



大肠杆菌合成中链脂肪酸研究进展

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摘要 中链脂肪酸(medium-chain fatty acids, MCFAs)及其衍生化学品在能源、医药、化工等诸多领域具有重要的应用价值。微生物发酵生产MCFAs是一条绿色、可持续的路线。大肠杆菌(*Escherichia coli*)可利用天然脂肪酸合成(fatty acid biosynthesis, FAB)路径和逆向 β 氧化(reversed β -oxidation, RBO)路径合成MCFAs。MCFAs生产存在链长难控制、合成效率低、细胞损伤大等问题。近年来, 工程大肠杆菌合成MCFAs取得了重大进展。通过链长控制蛋白的筛选与改造, MCFAs合成特异性得以提高; 通过增加前体供应和强化产物释放, 对合成路径进行“推-拉”, MCFAs合成效率得以改善; 通过膜工程改造、应激响应调控及适应性进化, 工程菌株对MCFAs的耐受性得以增强。本文基于大肠杆菌合成MCFAs的两种路径, 系统综述了近年来控制产物链长、优化合成路径和增强细胞耐受的工程化策略, 并展望了后续改善MCFAs合成的研究方向。

关键词 大肠杆菌, 中链脂肪酸, 代谢工程, 链长, 耐受

中链脂肪酸(medium-chain fatty acids, MCFAs)是碳原子数为6~12的直链脂肪酸的统称, 主要包括己酸($C_6H_{12}O_2$)、辛酸($C_8H_{16}O_2$)和癸酸($C_{10}H_{20}O_2$)等。MCFAs在食品、营养、医疗和化工行业有广泛的应用, 可直接用作香料、抗菌剂、润滑剂、增稠剂和橡胶加工助剂等^[1]。另外, MCFAs可以转化为中链烷烃用作汽油和航空燃油^[2], 或酯化为中链甘油三酯, 用作食品添加剂和营养辅助剂^[3]。因此, MCFAs具有巨大的市场需求和经济价值。目前MCFAs及其衍生物主要是从石油原料或植物生物质中提取而来, 存在可持续性差、环境不友好和效率低等问题^[4]。利用微生物以可再生物质为底物发酵生产MCFAs, 是一条可持续、绿色、高效的中链化学品合成路线, 有助于实现“2030年碳达峰、2060年碳中和”的目标。

大肠杆菌具有复制速度快、遗传清晰易驯服、工艺放大简单、基因操作工具丰富等特点, 是研究代谢机制和工程改造常用的模式微生物^[5~7]。某些大肠杆菌作为工业细胞工厂已用于生产“一般公认安全”(generally recognized as safe, GRAS)的物质, 如花青素、姜黄素、2'-岩藻糖基乳糖和L-蛋氨酸等^[8,9]。大肠杆菌可以利用天然脂肪酸合成(fatty acid biosynthesis, FAB)路径或逆向 β 氧化(reversed β -oxidation, RBO)路径合成MCFAs, 但是存在链长难控制、合成效率低、细胞损伤大等问题。几十年来, 随着代谢工程、合成生物学及高通量测序等技术手段的发展和完善, 工程改造大肠杆菌合成MCFAs已取得重大进展^[10]。本文简介MCFAs合成路径, 探讨MCFAs链长控制及细胞损伤机理, 系统综述MCFAs链长调控、路径优化、细胞耐受的研究,

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并提出后续工作的方向。

1 MCFAs合成路径

脂肪酸的合成通过碳链延伸和碳链解离过程完成。大肠杆菌中天然的碳链延伸路径并非倾向形成中链产物。大量研究通过调控碳链延伸和解离来实现MCFAs合成。目前常用的合成路径包括FAB路径和RBO路径。

1.1 天然脂肪酸合成路径

大肠杆菌使用II型脂肪酸合酶(fatty acid synthase, FAS)进行碳链延伸,形成脂酰-酰基载体蛋白(acyl carrier protein, ACP)。大肠杆菌FAB路径起始于二碳供体乙酰-CoA,乙酰-CoA羧化酶(ACC)消耗ATP羧化乙酰-CoA形成丙二酰-CoA,丙二酰-CoA由丙二酰-CoA:ACP转酰基酶(FabD)催化转酰基生成丙二酰-ACP。随后通过3-酮脂酰-ACP合酶(FabH)的缩合形成乙酰乙酰-ACP,经3-酮脂酰-ACP还原酶(FabG)还原成3-羟基丁脂酰-ACP,经3-羟基脂酰-ACP脱水酶(FabA或FabZ)催化为2-丁烯酰-ACP,经烯脂酰-ACP还原酶(FabI)催化为丁脂酰-ACP,丁脂酰-ACP与丙二酰-ACP再次缩合由此进入下一轮循环反应。3-酮脂酰-ACP合成酶(FabB或FabF)、3-酮脂酰-ACP还原酶(FabG)、3-羟基脂酰-ACP脱水酶(FabA或FabZ)和烯脂酰-ACP还原酶(FabI)催化接下来每轮碳链延伸中的四步反应。每一次循环加入一个丙二酰-ACP分子,脂酰-ACP碳原子数目增加

