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·微生物细胞合成·

蔡孟浩 华东理工大学生物工程学院教授、博士生导师，生物反应器工程国家重点实验室 PI，入选国家级青年人才计划、上海市青年科技启明星计划，主要从事微生物合成生物技术研究，通过新型微生物底盘创制与表达调控，实现重组蛋白药物、小分子天然产物、新型替代蛋白等功能分子的绿色、高效生产。以通信作者在 *Science Advances*、*Nucleic Acids Research*、*Metabolic Engineering* 等权威期刊发表论文 50 余篇，获得授权发明专利 17 项，研究成果实现多项合作应用与转让。

非常规酵母天然产物合成

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钱芷兰，宋丽丽，刘启，龚秀龙，康艺嘉，何子雨，龙浩雨，蔡孟浩. 非常规酵母天然产物合成[J]. 生物工程学报，2023，39(6): 2284-2312.
QIAN Zhilan, SONG Lili, LIU Qi, GONG Xiulong, KANG Yijia, HE Ziyu, LONG Haoyu, CAI Menghao. Biosynthesis of natural products by non-conventional yeasts[J]. Chinese Journal of Biotechnology, 2023, 39(6): 2284-2312.

摘 要：以解脂耶氏酵母(*Yarrowia lipolytica*)、巴斯德毕赤酵母(*Pichia pastoris*)、马克斯克鲁维酵母(*Kluyveromyces marxianus*)、圆红冬孢酵母(*Rhodosporidium toruloides*)、多形汉逊酵母(*Hansenula polymorpha*)为代表的非常规酵母凭借较广的底物利用谱、较强的环境耐受性等优势，已成功实现多种天然产物的高效生产。随着合成生物学及基因编辑技术的发展，针对非常规酵母代谢工程改造的工具和策略也逐渐丰富。本文介绍了几类常见的非常规酵母的生理特性、工具开发及应用现状，并总结归纳了天然产物合成优化中常用的代谢工程策略；最后讨论了现阶段非常规酵母作为天然产物合成细胞工厂的优势和不足，并对后续研究和发展趋势进行了展望。

关键词：天然产物；非常规酵母；细胞工厂；代谢工程

资助项目：国家重点研发计划(2022YFC2805102，2020YFA0907804)；国家自然科学基金(31870073，32201206)
This work was supported by the National Key Research and Development Program of China (2022YFC2805102, 2020YFA0907804) and the National Natural Science Foundation of China (31870073, 32201206).
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Received: 2022-11-16; Accepted: 2023-02-07; Published online: 2023-02-10

Biosynthesis of natural products by non-conventional yeasts

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Abstract: Non-conventional yeasts such as *Yarrowia lipolytica*, *Pichia pastoris*, *Kluyveromyces marxianus*, *Rhodosporidium toruloides* and *Hansenula polymorpha* have proven to be efficient cell factories in producing a variety of natural products due to their wide substrate utilization spectrum, strong tolerance to environmental stresses and other merits. With the development of synthetic biology and gene editing technology, metabolic engineering tools and strategies for non-conventional yeasts are expanding. This review introduces the physiological characteristics, tool development and current application of several representative non-conventional yeasts, and summarizes the metabolic engineering strategies commonly used in the improvement of natural products biosynthesis. We also discuss the strengths and weaknesses of non-conventional yeasts as natural products cell factories at current stage, and prospects future research and development trends.

Keywords: natural products; non-conventional yeasts; cell factories; metabolic engineering

天然产物通常是指自然生物体内的组成成分及其代谢产物。根据骨架结构的不同，天然产物可大致分为萜类、黄酮、聚酮、多糖、生物碱、脂肪酸等大类。这些天然产物被广泛应用于医药、保健、食品、化妆品等多个领域，尤其是作为新型药物分子开发的天然宝库，为多种疾病的治疗提供了更多可能[1-6]。

近年来，人们对天然产品的需求迅速增长，促进了高效合成方法相关的探究。自然提取天然产物的效率不高，主要受不稳定的自然环境限制，如植物提取化合物通常需要很长的周期，且有效产物含量有限。而化学合成则受限于大量有机试剂使用，容易造成污染，且对于复杂途径产物难以实现高效从头合成。相较而言，生物合成可使用廉价的大宗化合物作为底物，生产周期较短，具有低成本、高效率、可定向设计且环境友好的优势，为天然产物的挖掘与合成提供了新的发展方向，且近年来随着合成生物学技术的发展，无论是在新分子的挖掘及产物合成能力的提升上都有了很多突破与进展[7-10]。

酵母作为最常用的真核单细胞微生物，生长繁殖迅速，遗传操作较为简单，相对于原核表达宿主在异源酶表达上更具优势，在生物合成的应用中备受研究者的关注。其中，酿酒酵母(*Saccharomyces cerevisiae*)研究历史较为悠久、遗传背景清晰，目前已被广泛应用于天然产物的异源合成[7,11]。然而，酿酒酵母在高密度发酵及底物利用效率方面仍有一定局限，且可适应的培养条件并不宽泛，对于部分天然产物的生产能力上难有突破。

除酿酒酵母和粟酒裂殖酵母(*Schizosaccharomyces pombe*)外，其他一些非常规酵母(non-conventional yeasts)近年来也吸引了较多的研究和应用，例如，解脂耶氏酵母

(*Yarrowia lipolytica*)、巴斯德毕赤酵母(*Pichia pastoris*)、马克斯克鲁维酵母(*Kluyveromyces marxianus*)、圆红冬孢酵母(*Rhodosporidium toruloides*)及多形汉逊酵母(*Hansenula polymorpha*)等。相对于酿酒酵母而言，非常规酵母具有独特的生理代谢优势和良好的环境耐受性，如耐高温、耐低 pH 等，其中大部分还具有广泛的底物利用谱[12-14]。这些特性使得非常规酵母较酿酒酵母在一些特殊天然产物的合成上有独特的优势。随着合成生物技术的飞速发展，多种非常规酵母中已建立了高效且便捷的基因操作方法，为合成途径的组装和代谢工程的优化提供了有效工具[15-18]。目前基于非常规酵母合成天然产物的应用越来越多，产物类型日益丰富，且部分化合物产量也相当可观[19-22]。本文主要介绍部分非常规酵母的特性及相应的天然产物合成现状，并对促进目标化合物合成的常用改造策略进行了总结。

1 非常规酵母基本特性及发展现状

1.1 解脂耶氏酵母

解脂耶氏酵母(*Yarrowia lipolytica*)属于子囊菌门、酵母菌目、耶氏酵母菌属，于 1942 年首次被分离，被美国食品药品监督管理局(Food and Drug Administration, FDA)认定为一般认可安全(generally recognized as safe, GRAS)微生物，通常存在于乳制品、面包等发酵食品和土壤、海洋及油质污染等生态环境中[23-24]。解脂耶氏酵母是一种二态型酵母，可以作为圆形多极出芽细胞、假菌丝或带有隔膜菌丝的菌丝体生长，具体形态取决于生长条件[25]。其有较强的环境适应能力，可在高渗透压及较为极端的 pH 条件(2.5–9.5)下生长，可耐受的最高温度为 38 ℃[26]。解脂耶氏酵母生长所需的培养条件简单，底物利用谱广泛。除了能利用葡萄糖、果糖、甘露糖、甘油等碳源之外，解脂耶氏酵母还具有分解烷烃及脂肪酸等疏水性底物的能力，能够以废弃的工业油脂等廉价原料为底物[27]。代谢工程改造可进一步拓展解脂耶氏酵母的碳源利用谱，通过理性的人工改造可实现其利用木糖、半乳糖、菊粉、木质纤维素等进行生长及代谢[28-29]。

