORI1001, CEN1 | [46] |
| P-YaliA1 | ORI1001, CEN1-1 | [22] |
| ylAC | ORI3018, CEN3-1 | [45] |
| P-mtORI | mtORI | [47] |

道表明，增加 ARS 中复制起始位点和 CEN 之间的间隔序列可以提高游离质粒的稳定性[48]。Guo 等[45]在解脂耶氏酵母中设计和构建人工染色体(ylAC)，ylAC 是由 2 个端粒(telomere sequences, TEL)和 1 个包含着丝粒(CEN3-1)的 ARS 组成，确保在细胞分裂过程中的自主复制，可用于目的基因和染色体组装。最近，Cui 等[47]在解脂耶氏酵母线粒体 DNA 中鉴别到 516 bp 的线粒体 DNA 序列，并以此为人工复制起点构建了高稳定性质粒。

#### 1.2.2 基于同源重组整合

整合型表达载体较游离质粒表达更加稳定。在解脂耶氏酵母中修复 DNA 双链断裂的主要方式是非同源末端连接(non-homologous end joining, NHEJ)，为了在解脂耶氏酵母中实现高效的同源重组(homologous recombination, HR)，目前常运用敲除主要负责 NHEJ 的相关蛋白编码基因 *KU70* 的方式。将基因片段与整合位点的同源臂加长到 1 000 bp，能够有效地提高同源重组效率[51]。此外，通过在解脂耶氏酵母中表达酿酒酵母来源的介导同源重组的基因 *RAD52*，也能够进一步提高其同源重组效率[51]。

#### 1.2.3 基于非同源末端连接整合

在 NHEJ 整合过程中，DNA 片段不需要存在与基因组同源的序列片段，可以通过 NHEJ 整合到基因组的任何位点。目前，已经在解脂耶氏酵母中探索出同时整合多个 DNA 片段构建模块化基因表达库的潜力，在构建多种表型库时具有一定的优势[52]。Liu 等[53]通过 NHEJ 介导整合到基因组上，构建出过表达 MVA 途径和 α-法尼烯合成酶基因的菌株库；进一步经过筛选并优化策略合成了 25.55 g/L α-法尼烯。

基因编辑技术的快速发展为分子操作提供了新的手段，CRISPR-Cas9 系统的开发应用能够快速实现基因的敲除和敲入[46,54]。基于解脂耶氏酵母的倾向于 NHEJ 的特性，CRISPR-Cas9 系统能够在不提供 donor 质粒的情况下实现基因的高效敲除[55]。此外，工作人员通过 CRISPR-Cas9 系统在解脂耶氏酵母基因组中快速构建了番茄红素生物合成途径，实现了四萜复杂化合物路径的搭建[46]。

## 2 解脂耶氏酵母合成萜类化合物策略

近年来，人们以解脂耶氏酵母为底盘菌株，实现了一系列高附加值天然产物的高效合成[56]，如柠檬烯[57-58]、β-法尼烯[59]、α-石竹烯[60]、橙花叔醇[42]和积雪草酸[61]等(表 2)。

Dissook 等[127]通过液相色谱质谱法和 $^{13}C$ 标记同位素示踪等手段，在解脂耶氏酵母中发现了其潜在的内源 MEP 途径，然而关于天然的 MEP 途径的利用尚无进一步报道。为了实现解脂耶氏酵母中异源萜类化合物的高效合成，需要考虑基因的高效表达和代谢路径优化等因素。在解脂耶氏酵母细胞内，萜类化合物骨架异戊烯基焦磷酸(isopentenyl pyrophosphate, IPP)和二甲基烯丙基焦磷酸(dimethylallyl pyrophosphate, DMAPP)主要通过 MVA 途径合成(图 2)。

目前，代谢工程改造主要策略包括增加合成产物的代谢通量、抑制目标产物的竞争途径及平衡辅因子等。其中 MVA 途径的优化被广泛用于萜类化合物的合成，主要采用过表达限速酶和酶融合等方式。

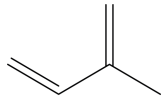
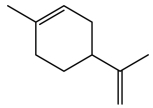
### 2.1 优化 MVA 途径

#### 2.1.1 HMGR 的表达强化

大量研究证明羟甲基戊二酰辅酶 A 还原酶(hydroxymethylglutaryl-CoA reductase, HMGR)是

**表 2　解脂耶氏酵母合成的萜类化合物及其他产物**

Table 2　Synthesis of terpenoids and other products reported by engineered *Yarrowia lipolytica*

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| 半萜 Hemiterpenes | | | | | | | | | |
| 异戊二烯 Isoprene |  Isoprene | Po1g | 葡萄糖 Glucose | *HMG*, *ERG13*, *IDI*, *PmISPS* | | | 530.4 μg/L | 密封小瓶 Sealed vials | [62] |
| 单萜 Monoterpenoids | | | | | | | | | |
| 柠檬烯 Limonene |  Limonene | Po1f | 葡萄糖，丙酮酸 Glucose, pyruvate | *TArLS*, *tSlNDPS1*, *HMG*, *ERG12* | | | 23.6 mg/L | 摇瓶 Shake flask | [63] |
| | | Po1g | 废弃食用油 Waste cooking oil | *HMG*, *ClLS* or *MsLS* | | | D-柠檬烯 D-limonene 11.7 mg/L L-柠檬烯 L-limonene 11.1 mg/L | 5 L 发酵罐 5 L bioreactor | [64] |
| | | Po1f | 柠檬酸，甘油 Citric acid, glycerol | *TArLS*, *tSlNDPS1*, *HMG*, *ERG12* | | | 165.3 mg/L | 发酵罐 Bioreactor | [65] |
| | | ATCC 20460 | 葡萄糖 Glucose | *HMG*, *ERG12*, *ACL1*, *SQS* *SeACS*, *IDI*, *ERG20*$^{F88W\text{-}N119W}$, *PfLS* | | | 35.9 mg/L | 玻璃管 Glass tube | [66] |
| | | Po1f | 木质纤维素水解产物，柠檬酸 Lignocellulose hydrolysis product, citric acid | *ssXR*, *ssXDH*, *XKS*, *TArLS*, *tSlNDPS1*, *HMG*, *ERG12* | | | 20.57 mg/L | 摇瓶 Shake flask | [57] |

(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| | | Po1f | 废弃食用油 Waste cooking oil | *ClLS* or *MsLS*, *HMG*, *IDI*, *tSlNDPS1* | | | D-柠檬烯 D-limonene 91.24 mg/L L-柠檬烯 L-limonene 83.06 mg/L | 摇瓶 Shake flask | [67] |
| | | Po1g | 葡萄糖 Glucose | *YALI0F19492* | | | 提高了 1.8 倍 | 摇瓶 Shake flask | [58] |
| 芳樟醇 Linalool | Linalool | Po1f | 柠檬酸，丙酮酸 Citric acid, pyruvate | *AaLIS*, *HMG*, *IDI*, *ERG20*$^{F88W\text{-}N119W}$ | | | 6.96 mg/L | 摇瓶 Shake flask | [68] |
| α-蒎烯 α-pinene | Alpha-pinene | Po1f | 废弃食用油，大豆油和木质纤维素水解物介质 Waste cooking oil, soybean oil and lignocellulosic hydrolysate | *HMG*, *tSlNDPS1*, *tPtPS*, *ERG8*, *ERG12*, *MBP-ERG12*, *AMPD* | | | 33.8 mg/L 36.6 mg/L 36.1 mg/L | 摇瓶 Shake flask | [69] |
| 倍半萜 Sesquiterpenes | | | | | | | | | |
| α-法尼烯 α-farnesene | Alpha-farnesene | Po1h | 葡萄糖，果糖 Glucose, fructose | *SctHMG*, *IDI*, *MdFS-L-ERG20* | | | 259.98 mg/L | 1.5 L 发酵罐 1.5 L bioreactor | [70] |
| | | Po1f | 葡萄糖 Glucose | *BpHMG*, *ERG13*, *ERG12*, *IDI*, *ERG8*, *ERG19*, *GPPS*, *MdFS-L-ERG20* | | | 25.55 g/L | 1 L 发酵罐 1 L bioreactor | [53] |
| | | Po1f | 葡萄糖，甘油 Glucose, glycerol | *FS-L-ERG20*, *IDI*, *SctHMG1*, *HMG*, *ERG19* | | | 2.57 g/L | 5 L 发酵罐 5 L bioreactor | [71] |