2(图1(a))。

生成的脂酰-ACP被硫酯酶水解,可使相应的脂肪酸链解离,形成游离脂肪酸(图1(a))。体外表征实验表明,大肠杆菌内源硫酯酶(TesA、TesB、YciA、FadM、YdiI和YbgC)的活性随着底物酰基-CoA碳链长度的增加而增加^[11]。延伸循环通常在合成长链(C16~C18)酰基-ACP后停止^[10],脂酰-ACP进入脂质合成过程,主要用于膜生物合成和细菌群体感应等其他过程^[12]。利用代谢工程过表达中链酰基-ACP特异性的硫酯酶或借助蛋白质工程改造大肠杆菌内源长链酰基-ACP特异性硫酯酶,可实现MCFAs合成^[13~15](表1)。

1.2 逆向 β 氧化路径

大肠杆菌利用 β 氧化路径将形成的脂肪酸降解为乙酰-CoA,典型的 β 氧化循环中每步酶催化反应都是可逆的,这为构建RBO路径以合成脂肪酸提供了可能性。Ramon Gonzalez课题组^[22]首次提出RBO路径,通过突变或敲除分解代谢阻遏物Crp、全局调节因子ArcA、乙酰乙酸代谢调节蛋白AtoC和脂肪酸代谢调节蛋白FadR编码基因,解除其对 β 氧化路径酶的调控,实现 β 氧化路径酶的组成型表达,并通过一轮RBO循环合成丁酰-CoA进而还原为正丁醇。另外,Ramon Gonzalez课题组^[11]通过蛋白纯化与体外表征实验确定了RBO路径的核心功能单元并合成了多种C4羧酸。

RBO路径以乙酰-CoA为延伸单元,是CoA依赖的

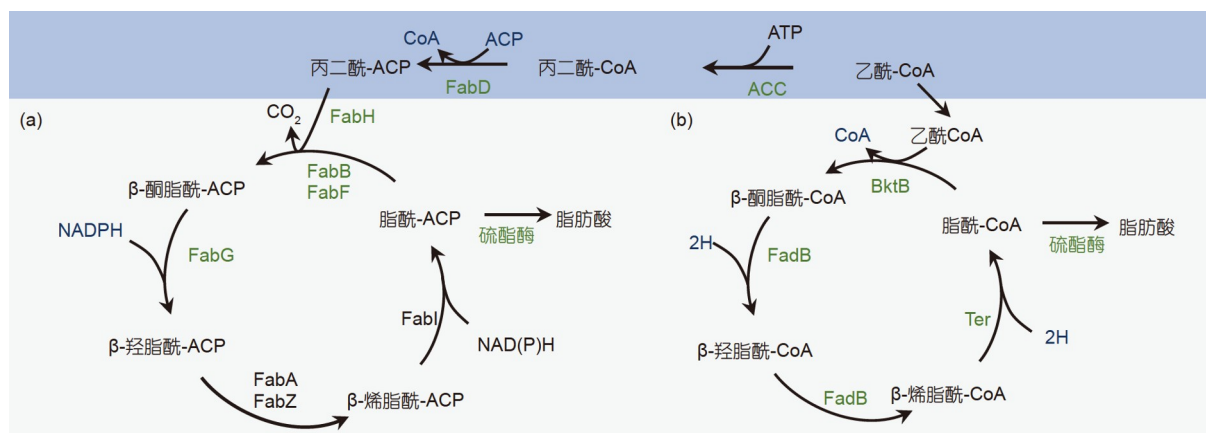


图1 (网络版彩色)大肠杆菌MCFAs合成路径。(a) FAB路径。ACC: 乙酰-CoA羧化酶; FabD: 丙二酰-CoA:ACP转酰基酶; FabH: 3-酮脂酰-ACP合酶; FabG: 3-酮脂酰-ACP还原酶; FabA/FabZ: 3-羟基脂酰-ACP脱水酶; FabI: 烯脂酰-ACP还原酶; FabB/FabF: β -酮脂酰-ACP合酶。(b) RBO路径。BktB: 硫酯酶; FadB: 3-羟基脂酰-CoA脱氢酶/烯脂酰-CoA水合酶; Ter: 脂酰-CoA脱氢酶/反式-烯脂酰-CoA还原酶

Figure 1 (Color online) MCFAs synthetic pathway in *E. coli*. (a) FAB pathway. ACC: Acetyl-CoA carboxylase; FabD: malonyl-CoA:ACP transacylase; FabH: β -keto-acyl-ACP synthase III; FabG: β -keto-acyl-ACP reductase; FabA/FabZ: β -hydroxy acyl-ACP dehydratase; FabI: enoyl-acyl-ACP reductase; FabB/FabF: β -keto-acyl-ACP synthase I/ β -keto-acyl-ACP synthase II. (b) RBO pathway. BktB: Thiolase; FadB: enoyl-CoA hydratase/ β -hydroxyacyl-CoA dehydrogenase; Ter: acyl-CoA dehydrogenase/trans-enoyl-CoA reductase