解脂耶氏酵母目前已表征了多种内源启动子，组成型启动子包括 P_{TEF1}、P_{TDH1}、P_{FBA1} 及 P_{GPM1}，诱导型启动子中有许多源自脂质代谢途径，如受油酸诱导的启动子 P_{POX2}、P_{POT1} 和 P_{LIP2}，此外还有 Cu^{2+}诱导型启动子 $P_{MT1\text{-}6}$、赤藓糖醇诱导型启动子 P_{EYK1} 等，在此基础上也有人工设计的杂合型启动子以实现定制化的基因表达需求[30-33]。解脂耶氏酵母中游离型质粒具有不稳定性，因此通常将目标基因序列整合到基因组进行基因表达，目前已有商用的整合型载体 pYLEX1 (胞内表达型)和 pYLSC1 (分泌表达型)。近年来 CRISPR 系统在解脂耶氏酵母中广泛应用，在基因的删除、整合、多基因编辑方面都展现了出色的效果[34-38]。此外，位点特异性重组 Cre-*loxP* 系统也已在解脂耶氏酵母实现了筛选标记的回收和多轮的基因整合[39-40]。解脂耶氏酵母中诸多转录元件的相继开发及 CRISPR 介导的调控系统的应用也加速了其遗传操作技术体系的发展，为产物的合成与生产奠定了坚实的基础[41-44]。

作为天然的产油微生物，解脂耶氏酵母的代谢系统非常适合油脂和脂肪酸的生产，胞内高浓度的脂质含量也为其他疏水性天然产物的合成与储存提供了得天独厚的条件[45]。解脂耶氏酵母是典型的 Crabtree 阴性酵母，相对于酿酒酵母，其在培养过程中基本不产生乙醇，可以避免发酵过程中乙醇积累对产物的影响，具

有很好的工业应用潜力[46]。此外，解脂耶氏酵母胞内有多条乙酰辅酶 A 合成途径，可为多种产物的合成提供充足的前体[47]。近几年基因组规模代谢模拟工具的开发进一步促进了解脂耶氏酵母合成代谢网络的解析及理性优化[48-49]。目前，解脂耶氏酵母在脂质、萜类化合物、黄酮化合物等多类天然产物的合成中已得到广泛应用，部分产物产量相当可观[50-51] (表 1)。

表 1　非常规酵母天然产物生产实例

Table 1　Natural products produced by non-conventional yeasts: representative cases

Yeast strain	Product	Titer	Substrate	Scale	References
Yarrowia lipolytica	Lipids				
	Arachidonic acid	118.10 mg/L	Glucose	Shake flask	[52]
	Conjugated linoleic acid	9.70 g/L	Glucose, volatile fatty acids	Bioreactor	[53]
	Docosahexaenoic acid	86.10 mg/L	Glucose	Bioreactor	[54]
	Dodecanedioic acid	11.00 g/L	Glucose	Shake flask	[55]
	Fatty acid	16.00 g/L	Volatile fatty acids	Bioreactor	[56]
	Fatty acid ethyl esters	1.18 g/L	Glucose	Shake flask	[57]
	Fatty acid methyl esters	98.90 g/L	Glucose	Bioreactor	[58]
	Fatty alcohol	5.80 g/L	Glucose	Bioreactor	[59]
	Fatty alkane	23.30 mg/L	Glucose	Shake flask	[60]
	Lipid	115.00 g/L	Acetic acid and acetate	Bioreactor	[61]
	Medium chain fatty acids	1.50 g/L	Glucose	Shake flask	[62]
	Odd chain fatty acid	0.82 g/L	Sucrose, glycerol	Bioreactor	[63]
	Oleic acid	56.00 g/L	Glucose	Bioreactor	[64]
	Poly-3-hydroxybutyrate	7.35 g/L	Acetic acid	Bioreactor	[65]
	Polyhydroxyalkanoates	2.90 g/L	Glucose	Shake flask	[66]
	Ricinoleic acid	12.00 g/L	Glucose	Shake flask	[67]
	α-linolenic acid	1.40 g/L	Glucose	Bioreactor	[68]
	γ-linolenic acid	71.60 mg/L	Glucose	Shake flask	[69]
	Terpenoids				
	(–)-α-bisabolol	4.40 g/L	Glucose	Bioreactor	[70]
	(+)-nootkatone	978.20 mg/L	Glucose	Shake flask	[71]
	Astaxanthin	3.30 g/L	Glucose	Bioreactor	[72]
	Abscisic acid	263.50 mg/L	Glucose	24-deep-well plate	[73]
	Amorphadiene	171.50 mg/L	Glucose	Shake flask	[74]
	Betulinic acid	51.87 mg/L	Glucose	Bioreactor	[75]
	Limonene	165.30 mg/L	Glycerol	Bioreactor	[76]
	Linalool	6.96 mg/L	Glucose	Shake flask	[77]
	Lupeol	411.70 mg/L	Glucose	Shake flask	[78]
	Lycopene	17.60 g/L	Glucose	Bioreactor	[79]
	Squalene	731.20 mg/L	Glucose	shake flask	[80]
	Valencene	113.90 mg/L	Glucose	Tube	[81]
	α-farnesene	25.55 g/L	Glucose	Bioreactor	[82]
	α-pinene	36.10 mg/L	Lignocellulosic hydrolysate	Shake flask	[83]

(待续)

(续表 1)

Yeast strain	Product	Titer	Substrate	Scale	References
	β-carotene	39.50 g/L	Glucose	Bioreactor	[79]
	β-farnesene	22.80 g/L	Glucose	Bioreactor	[84]
	β-ionone	4.00 g/L	Food waste hydrolysate	Shake flask	[85]
	Flavonoids and precursors				
	Eriodictyol	134.20 mg/L	Glucose	Shake flask	[86]
	Naringenin	898.00 mg/L	Glucose	Bioreactor	[87]
	Resveratrol	22.50 g/L	Glucose	Bioreactor	[88]
	p-coumaric acid	593.00 mg/L	Glucose	Shake flask	[89]
	Scutellarin	346.00 mg/L	Glucose	Bioreactor	[90]
	Taxifolin	110.50 mg/L	Glucose	Shake flask	[86]
	Alcohols				
	(Z)-7-dodecenol	0.10 mg/L	Glycerol	Shake flask	[91]
	(Z)-7-tetradecenol	0.48 mg/L	Glycerol	Shake flask	[91]
	(Z)-9-dodecenol	0.21 mg/L	Glycerol	Shake flask	[91]
	2-phenylethanol	2 669.50 mg/L	Glucose	Shake flask	[92]
	Campesterol	837.00 mg/L	Glucose	Bioreactor	[93]
	Retinol	4.86 g/L	Glucose	Bioreactor	[94]
	Others				
	Arbutin	8.60 g/L	Glucose	Bioreactor	[95]
	Bisdemethoxycurcumin	0.17 mg/L	Glucose	Shake flask	[87]
	Cordycepin	3 249.58 mg/L	Glucose, molasses	Shake flask	[96]
	Deoxyviolacein	55.00 mg/L	Glucose	Shake flask	[89]
	Ergothioneine	1.63 g/L	Glucose	Bioreactor	[97]
	Methyl ketone	314.80 mg/L	Glucose	Bioreactor	[98]
	Pentane	4.98 mg/L	Glucose	Shake flask	[99]
	Triacetic acid lactone	35.90 g/L	Glucose, sodium acetate	Bioreactor	[100]
	Violacein	366.00 mg/L	Glucose	Shake flask	[89]
	γ-decalactone	3.50 g/L	Castor oil	Shake flask	[101]
Pichia pastoris	Lipids				
	2-decenoic acid	33.70 mg/L	Glucose	Shake flask	[102]
	Fatty acid	23.40 g/L	Methanol	Bioreactor	[103]
	Fatty alcohol	2.00 g/L	Methanol	Bioreactor	[103]
	Long-chain α-alkenes	1.60 mg/L	Methanol	Shake flask	[104]
	Ricinoleic acid	171.44 mg/L	Methanol	Shake flask	[105]
	Terpenoids				
	(+)-ambrein	>100.00 mg/L	Methanol	Bioreactor	[106]
	(+)-nootkatone	208.00 mg/L	Methanol	Bioreactor	[107]
	Astaxanthin	3.70 μg/g DCW	Glucose	Shake flask	[108]
	Dammarenediol-II	0.10–1.07 mg/g DCW	Methanol	Shake flask	[109]
	Lycopene	714.00 mg/L	Methanol	Bioreactor	[110]
	Menaquinone-4	0.24 mg/g DCW	Glucose	Shake flask	[111]