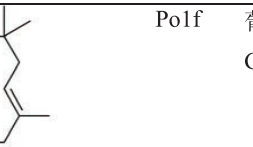
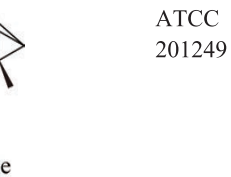
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| β-法尼烯 β-farnesene | (E)-beta-farnesene | ATCC 20460 | 葡萄糖 Glucose | *AaBFS*, *HMG*, *ERG12*, *ACL1*, *SeACS*, *IDI*, *ERG20* | | | 955 mg/L | 玻璃管 Glass tube | [66] |
| | | Po1f | 葡萄糖 Glucose | *AaBFS*, *tHMGR*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *IDI* | | *DGA1-2*, *GUT2*, *POX3-6*, | 22.8 g/L | 2 L 发酵罐 2 L bioreactor | [39] |
| | | Po1f | 油酸，废食用油 Oleic acid, waste cooking oil | *ERG20*, *AanFS*$^{K197T\text{-}F180H}$, *ERG8*, *ERG12*, *ERG19*, *IDI*, *GPPS* | | *DGA1-2* | 35.2 g/L 31.9 g/L | 5 L 发酵罐 5 L bioreactor | [72] |
| | | Po1f | 木质纤维素水解液 Lignocellulose hydrolysate | *AaBFS*, *tHMGR*, *spHMGR*, *ERG8*, *ERG19*, *ERG10*, *ERG13*, *ERG12*, *IDI*, *ERG20* | | | 7.38 g/L | 2 L 发酵罐 2 L bioreactor | [73] |
| | | Po1f | $CO_2$ 转化的甲酸和乙酸 $CO_2$-converted formic and acetic acids | *AaBFS*, *ScACS*, *CbFDH*, *PANK*, *RspPPK* | | | 14.8 g/L | 5 L 发酵罐 5 L bioreactor | [74] |
| | | Po1f | 葡萄糖 Glucose | *AaBFS*, *tHMGR*, *spHMGR*, *ERG8*, *ERG19*, *ERG10*, *ERG13*, *ERG12*, *IDI*, *ERG20*, *McMAE*, *MmACL*, *AMPD*, *YHM2* | | *PFK* | 28.9 g/L | 2 L 发酵罐 2 L bioreactor | [59] |

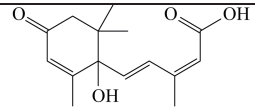
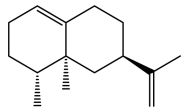
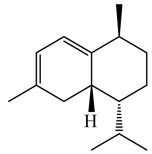
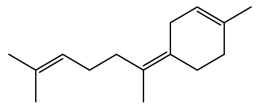
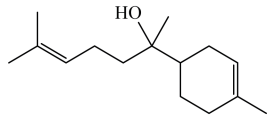
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| α-石竹烯 α-caryophyllene |  Alpha-caryophyllene | Po1f | 葡萄糖 Glucose | *POT1*, *AcACHS2-PTS*, *RpHMG-PTS*, *ANT1*, *ERG12-PTS*, *ERG8-PTS*, *ERG20-PTS*, *ERG10-PTS*, *ERG13-PTS*, *ERG19-PTS*, *IDI-PTS* | | | 3.2 g/L | 5 L 发酵罐 5 L bioreactor | [60] |
| | | Po1f | 废弃食用油 Waste cooking oil | *YALI0E32835*, *POT1*, *AcACHS2-PTS*, *RpHMG-PTS*, *ANT1*, *ERG12-PTS*, *ERG8-PTS*, *ERG20-PTS*, *ERG10-PTS*, *ERG13-PTS*, *ERG19-PTS*, *IDI-PTS* | *YALI0B21780*, *YALI0B21142* | | 5.9 g/L | 5 L 发酵罐 5 L bioreactor | [75] |
| | | Po1f | 葡萄糖 Glucose | *AcACHS2*, *tHMG1*, *AAD*, *ERG12*, *ERG8*, *ERG20*, *ERG10*, *ERG13*, *ERG19*, *IDI* | *ERG9* | | 21.7 g/L | 5 L 发酵罐 5 L bioreactor | [30] |
| α-檀香烯 α-santalene |  Santalene | ATCC 201249 | 葡萄糖 Glucose | *ClSTS*, *ERG8*, *tHMG* | | | 27.92 mg/L | 5 L 发酵罐 5 L bioreactor | [76] |

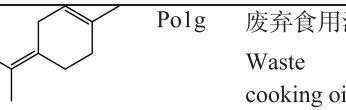
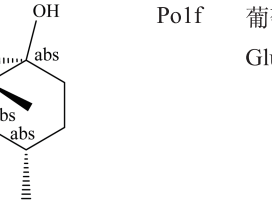
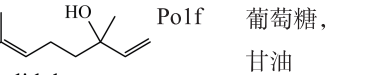
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| 脱落酸 Abscisic acid |  Abscisic acid | ATCC 20460 | 葡萄糖 Glucose | *HMG*, *ERG12*, *ACL1*, *SeACS*, *IDI*, *ERG20*, *BcABA1-4*, *BcCPR1*, *POS5*, *AtDTX50* | *SQS* | | 263.5 mg/L | 深孔板 Deep well plate | [5] |
| 瓦伦西亚烯 Valencene |  Valencene | ATCC 20460 | 葡萄糖 Glucose | *HMG1*, *ERG12*, *ACL1*, *SQS* *SeACS*, *IDI*, *ERG20*, CnVS | | | 113.9 mg/L | 玻璃管 Glass tube | [66] |
| 紫穗槐二烯 Amorphadiene |  Amorphadiene | Po1g | 葡萄糖 Glucose | *AaADS*, *HMG*, *ERG12*, *POT1*, *PAT1* | | | 171.5 mg/L | 摇瓶 Shake flask | [77] |
| | | Po1g Po1f | 葡萄糖 Glucose | *POT1*, *PAT1*, *MFE2*, *AaADS*, *ACS2*, *tHMG1*, *SQS1-ADS* | | *PAH1*, *DGA1-2* | 71.74 mg/L | 摇瓶 Shake flask | [78] |
| α-红没药烯 α-bisabolene |  Alpha-bisabolene | Po1g | 废弃食用油 Waste cooking oil | *HMG*, *GcABC-G1*, *Agα-BS* | | | 973.1 mg/L | 摇瓶 Shake flask | [79] |
| α-红没药醇 α-bisabolol |  Alpha-bisabolol | Po1f | 葡萄糖 Glucose | *POT1*, *MrBBS*, *tHMG*, *SQS* *ERG20* | | | 364.23 g/L | 摇瓶 Shake flask | [80] |

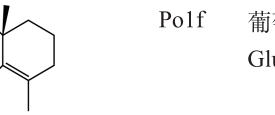
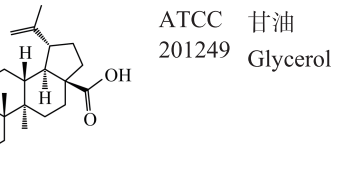
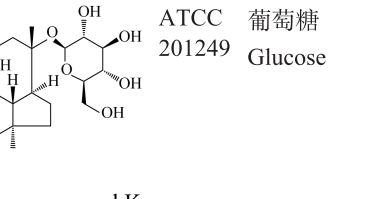
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| α-红没药烯 α-bisabolene |  Alpha-bisabolene | Po1g | 废弃食用油 Waste cooking oil | *Agα-BS*, *MLS-HMGR*, *MPC2*, *PDA1*, *MGM1*, *GcABC-G1* | | | 1 058.1 mg/L | 5 L 发酵罐 5 L bioreactor | [81] |
| | | JMY1212 | 葡萄糖 Glucose | *BiS*, *tHMG1*, *DGA2*, *GPD1* | | *POX1-6*, *TGL4* | 1 243 mg/L | 摇瓶 Shake flask | [82] |
| | | Po1g | 废弃食用油 Waste cooking oil | *Agα-BS*, *HMG1*, *DGA1*, *OLE1*, *GcABC-G1* | | | 1 954.3 mg/L | 摇瓶 Shake flask | [83] |
| 广藿香醇 Patchouli alcohol |   Patchouli alcohol | Po1f | 葡萄糖 Glucose | *PS1*, *tHMG1*, *IDI*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *ERG20-PS1* | *ERG9* | | 2.864 g/L | 5 L 发酵罐 5 L bioreactor | [84] |
| 橙花叔醇 Nerolidol |   Nerolidol | Po1f | 葡萄糖, 甘油 Glucose, glycerol | *HMG1*, *IDI*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *FaNES1-ERG20* | | | 11.1 g/L | 5 L 发酵罐 5 L bioreactor | [42] |
| 吉玛烯 A Germacrene A | Germacrene A | Po1f | 葡萄糖 Glucose | *dlGAS*, *dlGAS-ERG20*, *HMG1*, *IDI*, *ERG13*, *ERG12*, *ERG8*, *ERG19*, *FAA1* | *ERG9*, *ACC1* | | 39 g/L | 5 L 发酵罐 5 L bioreactor | [85] |

