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Review 综 述

基因工程微生物合成鼠李糖脂表面活性剂的研究进展

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摘要：鼠李糖脂是一类重要的生物表面活性剂。相比于化学合成的表面活性剂，其具有更优秀的理化性质及环境友好等特点，被广泛应用于微生物采油、环境污染修复等工程中。目前，鼠李糖脂的工业生产主要采用铜绿假单胞菌这一具有致病性的天然合成菌株，与此同时，受菌株遗传背景的限制，优化发酵过程等方法在产量提升方面遇到了一些瓶颈问题。利用基因工程方法对菌株进行改良有望进一步提高鼠李糖脂生产的安全性、产量、产物性能等多项指标，因此受到了越来越广泛的关注。本文综述了近年来利用基因工程方法优化鼠李糖脂生物合成的最新进展，讨论了异源合成、代谢通路改造、基因表达优化、蛋白质工程、底盘工程等多种策略的应用，并展望了一系列可行的研究方向。

关键词：鼠李糖脂，生物表面活性剂，代谢工程，合成生物学，微生物采油

鼠李糖脂(rhamnolipid)是一类研究时间长、应用技术成熟的生物表面活性剂。其包含 1−2 分子鼠李糖残基组成的亲水基团和 1–2 个 β-羟基脂肪酸单元组成的疏水基团，二者通过 β-糖苷键连接。鼠李糖脂具有丰富的结构多样性(糖基数量、脂链数量、脂链长度、饱和度等)，约 60 种鼠李糖脂同类物(congener)或同系物(homologue)已被鉴定出来[1]，其分子式可表述为 Rhl-C_x、Rhl-C_x-C_y、Rhl-Rhl-C_x 或 Rhl-Rhl-C_x-C_y (Rhl 为鼠李糖；x、y 通常为 8–16)。

与传统的化学表面活性剂相比，鼠李糖脂不但具有更优异的表面活性和稳定性，还具有无毒、无污染、能生物降解、生物相容性好等优点，从而在医药、食品、农业、石油开采、环境污染修复等众多领域展示了其独特的应用前景[2–3]。例如，鼠李糖脂已被成功应用于微生物采油

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(microbial enhanced oil recovery，MEOR)中[4–5]。鼠李糖脂能够通过降低油水界面张力、乳化分散残余油、改变岩石润湿性等多种机理提高水驱采油的效率，而且能在高温、高矿化度的油藏环境中保持其性能稳定性[6–7]。在使用时，地上生产的发酵液无需进行分离纯化，直接注入地下即可，大大降低了应用成本。随着人类社会的进步发展和人们环保意识的增强，生物表面活性剂将有更加广阔的前景，有望成为传统的化学合成表面活性剂的替代品。

提升鼠李糖脂产量主要通过基因工程改造和发酵工艺优化(如培养条件、培养基成分、过程控制等)两种途径。虽然发酵优化能够显著提升鼠李糖脂的产量[8–9]，但仍存在诸多限制，例如：转化效率、发酵底物选择、产物类型等重要生产指标仍受菌种遗传背景限制；发酵罐中的高产不代表在发酵罐外的复杂应用场景(如油藏、土壤等)中的高产，自然条件下的低氧[10]、高渗[11]、高温[12]胁迫均会对鼠李糖合成产生较大负面影响；主流生产菌株铜绿假单胞菌(*Pseudomonas aeruginosa*)具有较强致病性等。因此，利用基因工程开发更高效的鼠李糖脂合成菌株受到了越来越多的关注，与发酵工艺优化具有同等重要的地位。

随着代谢工程和合成生物学技术的发展，多种改造策略被用于优化鼠李糖脂生产(指标包括产量、生产安全性、产物类型定制化等)，如代谢通路改造[13]、酶工程[14]、定向进化[15]、底盘工程[16]、实验室适应进化[17]等，未来也将有更多技术应用于其中。本文以基因工程策略为线索，对近年来基因工程微生物合成鼠李糖脂的研究进展进行综述，并对其未来发展方向作出展望。

1 鼠李糖脂的合成通路及其调控

鼠李糖脂的合成通路目前研究得较为透彻，如图 1 所示。其亲水基团来自 dTDP-L-鼠李糖。首先，葡萄糖-6-磷酸在 AlgC 的作用下转化为葡萄糖-1-磷酸，随后在 RmlA、RmlB、RmlC 和 RmlD 的作用下依次经历活化、脱水、异构、还原四步合成 dTDP-L-鼠李糖。β-羟基脂肪酸提供鼠李糖脂的疏水基团，但其究竟来源于脂肪酸从头合成(以 β-羟脂酰 ACP 的形式，由 RhlG/FabG 合成)还是脂肪酸 β-氧化(以 β-羟脂酰 CoA 的形式，由 RhlYZ 合成)仍存在争议，早期研究支持前者[18]，而多项后续研究倾向后者[19-21]。RhlA 催化 2 个 β-羟基脂肪酸单体发生酯化反应形成 HAA (hydroxyalkanoyloxy-alkanoic acid)。RhlB 和 RhlC 依次在 HAA 上引入第一、二个鼠李糖基，形成单、双鼠李糖脂。