(待续)

(续表 1)

Yeast strain	Product	Titer	Substrate	Scale	References
	Trans-nootkatol	94.00 mg/L	Methanol	Shake flask	[112]
	α-farnesene	2.56 g/L	Oleic acid, sorbitol	Shake flask	[113]
	β-carotene	>5.00 mg/g DCW	Methanol	Shake flask	[114]
	Flavonoids and precursors				
	3′-hydroxygenistein	20.30 mg/L	Glucose, genistein	Shake flask	[115]
	Baicalein	401.90 mg/L	Ethanol	Shake flask	[116]
	Cinnamic acid	124.10 mg/L	Ethanol	Shake flask	[117]
	Naringenin	1 067.00 mg/L	Glycerol	Bioreactor	[118]
	Oroxylin A	339.50 mg/L	Ethanol	Shake flask	[116]
	p-coumaric acid	302.00 mg/L	Ethanol	Shake flask	[117]
	Resveratrol	1 825.00 mg/L	Glycerol	Bioreactor	[118]
	Polyketides				
	6-methylsalicylic acid	2.20 g/L	Methanol	Bioreactor	[119]
	Citrinin	0.60 mg/L	Methanol	Shake flask	[120]
	Lovastatin	419.00 mg/L	Methanol	Bioreactor	[121]
	Monacolin J	3.20 g/L	Ethanol	Bioreactor	[122]
	Alkaloids				
	Norcoclaurine	9.70 mg/L	Glycerol	Bioreactor	[118]
	Reticuline	292.00 μg/L	Glycerol	Shake flask	[118]
	Polysaccharides and proteoglycans				
	Chitin	2.23 g/L	Glucose	Bioreactor	[123]
	Chondroitin sulfate	2.60 g/L	Glycerol	Bioreactor	[124]
	Heparin	2.08 g/L	Methanol	Bioreactor	[125]
	Hyaluronic acid	1.70 g/L	Methanol	Bioreactor	[126]
	Others				
	Cytidine-5′-diphosphocholine	30.00 g/L	Glucose	Shake flask	[127]
	Riboflavin	175.00 mg/L	Glucose	Bioreactor	[128]
	S-adenosyl-l-methionine	13.50 g/L	Methanol	Bioreactor	[129]
Kluyveromyces marxianus	Astaxanthin	9 972.00 μg/g DCW	Galactose	Bioreactor	[130]
	Triacetic acid lactone	1.24 g/L	Xylose	Tube	[131]
	2-phenylethanol	26.50 g/L	L-Phenylalanine	Bioreactor	[132]
	2-phenylethylacetate	6.10 g/L	L-Phenylalanine	Bioreactor	[132]
	Fructose	234.44 g/L	Jerusalem artichoke tuber	Shake flask	[133]
Rhodosporidium toruloides	Lipid	89.40 g/L	Glucose	Bioreactor	[134]
	Linoleic acid	1.30 g/L	Galactose, raffinose	Shake flask	[135]
	Fatty acid ethyl esters	9.97 g/L	Glucose	Bioreactor	[136]
	Fatty alcohols	8.00 g/L	Sucrose	Bioreactor	[137]
	Carotenoid	1 136.70 μg/g DCW	Glucose	Shake flask	[138]
	Limonene	393.50 mg/L	Glucose	Tube	[139]
	Prespatane	1.17 g/L	Poplar hydrolysate	Bioreactor	[140]
	Epi-isozizaene	0.36 g/L	Poplar hydrolysate	Bioreactor	[140]
	Bisabolene	7.80 g/L	Organic acids	Bioreactor	[141]
	Ent-kaurene	1.44 g/L	Lignocellulosic hydrolysate	Bioreactor	[142]
	Triacetic acid lactone	28.00 g/L	glucose	Bioreactor	[143]
Ogataea polymorpha	Fatty acids	15.90 g/L	Methanol	Bioreactor	[144]
	Fatty alcohols	12.00 mg/L	Glucose	Shake flask	[145]
	Paclitaxel	1.00 mg/L	Glucose	Shake flask	[146]
	Gentiopicroside	2.20 mg/L	Glucose	Shake flask	[147]

1.2 巴斯德毕赤酵母

巴斯德毕赤酵母(*Pichia pastoris*, 又称*Komagataella phaffii*)属于子囊菌门、酵母菌目、*Komagataella* 属，最早从美国加利福尼亚州的橡树中分离得到[148]。其早年被归类于毕赤酵母属，Yamada 等[149]于 1995 年根据 18S 和 26S rRNA 的部分序列将其重新归为 *Komagataella* 属，Kurtzman 等[150]于 2005 年根据 26S rRNA 的部分序列将其细分为 *K. pastoris* 和 *K. phaffii*，而 Love 等[151]则进一步分析了这两种酵母的基因组和转录组差异。目前常用的毕赤酵母菌株 GS115 和 CBS7435 均属于 *K. phaffii*，且分别于 2009 年和 2011 年完成全基因组测序[152-153]。

毕赤酵母因其生长密度高、蛋白翻译后修饰适度、蛋白分泌能力强、培养工艺简单等优势，已逐渐成为最为常用的异源蛋白真核表达平台[154-155]。毕赤酵母被 FDA 认定为 GRAS 菌株，并获准用于制药和食品行业。据报道，目前已采用毕赤酵母表达了 5 000 多种蛋白，其中超过 70 种蛋白产品已经进入市场(http://pichia.com/)。

作为一种典型的甲基营养型酵母，毕赤酵母能够以甲醇为唯一碳源进行生长，最适生长温度为 28–30 ℃，可耐受 pH 范围为 3.0–7.0。其天然的醇氧化酶启动子 P_{AOX1} 具有非常高效的启动能力且受甲醇的严格诱导，甲醇诱导培养体系也已发展成为毕赤酵母最常用的发酵生产工艺。除甲醇外，葡萄糖、甘油、乙醇、山梨醇等也是毕赤酵母可用的碳源底物，其相关的各类天然启动子也在毕赤酵母中均有较好的应用，包括强度各异的诱导型启动子及组成型启动子等[114,156-158]。基于天然启动子构建的启动子突变文库以及人工设计的合成启动子也进一步丰富了毕赤酵母的转录调控工具库[159-160]。相比之下，终止子的开发相对有限，目前仍以醇氧化酶终止子 *AOX1tt* 为主。近年来，一些内源性终止子相继被鉴定，且一些外源终止子也被证实可在毕赤酵母中发挥功能[161]。目前，毕赤酵母已有多种商业化的表达载体可供直接使用，如 pPIC3.5K、pPIC9K、pPICZ 系列、pPICZα 系列和 pGAPZ 系列等。近年来，毕赤酵母中合成生物学工具的开发也取得重要进展，包括 Golden-Gate 组装、Cre-*loxP* 重组，以及基于 CRISPR/Cas9 的基因编辑技术等，大大降低了毕赤酵母中途径组装与代谢重构的遗传操作难度[162-165]。