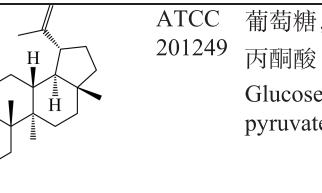
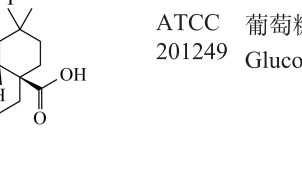
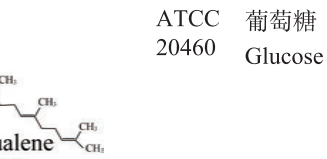
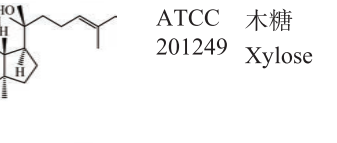
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| β-榄香烯 β-elemene | Beta-elemene | Po1f | 葡萄糖 Glucose | *LTC2*, *tHMG1*, *IDI*, *ERG20*, | *ERG9* | | 5.08 g/L | 5 L 发酵罐 5 L bioreactor | [86] |
| 二萜 Diterpenes | | | | | | | | | |
| 紫杉二烯 Taxadiene |  Taxadiene | Po1f | 葡萄糖 Glucose | *TASY*, *tHMG1*, *GGSP1*, *ERG20-GGPPS1*, *SUMO-TASY* | *ERG9* | | 101.4 mg/L | 5 L 发酵罐 5 L bioreactor | [87] |
| 三萜 Triterpenes | | | | | | | | | |
| 白桦脂酸 Betulinic acid |  Betulinic acid | ATCC 201249 | 甘油 Glycerol | *tHMG1*, *SQS*, *AtLUP1*, *MtCYP716A12-tAtATR1* | | | 26.53 mg/L | 摇瓶 Shake flask | [88] |
| | | ATCC 201249 | 葡萄糖 Glucose | *RcLUS*, *BPLO*, *LjCPR*, *SQS*, *SQE*, *HMG*, *MFE1* | | | 204.89 mg/L | 摇瓶中三萜总量 Total triterpenes in shake flask | [89] |
| 人参皂苷 Ginsenoside compound K |  Ginsenoside compound K | ATCC 201249 | 葡萄糖 Glucose | *tHMG*, *ERG20*, *SQS*, *PgDS*, *PgPPDS-L-tAtATR1*, *PgUGT1* | | | 161.8 mg/L | 5 L 发酵罐 5 L bioreactor | [90] |

(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| 羽扇豆醇 Lupeol |  Lupeol | ATCC 201249 | 葡萄糖，丙酮酸 Glucose, pyruvate | *RcLUS*, *HMG*, *SQS*, *SQE*, *OLE1* | | *PAH1*, *DGK1* | 411.72 mg/L | 摇瓶 Shake flask | [91] |
| 齐墩果酸 Oleanic acid |  Oleanic acid | ATCC 201249 | 葡萄糖 Glucose | *tHMG*, *ERG20*, *SQS*, *GgBAS*, *MtCYP716A12-L-tAtATR1* | | | 540.7 mg/L | 5 L 发酵罐 5 L bioreactor | [92] |
| 2,3-环氧角鲨烯 2,3-oxidosqualene |  2,3-oxidosqualene | ATCC 20460 | 葡萄糖 Glucose | *HMG*, *ERG12*, *ACL1*, *SeACS*, *IDI*, *ERG20*, *SQS*, *SQE* | *ERG7* | | 22 mg/L | 深孔板 Deep well plate | [66] |
| 原人参二醇 Protopanaxadiol |  (20R)-protopanaxdiol | ATCC 201249 | 木糖 Xylose | *SsXR*, *SsXDH*, *XKS*, *PgDS*, *PgPPDS-L-AtATR1*, *tHMG*, *ERG20*, *SQS*, *TKL*, *TAL*, *TX* | | *POX1-3* | 300.63 mg/L | 5 L 发酵罐 5 L bioreactor | [93] |
| 角鲨烯 Squalene | Squalene | Po1f | 葡萄糖，柠檬酸 Glucose, citric acid | *HMG*, *ACL1*, *SeACSL641p* | | | 10 mg/g DCW | 摇瓶 Shake flask | [94] |
| | | Po1f | 葡萄糖 Glucose | *CarB*, *Carp*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *tHMG*, *IDI* | | *GUT2*, *POX3-6* | 531.6 mg/L | 2 L 发酵罐 2 L bioreactor | [95] |

(待续)

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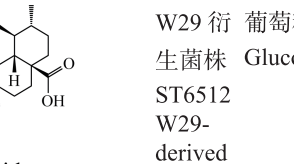
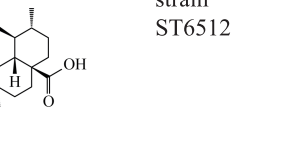
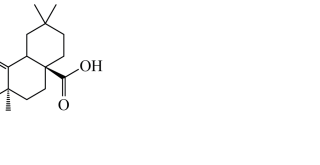
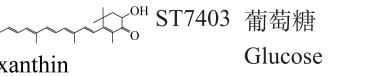
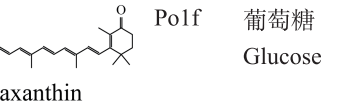
(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| | | ATCC 20460 | 葡萄糖 Glucose | HMG, ERG12, ACL1, SeACS, IDI, *ERG20*, SQS | *ERG7* | | 402.4 mg/L | 深孔板 Deep well plate | [66] |
| | | Po1g | 葡萄糖 Glucose | *SQS*, *HMG*, *MnDH2*, *ACL2* | | | 502.7 mg/L | 摇瓶 Shake flask | [96] |
| | | Po1f | 葡萄糖 Glucose | *HMG*, *DGA1* | | *PEX10*, *URE2* | 240.5 mg/L | 摇瓶 Shake flask | [97] |
| 积雪草酸、羟基积雪草酸和阿江榄仁酸 Asiatic acid, madecassic acid, arjunolic acid |  Asiatic acid  Madecassic acid  Arjunolic acid | W29 衍生菌株 ST6512 W29-derived strain ST6512 | 葡萄糖 Glucose | *CaCYP716C11p*, *CaCYP714E19p*, *CaCYP716E41p* | | | 0.12 mg/g 9.1 mg/g | 深孔板 Deep well plate | [61] |
| 四萜 Tetraterpenoids | | | | | | | | | |
| 虾青素 Astaxanthin |  Astaxanthin | ST7403 | 葡萄糖 Glucose | *XdcrtYB*, *XdcrtI*, *HMG*, *XdcrtE*, *PscrtW*, *PacrtZ* | *SQS* | | 54.6 mg/L | 深孔板 Deep well plate | [98] |

(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| | | ST7403 | 葡萄糖 Glucose | *XdcrtYB*, *XdcrtI*, *HMG*, *XdcrtE*, *SsGGPPS*, *HpBKT*, *HpCrtZ* | *SQS* | | 285 mg/L | 1 L 发酵罐 1 L bioreactor | [99] |
| | | ST7403 | 红花油 Safflower oil | *XdcrtYB*, *XdcrtI*, *HMG*, *XdcrtE*, *PscrtW*, *PacrtZ* | *SQS* | | 167 mg/L | 1.5 L 发酵罐 1.5 L bioreactor | [100] |
| | | Po1f | 葡萄糖 Glucose | *PsCrtW-HpCrtZ-SKL*, *PsCrtW-HpCrtZ-OLE OSIN*, *PsCrtW-HpCrtZ-KDE L*, *SaGGPPS*, *McCarRP*, *McCarB* | | | 858 mg/L | 摇瓶 Shake flask | [101] |
| 角黄素 Canthaxanthin |  Canthaxanthin | Po1f | 葡萄糖 Glucose | *BsCrtW*, *McCarB*, *McCarRP*, *GGPPS* | | | 36.1 mg/L | 摇瓶 Shake flask | [102] |
| β-胡萝卜素 β-carotene | Beta-carotene | Po1f | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *GGPPS*, *tHMG*, *IDI* | | *POX3-6* | 4 g/L | 2 L 发酵罐 2 L bioreactor | [95] |
| | | ATCC 20460 | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *HMG*, *GGPPS*, *DGA2*, *GPD1* | | *POX1-6*, *TGL4* | 6.5 g/L | 5 L 发酵罐 5 L bioreactor | [103] |
| | | Po1g | 葡萄糖 Glucose | *EcAtoB*, *ScERG13*, *HMG*, *ERG8*, *ERG12*, *ERG19*, *ERG20*, *IDI*, *GGPPS*, *McCarB*, *McCarRP* | | | 12.1 mg/g DCW | 摇瓶 Shake flask | [52] |
| | | S11073 | 葡萄糖 Glucose | *McCarB*, *McCarRP* | | | 75 mg/L | 摇瓶 Shake flask | [104] |