在天然鼠李糖脂合成菌株中，鼠李糖脂的合成通常受到群体响应(quorum sensing，QS)系统调控，即细胞达到一定密度时(例如指数生长后期)才开始合成鼠李糖脂[22]。*P. aeruginosa* 中，至少有 4 套 QS 系统通过复杂的相互作用协调各个鼠李糖脂合成基因的表达，但相关机制尚未被研究清楚，详细内容可参见相关综述[23–24]。虽然细胞密度依赖的鼠李糖脂合成对于微生物适应复杂环境和执行群体行为中具有重要意义，但这在工业生产中并不是一个好性状，因为人们希望工业菌株能在整个发酵过程中持续合成鼠李糖脂。群体响应使得细菌通常在指数生长后期才合成鼠李糖脂，导致了鼠李糖脂的发酵周期过长、转化率低、生产速率低，阻碍了鼠李糖脂的工业化开发。而且，群体响应背后复杂的基因表达调控也加大了基因工程改造的难度。

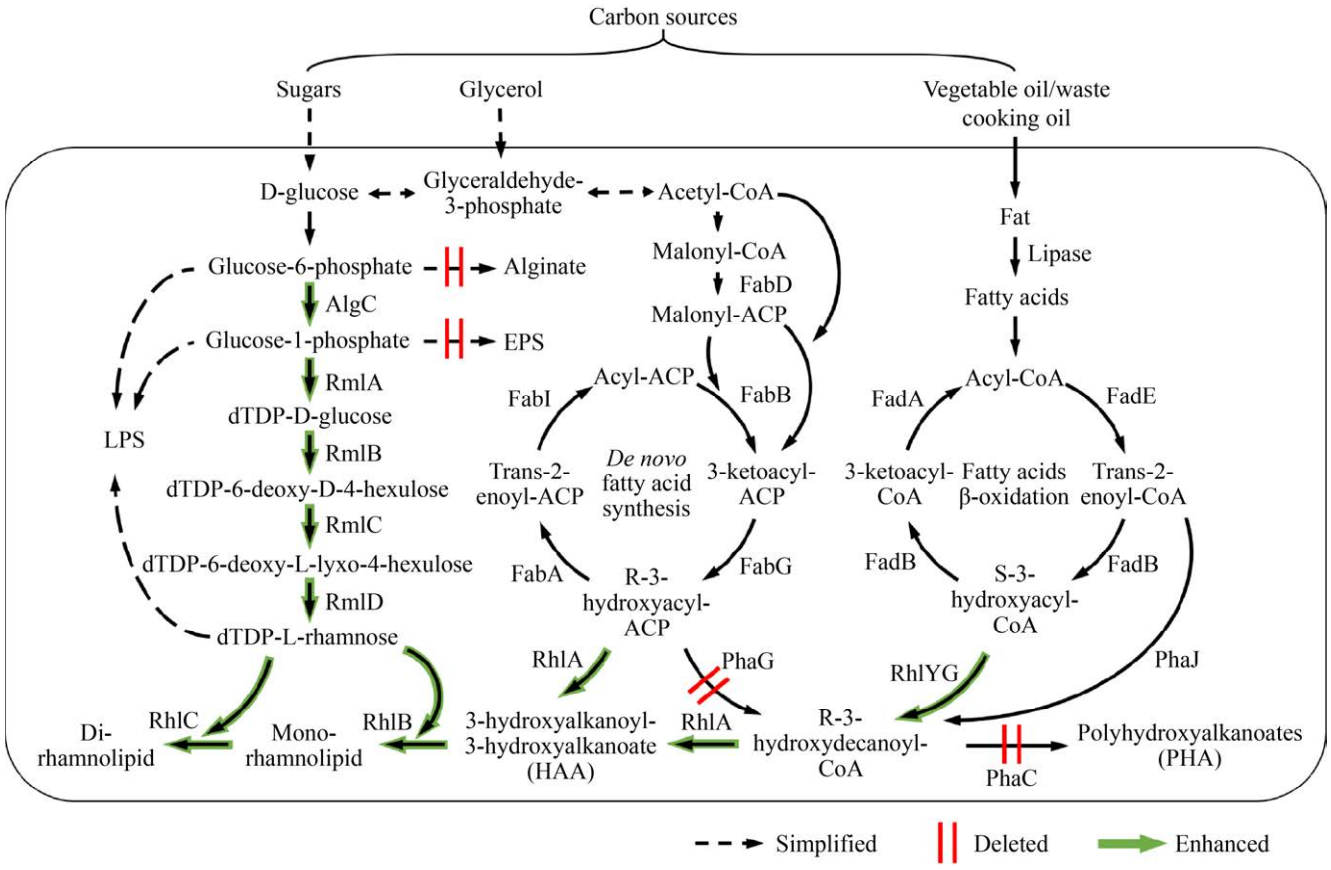

图 1. 鼠李糖脂合成通路和改造靶点

Figure 1. Rhamnolipid biosynthesis pathways and genetic engineering targets. Sugar, glycerol, and vegetable oil are most frequently used as carbon resources in research and commercial production. Precursor dTDP-L-Rhamnose derives from D-glucose. 3-hydroxy fatty acid can be synthesized via *de novo* fatty acid synthesis (in -ACP form) or fatty acid β-oxidation (in -CoA form). RhlA condenses two 3-hydroxy fatty acids to form HAA. RhlB and RhlC sequentially incorporate two rhamnose groups to the lipid part. To increase the yield, some steps can be enhanced[16,33,45,50–52] (thick arrow), while some competitive pathways can be deleted (double lines), including alginate[45], extracellular polysaccharide[13,45] (EPS), and PHA[13,16,41] biosynthesis pathways.