表 1 大肠杆菌FAB路径合成MCFAs研究进展

Table 1 The research progress of MCFAs biosynthesis by FAB pathway in *E. coli*

培养基主要成分 (碳源) ^{a)}	菌株	硫酯酶来源	MCFAs产量 (链长、发酵体系)	参考文献
LB(甘油)	MG1655	<i>Umbellularia californica</i>	0.4 g/L(50 mL)	[16]
M9(葡萄糖/甘油)	BL21(DE3)	<i>Acinetobacter baylyi</i>	约1.6 g/L(5 L)	[15]
LB(甘油)	MG1655	<i>Escherichia coli</i>	0.35 g/L C12、0.32 g/L C8(25 mL)	[17]
MOPS-1(葡萄糖)	MG1655	<i>Anaerococcus tetradius</i>	0.043 g/L(10 mL)	[18]
M9(葡萄糖)	MG1655	<i>Acinetobacter baylyi</i>	0.045 g/L(1 mL)	[19]
M9补充CaCl ₂ 、生物素、硫胺素 (葡萄糖)	K27	<i>Cuphea hookeriana</i>	0.038 g/L C8、0.01 g/L C10(50 mL)	[20]
MOPS-2(甘油)	RL08ara	<i>Cuphea palustris</i>	1.75 g/L C8(50 mL)	[21]
MOPS-1补充酵母抽提物(葡萄糖)	BL21(DE3)	<i>Escherichia coli</i>	2.7 g/L(5 L)	[13]

a) LB: 酵母抽提物、胰蛋白胨、NaCl; M9: Na₂HPO₄、KH₂PO₄、NH₄Cl、NaCl、MgSO₄; MOPS-1: MOPS、Na₂HPO₄、KH₂PO₄、NH₄Cl、NaCl、MgSO₄、CaCl₂、硫胺素、微量元素((NH₄)₆Mo₇O₂₄·7H₂O、H₃BO₃、CoCl₂·6H₂O、CuSO₄·5H₂O、MnCl₂·4H₂O、ZnSO₄); MOPS-2: MOPS、胰蛋白胨、酵母抽提物、泛酸钙、Na₂HPO₄

碳链延伸路径, 包括硫解、还原、水合、还原四步反应(图1(b)). 循环第一步为硫解酶催化脂酰-CoA(第一个循环为乙酰-CoA)与乙酰-CoA结合并游离出一分子CoA. 上一步生成的β-酮脂酰-CoA由3-羟脂酰-CoA脱氢酶还原为反式β-羟脂酰-CoA; 烯脂酰-CoA水合酶催化反式β-羟脂酰-CoA脱水转化为反式-烯脂酰-CoA; 反式-烯脂酰-CoA再由脂酰-CoA脱氢酶/反式-烯酰-CoA还原酶还原为脂酰-CoA, 碳原子数目增加2. 最后, 形成的脂酰-CoA经硫酯酶催化形成脂肪酸. 3-羟脂酰-CoA脱氢酶、烯脂酰-CoA水合酶通常使用大肠杆菌的FadB, 常用的脂酰-CoA脱氢酶/反式-烯脂酰-CoA还原酶为来自纤细裸藻(*Euglena gracilis*)的EgTer^[23]. 催化起始反应的硫解酶和控制解离反应的硫酯酶是RBO路径中最关键的酶. 大肠杆菌内源的硫解酶主要包括AtoB、YqeF、FadA和FadI, 其中AtoB对短链脂酰-CoA

具有高特异性, FadA对各种链长脂酰-CoA选择性低, 对乙酰-CoA效率低^[11]. 目前研究中常用的硫解酶为大肠杆菌的AtoB/FadA或罗尔斯通氏菌(*Ralstonia eutropha*)的ReBktB, 其特异性底物为C4和C6链长的脂酰-CoA^[23]. 类似于硫酯酶, 广泛筛选或工程改造硫解酶也是开发MCFAs合成潜力的关键手段(表2). 其中, Ramon Gonzalez课题组^[11]联合应用AtoB和FadA两种不同链长特异性的硫解酶, 实现β氧化循环多轮逆转, 成功合成C12脂肪酸.

1.3 FAB路径与RBO路径的比较

为了最优化大肠杆菌发酵合成MCFAs, 精准选择代谢路径并合理优化发酵极其重要, 而这基于对FAB路径和RBO路径之间差异的充分理解. FAB路径和RBO路径中功能酶完全不同, 使得两个路径在物质、

表 2 大肠杆菌RBO路径合成MCFAs研究进展

Table 2 The research progress of MCFAs biosynthesis by RBO pathway in *E. coli*

菌株	路径酶			MCFAs产量 (发酵体系)	参考文献
	硫解酶	硫酯酶	其他酶		
MG1655	FadA	YqeF、TesA、TesB	FadB	0.1~0.5 g/L(20 mL)	[22]
MG1655	AtoB	TesA	FadB、Ter	约3.3 g/L(20 mL)	[11]
MG1655	BktB	YdiI、TesB	FadB、Ter	约1.1 g/L(20 mL)	[24]
MG1655	BktB	’TesA	FadB、Ter	约1.3 g/L(15 mL)	[14]
MG1655	BktB	FadM	FadB、Ter	0.9 g/L(15 mL)	[25]
BL21(DE3)	BktB	YdiI	FadB、Ter	3.8 g/L(50 mL)	[26]
BL21(DE3)	BktB	YdiI	FadB、Ter	4.7 g/L(50 mL)	[27]
BL21(DE3)	BktB	YdiI	FadB、Ter	15.7 g/L(3 L)	[23]