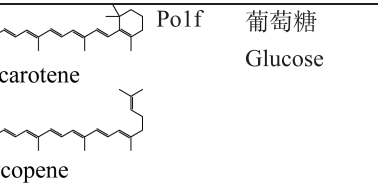
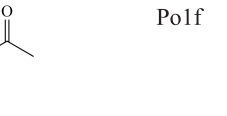
(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| | | ATCC 20460 | 葡萄糖 Glucose | *HMG*, *ERG12*, *ACL1*, *SeACS*, *IDI*, *GGPPS*, *XdcrtYB*, *XdcrtI* | *SQS* | | 164 mg/L | 深孔板 Deep well plate | [66] |
| | | Po1f | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *GGPPS*, *HMG*, *ERG13* | | *POX2-3*, *MFE* | 4.5 g/L | 5 L 发酵罐 5 L bioreactor | [105] |
| | | Po1f | 葡萄糖 Glucose | *tHMG*, *BtCarB*, *BtCarRA*, *GGPPS*, *Hxk*, *ERG13* | | *GUT2* | 2.4 g/L | 50 L 发酵罐 50 L bioreactor | [106] |
| | | Po1f | 葡萄糖 Glucose | *tHMG*, *CarB*, *CarRP*, *GGPPS*, *DID2* | | *GUT2* | 2.01 g/L | 5 L 发酵罐 5 L bioreactor | [107] |
| | | IMUFRJ 50682 | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *GGPPS* | | | 50.1 mg/L | 摇瓶 Shake flask | [108] |
| | | Po1f | 葡萄糖 Glucose | *tHMG*, *BtCarB*, *BtCarRA*, *GGPPS*, *DID2* | | *GUT2* | 2.6 g/L | 5 L 发酵罐 5 L bioreactor | [109] |
| | | Po1f | 葡萄糖 Glucose | *tHMG*, *GGPPS*, *BtCarRA*, *BtCarB* | | | 1.7 g/L | 5 L 发酵罐 5 L bioreactor | [110] |
| | | Po1f | 葡萄糖 Glucose | *McCarB*, *McCarRP* | | *NDT80* | 12.5 mg/g DCW | 摇瓶 Shake flask | [111] |
| | | Po1f | 葡萄糖 Glucose | *AfGGPS*, *IDI*, *ERG8*, *ERG10*, *ERG12*, *ERG19*, *ERG20*, *VHb*, *McCarRP*, *GGPPS*, *McCarB*, *ScERG13*, *HMG* | | *CLA4*, *MHY1* | 7.6 g/L | 1 L 发酵罐 1 L bioreactor | [112] |
| | | Po1f | 葡萄糖 Glucose | *XdcrtI*, *XdcrtE*, *XdcrtYB*, *ACC1*, *tHMGR* | | | 2.7 g/L | 5 L 发酵罐 5 L bioreactor | [113] |

(待续)

(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| β-胡萝卜素、番茄红素 β-carotene, lycopene |  Beta-carotene Lycopene | Po1f | 葡萄糖 Glucose | *ScCK*, *AtIPK*, *ERG12*, *tHMG*, *ERG20*, *IDI*, *XdcrtE*, *CarB*, *McCarRP*$^{Y27R}$, *McCarRP*$^{E78K}$ | | | β-胡萝卜素 β-carotene 39.5 g/L 番茄红素 lycopene 17.6 g/L | 3 L 发酵罐 3 L bioreactor | [114] |
| 番茄红素 Lycopene | Lycopene | H222 | 葡萄糖 Glucose | *PaCrtB*, *PaCrtI*, *GGPPS*, *HMG* | | *POX1-6*, *GUT2* | 16 mg/g DCW | 摇瓶 Shake flask | [115] |
| | | Po1f | 葡萄糖，果糖 Glucose, fructose | *PaCrtE*, *PaCrtB*, *PaCrtI*, | | | 242 mg/L | 1.5 L 发酵罐 1.5 L bioreactor | [116] |
| | | Po1f | 葡萄糖 Glucose | *HMG*, *PaCrtE*, *PaCrtB*, *PaCrtI*, *ERG8*, *ERG19* | | | 213 mg/L | 1 L 发酵罐 1 L bioreactor | [117] |
| | | Po1f | 葡萄糖 Glucose | *PaCrtE*, *PaCrtB*, *PaCrtI*, *AMPD* | | | 745 mg/L | 5 L 发酵罐 5 L bioreactor | [118] |
| | | Po1f | 葡萄糖，棕榈酸 Glucose, palmitic acid | *PvIDI*, *LpCrtE*, *LpCrtB*, *LpCrtI*, *AtIPK*, *ScCHK*, *ERG20* | | | 4.2 g/L | 3 L 发酵罐 3 L bioreactor | [119] |
| 类胡萝卜素 Carotenoids | | | | | | | | | |
| β-紫罗兰酮 β-ionone |  Beta-ionone | Po1f | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *OfCCD1*, *SsNphT7*, *HpIDI*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *tHMG*, *GPS*, ERG20-GGPPS | | | 380 mg/L | 2 L 发酵罐 2 L bioreactor | [120] |

(待续)

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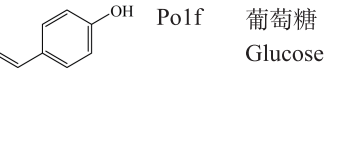
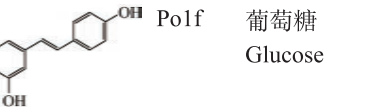
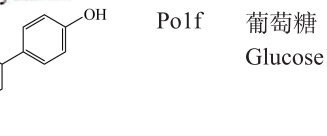
(续表 2)

| 化合物 Compound | 结构式 Structural formula | 菌株 Strain | 碳源 Carbon source | 萜类合成策略 Strategies for terpenoid synthesis | | | 浓度 Concentration | 发酵条件 Fermentation condition | 参考文献 Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 上调或表达基因 Up-regulated or expressed gene | 下调基因 Down-regulated gene | 敲除基因 Knockout gene | | | |
| | | Po1f | 葡萄糖 Glucose | *McCarB*, *McCarRP*, *PhCCD1*, *GGPPS*, *tHMG*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *IDI*, *BbPK*, *BsPTA* | | | 0.98 g/L | 3 L 发酵罐 3 L bioreactor | [121] |
| | | Po1f | 有机废水 Organic waste hydrolysates | *McCarB*, *McCarRP*, *GGPPS*, *tHMG*, *ERG8*, *ERG10*, *ERG12*, *ERG13*, *ERG19*, *ERG20*, *IDI*, *BbPK*, *BsPTA*, *PhCCD1* 突变体 | | | 4 g/L | 3 L 发酵罐 3 L bioreactor | [122] |
| 其他 Others | | | | | | | | | |
| 白藜芦醇 Resveratrol |  Resveratrol | Po1f | 葡萄糖 Glucose | *FjTAL*, *Pc4CL1*, *ARO4*$^{K221L}$, *ARO7*$^{G139S}$, *YlARO1*, *YlARO3*$^{K225L}$, *Pc4CL1-VvSTS*, *AtPAL-AtC4H*, *YlCYB5*, *AtATR2*, *CaFPK*, *BsPTA* | | *DGA1* | 22.5 g/L | 5 L 发酵罐 5 L bioreactor | [123] |
| 虎杖苷 Polydatin |  Polydatin | Po1f | 葡萄糖 Glucose | *RgTAL*, *At4CL*, *VvST1*, *AroG**, *TyrA**, *BbPK* *BsPTA*, *R3GAT* | | *MHY1*, *DGA1-2*, *BGL2*, *EXG1* | 6.88 g/L | 摇瓶 Shake flask | [124] |
| 柚皮素 Naringenin |  Naringenin | Po1f | 葡萄糖 Glucose | *G2PS1*, *HsPKS1*, *AhSTS*, *OsCUS*, *RpBAS*, *SeSam8*, *Nt4CL*, *ARO4* 突变体 | | | (898±19) mg/L | 3 L 发酵罐 3 L bioreactor | [125] |
| | | Po1f | 葡萄糖，木糖 Glucose, xylose | *AtCHI*, *AtCHS*, *At4CL*, *RgTAL*, *XT*, *XR*, *XDH*, *XKS* | | *DGA1* | (715.3±12.8) mg/L | 摇瓶 Shake flask | [126] |