毕赤酵母在天然产物合成方面主要以聚酮、萜类为主，其他还包括黄酮、多糖以及脂肪酸衍生物[148,166] (表 1)。甲醇、甘油或葡萄糖一般被用作主要的碳源底物，而近年来乙醇也被作为碳源在聚酮及黄酮合成方面表现出了明显优势[116-117,122]。此外，强化前体供应、途径区室化、辅因子工程及途径精细调控等代谢工程调控策略也已有较多研究，可以有效促进毕赤酵母中的产物合成。例如，本课题组利用碳源优化及途径改造提高前体供给、菌群共培养缓解细胞负担、构建转录器件库精细调控途径平衡以及构建产物外排泵释放细胞压力等策略，能够实现毕赤酵母高效从头合成聚酮药物莫纳克林 J 和洛伐他汀[121-122,160,167]。

1.3 马克斯克鲁维酵母

马克斯克鲁维酵母(*Kluyveromyces marxianus*)属于子囊菌门、酵母菌目、克鲁维酵母菌属，最初由葡萄中分离获得，其广泛存在于植物和乳制品中，产生的芳香化合物可以为乳制品以及酒类增加特殊风味[168]。马克斯克鲁维酵母不仅是 FDA 认证的 GRAS 级别微生物，还通过了欧盟食品安全监督局(European Food Safety Authority, EFSA)的安全认证(qualified presumption of safety, QPS)，在 2013 年被中国国家卫生和计划生育委员会批准为新食品原料[168]。

马克斯克鲁维酵母独特的生理特性主要体现在耐高温、生长速率高以及具有利用多种碳源的能力。菌株普遍都能在 40 ℃条件下生长，生长速率可达 0.86–0.99/h，远高于其他酵母，其中部分菌株最高可耐受 50 ℃以上的温度[12]。高温发酵可以大幅降低冷却成本及染菌风险，且更利于一些在高温下活性更好的酶进行催化反应。除了葡萄糖以外，马克斯克鲁维酵母还可以利用一些其他糖类作为单一碳源进行生长，包括果糖、木糖、阿拉伯糖、半乳糖、乳糖和菊糖等，因此这些糖类来源的许多廉价农业及食品业副产物都可以作为其发酵碳源[169-172]。

许多酿酒酵母来源的启动子都能在马克斯克鲁维酵母中发挥作用，主要以组成型为主[173]。研究较多的内源启动子以糖诱导型为主，包括 β-半乳糖苷酶的启动子 P_{LAC4} 以及菊糖酶的启动子 P_{INU1} 等[174-175]。鉴于马克斯克鲁维酵母的耐热特性，一些内源启动子的强度还会受到温度的影响和调控[176]。马克斯克鲁维酵母的常用抗性标签为 G418 和博来霉素，常用的营养缺陷型标签为 *URA3*、*LEU2* 以及 *TRP1*[176-177]。其常用质粒主要由果蝇克鲁维酵母(*Kluyveromyces drosophilarum*)的 pKD1 质粒以及粟酒裂殖酵母的 pDblet 质粒衍生而来，也有由内源元件组装而成的类型[171]。马克斯克鲁维酵母中天然的同源重组效率很低，实现基因的替换或缺失通常需要 500 bp 以上的同源臂。目前 Cre-*loxP* 以及 CRISPR 系统都有在马克斯克鲁维酵母中实现基因敲除的成功应用[178-180]。

天然马克斯克鲁维酵母可以生产苯乙醇以及乙酸乙酯等化合物。通过基因工程改造，马克斯克鲁维酵母还能生产果糖糖浆、虾青素以及三乙酸内酯等，由于分子操作技术以及相关代谢背景的限制，目前常用的产物提高策略仍以关键基因过表达以及旁路基因的敲除为主[181-183] (表 1)。

1.4 圆红冬孢酵母

圆红冬孢酵母(*Rhodotorula toruloides* 或 *Rhodosporidium toruloides*)属于担子菌门、锁掷酵母目、红冬孢酵母属(*Rhodosporidium*)，也有研究依据系统发育分类将该属划归为红酵母属(*Rhodotorula*)[184]。圆红冬孢酵母在自然界中分布广泛，且抗逆性较强，能利用多种工农业废弃物作为碳源，如粗甘油、木质纤维素水解物、挥发性脂肪酸、甘蔗糖蜜以及稻壳废弃物等[14]。作为有较强油脂累积能力的微生物，其在不同培养条件下的细胞油脂含量可占干重的 20%–79%，是生产食用油脂及生物柴油原料的潜力微生物[14]。此外，红冬孢酵母还被用于合成多种类胡萝卜素、脂肪酸衍生物以及萜类化合物等[14,185-186] (表 1)。

2011 年至今，已有多株圆红冬孢酵母完成了全基因组测序，随后多组学分析被广泛用于探究其碳源利用、胁迫响应及油脂等的相关代谢[14,187]。在此基础上，多个研究团队构建完善了圆红冬孢酵母详细的油脂代谢网络，为进一步优化菌株的油脂生产提供了良好的代谢背景基础[188-190]。目前红酵母中最常用的转化法为农杆菌介导的转化法(*Agrobacterium tumefaciens*-mediated transformation, ATMT)，此外电穿孔转化法和原生质体转化法等也有应用[191-192]。在元件挖掘方面，多种组成型和诱导型的天然启动子、终止子均已被鉴定，一些外源的启动子也被证实可以在圆红冬孢酵母中发挥作用[14,192]。

传统的化学、物理诱变方法一直是获得高产圆红冬孢酵母菌株的常用方法，经典的代谢工程策略也常结合 ATMT 应用于提高油脂及类胡萝卜素产量[14,138,186,193]。但是，圆红冬孢酵母本身同源重组效率过低，这很大限制了其在菌株改造及异源合成方面的发展。随着基因操作

技术的发展，近年来多个课题组已成功在红酵母中建立了 CRISPR/Cas9 系统[194-196]。目前，ATMT 以及 CRISPR 等技术均可在圆红冬孢酵母中实现基因缺失操作，RNAi 也能实现基因转录抑制[194,196-198]。这些工具的建立进一步促进了圆红冬孢酵母在化合物生产上的开发应用。

1.5 多形汉逊酵母

多形汉逊酵母(*Hansenula polymorpha*, 也称 *Ogataea polymorpha*)属于子囊菌门、内孢霉目、汉逊酵母属，是典型的甲基营养型微生物，可以利用甲醇为唯一碳源进行高密度生长，具有耐热的特性，适合在 37–43 ℃的条件下生长，最高可耐受 49 ℃。其甲醇利用途径与巴斯德毕赤酵母较为相似，同样在过氧化物酶体中进行，区别在于多形汉逊酵母仅拥有一个甲醇氧化酶 Mox，且甲醇调控不如毕赤酵母严格，在低浓度的甘油和葡萄糖中也能实现一定强度的 Mox 表达，常用的发酵碳源以甘油及甲醇为主[199-201]。