反应及其关联的细胞需求方面各有特点。首先, RBO路径以乙酰-CoA为延伸单元, 而FAB路径以丙二酰-ACP为延伸单元, 需两分子乙酰-CoA羧化后转酰为一分子丙二酰-ACP, 此过程消耗ATP并吸收一分子二氧化碳, 后续碳链延伸过程中, β -酮脂酰-ACP合成酶的催化过程会释放一分子二氧化碳。所以, RBO路径较FAB路径有更高的能量利用效率。其次, FAB路径和RBO路径中的限速步骤均为第一步反应, 分别为ATP依赖性的乙酰-CoA羧化反应和热力学不利的3-酮脂酰-CoA生成反应($\Delta G=+29$ kJ/mol)。通过“推-拉”策略或动态调控手段来解除限速步骤的控制是改造大肠杆菌合成脂肪酸的有效手段^[28]。最后, FAB路径酶的辅因子为NADPH/NADH, RBO路径酶则以NADH/FAD为辅因子。因此, 基于不同路径酶的辅因子需求进行特定辅因子工程改造也是提高路径效率的重要手段。

另外, FAB路径受到细胞严格的监管, 可操纵部分较少^[29], 但有些生物环境对FAB路径具有偏好性, 如有研究提出, 将木糖转化为己酸的主要途径是FAB, 而不是RBO^[30]。RBO路径不需额外消耗能量, 但由于其整体可逆, 容易积累中间产物, 对上游基因产生反馈抑制作用或者用于合成其他不需要的副产物^[28]。此外, 多轮RBO路径合成MCFAs的同时也会伴随短链脂肪酸的生成^[31]。大肠杆菌中同时改造FAB和RBO路径合成MCFAs的研究较少。目前, 利用大肠杆菌RBO路径合成MCFAs的相关研究中, FAB和RBO两种路径共存于细胞中, 但是FAB路径用于正常细胞生长需求, 而不是合成MCFAs。很多研究利用混菌体系共用FAB和RBO两种路径合成MCFAs^[32,33], 比如, 通过微生物群落宏基因组分析发现, FAB和RBO路径都参与转化污泥(waste

activated sludge, WAS)碱性发酵液和乙醇生产MCFAs的过程^[32]。所以, 在特定需求条件下需综合评估选定路径并进行优化。

2 链长控制

利用FAB或RBO路径经多轮碳链延伸形成的产物是多种成分的混合物。因此, 链长控制是合成MCFAs的关键, 不仅能提高产物产量, 还能改善产物纯度, 由此最小化下游分离成本^[34]。产物链长主要由合成路径中起始缩合和终止水解步骤中相关蛋白的底物特异性决定。通过引入异源的中链底物特异性调控蛋白或改造内源蛋白的底物特异性, 可实现MCFAs的链长控制^[13]。

2.1 链长控制机制

链长控制相关的蛋白包括FAB路径中 β -酮脂酰-ACP合成酶和ACP、RBO路径中硫酯酶、控制终止水解的硫酯酶。目前主要通过探索这些蛋白中酰基结合部位氨基酸的种类、性质及位置来解析链长控制的机制。硫酯酶的底物特异性也是决定产物链长的重要因素, 然而该酶的链长控制机制尚不清晰, 下面给出其他3个蛋白的例子(图2)。

大肠杆菌硫酯酶I(TAP)与底物结合界面的疏水相互作用受底物酰基链长度调控, 从而影响TAP构象及催化活性。TAP中底物结合缝隙两侧主要由疏水残基组成, 包括Leu11和Pro110两个分离的疏水簇, 在TAP与辛酸结合形成的TAP-OCA复合物结构中, 辛酸的C3~C4原子以及C5~C8原子分别与Leu11和Pro110疏水簇残基紧密结合, 形成连续的疏水表面, 诱发TAP构象变化。但是, 短链酰基底物只能与单侧疏水簇结合, 疏

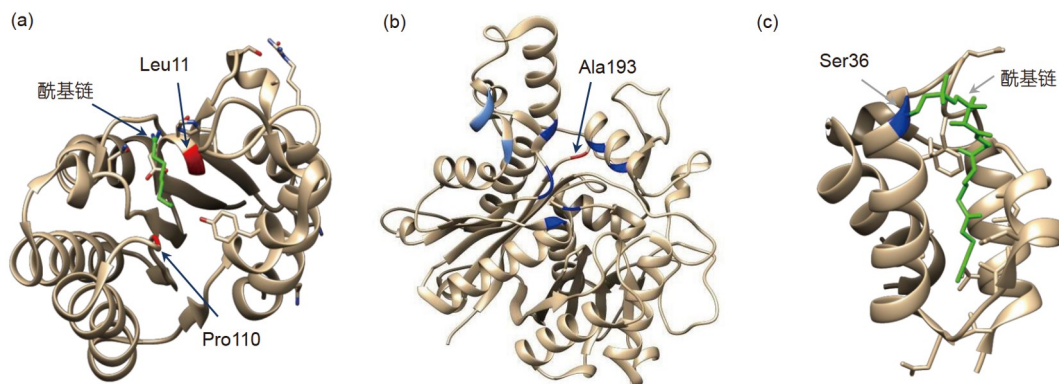


图2 (网络版彩色)MCFAs链长控制相关蛋白。(a) 硫酯酶I; (b) β -酮脂酰-ACP合成酶; (c) 酰基载体蛋白(ACP)

Figure 2 (Color online) The proteins controlling MCFAs chain-length. (a) Thioesterase I; (b) β -keto-acyl-ACP synthase; (c) acyl carrier protein, (ACP)

水性相互作用较弱,不能诱发TAP构象变化^[35](图2(a)).