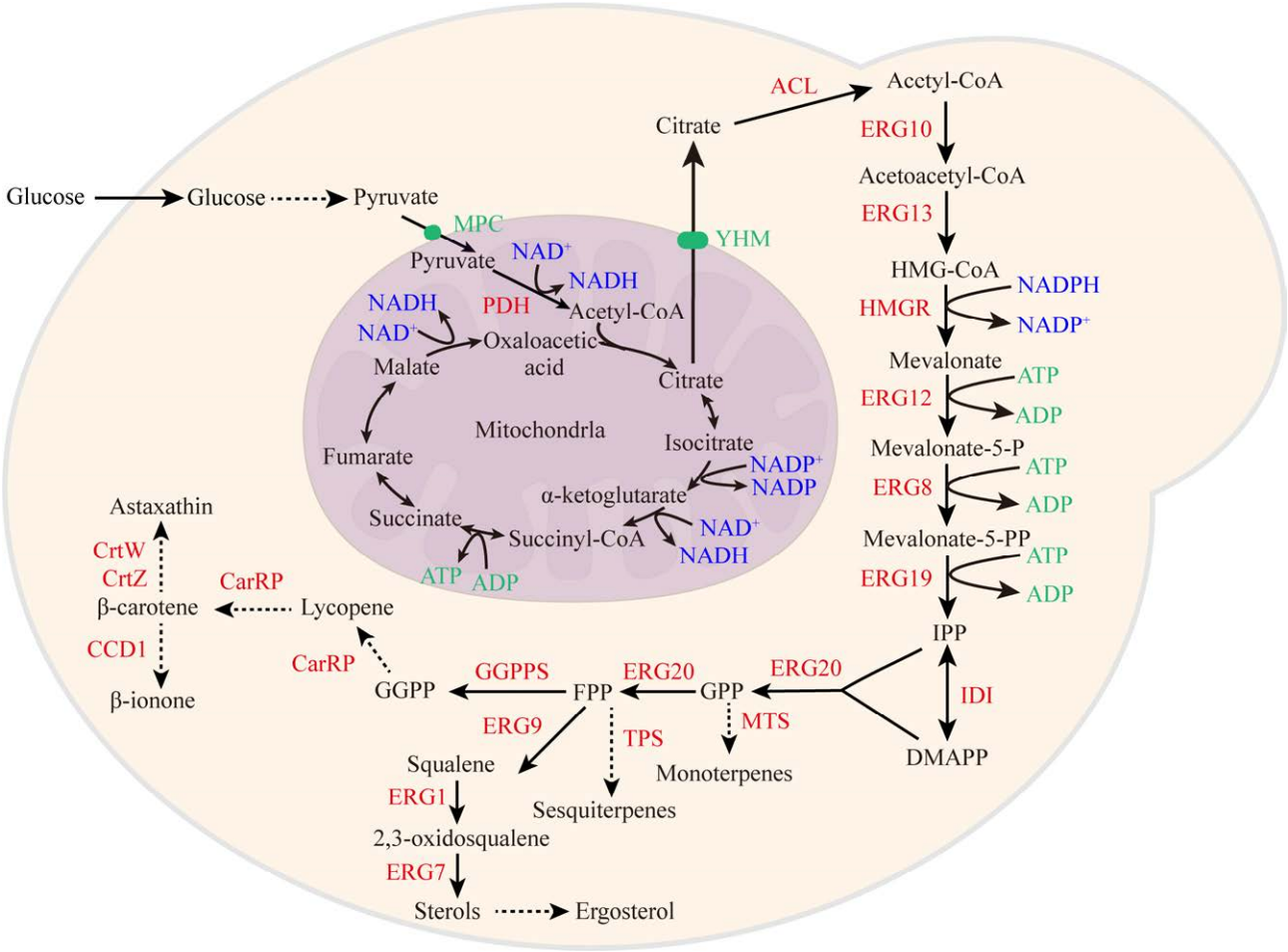



**图 2　在解脂耶氏酵母中构建的萜类化合物的生物合成途径**　MPC：线粒体丙酮酸转运蛋白；PDH：丙酮酸脱氢酶复合体；YHM：线粒体柠檬酸转运蛋白；ACL：ATP-柠檬酸裂解酶；ERG10：乙酰辅酶 A 乙酰基转移酶；HMGR：羟甲基戊二酰辅酶 A 还原酶；ERG20：法尼基焦磷酸合成酶；MTS：单萜合成酶；TPS：倍半萜合成酶；ERG9：法尼基焦磷酸法尼基转移酶；ERG1：角鲨烯环氧化酶；ERG7：羊毛甾醇合成酶；GGPPS：香叶基香叶基二磷酸合酶；CarRP：八氢番茄红素合成酶/番茄红素环化酶；CCD1：类胡萝卜素裂解双加氧酶 1；CrtW：β-胡萝卜素酮醇酶；CrtZ：β-胡萝卜素羟化酶

Figure 2　Terpenoid biosynthesis pathway constructed in *Yarrowia lipolytica*. MPC: Mitochondrial pyruvate carrier; PDH: Pyruvate dehydrogenase; YHM: Mitochondrial citrate carrier; ACL: ATP-citrate lyase; ERG10: Acetyl-CoA C-acetyltransferase; HMGR: Hydroxymethylglutaryl-CoA reductase; ERG20: Farnesyl diphosphate synthase; MTS: Monoterpene synthase; TPS: Sesquiterpene synthases; ERG9: Farnesyl-diphosphate farnesyltransferase; ERG1: Squalene epoxidase; ERG7: Lanosterol synthase; GGPPS: Geranylgeranyl-diphosphate synthase; CarRP: Phytoene synthase/lycopene cyclase; CCD1: Carotenoid cleaving dioxygenase 1; CrtW: β-carotene ketolase; CrtZ: β-carotene hydroxylase.

MVA 途径的限速酶，通过增强 HMGR 的表达有利于增加 MVA 途径通量，提高萜类化合物的合成[42,85]。研究显示，HMGR 定位于内质网膜上，包含了 N 端膜锚定序列和 C 端催化结构域[16,128]。相关研究表明，通过表达 N-末端截短的 HMGR (tHMGR)能够有效地增强萜类化

合物的合成[39,94]。tHMGR 游离于细胞质中可能有利于延缓其降解[113]。

Peng 等[84]研究发现，广藿香醇产量随着 *tHMGR* 拷贝数的增加而提高。Lu 等[121]研究报道中，在解脂耶氏酵母中同时过表达基因 *tHMGR* 和 *GGPPS* 的菌株，较对照菌株 β-紫罗兰酮增加了 15 倍。然而，对于过表达 *tHMGR* 是否能够有效地提高产物产量，不同的研究似乎有着不同的结论。Kildegaard 等[98]比较了 HMGR 和 tHMGR 的性能，发现只有过表达基因 *HMGR* 才有利于 β-胡萝卜素生成。在橙花叔醇、α-红没药烯和吉玛烯 A 等相关研究中也表明过表达基因 *HMGR* 比 *tHMGR* 更有利于化合物的生成[42,83,85]。

**2.1.2 ERG20 改造策略**

法尼基焦磷酸合成酶(farnesyl diphosphate synthase, ERG20)是 FPP 产生的关键酶，其过表达有利于萜类化合物合成[42,84-85]。此外，其与产物合成酶之间的距离是发挥催化效果的重要因素之一。将 ERG20 和萜类合成酶融合表达有利于萜类合成，如 Liu 等[85]研究发现，dlGAS-ERG20 融合表达较单独过表达基因 *ERG20* 和 *dlGAS* 的菌株的产量高 10.6%，生成了 95.9 mg/L 吉玛烯 A。然而，酶融合表达的位置差异也会导致不同的结果[42,84]，这可能是由于合成酶活性位点差异导致在空间上催化效果不同。Liu 等[42]研究显示，FaNES1-ERG20 的融合表达能够将反式橙花叔醇的产量提高到 221 mg/L，ERG20 的 C 末端或 FaNES1 的 N 末端对发挥其生物功能至关重要。研究者们在比较了不同的连接方式并预测蛋白质的三维结构后发现，ERG20-PTS 融合表达时，两者活性位点之间的距离最小，活性位点距离的缩短有助于广藿香醇的合成[84,129]。

天然的 ERG20 倾向于生成 FPP，不利于合成单萜类化合物。相关研究表明，构建突变体是一种有效的策略。Cao 等[68]在合成芳樟醇的研究中引入 $ERG20^{F88W\text{-}N119W}$ 突变体，当同时过表达 *HMG1*、异戊烯基二磷酸异构酶(isopentenyl-diphosphate, IDI)基因和 $ERG20^{F88W\text{-}N119W}$ 时，能显著提高芳樟醇产量。Muñoz-Fernández 等[130]在一种丝状真菌棉阿舒囊霉(*Ashbya gossypii*)中构建 *ERG20* 的突变体，发现 $ERG20^{F95W}$ 较野生型 *ERG20* 显著增加柠檬酸产量。

**2.1.3 过表达 MVA 途径其他基因**

除了 *HMGR*，MVA 途径中其他基因的过表达也是增加 MVA 通量的因素。Zhao 等[79]发现，*ERG10*、*ERG13*、*ERG12*、*ERG8* 和 *ERG19* 的单独过表达均能够提高红没药烯的产量。还有研究显示，在过表达 *ERG13* 的情况下，对 β-胡萝卜素的生成有效[105]。Qiang 等[106]发现，相较于对照菌株，过表达 *ERG13* 的菌株的 β-胡萝卜素产量提高了 181%，并且在增加 *ERG13* 的拷贝后产量比亲本菌株高 259%。

IDI 催化 IPP 和 DMAPP 之间的转化，IPP 与 DMAPP 在香叶基焦磷酸(geranyl pyrophosphate, GPP)和法尼基焦磷酸(farnesyl pyrophosphate, FPP)的产生中起着至关重要的作用[16,68]。在过表达 *HMGR* 的基础上过表达 *IDI*，能够有效增强萜类化合物的合成[68,70]。Yang 等[70]报道，*IDI* 过表达使 α-法尼烯的产量增加到 57.08 mg/L，较对照菌株高 2.67 倍。

此外，引入合成 IPP 和 DMAPP 的异源途径也是提高产量方式之一。Luo 等[119]引入异戊二烯醇利用途径(AtIPK-ScCHK)，以增加 IPP 和 DMAPP 的供应。相较于单独的 MVA 途径，引入 AtIPK-ScCHK 途径将 IPP 和 DMAPP 的总水平提高了 15.7 倍，这进一步将番茄红素产量提高到 120 mg/g[119]。因此，参与 MVA 途径的基因被认为是增强萜类化合物生产的工程靶标。