目前，常用多形汉逊酵母细胞菌株主要为 DL1、CBS4732 以及 NCYC495。其基因表达元件在近几年也有较多开发，包括应用较早的甲醇诱导型启动子 P_{MOX}、P_{FMD}、P_{DHAS}，硝酸盐诱导型启动子 P_{YNT1}、P_{YNT1l}、P_{YNR1}，以及组成型启动子 P_{GAP}、P_{PMA1}、$P_{TEF1/2}$[199-201]。Zhou 等表征了前体供应途径、甲醇利用途径以及活性氧(reactive oxygen species, ROS)防御途径中的数十个启动子，包括不同强度的甲醇诱导型和组成型启动子、乙醇诱导型启动子 P_{ICL}、鼠李糖诱导型启动子 $P_{LRA3/4}$、双向启动子 P_{Mal}-P_{Per}，并且还拓展构建了一系列杂合型启动子[202-204]。多形汉逊酵母常用的转化方法为电穿孔法，此外也有原生质体法和乙酸锂法。筛选标记有营养缺陷型标记 *URA3*、*LEU1*、*ADE11* 和 *TRP3*，也可使用抗生素 G418、腐草霉素或博来霉素进行筛选[199-201]。多形汉逊酵母在进行外源片段整合时多偏向非同源重组，且有很大概率串联多拷贝整合，而其本身的同源重组效率较低。Zhou 等[145,205]通过下调 Ku80 的表达及过表达酿酒酵母来源的 Rad 蛋白，有效提高了 CRISPR-Cas9 在多形汉逊酵母体内的基因编辑效率，并基于 CRISPR-Cas9 技术验证筛选到了 17 个中性整合位点。

多形汉逊酵母多应用于异源蛋白的表达，已商业化的产品包括重组乙型肝炎疫苗、胰岛素以及植酸酶等[201]。目前，在化合物合成方面的应用并不多，主要产物包括脂肪酸、脂肪醇、龙胆苦苷以及紫杉醇等[144-147,206] (表 1)。通过适应性进化和理性代谢改造，可实现由甲醇为唯一碳源高效生产脂肪酸，产量可达 15.9 g/L[144]。另外，通过培养条件的优化，以葡萄糖为碳源生产脂肪酸产量也可达到 18 g/L[206]。

2 碳源底物的选择及优化利用

2.1 基础碳源的利用

底物的选择直接影响菌体的生长及生产，并在生产成本中占有较大比例。酵母常用的碳源主要包括以葡萄糖为代表的一系列单糖以及甘油，其中各酵母由于自身代谢特性会有相应的优势利用碳源。选择甲基营养型酵母作为底盘时，甲醇通常是首选碳源，在毕赤酵母、汉逊酵母等底盘宿主中利用甲醇生产天然产物已有许多成功案例[207-209]；马克斯克鲁维酵母除了能利用乳糖以外，还有较强的五碳糖以及菊糖(果糖聚合物)代谢能力，因此木糖和菊糖是该酵母生产中应用较多的底物[171-172]。

不同的底物在前体合成以及能量供应上也会有所差异，选择合适的碳源能够对产物合成起到良好的促进作用。作为合成聚酮、黄酮、萜类以及脂肪酸等天然产物的重要前体，胞质中乙酰辅酶 A 的供应一直都是代谢途径优化的

研究重点。选择能够在胞质中快速积累乙酰辅酶 A 的前体碳源，对于上述产物的合成非常重要。相比其他代谢途径复杂的常规碳源，乙醇在酵母中仅需 3 步即可合成胞质乙酰辅酶 A，对于前体的供应非常有利。本课题组在毕赤酵母中过表达乙醇脱氢酶基因 *adh2* 及单位点突变型乙酰辅酶 A 合酶基因 *acs1**，强化利用乙醇作为唯一碳源高效合成了真菌聚酮药物分子莫纳可林 J (3.2 g/L)[122]；Qian 等[116]则发现乙醇相比葡萄糖作为碳源能显著提高植物源黄酮分子的产量，且能缓解产物对生长造成的抑制作用。乙酸盐相比乙醇可通过乙酰辅酶 A 合酶(Acs)一步生成乙酰辅酶 A，作为底物的生产效率更高，已在解脂耶氏酵母中被用于生产三乙酸内酯及脂质等[61,210]。甘油相比葡萄糖合成酪氨酸前体所需的反应步骤更少，因此 Kumokita 等[118]选择甘油和粗甘油作为毕赤酵母合成酪氨酸衍生产物的碳源，在反应器水平可高效生产白藜芦醇(1 825 mg/L)和柚皮素(1 067 mg/L)。

2.2 废弃碳源的利用

考虑到成本因素以及对环境废弃资源的有效利用，近年来利用工业、农业、林业废料作为碳源底物合成高附加值产品的研究也越来越多(图 1)。粗甘油是生物柴油生产过程中的主要副产物，成分包括甘油、灰分、甲醇等。粗甘油的化学精制成本较高，利用微生物将其转化为高附加值化合物是拓宽粗甘油利用途径的新思路。多种非常规酵母在粗甘油条件下的生长水平与葡萄糖条件下相似或者更优[211]。解脂耶氏酵母可以利用多种废料来源的粗甘油作为碳源，在油脂的生产中展现出了良好的应用前景[212-213]。糖蜜作为制糖行业的主要副产物，包含大量蔗糖和少量其他糖类、有机酸及矿物质。圆红冬孢酵母利用糖蜜可生产 39.2 g/L 的脂质，细胞生长及产量相比于葡萄糖为碳源时均有提升[214]。木质纤维素类生物质来源于农林业副产物，作为过量且可再生的自然资源，其开发利用策略一直都是研究热点。木质纤维素无法被直接利用，通常需要经水解后生成木糖、葡萄糖、半乳糖等糖类后再被酵母利用。圆红冬孢酵母利用玉米秸秆水解物占比 75%的培养基生产二萜化合物，经过“设计-合成-测试-学习”(design-build-test-learn, DBTL)优化后，反应器水平可生产 1.44 g/L 的贝壳杉烯[142]。利用碱处理的玉米秸秆水解物作为唯一碳源，圆红冬孢酵母的生长及生产均优于其在相应纯碳源中的情况，反应器水平可生产红没药烯 680 mg/L[215]。

虽然对于废弃物料的利用可以有效降低成本且符合环保理念，但是物料的复杂性易于导致其中的某些有害物质对酵母细胞的生长产生抑制作用，如工业废水以及木质纤维素水解液中的呋喃、苯酚以及某些羧酸都不利于酵母生长。目前，针对这些化合物具体抑制作用机制的相关研究还较少，可以通过适应性进化增加菌株耐受、增加有毒化合物降解或转化，以及拓宽有毒化合物外排转运等策略缓解复杂底物的抑制作用[14,171]。

3 代谢工程改造策略

3.1 途径功能酶的筛选与优化

在非常规酵母中组装天然产物合成途径时，功能酶元件的选择是直接关系产物类型及产量的重要环节(图 1)。对于合成途径已较为明确的化合物来说，选择原宿主来源的酶元件是目前较为普遍的方法。实际上，在非常规酵母中表达异源蛋白时，来源不同的功能酶展示的胞内活性也会有很大差异。Liu 等[113]比较了 20 种法尼烯合成酶(Afs)在毕赤酵母中的活性，得到法尼烯产量为 64.92–437.15 mg/L。Petkevicius 等[91]在解脂耶氏酵母中合成昆虫信息素前体