2 底盘微生物的选择

2.1 鼠李糖脂天然合成菌株

鼠李糖脂合成酶系天然存在于多种生物中[25]。其中，人们对假单胞菌属(*Pseudomonas*)和博克霍尔德氏菌属(*Burkholderia*)的鼠李糖脂合成研究较为深入。*P. aeruginosa* 在好氧条件下合成鼠李糖脂产量较高，是当前工业生产的主流菌株。从油藏中分离出的 *P. aeruginosa* SG 菌株能够在厌氧条件下生长并合成鼠李糖脂，在原位微生物采油(*in situ*-MEOR)中具有很好的应用前景[26]。然而，*P. aeruginosa* 作为一种条件致病菌，生物安全性较差。*Burkholderia* 属的某些菌种虽然生物安全性较好，且能够合成表面活性更优的长链鼠李糖脂，但其发酵速度慢、产量较低、且遗传背景不够清晰，很少真正用于工业生产中。

2.2 鼠李糖脂的异源合成

由于天然合成菌株致病性等一些缺点，鼠李糖脂在公认为安全级(generally recognized as safe，GRAS)微生物中的异源合成受到了广泛关注。鼠李糖脂的两个重要前体 dTDP-L-鼠李糖和 β-羟基脂肪酸合成通路在各种微生物中普遍存在，理论上只需异源表达 *rhlABC* 基因即可实现鼠李糖脂的合成。

异源合成的首选是假单胞菌属的其他菌种。德国亚琛工业大学 Lars M. Blank研究组致力于利用恶臭假单胞菌(*Pseudomonas putida* KT2440)合成鼠李糖脂。*P. putida* 作为假单胞菌属模式菌株，被广泛用作细胞工厂底盘(chassis)，具有较好的生物安全性[27]。研究发现，*P. putida* 能耐受高达 90 g/L 的鼠李糖脂，而且人工引入的鼠李糖脂合成通路不受 QS 的调控，能够实现在各个阶段合成鼠李糖脂，而非仅在生长后期合成[28]。中国科学院沈阳应用生态研究所张颖研究组在利用厌氧微生物合成鼠李糖脂上进行了一系列研究工作[29]。Zhao 等在施氏假单胞菌 *Pseudomonas stutzeri* DQ1 中引入 *rhlABRI* 基因，首次实现了用厌氧微生物异源合成鼠李糖脂，厌氧条件下产量可达 1.61 g/L[30]。还有一些工作利用绿针假单胞菌[31] (*Pseudomonas chlororaphis*)、防御假单胞菌[32] (*Pseudomonas protegens*)等底盘合成鼠李糖脂。值得一提的是，上述两个菌种不但安全性好，还可用于生物防治，有望扩展鼠李糖脂在农业中的应用。

大肠杆菌(*Escherichia coli*)是应用最广泛、基因工程操作最为成熟的底盘微生物。中国科学院天津工业生物技术研究所王钦宏研究组在异源合成鼠李糖脂上取得了一系列进展[15,33–34]。虽然大肠杆菌获得的鼠李糖脂产量较低(< 1 g/L)，但便于快速验证一些概念，能够为其他高产菌种的改造工作提供重要参考。

除了原核生物，在真核生物酿酒酵母(*Saccharomyces cerevisiae*) 中异源合成鼠李糖脂的概念也得到了初步验证[35]。

3 合成生物学策略在鼠李糖脂合成中的应用

合成生物学是指在工程学思想指导下，按照特定目标理性设计、改造乃至从头合成人工生物体系[36]。细胞工厂的设计和构建是合成生物学的一个重要研究方向，目前已发展了从分子、途径到基因组各个层次的微生物细胞工厂设计和工程化构建策略，相比于传统的筛选、诱变育种等非理性设计方式，创制效率大幅提升[37]。对于代谢途径中的关键酶，合成生物学提供了多种蛋白质工程手段来优化其催化性能[38]；在代谢途径层面，合成生物学提供了大量基因表达调控元件来精细调控甚至动态调控各个途径酶的表达量[39]；蓬勃发展的基因编辑和 DNA 合成技术使大规模改造甚至从头合成宿主基因组成为可能，将为细胞工厂创造更优良的底盘生物[40]。在上述策略的指导下，产鼠李糖脂细胞工厂的构建近年来也取得了长足进步(图 2，表 1)。

3.1 代谢通路优化提升前体物质供给

删除竞争旁路或过表达通路中的关键酶是提升目标通路中代谢流的常用方法。对于鼠李糖脂合成，提升脂前体 β-羟基脂肪酸和糖前体 dTDP-L-鼠李糖合成通路中的代谢流都可以提高鼠李糖脂产量。