β -酮脂酰-ACP合成酶催化脂酰-ACP与丙二酰-ACP羧化为增加两个碳原子的脂酰-ACP,其主要是通过疏水性口袋来结合脂酰-ACP底物. Ala193突变为更大的Met会使该疏水性口袋变小,从而限制长链脂酰-ACP底物的结合. 该突变体对C6脂酰-ACP的催化活性增加25%~30%,对C8及更长碳链脂酰-ACP的催化活性降低12倍以上^[36](图2(b)).

ACP是细胞质中富含的较小酸性蛋白质,序列相似性低,但结构高度保守,ACP疏水口袋的大小决定结合底物的链长^[12,37]. ACP有4个 α 螺旋,主螺旋I、II和IV相互平行,包围着可以结合酰基链的疏水口袋,酰基链以保守的Ser连接到4-磷酸泛乙炔,进入螺旋II和III之间的疏水口袋,其中C8脂酰基链可以完全结合在口袋中. 将口袋内氨基酸残基替换为更大的氨基酸,可以增加酰基链的空间位阻,使得脂肪酸链变短^[37](图2(c)).

2.2 关键蛋白的筛选与改造

利用代谢工程手段过表达某些细菌或植物来源的链长控制蛋白可以实现MCFAs的特异性合成. 植物与细菌来源的硫酯酶具有较强的底物特异性,加州月桂(*Umbellularia californica*)、萼距花(*Cuphea hookeriana*)来源的硫酯酶分别更倾向于以C12和C8脂酰-ACP为底物,具体研究总结见表1. 罗尔斯通氏菌来源的硫解酶ReBktB比大肠杆菌内源硫解酶AtoB更倾向于合成C6~C10脂肪酸^[24],共表达此硫解酶ReBktB、烯酰-CoA还原酶EgTer以及长链脂酰-CoA硫酯酶FadM,工程大肠杆菌的癸酸产量达2.1 g/L^[25].

对链长调控蛋白进行蛋白质工程改造也能实现特定MCFAs的合成. 大肠杆菌硫酯酶TesA底物结合口袋中氨基酸残基突变为疏水性更强的残基,可以提高该酶对C8脂酰-ACP的选择性,在敲除 β 氧化路径的基础上过表达该突变体辛酸产量比野生型提高约10倍,在5 L发酵罐进行补料分批发酵,辛酸产量达2.7 g/L^[13]. 萼距花来源的硫酯酶ChFatB2中底物结合口袋的I198残基位于催化活性中心附近, I198E突变体使得其与ACP形成的疏水相互作用变弱,合成产物中辛酸的比例增加至57.6%,产量提高1倍以上. 将长聚球藻(*Synechococcus elongatus*)来源ACP中较小的疏水侧链残基(如Ile或Leu)替换为较大的氨基酸(如Phe、Met、Tyr或Trp),其中I75W和I75Y突变体的脂肪酸链变短,在过表达加州月桂硫酯酶的基础上过表达这两个ACP突

变体,工程大肠杆菌C12脂肪酸的产量提高约6倍,达140 mg/L^[38].

3 合成路径优化

大肠杆菌利用FAB路径或RBO路径合成MCFAs受到细胞严格调控,导致MCFAs产量很低. 大量研究通过适配硫酯酶、增强辅因子或前体供应对合成路径进行“推-拉”,由此提高路径合成效率.

3.1 FAB路径的“推-拉”策略

FAB路径已广泛用于长链脂肪酸的合成,提高产量的策略包括增加前体乙酰-CoA的供应(“推”)、敲除 β 氧化路径等,这些策略也适用于MCFAs的合成. 除此之外,利用FAB路径高效合成MCFAs的关键在于调控硫酯酶的表达(“拉”). 植物和细菌普遍使用II型FAS系统合成脂肪酸,其中多种具有中链脂酰-ACP特异性的硫酯酶已广泛用于大肠杆菌合成MCFAs. 在大肠杆菌中表达香樟(*Cinnamomum camphora*)来源的CcFatB1, MCFAs产量达到约144 mg/L^[39]. 在敲除 β 氧化路径的基础上过表达加州月桂来源的硫酯酶BTE, MCFAs产量增加约7倍,产物链长以C12为主^[16]. 过表达萼距花来源的硫酯酶ChFatB2,发酵24 h积累约50 mg/L的MCFAs,产物链长以C8为主^[20]. 在大肠杆菌中过表达贝氏不动杆菌(*Acinetobacter baylyi*)的硫酯酶AcTesA,脂肪酸总产量达501.2 mg/L,5 L发酵罐进行补料分批发酵, MCFAs产量可达1.6 g/L^[15]. 此外,大肠杆菌内源硫酯酶经工程改造也能用于合成MCFAs. 大肠杆菌内源硫酯酶TesA突变体TesA^{RD-2}对C8脂酰-ACP具有高特异性,过表达此突变体的工程菌株于5 L发酵罐进行补料分批发酵产出2.7 g/L辛酸^[13].