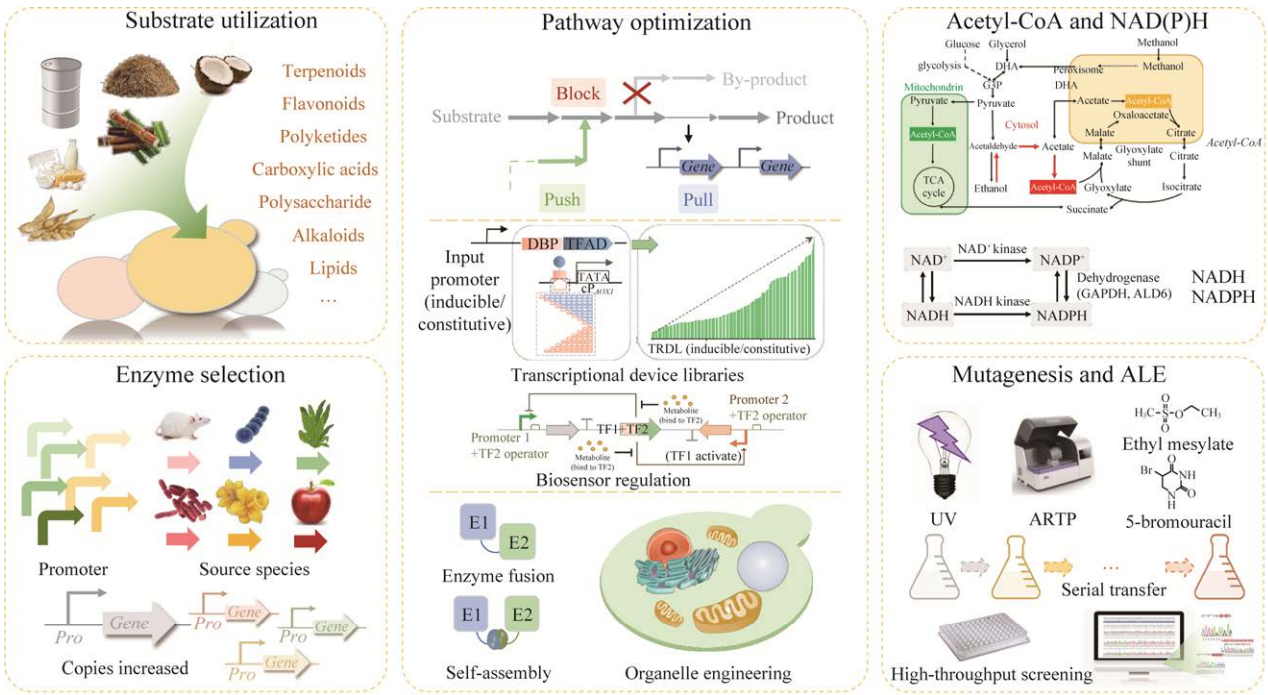

图 1　非常规酵母天然产物合成优化策略

Figure 1　Optimization strategies for natural products synthesis in non-conventional yeasts.

时，将两种来源的脂肪酰辅酶 A 去饱和酶(Fad)和 11 种来源的过氧化物酶体氧化酶(Pox)组合表达，结果各菌株产生的甲酯不饱和脂肪酸含量及组成有很大差异。细胞色素 P450 家族(CYP450)酶的活性对于黄酮、萜类以及生物碱等复杂天然产物的异源合成均有关键影响。共表达细胞色素 P450 还原酶(CPR)是优化 CYP450 的常用策略，在毕赤酵母以及解脂耶氏酵母中尝试不同来源的 CYP450 以及 CPR 都能发现其催化活性的显著差异，从而导致不同的产物合成能力[74,116,186]。根据具体宿主的密码子偏好性，对功能基因进行密码子优化也是提高酶活的有效策略。在毕赤酵母中构建软骨素合成途径时，通过对软骨素聚合酶(KfoC)、UDP-N-乙酰氨基葡萄糖 4-差向异构酶(KfoA)和 UDP 葡萄糖脱氢酶(TuaD)等编码基因的密码子优化，软骨素产量由 5.5 mg/L 提高至 102.5 mg/L[216]。

除了对酶的来源进行筛选，增加拷贝数和更换启动子也是提高产物合成效率的有效策略。在毕赤酵母中利用抗生素浓度梯度筛选是构建多拷贝菌株的普遍策略。He 等[129]利用 G418 浓度梯度筛选到了 S-腺苷甲硫氨酸(S-adenosylmethionine, SAM)合成酶基因 *sam2* 多拷贝整合的菌株，将 SAM 产量由 0.04 g/L 提高至 0.74 g/L。此外，构建表达盒多拷贝质粒以及利用基因组上的重复序列作为整合位点也是筛选多拷贝菌株的有效手段[111,217-218]。Gao 等[218]在解脂耶氏酵母中通过更换强启动子及多次整合增加限速酶的拷贝数，使 β-胡萝卜素产量提高了 100 倍。Liu 等[88]利用解脂耶氏酵母基因组上的核糖体 26S rDNA 以及 ZETA 多拷贝序列作为外源基因的整合位点，通过两轮筛选获得了白藜芦醇产量可达 632.1 mg/L 的菌株。启动子的选择对于酶转录层面的活性起着关键作

用，目前许多非常规酵母都已鉴定了组成型以及诱导型的多种启动子，一些酿酒酵母来源的启动子也已被证实可以用在部分非常规酵母中。在此基础上，也有针对不同应用场景定向设计的人工启动子以及为了获得多样化的表达强度而构建的启动子库[32,160]。例如，Vogl 等[159]设计了 168 个人工构建的双向启动子，并成功用于优化紫杉烯和 β-胡萝卜素合成基因的共表达。

3.2 代谢途径的调控与平衡

3.2.1 “推-拉-敲”调控策略

作为代谢工程改造中的经典策略，增加前体供应(“推[push]”)、提高限速步骤速率(“拉[pull]”)以及阻断或减弱副产物合成及代谢旁路的分流(“敲[block]”)均可有效提高目标产物的合成，在非常规酵母的代谢工程研究中有非常广泛的应用[64] (图 1)。例如，强化丙二酰辅酶 A 的合成可以有效促进脂肪酸以及聚酮等产物的合成通量，通过过表达乙酰辅酶 A 羧化酶(Acc)能够增强丙二酰辅酶 A 的供应，在多种非常规酵母中都被证实能显著提高产物产量[122,134,210,219]。同时，减弱降解脂肪酸的 β 氧化以及合成的逆反应则可以有效提高油脂产物的累积量，在解脂耶氏酵母以及毕赤酵母中通过敲除 Pox 或脂肪酰辅酶 A 合成酶(Faa)都能有效提高油脂类产物的合成[103,206,219-220]。黄酮类化合物的主要合成前体酪氨酸和苯丙氨酸受限于途径负反馈抑制，胞内累积量有限，因此许多研究者在多种酵母底盘中过表达外源或内源的 3-脱氧-D-阿拉伯糖七磷酸合酶及分支酸变位酶的反馈抑制不敏感突变体如 $Aro4^{K229L}$ 及 $Aro7^{G141S}$ 等以增强前体供应[88-89,118]。而对于萜类化合物来说，其前体主要依靠甲羟戊酸(mevalonate pathway, MVA)途径合成，对参与该途径中的酶，特别是关键限速酶 HMG-CoA 还原酶(HmgR)和异戊二烯基二磷酸合酶(Idi1)进行过表达，可以有效强化萜类产物的合成[80,221-222]。由于萜类化合物合成途径分支较多，因此减弱代谢旁路也是增强主途径通量的有效方法。法尼基焦磷酸(farnesyl pyrophosphate, FPP)是萜类化合物的重要前体，Shi 等[84]在解脂耶氏酵母中通过敲除整个 β-胡萝卜素合成途径的相关基因，使得共用萜类前体的 β-法尼烯的产量由 8 mg/L 提升至 3 145 mg/L。Kildegaard 等[223]通过截短及更换启动子减弱角鲨烯合酶(Sqs1)的表达，下调角鲨烯合成通量从而将 β-胡萝卜素产量提高了 2.5 倍。