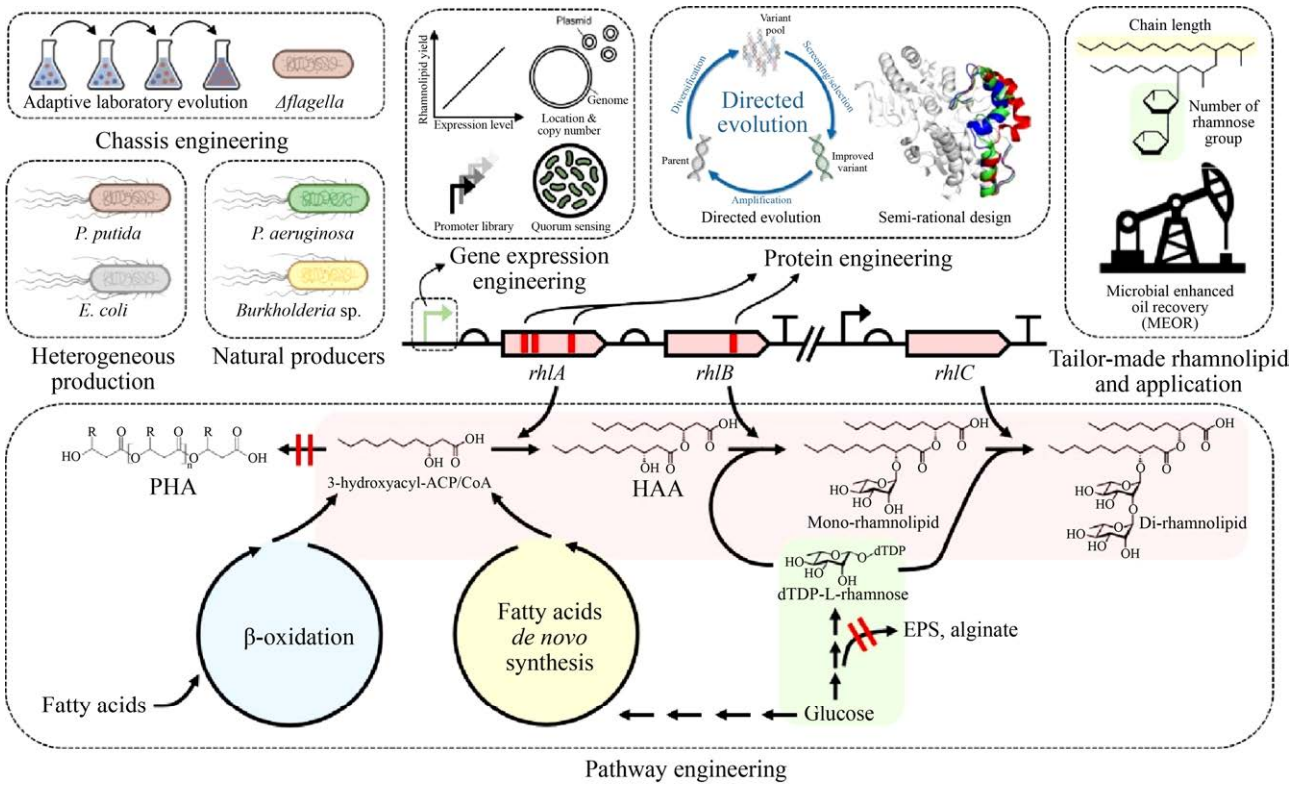

图 2. 基因工程微生物合成鼠李糖中应用的代谢工程策略

Figure 2. Overview of the engineering strategies used to optimize rhamnolipid production. These strategies include heterogeneous production (mainly *P. putida*[16,28,50,51,70–71], *E. coli*[15,33–34]), pathway engineering, gene expression engineering[50–54,56], chassis engineering[16–17,60], and protein engineering[14–15]. Besides increasing the yield, tailor-made rhamnolipid production[69–71] also draw more and more attentions.

假单胞菌中，聚羟基脂肪酸酯(polyhydroxyalkanoates, PHA)合成与鼠李糖脂合成共享 β-羟基脂肪酸前体。多项工作涉及删除 PHA 合成通路，将代谢流引向鼠李糖脂合成[13,16,41]。在 *P. aeruginosa* 中过表达脂肪酶 EstA[42–43]和 LipC[44]也可提升鼠李糖脂的产量，但机制尚不明确，猜测可能与脂肪代谢有关。

胞外多糖(exopolysaccharide，EPS)的合成消耗 dTDP-L-鼠李糖合成通路中的 dTDP-L-葡萄糖。Lei 等敲除了 *P. aeruginosa* SG 的胞外多糖合成基因 *pslAB*，鼠李糖脂产量较野生型提升 21%，在此基础上再敲除 *phaC1DC2*，产量较野生型提升 69.7%，达到 21.496 g/L[13]。

Du 等在 *E. coli* 中优化 *rhlABC* 的表达量，并引入额外 *rfbD* 基因(同假单胞菌中 *rmlD* 基因)拷贝以提高前体物质 dTDP-L-鼠李糖的供给，鼠李糖脂的产量从 0.446 g/L 提高至 0.64 g/L，创造了利用大肠杆菌合成鼠李糖脂的最高产量[33]。

组合使用多种策略在一定程度上可以产生叠加效果。一项来自中国科学院微生物研究所的专利将 *P. aeruginosa* PAO1 一系列消耗前体物质的旁路基因(胞外多糖合成基因簇 *pslA-pslO* 和 *pelA-pelG*、藻酸盐合成基因簇 *algD-alg8-alg44-algKEGXLIJF*、PHA 合成基因簇 *phaC1-D-C2*)逐步替换为有助于