3.2 RBO路径的“推-拉”策略

大肠杆菌自身没有RBO路径,RBO路径的限速步骤是以乙酰-CoA为底物合成 β -酮脂酰-CoA的过程. 因此,在利用此路径合成MCFAs时,除了表达硫酯酶(“拉”),还需采取多种策略来推动此路径的顺利高效运转,包括筛选异源路径酶,提高氧化还原辅因子供应,抑制底物乙酰-CoA分流(“推”)等^[40]. Ramon Gonzalez团队^[14]通过表达硫解酶BktB和硫酯酶YdiI实现 β -氧化循环的多轮逆转,工程大肠杆菌菌株合成1.1 g/L MCFAs,更换硫酯酶为内源截掉前导肽的硫酯酶TesA, C6~C10脂肪酸产量提高至1.3 g/L. 过表达乙酰-CoA合

成酶和硫解酶分别增强乙酰-CoA供应与转化,同时利用CRISPRi抑制乙酰-CoA分流路径,工程菌株可产生3.8 g/L MCFAs,产物链长以C6~C10为主^[26]。在RBO路径中,氧化还原辅因子也是影响路径效率的因素之一,过表达大肠杆菌的乙酰-CoA合成酶和甲醇酵母(*Candida boidinii*)来源的甲酸脱氢酶,以增强乙酰-CoA和NADH的供应,工程大肠杆菌菌株的MCFAs产量达到4.7 g/L^[27]。

4 耐受性改造

MCFAs会造成酸性环境胁迫和细胞膜损伤,影响细胞生长和活性,进而制约其高效合成。增强细胞对MCFAs的耐受性是提高工程大肠杆菌MCFAs合成能力的重要手段。现有研究中大肠杆菌耐受性改造策略主要包括膜工程改造、应激响应调控和适应性进化。

4.1 损伤机制

多项研究表明,过量合成MCFAs会产生严重的细胞毒性。外源添加40 mmol/L己酸、辛酸或20 mmol/L癸酸会显著抑制大肠杆菌生长^[41]。另有研究表明,内源合成0.3 g/L C8~C14脂肪酸比外源添加0.5 g/L C12脂肪酸使大肠杆菌细胞膜渗透性升高更多,对细胞损伤更严重^[42]。目前对MCFAs细胞损伤机制的研究主要涉及两个方面:MCFAs的存在会降低胞内pH^[43];MCFAs会破坏细胞膜结构^[41](图3(a))。

胞内pH的下降会破坏胞质稳态,从而影响细胞的正常生长及活性。当胞内积累过多MCFAs时,解离出的 $R-COO^-$ 和 H^+ 量会增加,引起大量阴离子堆积,破坏细胞正常渗透压,细胞需向胞内转运 K^+ 并外排谷氨酸盐,造成谷氨酸含量降低,阻碍胞内pH的恢复,进而影响细胞生长^[44,45]。另外,胞内堆积的质子泵出细胞需要消耗额外的ATP,由此造成细胞更大的生存压力^[44]。其次,多数蛋白质在酸性环境中会出现错误折叠、变性等问题,导致蛋白功能丧失,进而影响细胞活性^[46]。

细胞膜可以作为屏障将细胞与外部环境不完全地隔离开,膜成分或膜结构的改变会影响膜的完整性、渗透性、流动性等性质,进而影响细胞的耐受性^[47]。大肠杆菌细胞膜膜脂成分主要是C12~C18的长链膜脂,过量生产MCFAs工程菌株中,较短的MCFAs会经2-酰基甘油磷酸乙醇胺酰基转移酶/酰基ACP合成酶Aas催化并入膜脂中,导致细胞膜平均膜脂长度变短、结构变得疏松,造成细胞内物质外漏、胞内渗透压失衡,破坏内环境稳态,从而降低细胞活力^[10,48]。

4.2 膜工程改造

对细胞膜成分的调控和改造是损伤性产物合成的重要研究方向。大肠杆菌耐受MCFAs的膜工程改造主要集中在细胞膜磷脂头部、磷脂尾部和膜外排蛋白。过表达磷脂酰丝氨酸合酶PssA可以增加膜脂头部磷脂