3.2.2 途径平衡

代谢途径的通量平衡对于目标产物的合成效率有着关键影响，途径通量不匹配容易造成中间产物的积累及碳源前体的浪费。调控途径中各功能酶的相对表达强度，可以优化整个酶系的转化效率，使通量高效流向终产物(图 1)。丰富且功能多样化的表达调控元器件是进行途径精细调控的前提。Vogl 等[114]基于毕赤酵母甲醇利用途径、磷酸戊糖途径以及活性氧防御筛选表征了一系列启动子和终止子，并应用于 β-胡萝卜素合成的高通量筛选，使菌株产量提升可达 10.2 倍。Li 等[70]构建了用于解脂耶氏酵母 DNA 组装的 Golden-Gate 模块化克隆系统 YALIcloneNHEJ，用一系列启动子和终止子组合表达倍半萜合成途径功能酶，筛选到了可产 4.4 g/L α-红没药醇的菌株。本课题组 Zhu 等[160]基于转录调控元件的筛选鉴定和理性设计，构建了组成型人工转录器件库 cTRDL 以及甲醇诱导型人工转录器件库 iTRDL，强度分别为常用启动子 P_{AOX1} 的 16%–520%和 30%–500%，并将其应用于莫纳可林 J 合成途径的通量调控与平衡，使最终产量提升 3 倍。

通过转录型生物传感器可以实现化合物生产菌株的高通量筛选以及代谢通路的动态调

控。随着针对不同化合物可结合的天然转录因子的挖掘及人工改造，转录传感器在代谢工程研究中已有数千个应用案例[224]。本课题组 Wen 等[225-226]利用转录阻遏因子 FapR 在毕赤酵母中设计了可响应丙二酰辅酶 A 浓度从而实现途径通量动态调控的系统，为丙二酰辅酶衍生物的合成优化提供了新的调控工具。Lv 等[227]则利用 CRISPRi 技术和脂肪酸诱导启动子实现了丙二酰辅酶 A 的重新分配，利用黄酮特异性结合的转录激活因子 FdeR 控制关键营养因素的合成，以促进柚皮素高产菌株的性状稳定。通过甲磺酸乙酯介导的随机诱变与可响应脂肪酸的生物传感器结合，Park 等[228]实现了对生产 ω-羟基棕榈酸的解脂耶氏酵母菌株的高通量筛选。

3.2.3 途径的区域化设计

对合成途径进行区域化分配与设计可以有效促进底物在功能酶之间的快速转化，提高合成效率，降低旁路损失[229] (图 1)。多酶融合表达设计简单且应用普遍，不同的连接肽往往会影响融合蛋白的构型及活性。Duan 等[96]使用不同的 linker，将负责虫草素合成的氧化还原酶/脱氢酶(Cns1)和金属依赖性磷酸水解酶(Cns2)在解脂耶氏酵母中进行融合表达，效果最好的 $(EAAAK)_4$ 可以将产量提高 1.7 倍。Liu 等[88]将对香豆酸辅酶 A 连接酶(4cl)和白藜芦醇合酶(Sts)通过 8 种不同的连接肽以实现融合表达，其中两个蛋白通过 EAAAK 连接时能显著促进对香豆酸至白藜芦醇的转化。融合蛋白的复杂结构限制了适用的酶数量，而酶自组装区别于蛋白融合表达在基因层面的连接，由功能酶单独表达后在胞内通过末端携带的短肽相互结合，这种自组装机制能更加精准地实现针对区域定位与计量变化的需求。Zhao 等[230]利用 PDZ 蛋白结构域及其相应配体 PDZlig 让达玛烯二醇 II 合成途径中的两个关键酶实现毕赤酵母胞内自组装，将产量提高了 2.1 倍。由此机制还有衍生的蛋白支架应用策略，将整个酶系结合在特定结构的骨架蛋白上，在胞内形成空间上的底物转化通道[231]。

区域性更强的细胞器定位能够缓解产物累积对细胞生长的抑制，并提供更充足的前体和能量。相比于细胞质，线粒体可以提供更为丰富的乙酰辅酶 A 以及能量，是天然产物合成的优选区域[232]。Zhu 等[233]将萜类合成途径同时构建在解脂耶氏酵母的胞质与线粒体中，反应器规模以废弃食用油为碳源可合成 α-红没药烯 1 058.1 mg/L。但是由于线粒体在能量代谢方面的重要作用，对应的改造设计需要考虑对细胞自身的生长代谢损害。过氧化物酶体通过脂肪酸 β 氧化供应乙酰辅酶 A，并参与过氧化氢信号转导与细胞解毒，是以乙酰辅酶 A 为前体及有毒性的化合物合成的理想场所。同时，过氧化物酶体具有储存疏水性化合物的能力，在促进产物合成的同时还能增强细胞耐受[234]。目前，在非常规酵母中过氧化物酶体定位已成功促进了类胡萝卜素、法尼烯、昆虫信息素前体等天然产物的合成[91,113,235]。Xu 等[60]将负责转化酰基辅酶 A 以及酰基 ACP 的酶分别靶向细胞质、过氧化物酶体以及内质网，两个细胞器定位显著提高了脂肪酸乙酯和脂肪烷烃的产量。Ma 等[236]将合成途径分别以组合定位于脂质体、内质网以及过氧化物酶体，同时 3 处定位的菌株能将虾青素产量由 28.9 mg/L 提升至 139.4 mg/L。增加细胞器的数量或者体积能有效提高储存容量，增加产物累积。Liu 等[113]通过利用油酸与山梨醇混合碳源，有效提高了毕赤酵母中过氧化物酶体定位的法尼烯合成。

3.3 乙酰辅酶 A 及 NAD(P)H 的供应优化

乙酰辅酶 A 作为细胞合成代谢的枢纽分子，途径涉及胞质、线粒体、过氧化物酶体以及细胞

核，是大部分天然产物合成的前体(图 1)。其通过 MVA 途径产生萜类的关键前体异戊烯基二磷酸酯(IPP)和二甲基烯丙基二磷酸酯(DMAPP)；或经 Acc 形成丙二酰辅酶 A 后进入脂肪酸、黄酮、聚酮合成途径。Huang 等[237]基于基因组规模的代谢网络分析重新分配丙酮酸通量，强化多条合成途径基因表达以及减弱流向过氧化物酶体的通量构建了多个解脂耶氏酵母菌株，最佳菌株过表达肠道沙门氏菌(*Salmonella enteric*)的 *acs**以及内源的 ATP 柠檬酸裂解酶基因 *acl1*，有效提高了 50%的乙酰辅酶 A 累积，使角鲨烯产量增加 16.4 倍。如上文所述，选择合成乙酰辅酶 A 路径更短的乙醇及乙酸盐作为底物、强化胞质合成途径以及强化线粒体或过氧化物酶体中乙酰辅酶 A 的合成也是有效的优化策略[75]。