表 1. 基因工程微生物合成鼠李糖脂研究工作概览

Table 1. Overview of engineered strains for rhamnolipid production

Chassis	Engineering strategies	Medium and carbon source	Maximum yield	Increased/%	References
P. aeruginosa SG	Blocking exopolysaccharide and PHA synthesis pathways	60 g/L glycerol	21.496 g/L	69.7	[13]
P. putida KT2440	Chromosomally integrated *rhl* genes expressed by constitutive promoter P_{14ffg}; Deleting flagella machinery	Delft medium with 10 g/L glucose	~1.1 g/L	130	[16]
P. putida KT2440	Chromosomally integrated *rhl* genes expressed by constitutive promoter P_{14ffg}; Blocking PHA synthesis	Delft medium with 10 g/L glucose	~1 g/L	115	[16]
P. putida KT2440	Chromosomally integrated *rhl* genes expressed by salicylate inducible promoter (NagR/P_{nagAa})	LB medium with 10 g/L glucose	~1 g/L (high genetic stability)	–	[16]
P. aeruginosa PA14	Blocking PHA synthesis and over-expressing the rhlAB-R operon	PPGAS medium	~1500 μmol/L	59	[41]
P. aeruginosa PAO1	Overexpressing EstA	PPGAS medium (0.5% glucose)	1.7 g/L	325	[42]
P. aeruginosa PAO1	Overexpressing EstA	42 g/L glycerol	22 g/L	290	[43]
P. aeruginosa PAO1	Overexpressing LipC	M9-minimal broth medium (4 g/L glucose)	0.8 g/L	100	[44]
E. coli BL21 (DE3)	Co-expressing *rhlABC* and *rfbD*	LB medium with 0.4% Glucose	0.64 g/L	43	[33]
P. aeruginosa PAO1	Blocking exopolysaccharide, alginate, PHA synthesis and overexpressing *rmlBDAC*, *rhlYZ*, *algC*, *fadD4*, *lipC/estA*	MS medium (75 g/L palm oil)	43.7 g/L	108	[45]
E. coli BL21 (DE3)	Directed evolution of RhlB (L168E)	Mineral salt or LB medium with 0.4% glucose	Surface tension: from 0.26 to 0.021 mN/m	–	[15]
P. aeruginosa PA14	Semi-rational evolution of RhlA (R202K)	MSM-glycerol broth (15 g/L glycerol)	800 mg/L·OD_{600}	101	[14]
P. putida KT2440	Using synthetic promoter library to drive the expression of *rhlA-rhlB-gfp* operon (biofilm as the production platform)	FAB medium with 1 mmol/L sodium citrate	Yield linearly correlated with enzyme level	–	[50]
P. putida KT2440	Synthetic promoter library	LB medium with 10 g/L glucose	2.8 g/L	–	[51]
P. aeruginosa SG	Plasmid-encoded *rhlAB* fused with the strong *oprL* promoter	Glycerol-nitrate medium (45 g/L glycerol)	20.98 g/L	183	[52]
B. thailandensis E264	Deleting three QS systems (TΔ*btaI1*Δ*btaI2*Δ*btaI3*)	Nutrient broth with 4 g/L glycerol	4.46 g/L	374	[53]
B. thailandensis E264	Releasing QS's repression on *rhl* genes by deleting *scmR*	Nutrient broth (NB) medium with 2 or 4% glycerol	3 g/L	200	[54]
P. aeruginosa PAK	Mutating the 5′UTR of *rhlI* to release repression by sRNA P27	M8-based agar plate with 0.2% glucose	Diameter ratio from 1.72 to 1.95 (CTAB agar test)	–	[56]
P. aeruginosa (NRRL B–771)	Expressing the *Vitreoscilla* hemoglobin gene (vgb)	MM media with 1% glucose	8.373 g/L	100	[60]
P. putida KT2440	Performing adaptive laboratory evolution in order to use ethanol as carbon source and defoamer	Modified M9 medium with 0.96% ethanol	0.94 g/L	47	[17]

–: none.

提升产量的基因(*rmlBDAC*、*rhlYZ*、*algC*、*fadD4*、*lipC*/*estA*),产量从 21 g/L 逐步提升至 43.7 g/L[45]。

3.2 蛋白质工程优化鼠李糖脂合成酶

除了优化代谢通路提升目标通路上的代谢流量，也可采取蛋白质工程手段直接对酶进行改造。蛋白质工程经历了从定向进化到计算设计的发展历程，前者需要进行多轮大规模的突变和筛选从而引导蛋白质朝着人类想要的方向进化，而后者基于对蛋白质结构和机理的研究以及高性能计算方法从头设计蛋白质[46]。

由于 RhlA、RhlB、RhlC 均缺少实验获得的晶体学结构数据，已有工作均采用定向进化[47] (directed evolution)和半理性设计[48] (semi-rational design)两种较为初级的方式对鼠李糖脂合成酶进行改造。2014 年，Han 等首次将定向进化的方法引入鼠李糖脂代谢工程研究中。该研究对 *rhlB* 基因进行易错 PCR，并利用鼠李糖脂的抗菌性设计了一种通量较高的筛选方案：在发酵上清液中培养枯草芽孢杆菌，*OD* 值小说明鼠李糖脂合成量高。该方案筛选出了 L168 这一关键位点，将其突变为谷氨酸后，产物中长链鼠李糖脂比例高，表面活性和模拟驱油能力都有所提升[15]。2019 年，Dulcey 等对 RhlA 进行了“半理性”改造。该研究利用同源建模(homology modeling)、点突变、构建嵌合酶等方式鉴定了一系列参与催化和底物识别的重要位点，其中，针对负责底物识别的 cap-domain 的一些突变可以提升酶对底物的识别效率(尤其是长链底物)，从而提升产量以及产物中长链鼠李糖脂的比例。其中，Y165F 和 R202K 突变使产量提升约一倍[14]。

3.3 基因表达层面的优化

在一定范围内，细胞工厂产量与关键合成酶的表达量直接相关[49]。通过采用强启动子、增加基因拷贝数等方式提高合成酶的表达量也是提高鼠李糖脂产量的一种方式。

Wigneswaran 等在 *P. putida* 中构建了人工合成启动子文库(synthetic promoter library，SPL)，用于驱动 *rhlA-rhlB-gfp* 多顺反子的表达。用高效液相色谱-高分辨率质谱(UHPLC-HRMS)和流式细胞仪分别对鼠李糖脂产量与鼠李糖脂合成酶表达量进行精确定量，发现二者在很大范围内具有线性相关性[50]。