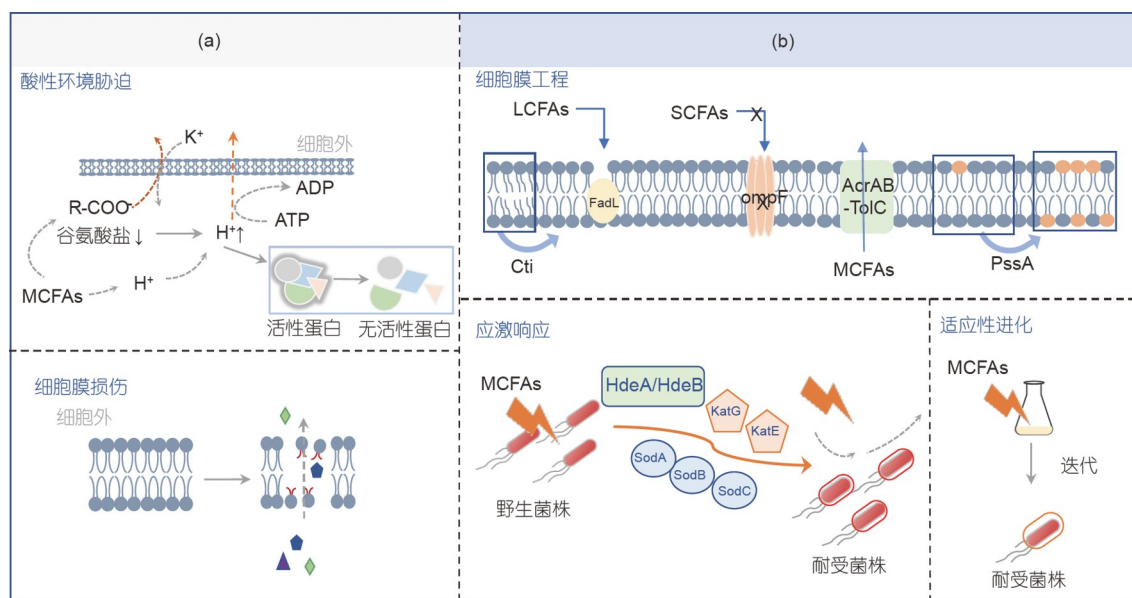


图3 (网络版彩色)MCFAs的损伤机制和耐受策略。(a) 损伤机制;(b) 耐受策略

Figure 3 (Color online) The injury mechanisms of MCFAs and the tolerance strategies. (a) The injury mechanisms; (b) the tolerance strategies

酰乙醇胺(phosphatidylethanolamine, PE)的丰度, 进而提高膜厚度及疏水核心厚度, 工程菌株在含20 mmol/L 辛酸的MOPS培养基中比生长速率提高29%, 辛酸产量提高46%, 达220 mg/L^[49]. 过表达铜绿假单胞菌(*Pseudomonas aeruginosa*)来源的顺反异构酶Cti, 将膜脂尾部中顺式不饱和脂肪酸转化为反式不饱和脂肪酸, 使得工程菌株膜流动性显著降低, 在10 mmol/L 辛酸中比生长速率提高约10%, 辛酸产量提高35%^[50]. 膜外排蛋白AcrAB-TolC负责分泌脂肪酸到胞外, 从而减轻其对胞内稳态环境的破坏, 敲除AcrAB的工程菌株对辛酸和癸酸的耐受从5 g/L分别降低到4和0.5 g/L^[51]. 另外, 敲除转运蛋白OmpF并且过表达FadL的工程菌株在10 mmol/L 辛酸中的比生长速率提高18%, 膜完整性提高37%, 脂肪酸产量提高约50%, 达2.3 g/L^[52].

4.3 应激响应调控

应激响应是机体在压力源存在的情况下发生的一系列基因表达调控, 对细胞应激响应进行调控也是提高工程菌株耐受性以及产物合成能力的重要手段^[53,54]. 大肠杆菌的耐酸性相关蛋白HdeA在酸性环境中可暴露疏水表面, 防止酸诱导的周质蛋白聚集, 从而明显地提高细胞活力^[53]. 大肠杆菌的氧化应激蛋白包括超氧化物歧化酶(SodA、SodB和SodC)、过氧化氢酶(KatE和KatG)等, 可降低胞内活性氧水平, 从而增强大肠杆菌对有机溶剂的耐受性^[54]. 另外, 转录组学分析发现, 高产MCFAs的大肠杆菌菌株中噬菌体休克蛋白PspABCDEG、调控子MarA/Rob/SoxS以及有氧呼吸操纵子nuo/cyo的表达显著增加, 并且敲除Rob后细胞生长严重受损, 活细胞计数从 7.3×10^8 减少到 3.3×10^8 (CFU/mL), 这些结果为MCFAs高度耐受和高效合成提供可用靶点^[42].

4.4 适应性进化

适应性进化是菌株在含胁迫因子的环境中产生自发突变得以耐受胁迫环境, 这种非理性策略结合高通量测序手段可用于研究细胞胁迫耐受机制并指导工程改造^[55,56]. 通过外源添加辛酸进行大肠杆菌MG1655的适应性进化, 最终获得一株对辛酸以及己酸、癸酸、

正丁醇和异丁醇耐受性都增强的菌株, 该菌株的细胞膜流动性和渗透性降低、平均膜脂长度增加、膜脂中饱和脂肪酸比例下降, 可产出 180 ± 30 mg/L 辛酸, 比亲本菌株提高4.6倍^[55].

5 展望

MCFAs具有较高的挥发性和较低的黏度, 广泛用作汽车和喷气式飞机的燃料, 展现出巨大的经济前景. 大肠杆菌生长速度快, 发酵批次周转数远高于其他可用宿主; 具有明确的遗传背景, 全基因组序列清晰; 具备极为丰富的代谢元件库和完备的基因组编辑、调控技术. 因此, 大肠杆菌在概念性验证、大规模代谢优化等方面具有明显优势. 但是, 与一些非常规微生物相比, 大肠杆菌抗逆性相对较差, 40 mmol/L (约4.6 g) 己酸可严重抑制大肠杆菌生长, 而合成8.5 g/L 己酸的开放式发酵体系中*Caproiciproducens* ssp. 是优势微生物^[57]. 另外, 大肠杆菌不能天然利用廉价而丰富的二氧化碳或光能, 而自养型微生物蓝藻(*Cyanobacteria*)具有将二氧化碳转化为MCFAs的潜力^[58].