#### 2.1.4 下调竞争途径

FPP 流向角鲨烯是以 FPP 为中间体的萜类化合物合成中的竞争途径，如在倍半萜和胡萝卜素等化合物合成中下调角鲨烯合酶(farnesyl-diphosphate farnesyltransferase, ERG9)，减少 FPP 至角鲨烯的流量一直受研究者们的青睐。因为角鲨烯是合成麦角固醇的前体，也是细胞生长的必要化合物，所以只能相对减少角鲨烯合成。

目前，常采用更换弱启动子或截短 *ERG9* 内源性启动子使蛋白质表达水平下降[98]。Xu 等[87]在合成紫杉二烯的研究中，采用相对较弱的启动子 $P_{ERG11}$ 取代内源性 $P_{ERG9}$，将紫杉二烯产量从 0.27 mg/L 提高到 0.31 mg/L。截短 *ERG9* 启动子策略的运用能相对提高虾青素和 α-红没药醇的产量[66,80]，此外，Liu 等[85]将 $P_{ERG9}$ 截短至 50 个碱基对的工程菌株产生了 787.5 mg/L 吉玛烯 A，较对照菌株提高了 49%。另外，基于麦角固醇的反馈抑制系统，Li 等[86]采用 $P_{ERG1}$ 替换内源性 $P_{ERG9}$ 来调节 *ERG9* 的表达；工程菌株生成的 β-榄香烯较对照菌株增加了 6%，达到了 420 mg/L。

Guo 等[30]应用截短 *ERG9* 天然启动子，发现能够减少角鲨烯量，但是 α-石竹烯浓度提高不明显；其采用 $Cu^{2+}$抑制型启动子 $P_{CTR1}$ 替换 $P_{ERG9}$，并在发酵培养液中添加 $CuSO_4$ 以减少角鲨烯浓度；相较于对照菌株，工程菌株将 α-石竹烯浓度提高了 54.2%。Peng 等[84]在后续研究中也成功运用 $Cu^{2+}$抑制型启动子提高了广藿香醇浓度。ERG9 的 C 端定位于细胞质，Marsafari 等[78]在合成紫穗槐二烯的研究中，将 ERG9 的 C 端与紫穗槐二烯合成酶结合，更有利于发挥合成酶活性从而提高紫穗槐二烯的产量。此外，增加前体供应以扩大合成萜类化合物路径的代谢流量也是常用的策略[17]。

### 2.2 增强乙酰辅酶 A 供应

乙酰辅酶 A 是生物体内重要的中间代谢物，是生物体利用 MVA 途径合成萜类化合物的前体物质。相关研究证明，增强胞质中乙酰辅酶 A 的供应有助于萜类化合物合成[59]。

#### 2.2.1 增强内源途径

一磷酸腺苷脱氨酶(adenosine monophosphate deaminase, AMPD)在碳源过量或缺乏氮的培养条件下被激活，并引起细胞内一磷酸腺苷的下降，抑制 TCA 中的异柠檬酸脱氢酶活性从而使得柠檬酸积累[131]。线粒体柠檬酸转运载体(mitochondrial citrate carrier, YHM2)可以将柠檬酸从线粒体转运至胞质，胞质中的柠檬酸可以进一步在 ATP-柠檬酸裂解酶(ATP citrate lyase, ACL)的转化下形成乙酰辅酶 A[131-133]。

最近，Bi 等[59]过表达解脂耶氏酵母内源基因 *ACL*，将 β-法尼烯产量提升至 613 mg/L；后续分别表达基因 *AMPD*、*YHM2* 和 *AMPD-YHM2*，发现均有利于提高 β-法尼烯产量，并且两者联合表达效果最好；后续进一步表达异源基因 *MmACL* 来增加胞质乙酰辅酶 A 的供应，使 β-法尼烯产量进一步增加到 697 mg/L。

此外，减少乙酰辅酶 A 流向脂质合成的通量，并增强 β-氧化提高脂质代谢也是常用策略之一。为减少脂质合成，Liu 等[85]通过构建乙酰辅酶 A 羧化酶 1 (acetyl-CoA carboxylase 1, ACC1)突变体降低其活性；并过表达脂肪酰辅酶 A 合成酶 1 (fatty acid-CoA synthetase 1, FAA1)和甘油三酰脂肪酶 4 (triacylglycerol lipase 4, TGL4)，通过增加脂质代谢提高乙酰辅酶 A 量，从而提高吉玛烯 A 的产量。有研究者经比较过表达 β-氧化途径基因 *FAA1*、*POX2*、*POX3*、*MFE1*、*PAT1* 等发现，通过过表达 3-酮脂酰辅酶 A 硫解酶(3-ketoacyl-CoA thiolase, POT1)增加 β-氧化途径以提供更多乙酰辅酶 A，工程菌株合成 β-法尼烯产量较对照菌株增加了 69%[72]。

#### 2.2.2 引入异源乙酰辅酶 A 合成途径

除了强化解脂耶氏酵母内源乙酰辅酶 A 合成路径，引入异源胞质乙酰辅酶 A 合成路径也被证明是一种有效的提高合成效率的策略[121]。两歧双歧杆菌(*Bifidobacterium bifidum*)的磷酸转酮酶(BbPK)和枯草芽孢杆菌(*Bacillus subtilis*)的磷酸转乙酰酶(BsPTA)组成的 PK-PTA 途径，可以成为增加胞质乙酰辅酶 A 供应的策略之一。Lu 等[121]将 PK-PTA 途径整合至解脂耶氏酵母基因组不同的位点，均有提高 β-紫罗兰酮产量的效果。Bi 等[59]在整合异源 PK 和 BsPTA 后，较对照菌株产量提高了 20%，β-法尼烯产量达到 810 mg/L。此外，在合成聚酮类化合物的报道中也证明 PK-PTA 途径对提高产物有效[123-124]。也有研究表明，PK-PTA 途径对有些萜类的合成无明显促进效果[30,85]。

### 2.3 平衡辅助因子代谢

细胞辅因子是保护细胞免受氧化应激的重要还原因子，也是参与酶促反应并维持代谢通量平衡的辅助因子。因此，通过调节细胞内的辅助因子水平，能够维持 MVA 途径通量并有效地促进目标产物的合成[60]。

在酵母中过表达玫瑰杆菌(*Silicibacter pomeroyi*)的 NADH 依赖性 HMGR (SpHMGR)，使碳流量进入 MVA 途径同时减轻对辅因子 NADPH 的依赖[134]。Guo 等[60]通过引入异源的 NADH 依赖性 HMGR，发现其有利于 α-石竹烯的合成。Bi 等[73]在解脂耶氏酵母中同时过表达基因 *tHMGR* 和 *SpHMGR*，有助于同时利用细胞内的 NADH 和 NADPH 进行 β-法尼烯的合成，整合的工程菌株中 β-法尼烯产量增加了 18.7%。

苹果酸酶(malate synthase, MAE)对维持解脂耶氏酵母中辅因子的平衡有显著影响[135]。相关研究表明，MAE 能够分别利用 $NAD^+$和 $NADP^+$进行催化，但内源的 MAE 对 $NAD^+$具有更高的亲和力[135]。卷枝毛霉(*Mucor circinelloides*)的苹果酸酶(McMAE)是一种 $NADP^+$依赖性苹果酸酶。Bi 等[59]过表达基因 *McMAE* 的工程菌株生成了 552 mg/L β-法尼烯，较对照菌株产量增加了 27.5%。除上述常用的优化合成策略之外，亚细胞结构的诸多优势逐渐引起研究者的关注。

### 2.4 发挥亚细胞结构优势

由于酵母细胞内含有多种细胞器，如内质网、高尔基体和线粒体等。这些细胞器中一方面含有丰富的乙酰辅酶 A 等物质；另一方面能够有效地将内部的反应与胞质分隔开，从而减少竞争途径对底物的竞争。研究者通过靶向序列将合成路径定位到特定的细胞器中，如过氧化物酶体[60]、内质网[78]和线粒体[81]等，实现了目标产物的高效合成。

#### 2.4.1 过氧化物酶体

过氧化物酶体由单层膜构成，可允许低分子量化合物通过，是生物体中脂肪酸发生 β-氧化的场所。其也起到隔离细胞中有毒分子和减缓底物抑制的作用。解脂耶氏酵母中丰富的脂滴经过过氧化物酶体的 β-氧化可以产生大量的乙酰辅酶 A。

Guo 等[60]将 α-石竹烯生成途径与过氧化物酶体靶向标签(peroxisome targeting signal, PTS)融合，构建了在过氧化物酶体中生成 α-石竹烯的菌株；并进一步过表达过氧化物酶体腺嘌呤核苷酸转运蛋白 1 (adenine nucleotide transporter 1, ANT1)，以提高 ATP 并增加乙酰辅酶 A 的量，将 α-石竹烯产量从(5.6±0.3) mg/L 提高至(565.0±21.1) mg/L。Liu 等[42]将反式橙花叔醇合成途径的基因引入过氧化物酶体中，其获得的工程菌株生成了 930 mg/L 反式橙花叔醇，较对照菌株提高了 31%。这些研究均表明，在解脂耶氏酵母中过氧化物酶体定位策略是适用于萜类化合物生成的策略之一。