Tiso 等以鼠李糖脂合成为例验证了一种“需求驱动” (driven-by-demand)的代谢工程策略。该策略基于“外周代谢途径受转录调控，而中心碳代谢途径在代谢反应层面被调控”这一事实。在 *P. putida* 中，通过较强的组成型启动子提高 *rhlAB* 基因的表达量后，为鼠李糖脂合成提供前体物质的中心碳代谢途径上的代谢流也随之提升，以满足鼠李糖脂合成这一外周代谢途径的需求，解释了产量与基因表达量的相关性[51]。

除了启动子强度，基因拷贝数可以通过剂量效应(gene dosage effect)影响基因表达水平。Zhao 等在能够厌氧合成鼠李糖脂的 *P. aeruginosa* SG 中引入质粒编码的 *rhlAB* 基因，并由 *oprL* 基因的组成型启动子驱动表达，鼠李糖脂产量从 11 g/L 提升至 21 g/L[52]。

质粒虽然由于剂量效应表达量高，但稳定性较差，需要抗生素来维持以避免丢失，不利于工业生产。Tiso 等利用 Tn7 转座子将鼠李糖脂合成基因整合入 *P. putida* 基因组中，解决了这一问题。

然而，即使在基因组上，过强的组成型表达也会为宿主造成代谢压力，导致基因组不稳定，自发地将目的基因丢失。采用水杨酸诱导型启动子驱动鼠李糖脂合成基因表达不但增强了工程菌株的稳定性，还获得了更好的产量[16]。

随着人们对群体响应(QS)控制鼠李糖脂合成机制理解的加深，QS 系统成为了提升鼠李糖脂产量的新靶标。在 *Burkholderia thailandensis* 中，QS 系统在指数生长期抑制鼠李糖脂合成酶基因的表达。敲除 *B. thailandensis* 3 条 QS 途径的信号分子合成酶，鼠李糖脂产量从 0.94±0.06 g/L 提升至 4.46±0.345 g/L[53]。后续研究发现，在指数生长期，QS 系统通过激活 *scmR* 基因抑制鼠李糖脂合成基因表达，同时促进 PHA 合成基因的表达，因此，*ΔscmR* 菌株能更早地开始合成鼠李糖脂，同时将更多代谢流从 PHA 合成引向鼠李糖脂，具有更高产量[54]。然而，不同菌种的 QS 系统对鼠李糖脂合成的调控模式不同。例如，在 *P. aeruginosa* 和 *Burkholderia glumae* 中，QS 对鼠李糖脂合成基因起正调控作用，敲除后会降低产量[22,55]，若要提升产量，则需要上调群体响应水平。*P. aeruginosa* 中，小 RNA (small RNA，sRNA)在群体响应调控中扮演着重要角色，sRNA P27 通过与群体响应关键组分 *rhlI* mRNA 的 5′UTR 相互作用抑制群体响应，从而下调鼠李糖脂合成，通过点突变解除 sRNA P27 对 *rhlI* mRNA 的抑制作用可以提高鼠李糖脂产量[56]。值得注意的是，对于铜绿假单胞菌社会行为的基础研究表明，将鼠李糖脂合成与 QS 解偶联后，利用群体生产的鼠李糖脂而自身不合成鼠李糖脂的“欺骗者”的比例会逐渐提高[57]，这种现象对时间跨度较长的发酵过程可能产生一定影响。

3.4 底盘工程

细胞工厂的产量与宿主的生理活动密切相关[58]。在不改变合成线路的情况下对底盘生物进行优化，也能够间接提升细胞工厂的产量。

透明颤菌血红蛋白(*Vitreoscilla* hemoglobin，VHb)能够提升细胞对氧气的摄取能力，全局性地优化细胞生长，被广泛应用于代谢工程中[59]。在 *P. aeruginosa* 中引入 VHb 的同时增加供氧量，鼠李糖脂产量从不足 4 g/L 提升至 8.373 g/L[60]。

实验室适应性进化(adaptive laboratory evolution，ALE)也是一种常用的菌种改造手段[61]。Bator 等对 *P. putida* KT2440 进行实验室适应性进化，获得了能高效利用乙醇为碳源合成鼠李糖脂的菌株，同时，乙醇作为消泡剂解决了发酵过程中的泡沫问题，通过对进化而来的菌株进行基因组重测序，乙醇适应性产生的机理也得到揭示[17]。

基因组中许多基因虽然不直接与目标合成通路竞争底物，但其表达和复制消耗了大量能量，对基因组进行精简有助于提高细胞工厂产量[62]。Tiso 等删除鞭毛相关基因，使更多能量能够用于鼠李糖脂合成，*P. putida* 产量从 0.47 g/L 提高了 130%[16]。

4 鼠李糖脂的“定制化”合成

微生物合成的鼠李糖脂是由多种同系物组成的混合物，不同类型的鼠李糖脂具有不同的性质。例如，在微生物强化采油中，单、双鼠李糖脂具有不同的界面活性、润湿性能、洗油效率等性质，在总量不变的情况下，优化配比也能显著提升采油性能[63–66]。此外，单、双鼠李糖脂的比例还影响鼠李糖脂的抗菌性[67]，烃链长度也和表面活性呈现正相关性[68]。基于不同应用场景对于