为了进一步提高大肠杆菌合成MCFAs能力, 未来的研究还可聚焦于以下方面: (1) 发展和完善用于指导蛋白质工程的计算方法, 强化MCFAs链长控制. 计算机预测蛋白质结构迄今主要集中在靠近功能位点的热点残基上, 开发精确定位关键远端热点的工具会拓宽对蛋白质结构及性能关系的研究. (2) 深入研究MCFAs毒性及耐受相关膜调控机制, 增强大肠杆菌抗逆性. 细胞膜包括内膜、外膜、膜蛋白等多种结构, 涉及疏水性、流动性、极性、完整性等多种性质, 细胞膜是流动的, 这些结构与性质是实时变化的, 对细胞膜的组合调控及实时动态研究会为MCFAs毒性机制研究及耐受性改造提供多层次视角. (3) 高通量识别外源和内源可用靶点, 进一步提升大肠杆菌底物利用潜力、抗逆性能和MCFAs合成能力. 利用KEGG、BRENDA、UniProt、NCBI等数据库, 筛选新型、高效的代谢路径、关键酶和基因簇等. 基于CRISPR相关技术构建全基因组编辑或调控文库, 利用生长压力、G蛋白偶联受体的MCFAs传感器^[59]等筛选方法, 结合高通量测序, 识别更多内源潜力基因靶点, 探索MCFAs耐受或高产的新机制.

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Summary for “大肠杆菌合成中链脂肪酸研究进展”

Advances on medium-chain fatty acids synthesis in *Escherichia coli*

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Medium-chain fatty acids (MCFAs), straight-chain fatty acids with 6–12 carbons, can be transformed into aliphatic hydrocarbons, triglycerides, and other oleochemicals. MCFAs and their derivatives show strong potential as useful materials in the energy, medicine, food and chemical industries. Compared with the conventional extraction methods based on petroleum or plant biomass, microbial fermentation is a green and sustainable alternative for MCFA production. *Escherichia coli* are frequently used as model microorganism for the analyses of metabolic mechanisms and industrial processes owing to the factors: rapid replication, clear genetics, ease of rearing in the laboratory, simple process for amplification and abundance of gene manipulation tools. In *E. coli*, MCFAs can be synthesised through the fatty acid biosynthesis (FAB) pathway and the reverse β -oxidation (RBO) pathway. However, there are still many challenges that severely limit MCFAs biosynthesis in *E. coli*, such as chain-length control, synthesis efficiency and cytotoxicity. With the development and improvement of metabolic engineering, synthetic biology and high-throughput sequencing technology, great progress has been made in recent years in the engineering of *E. coli* for MCFAs overproduction.

In this review, we first introduce the MCFAs synthetic pathways in *E. coli*, including the FAB and RBO pathways, and compare these two pathways in terms of extension unit, rate-limiting step and cofactors. We then summarise the challenges, advances and representative studies in MCFAs production, with a focus on three aspects: chain-length control, synthetic pathway optimisation and tolerance improvement. Chain-length control is the key to the directed synthesis of MCFAs, as multiple rounds of cycles in the synthetic pathway tend to generate mixtures of various chain lengths. We discuss the catalysis mechanism of functional protein in the synthetic pathways to achieving chain-length control, including thioesterase, thiolase, and ketoacyl synthase. Protein and metabolic engineering of these key enzymes for targeted synthesis of MCFAs are also summarised in this review. Furthermore, synthetic pathway optimisation is an effective strategy for the enhancement of MCFAs biosynthesis. The “Pull-Push” of the FAB and RBO pathways, a process frequently used to increase precursor supply and improve product release, is reviewed here. Tolerance engineering is extremely critical for MCFAs overproduction, owing to the cell toxicity of MCFAs. We discuss in this review the injury mechanisms associated with MCFAs production and the strategies for improving cellular tolerance. Specifically, MCFAs production reduces intracellular pH and changes the composition of the cell membrane, leading to disruption of cytoplasmic homeostasis and destruction of membrane structure and properties. Membrane engineering, stress response regulation and adaptive evolution are generally employed for improving cellular tolerance of MCFAs.

In summary, we cover in this review the recent advancements in the bio-engineering of *E. coli* for MCFAs production, based on the FAB and RBO biosynthetic pathways. Protein, metabolic and membrane engineering are generally applied to control the chain length, optimise the synthetic pathway and improve cellular tolerance. We also provide some perspectives for further strengthening MCFAs biosynthesis, including improvement of computer technology for protein assessment, in-depth and full perspective analysis of MCFAs toxicity and tolerance mechanisms and the mining of useful targets from exogenous and endogenous species using high-throughput screening.

***Escherichia coli*, medium-chain fatty acids, metabolic engineering, chain-length, tolerance**

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