#### 2.4.2 脂质体

由于解脂耶氏酵母具有优秀的脂质合成和积累能力,胞内脂质含量可以通过代谢调节进一步增加[95,136]。研究表明，提高脂质含量可以促进亲脂性萜类化合物在脂质体内的储存[103,117]。Lu 等[83]通过过表达基因 *DGA1*，有效提高了解脂耶氏酵母胞内脂质的积累量；同时实现了 844.6 mg/L α-红没药烯的产量，相较于对照菌株提升了 3.7 倍。然而，脂质的积累需要消耗乙酰辅酶 A，过度的积累也不利于萜类化合物的生物合成。相关研究表明，敲除解脂耶氏酵母脂质合成的基因 *DGA1* 和 *DGA2* 可以降低总脂质含量，进一步提高 β-法尼烯产量[39,72]。

#### 2.4.3 线粒体

线粒体是细胞的亚细胞结构之一，也是能量产生的主要场所。线粒体中具有高通量的 TCA 和乙酰辅酶 A，可以为萜类化合物合成提供前体。有研究者比较了线粒体和胞质中生产红没药烯的差异，发现采用线粒体合成比胞质合成产量提高了 306 倍[81]。线粒体能够提供丰富前体和减少副产物的生成，但是也会造成线粒体代谢负担而影响功能。未来，可以尝试多细胞器串联运用策略，优化萜类化合物的合成。

## 3 利用木糖和甲醇进行代谢

构建微生物细胞工厂，利用可再生原料生产高价值化合物是绿色环保生物制造的目标。目前，在解脂耶氏酵母中已有利用多种底物合成产物的报道，包括利用甘油[137-138]合成脂肪酸、异丙醇，利用废弃食用油生产红没药烯[79,81]，利用醋酸[139]、木质纤维素水解液生成脂质[140-141]。近年来，研究者逐渐关注到更加廉价并且易获取的木质纤维素，其主要成分包括木质素、纤维素和半纤维素[142]。木质纤维素通常来源于农业秸秆，具有廉价、丰富、可再生的特点，降解后能产生多种碳源，利用其生成高附加值化合物对可持续发展具有重要意义[143]。

### 3.1 利用木糖

半纤维素是木质纤维素的主要成分之一，其水解可以产生多种碳源，包括木糖、半乳糖、阿拉伯糖等，其中木糖含量最为丰富[144-145]。木糖的转化利用主要有两种方式，即木糖氧化还原途径和木糖异构酶途径(图 3)。木糖代谢过程产生的 6-磷酸果糖、3-磷酸甘油醛可进入糖酵解途径以及生成乙酰辅酶 A。野生型解脂耶氏酵母中木糖氧化还原途径的基因 *XR*、*XDH*、*XK* 处于“沉默”状态，在以木糖为唯一碳源的培养基中不能生长。然而，在木糖醇上可以少量生长[146-148]，只过表达 *XDH* 的工程菌株生长缓慢[149]，这表明解脂耶氏酵母中有部分木糖代谢途径的活性。Li 等[150]通过过表达树干毕赤酵母 (*Scheffersomyces stipites*) 的基因 *SsXR* 和 S*sXDH* 构建的工程菌株无法利用木糖。Prabhu 等[151]同时过表达内源基因 *XR*、*XDH*、*XK*，工程菌株能利用木糖生长；在过表达 *SsXR*、*SsXDH* 和内源基因 *XK* 后，工程菌株利用木糖的最高 $OD_{600}$ 与利用葡萄糖的生长水平相近。因此，过表达异源和内源木糖氧化还原途径的酶可促使菌株利用木糖进行代谢生长。

Wu 等[93]引入木糖氧化还原途径和原人参二醇合成途径，构建解脂耶氏酵母的工程菌株，其能利用木糖生产 18.18 mg/L 原人参二醇。Sun 等[141]将代谢工程和适应性实验室进化相结合，构建了一株能够有效地从葡萄糖和木糖中产生 6.25 g/L 脂质的工程菌株。此外，木糖和葡萄糖的共利用被证明有利于合成紫穗槐二烯[78]和柚皮素[126]。这些工作均揭示了工程解脂耶氏酵母菌株利用木质纤维素的潜力。

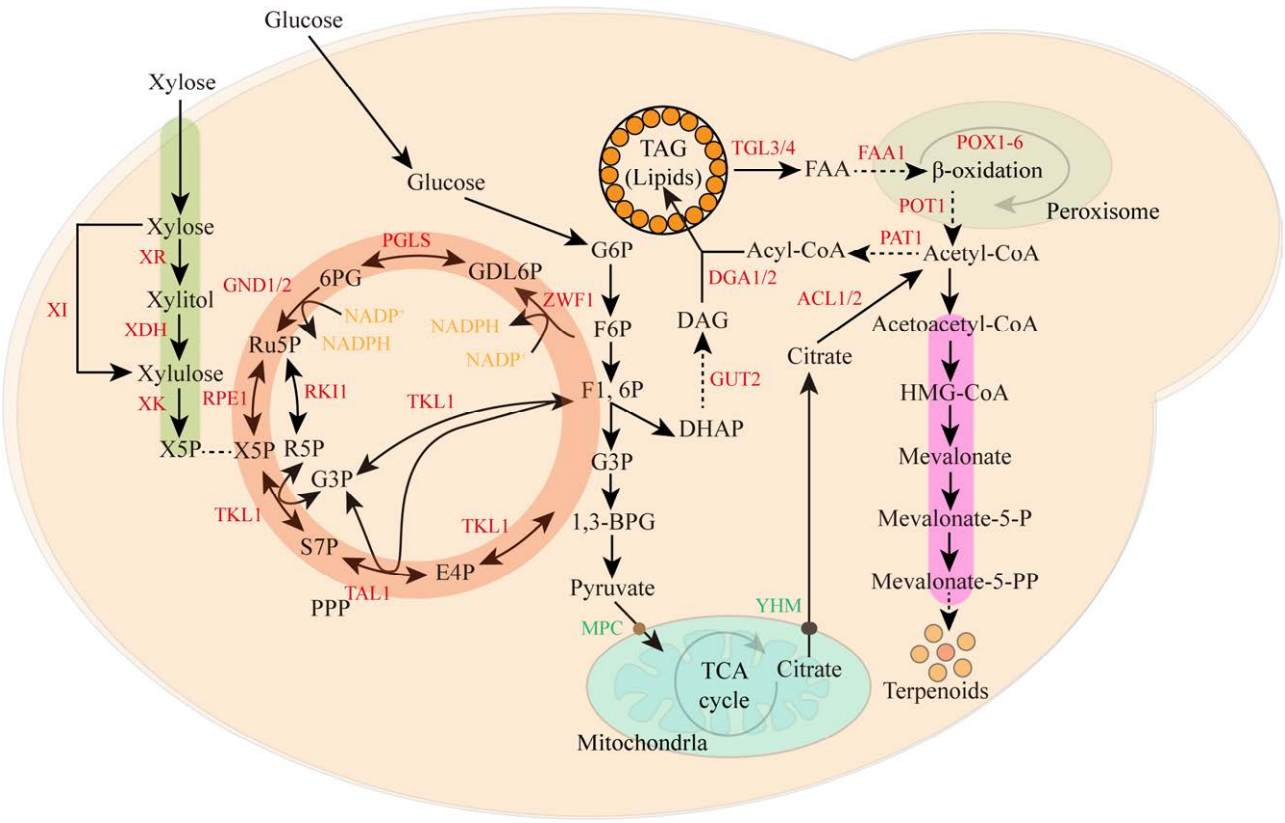



**图 3 在解脂耶氏酵母中利用木糖和葡萄糖为底物并结合脂质代谢合成萜类的路径** XR：木糖还原酶；XDH：木糖醇脱氢酶；XK：木酮糖激酶；XI：木糖异构酶；GUT2：甘油-3-磷酸脱氢酶 2；DGA1/2：甘油二酰酰基转移酶 1/2；TGL3/4：甘油三酰脂肪酶 3/4；POX1-6：酰基辅酶 A 氧化酶 1-6；POT1：3-酮脂酰辅酶 A 硫解酶 1；PAT1：乙酰辅酶 A 乙酰转移酶 1；FAA1：脂肪酸辅酶 A 合成酶 1；ZWF1：葡萄糖-6-磷酸脱氢酶；PGLS：6-磷酸葡萄糖酸内酯酶；GND1/2：6-磷酸葡萄糖酸脱氢酶 1/2；RPE1：磷酸核酮 3-差向异构酶 1；RKI1：核糖-5-磷酸异构酶 1；TKL1：转酮醇酶 1；TAL1：转醛酶 1

Figure 3 Pathways for terpene synthesis using xylose and glucose as substrates and in combination with lipid metabolism in *Yarrowia lipolytica*. XR: Xylose reductase; XDH: Xylitol dehydrogenase; XK: Xylulose kinase; XI: Xylose isomerase; GUT2: Glycerol-3-phosphate dehydrogenase 2; DGA1/2: Diacylglycerol acyltransferase 1/2; TGL3/4: Triacylglycerol lipase 3/4; POX1-6: Peroxisome acyl-CoA oxidase 1-6; POT1: 3-ketoacyl-CoA thiolase; PAT1: Acetyl-CoA C-acetyltransferase 1; FAA1: Fatty acid-CoA synthetase 1; ZWF1: Glucose-6-phosphate dehydrogenase; PGLS: 6-phosphogluconolactonase; GND1/2: 6-phosphogluconate dehydrogenase 1/2; RPE1: D-ribulose-5-phosphate 3-epimerase 1; RKI1: Ribose-5-phosphate ketol-isomerase 1; TKL1: Transketolase 1; TAL1: Transaldolase 1.