不同类型鼠李糖脂的需求, Wittgens 等提出了“定制化”合成鼠李糖脂(tailor-made rhamnolipids)的概念[69]。

目前，基因工程方法能够控制鼠李糖脂的糖基数量和脂链长度两个参数。仅表达 *rhlA* 和 *rhlB* 合成单糖鼠李糖脂, 再表达 *rhlC* 则可合成双糖鼠李糖脂。脂链长度与酶的底物选择性有关。来自 *P. aeruginosa* 的合成酶能够合成中等脂链长度(C_8 至 C_{12})的鼠李糖脂，以 C_{10}-C_{10} 为主，而来自 *Burkholderia* 属(如 *B. glumae*)的合成酶能合成 C_{12} 至 C_{16} 的长链鼠李糖脂。在 *E. coli* 中转入来自 *P. aeruginosa* 和 *B. pseudomallei* 的 RhlAB 和 RhlC，获得了烃链长度分布不同的单、双鼠李糖脂[33]。在 *P. putida* 中组合来自 *P. aeruginosa* 和 *B. glumae* 的 *rhlA* 和 *rhlB*，获得了烃链长度分布不同的单鼠李糖脂[70–71]。前文中提到的利用半理性设计和定向进化手段对 RhlA 和 RhlB 酶的改造都增大了底物口袋，使得长链底物更好进入，提高了长链鼠李糖脂的比例[14–15]。

5 展望

本课题组长期致力于微生物产鼠李糖脂表面活性剂在石油开采中的应用，目前已经获得比较理想的效果[63]，对不同条件和性质的油藏也有较好的适应性[7]，但是其大规模应用还要进一步降低生产成本。由于鼠李糖脂在油田中使用时不需要进行提取和纯化，直接注入发酵菌液即可，而且可以采用废弃油脂等低成本原料作为培养基[72–73]，因此生产成本主要由产率决定。而产率的决定性因素是菌株本身，所以通过基因工程手段提升产率对于鼠李糖脂在石油开采中的应用具有重要意义。

上述利用基因工程微生物合成鼠李糖脂的工作主要集中在异源合成、产量提升、定制化合成 3 个方面。虽然应用了多种改造策略，且取得了一些研究进展，但多数研究工作仍围绕基因敲除与过表达等传统代谢工程手段。快速发展的合成生物学技术有望为鼠李糖脂合成带来新的工具和思路，进一步提升鼠李糖脂的产量(图 3)。

(1) 应用基于 CRISPR 的基因编辑工具。迄今为止，所有对产鼠李糖脂微生物进行基因改造的研究工作均采用转座子插入[74–75]和同源重组[76–77]两种方式, 费时费力。近年来, 基于 CRISPR 的基因编辑技术发展迅速：假单胞菌属的 CRISPR 基因编辑工具箱能够以接近 100%的效率进行基因突变、删除、插入以及单碱基编辑[78]；CRISPR 干扰系统[79] (CRISPR interference, CRISPRi)也在假单胞菌中成功建立[80]，无需对靶基因进行突变即可实现对任意基因表达的控制。上述方法将大大加速增产基因筛选及工程菌构建的速度。

(2) 采用日益丰富的基因表达调控元件。如前文所述，鼠李糖脂产量与合成酶的表达量密切相关。近年来，*P. putida* 的常表达启动子库被成功构建[74,81], 能在很大范围内调节基因的表达量。此外, 来自噬菌体的 T7 或类 T7 聚合酶表达系统具有表达强度高、可调性好、能跨物种使用等优点[82]，有望在非模式微生物中实现高产鼠李糖脂。

(3) 利用合成蛋白支架 (synthetic protein scaffold)。合成支架可以将一条通路中的多个酶在空间上共定位，通过提高酶和反应物的局部浓度，产生“底物通道”效应，提升反应效率[83–84]。利用该支架将鼠李糖脂合成通路中的某些酶共定位(如 RmlD、RhlG、RhlYZ、RhlA、RhlB)，有望提高鼠李糖脂产量。