充足的辅因子供给是维持细胞生长及提高整体转化效率的重要因素，非常规酵母中研究和改造优化的辅因子主要集中为 NAD(P)H (图 1)。NAD(P)H 作为主要的生物还原当量，可以保护细胞免受氧化应激和延长碳-碳骨架，也是脂肪酸合成的主要限速因子。增强相应依赖型脱氢酶的表达是常用的 NADPH 强化手段，在解脂耶氏酵母中增强琥珀酸半醛脱氢酶(Uga)、6-磷酸葡萄糖酸脱氢酶(Gnd)、苹果酸酶(Mae)及甘露醇脱氢酶(Mndh)可以增强萜类化合物、聚酮化合物以及脂质的生产[210,238-240]。NADH 和 NADPH 虽然在结构上相似，但是参与的具体途径并不一致，比如，某些 CYP450s 对底物的转化可以通过提高 NADPH 水平来促进，但提高 NADH 水平却无法达到相同的效果[241]。Jin 等[75]通过过表达苹果酸脱氢酶(Emc、Emt 和 Rtme)增加 NADPH 供应可使解脂耶氏酵母合成三萜化合物的产量有所提升，但为提高 NADH 供应而过表达甘油醛-3 磷酸脱氢酶(Gapc)却无明显效果。通过过表达或者引入异源激酶促进 NADH 转化为 NADPH 也是一种有效的辅因子改造策略。Qiao 等[58]设计了多条途径将解脂耶氏酵母胞质 NADH 转化为脂质生物合成所需的 NADPH 或乙酰辅酶 A，使最佳菌株脂质水平提高了 25%。其他改造手段还包括通过突变改变酶的辅因子依赖型以及额外供应辅因子合成的前体等，目前主要在其他物种中应用较多[242-244]。

4 诱变育种与适应性进化

对于部分遗传背景不清楚或遗传操作平台不成熟的非常规酵母，诱变育种和适应性进化是通用性较强且效果较好的菌株改造策略(图 1)。诱变手段根据诱变原理可基本分为物理诱变和化学诱变。物理诱变中常用的为紫外线(ultraviolet, UV)以及常压室温等离子体(atmospheric room temperature plasma, ARTP)，化学诱变中烷化剂和核酸碱基类似物是使用频率较高的试剂。为了增加圆红冬孢酵母对木质纤维素水解物中抑制物的耐受性，Qi 等[193]采用氦源的 ARTP 筛选到了能利用木质纤维素水解液生长并累积油脂的 3 株突变株，其对 5-羟甲基糠醛、香兰素、甲酸、乙酸以及乙酰丙酸的耐受水平有明显提高。随着酵母胞内基因组操作技术的发展，直接通过分子层面对基因组进行随机整合或缺失是更高效的优势菌株筛选方法。Li 等[245]通过非同源末端连接(non-homologous end joining, NHEJ)介导的整合在解脂耶氏酵母基因组上产生随机突变，筛选到了可以显著提高脂肪醇积累的新蛋白靶点。

适应性实验室进化(adaptive laboratory evolution, ALE)是在实验室条件下模拟自然进化，借助人工选择压力实现微生物定向进化的方法。相比常规诱变方法的随机性，ALE 通过循序渐进施加压力促进细胞基因突变，因此定向性往往更强。在非常规酵母中，ALE 主要被应

用于改造菌株增加耐受及底物利用。Gao 等[144]利用多形汉逊酵母生产脂肪酸时发现细胞无法在甲醇中正常生长，通过 ALE (传代 18 次)成功恢复了细胞在甲醇为单一碳源中的生长，并基于基因型分析，确定了 *lpl1* 和 *izh3* 的缺失可以明显缓解甲醇代谢压力，在此基础上进行代谢优化后的脂肪酸产量可达到 15.9 g/L。Gassler 等[246]通过代谢改造赋予毕赤酵母固定并利用 CO_2 为碳源的能力，然后通过 ALE (传代 27–29 次)将 CO_2 的利用效率进一步提高，使细胞最大比生长速率 μ_{max} 由 0.008/h 提高至 0.018/h。Díaz 等[247]则利用 ALE (传代 4 个月)使原本在麦草水解物中无法生长的圆红冬孢酵母 0013-09 菌株成功恢复了 80%的生物量，结合代谢改造后可在反应器水平生产 39.5 g/L 脂质。随着分子操作技术以及高通量筛选技术的发展，将诱变技术或者分子水平的基因突变技术与 ALE 相结合的应用也越来越普遍，菌株通量也逐渐呈规模化发展，这也是非常规酵母菌种选育方面的重要发展方向[228]。

5 总结与展望

非常规酵母凭借自身的特性优势，已用于多种化合物以及蛋白质产品的工业化生产[20,248-250]。产油类酵母在油脂生产方面强大的合成及储存能力、甲基营养型酵母独特的甲醇利用能力及醇氧化酶强诱导型启动子、马克斯克鲁维酵母耐高温特性及高效的五碳糖代谢能力等都使得这些非常规酵母在部分化合物生产方面展现了独特的优势[21,171,209]。另外，广泛的底物利用谱以及对环境的高耐受性也使得非常规酵母在工业应用时，可以有效促进废弃资源利用并降低生产成本。近年来，合成生物学的快速发展使得非常规酵母中开发的表达工具及调控策略日益丰富，如具有多样化调控模式的转录器件工具库、生物传感器驱动的高通量筛选、理性设计的多细胞共培养等[160,162,167,251]。虽然很多策略目前仅在少数酵母中实现应用，不过其对产物合成水平出色的提升效果也展现了非常规酵母底盘的应用潜力。

相对酿酒酵母来说，非常规酵母在产物合成种类及产量方面仍有很大差距，主要限制在于基因编辑工具开发利用、基因表达元件挖掘表征及基础代谢背景研究等方面的缺乏。CRISPR 技术的发展为非常规酵母的基因操作提供了新的有效策略，但由于非常规酵母体内的 DNA 修复机制是以不精确的 NHEJ 机制为主，因而往往难以进行高效的外源基因整合及内源途径改造。通过敲除 Ku70/Ku80、Nej1、Dnl4 等 NHEJ 相关蛋白及过表达同源重组(homologous recombination, HR)相关蛋白(如 Rad51/52/59)等方式虽然能显著提高 HR 效率，但与酿酒酵母相比，在实际应用、优化策略以及衍生作用开发等方面仍有明显滞后[18,252]。基因编辑低效会直接影响产物途径优化的周期并增加菌株构建的工作量，因此虽然非常规酵母近几年在多种天然产物的合成上展现出了潜力，但总体上仍无法比拟酿酒酵母在异源产物合成中新工具及策略的应用速度及产量规模的突破提升。在基因表达元件方面，虽然部分代表性的酵母菌株已经完成了全基因组测序，但内源功能元件的开发表征在大部分酵母中依旧有限，尤其是强效诱导型启动子以及高效整合位点的匮乏。关于代谢网络的探究也主要局限于几条代表性途径通路，对于未知途径及功能酶的探索基本都需要借鉴酿酒酵母的背景展开，这种思路有时会影响非常规酵母内源特异性途径或机制的探究，限制了非常规酵母在天然产物合成中的表达调控优化及全局代谢改造。基于现状，为了充分发挥非常规酵母作为天然产物合成底盘的

潜力，需要对细胞代谢调控背景开展更多的研究和探索，仔细挖掘宿主在具体产物合成方面所能带来的特殊优势；另一方面，仍有必要进一步开发非常规酵母合成生物学工具，包括高效的基因组操作体系和灵活的表达调控工具。在此基础上，以非常规酵母自身独特的生理代谢机制为出发点，理性设计合成途径，结合多组学分析、高通量筛选、机器学习等先进的生物学技术优化产物合成，以实现非常规酵母在天然产物合成中高效、经济的工业化应用。

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