### 3.2 利用甲醇

甲醇是一种成本低且可再生的碳源，具有还原性强、能量高等特点[152-153]，微生物通过单磷酸核酮糖(RuMP)途径、单磷酸木酮糖(XuMP)途径或丝氨酸途径进行甲醇同化[154]。甲基营养酵母，如毕赤酵母(*Pichia pastoris*)、多形汉逊酵母(*Ogataea polymorpha*)等能够天然利用甲醇。目前有相关研究表明，在多形汉逊酵母和毕赤酵母中可以利用甲醇为底物进行代谢并合成化合物[155-157]。并且，在酿酒酵母中引入毕赤酵母甲醇代谢基

因可以实现其甲醇代谢[158]。

解脂耶氏酵母不能够直接利用甲醇，Wang 等[154]通过比较来源于毕赤酵母或大肠杆菌的甲醇利用途径，初步实现了其对甲醇的利用，但在细胞质中表达甲醇同化途径的菌株消耗甲醇的量较少。进一步构建过氧化物酶体甲醇同化途径，并过表达糖酵解途径和磷酸戊糖途径的相关基因增加了甲醇利用[154]。在此基础上，通过结合木糖代谢途径，菌株细胞生长提高了 28%；但解脂耶氏酵母的甲醇利用能力并未增强[154]。另一研究中，Zhang 等[159]将毕赤酵母的甲醇同化模块引入解脂耶氏酵母的过氧化物酶体中，在额外添加酵母提取物的甲醇培养基中实现了工程菌株的生长代谢。此外，因甲醇代谢需要 5-磷酸木酮糖(Xu5p)参与，其进一步构建木糖利用模块和过氧化物酶体的 Xu5p 代谢途径，为中间产物甲醛的代谢提供更多的代谢受体，进一步提高了菌株对甲醇的利用效率[159] (图 4)。

通过微生物利用甲醇合成高附值产物是一种经济而且环保的方式，在解脂耶氏酵母中甲醇代谢途径的引入以及生成萜类化合物尚须进一步研究探讨。

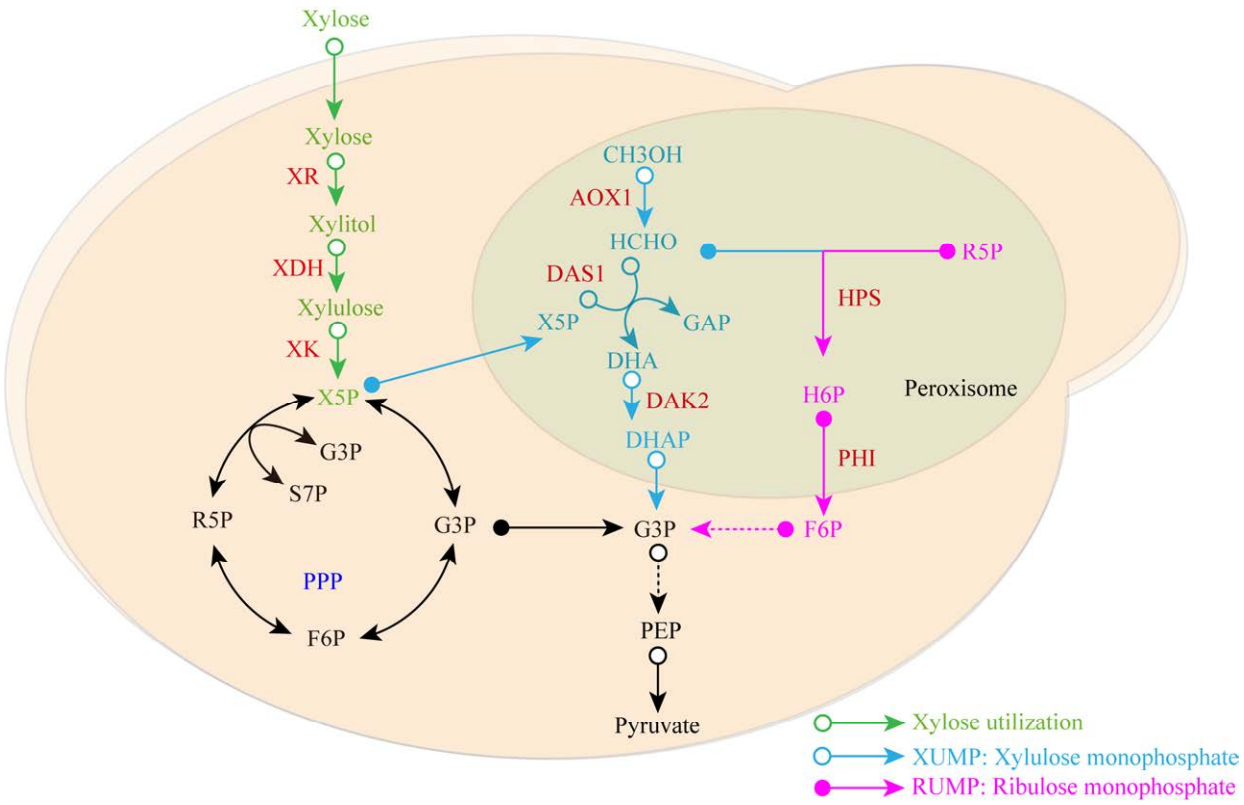



**图 4 解脂耶氏酵母中构建的木糖和甲醇代谢途径** XR：木糖还原酶；XDH：木糖醇脱氢酶；XK：木酮糖激酶；AOX：醇氧化酶；DAS1：二羟基丙酮合成酶；DAK2：二羟基丙酮激酶；HPS：己酮糖-6-磷酸合成酶；PHI：6-磷酸己酮糖异构酶

Figure 4 Xylose and methanol metabolic pathways constructed in the *Yarrowia lipolytica*. XR: Xylose reductase; XDH: Xylitol dehydrogenase; XK: Xylulose kinase; AOX: Alcohol oxidase; DAS1: Dihydroxyacetone synthase; DAK2: Dihydroxyacetone kinase; HPS: 3-hexulose-6-phosphate synthase; PHI: 6-phospho-3-hexuloisomerase.

## 4 总结与展望

已经有许多研究表明在解脂耶氏酵母中合成萜类化合物的可行性。基因编辑技术的快速发展和基因编辑工具的完善，促进了解脂耶氏酵母在合成生物学中的应用。但表达载体的整合效率仍有待进一步提高，CRISPR/Cas9 等基因编辑技术的应用及优化，在一定程度上缓解了同源重组效率低的局限，但较酿酒酵母等模式生物仍有较大差距，因而仍须开发更为高效的基因编辑工具。

此外，优化菌株的蛋白质折叠效率也是未来构建萜类细胞工厂的潜在策略。构建萜类化合物生物合成的酵母细胞工厂往往需要表达大量内源或异源蛋白质，蛋白质的大量表达会增加内质网的负荷进而影响蛋白质量。在酵母细胞中未折叠蛋白反应(unfolded protein response, UPR)受到 HAC1 等多种转录因子的调节[160]。通过调控 UPR 相关转录因子的表达，促进蛋白质的高效折叠及构象修饰，将有助于解脂耶氏酵母微生物细胞工厂的构建。

优化碳源利用也是合成生物学较为关注的一方面，对于实践可持续发展策略具有重要意义。另一方面，通过构建动态调控系统，实现菌株生产的精准调控，也是值得关注的研究方向。基于转录因子的动态调控系统，已经被用于调控柚皮素等化合物的生产。研究者通过结合类黄酮转录激活因子 FdeR 及其操纵子 FdeO 调控亮氨酸生物合成，在解脂耶氏酵母中实现了柚皮素高产菌株的快速筛选[161]。截至目前，在解脂耶氏酵母中运用动态调控策略合成萜类化合物的相关报道较少。

对于萜类化合物生产，结合提高乙酰辅酶 A 等前体供应、下调竞争途径通量、构建细胞器合成策略及转运蛋白发掘等策略能够有效地提高萜类产量，但不同产物之间的合成策略仍存在较大差异。未来合理运用基因组规模代谢网络模型将更好地帮助我们深入理解菌株代谢特点，并有助于挖掘萜类化合物合成的普适性策略。

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