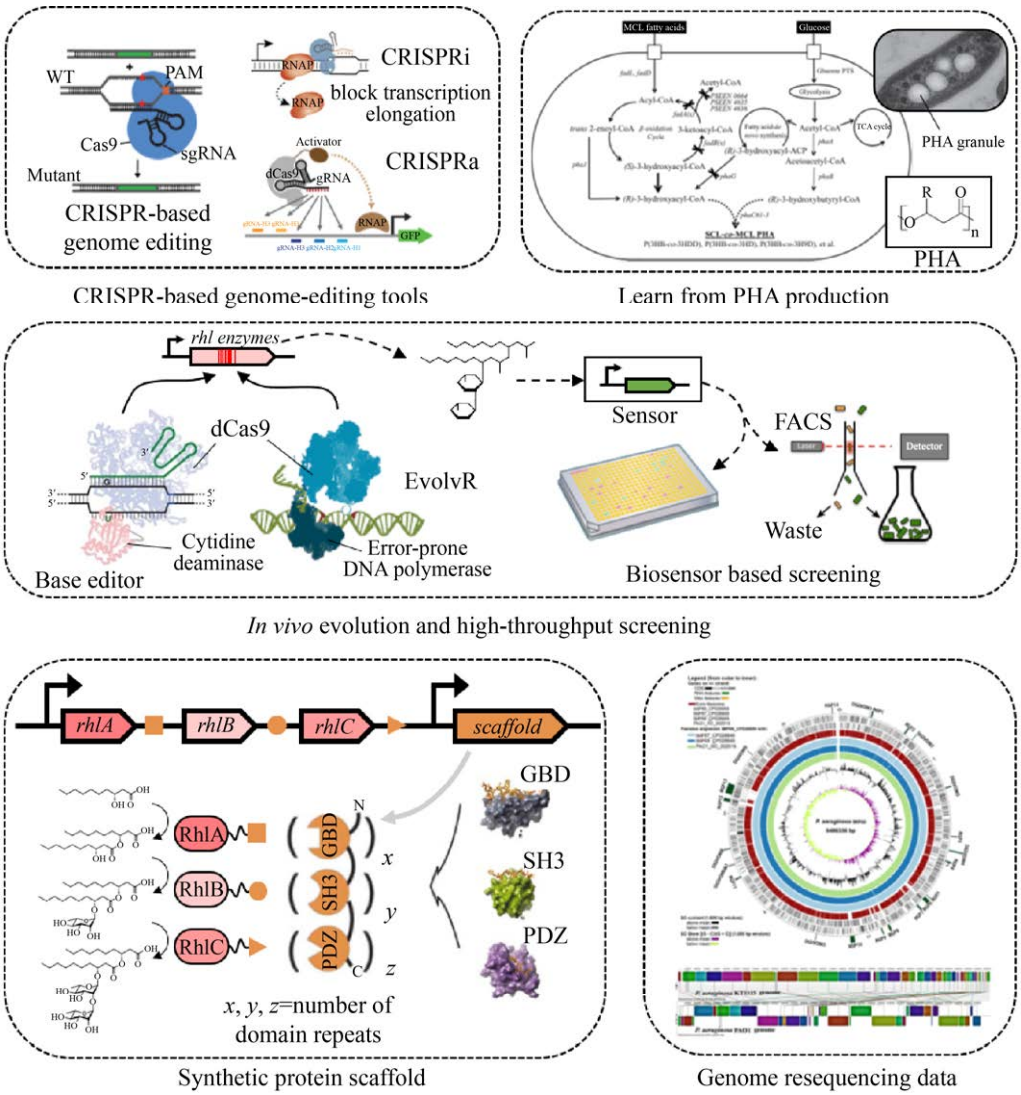

图 3. 可用于提升鼠李糖脂产量的潜在策略

Figure 3. Potential strategies that can be used to optimize rhamnolipid production. Available tools include the CRISPR-based genome engineering toolkit[78–80], *in vivo* directed evolution platform[78,87], synthetic protein scaffold[83–84], etc. Besides, we can learn from medium-chain PHA production[86], which shares same precursors with rhamnolipid, and genome resequencing data of rhamnolipid high-yield strains[90–91].

(4) 借鉴 PHA 增产思路。PHA 与鼠李糖脂在代谢通路上具有高度相关性[85]。作为一种性能优秀的生物聚合物生物产品，PHA 的研究和产业化比鼠李糖脂要成熟得多，多种合成生物学策略都已成功应用于 PHA 生产中[86]。国内，清华大学陈国强研究组和蓝晶微生物科技有限公司(Bluepha Co.,Ltd.)在 PHA 合成领域处于世界领先地位。PHA 的增产策略有望为鼠李糖脂提供思路。

(5) 构建体内定向进化及高通量筛选平台。3.2 中提到的两项改造鼠李糖脂合成酶的工作[14–15]均需要人工在体外构建突变体库，费时费力。而体内进化方法能在特定区域高效引入突变。例

如，一种名为 EvolvR 的系统可对长达 250 bp 的目标窗口进行高效突变[87]。单碱基编辑器可对较小的突变窗口(3–8 bp)进行突变[78]。除突变外，定向进化的另一个要素是筛选。传统的鼠李糖脂定性、定量检测方法[88]难以满足定向进化对高通量筛选的需求[14]，开发一种鼠李糖脂生物传感器或许是一种可行方法[89]。

(6) 挖掘高产菌株的基因组学数据。Liu 等对一株高产双鼠李糖脂的铜绿假单胞菌进行重测序，发现其特异地表达多种脂肪酶基因，猜测与该菌株能够高效利用油脂合成鼠李糖脂有关，同时还在 *rhlI*、*rhlA*、*rmlA*、*rmlC* 基因上发现了一些单核苷酸多态性位点，可能与酶的高催化活性有关[90]。Xu 等对油田中分离的 3 株具有不同鼠李糖脂产量的 *P. aeruginosa* 进行重测序，发现群体响应强度对鼠李糖脂合成影响很大[91]。这些重测序数据为鼠李糖脂工程菌的改造提供了宝贵的思路。

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Recent advances in the production of rhamnolipid biosurfactant by genetically engineered microorganisms

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Abstract: Rhamnolipid is an important family of biosurfactant synthesized by many natural or engineered bacteria. Due to its excellent chemo-physical properties and environmental friendliness, it has been extensively used in many application scenarios (e.g., microbial enhanced oil recovery (MEOR), bioremediation), in place of its chemically synthesized counterparts. In commercial production, rhamnolipid is mainly produced by *Pseudomonas aeruginosa*, a natural producer with pathogenicity. Its genetic context also limits further enhancement of production using fermentation optimization approach. As a solution, genetic engineering approach can improve the bio-safety, yield, and product performance of rhamnolipid production, which has attracted increasing attention. However, few review articles systematically discussed this topic. Herein, we summarized recent advances in rhamnolipid production using genetically engineered microorganisms, particularly focusing on engineering strategies applied (e.g., heterogeneous production, pathway engineering, gene expression engineering, protein engineering, chassis engineering). Other than high yields, tailor-made rhamnolipid production (producing rhamnolipid mixture of specific composition) was also discussed. Finally, we proposed several potential strategies which may benefit rhamnolipid production: (1) using CRISPR-based tools for genome engineering; (2) gene expression optimization using synthetic promoter library and T7 polymerases; (3) synthetic protein scaffold for enzyme colocalization; (4) learning from polyhydroxyalkanoates (PHA) bioengineering; (5) development of *in vivo* directed evolution platform and rhamnolipid biosensor; (6) analysis of genome resequencing data.

Keywords: rhamnolipid, biosurfactant, metabolic engineering, synthetic biology, microbial enhanced oil recovery

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