代谢工程改造酿酒酵母底盘细胞

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摘要 酿酒酵母是合成多种天然产物的微生物细胞工厂, 合理利用酿酒酵母底盘细胞内源的代谢途径可生产高附加值的生物医药、食品保健和精细化学品类产物. 如何精细调控和优化酿酒酵母胞内代谢流是实现目标化合物高产量、高产率和高转化的关键问题. 乙酰辅酶A是中心代谢和天然产物合成的基本前体, 精细调控乙酰辅酶A的合成是实现目标化合物高产的重要策略; 改造酿酒酵母的甲羟戊酸途径, 引入外源途径酶, 表达萜类合成酶生产不同种类的萜类化合物; 优化脂肪酸合成途径合成特定链长的脂肪酸及脂肪酸衍生物. 本文总结了强化酿酒酵母中乙酰辅酶A积累的代谢工程策略, 重构甲羟戊酸途径、脂肪酸途径从头合成天然萜类化合物和脂肪酸衍生物的研究进展, 为利用酿酒酵母底盘细胞生产天然产物的相关研究提供代谢工程改造策略.

关键词 酿酒酵母, 合成生物学, 乙酰辅酶A, 萜类, 脂肪酸

酿酒酵母(*Saccharomyces cerevisiae*)具有遗传操作简单、代谢网络清晰、生长速率快、翻译后修饰且具有真核细胞特有的亚细胞结构等特性, 随着合成生物学技术的应用, 研究者将酿酒酵母发展成为重要的细胞工厂, 应用于精细化学品及天然产物的生产. 目前, 酿酒酵母合成的天然产物超过100种, 研究者针对不同天然产物开发了特有的代谢改造途径. 同时, 这些代谢途径大多数以乙酰辅酶A为起点. 乙酰辅酶A是酿酒酵母中心碳代谢的关键分子, 参与脂肪酸、甾体的合成以及线粒体中的三羧酸循环, 为细胞供能. 以乙酰辅酶A为代谢前体的甲羟戊酸途径是植物萜类的直接合成途径. 植物来源的萜类化合物在生物医药、食品保健、化妆品有重要的应用, 如青蒿素、紫杉醇、大麻素和人参皂苷. 它们具有镇痛、治疗疟疾、抗肿瘤、治疗癫痫和保健等功效. 其中大麻素的全球市场份额

预计在2020年超过2000亿美元. 近年来, Luo等人^[1]通过对酿酒酵母的甲羟戊酸途径进行工程改造强化香叶基焦磷酸(geranyl pyrophosphate, GPP)前体的积累, 引入大麻植物来源的大麻素合成途径, 实现了四氢大麻酸和大麻二酚酸在酿酒酵母中的从头合成. 酿酒酵母的鲁棒性较强, 通过设计、构建、试错和学习的定量工程化改造驯化可实现天然代谢产物的定制生产. Yu等人^[2]依据产油解脂耶氏酵母(*Yarrowia lipolytica*)的中心代谢途径, 全局重构了酿酒酵母的乙酰辅酶A合成途径、磷酸戊糖途径、三羧酸(tricarboxylic acid, TCA)循环和辅因子能量的代谢流, 得到了高产脂肪酸的酿酒酵母菌株(产量为33.4 g/L). 因此, 本文对酿酒酵母胞内的乙酰辅酶A、甲羟戊酸和脂肪酸的代谢途径改造的研究进展进行总结, 并对酿酒酵母的合成生物学研究方向进一步展望.

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1 乙酰辅酶A途径的代谢工程改造

1.1 乙酰辅酶A的合成途径

乙酰辅酶A是酿酒酵母中心代谢和异源代谢途径中的关键代谢前体。乙酰辅酶A参与酿酒酵母胞内代谢的TCA循环、脂肪酸合成以及对蛋白质的乙酰化修饰。其主要合成途径是通过糖酵解途径合成的丙酮酸进入线粒体,随后被丙酮酸脱氢酶复合物(pyruvate dehydrogenase, PDH)催化合成乙酰辅酶A后进入三羧酸循环^[3]。在细胞质中存在丙酮酸脱羧酶支路,丙酮酸被胞质定位的丙酮酸脱羧酶催化形成乙醛,进而被酿酒酵母中的乙醛脱氢酶(acetaldehyde dehydrogenase, ALD)催化生成乙酸。同时,酿酒酵母胞内的乙酰辅酶A合成酶(acetyl-CoA synthetase, ACS)将乙酸和辅酶A缩合产生乙酰辅酶A^[4]。在过氧化物酶体中脂肪酸降解途径产生乙酰辅酶A,长链脂肪酸的酰基辅酶A被过氧化物酶体膜上的转运蛋白Pxa1和Pxa2转运至过氧化物酶体内进入 β -氧化途径产生乙酰辅酶A^[5]。同时,中短链的游离脂肪酸进入过氧化物酶体后被内膜蛋白Faa2酰基化后进入 β -氧化途径生成乙酰辅酶A。细胞核质中的乙酰辅酶A合成酶2(acetyl-CoA synthetase 2, ACS2)催化乙酸产乙酰辅酶A,维持组蛋白的乙酰化修饰,并参与转录调控^[6]。乙酰辅酶A在不同亚细胞器中是高度区域化的,如何提高胞质中乙酰辅酶A合成途径是高效合成代谢产物的关键。

1.2 强化酿酒酵母内源乙酰辅酶A合成途径

基于乙酰辅酶A的合成途径和降解支路,对酿酒酵母进行代谢工程改造以增强乙酰辅酶A的合成,进而提高目标化合物的产量。丙酮酸脱羧酶支路,从丙酮酸到乙醛,再经乙醛脱氢酶生成乙酸,最后经乙酰辅酶A合成酶生成乙酰辅酶A。整个反应消耗1分子葡萄糖,产生2分子乙酰辅酶A、4分子NAD(P)H和2分子CO₂。Shiba等人^[7]在过表达乙醛脱氢酶ALD6的基础上比较了酿酒酵母内源的乙酰辅酶A合成酶ACS1、ACS2和沙门氏菌(*Salmonella enterica*)来源的乙酰辅酶A合成酶Se_ACS,其中组合表达ALD6和Se_ACS^{L641P}将催化乙酸底物合成乙酰辅酶A的转化率提高3倍,进而将碳流引入甲羟戊酸途径使得紫穗槐二烯的产量提高了4倍。Lian等人^[8]在此基础上将乙醇脱氢酶(alcohol dehydrogenase)ADH2的表达启动子替换为P_{HTX1}启动子以提高ADH2的表达,使乙醇转化为乙酰辅酶A,从而减少碳

溢流。线粒体中生成的乙酰辅酶A进入三羧酸循环后与草酰乙酸合成柠檬酸。柠檬酸可以跨线粒体膜转运到细胞质中,产油酵母(*Y. lipolytica*)中存在柠檬酸裂解酶(ATP-dependent citrate lyase, ACL),将柠檬酸分解为乙酰辅酶A和草酰乙酸。在酿酒酵母中引入*Y. lipolytica*酵母来源的ACL,将n-丁醇的产量提高了2倍^[8]。Yu等人^[2]在产脂肪酸的酿酒酵母工程菌株中引入AnACL(*Aspergillus nidulans*来源的ACL酶),重构乙酰辅酶A的合成途径,并结合亚细胞代谢流转运、优化NADPH和ATP供给以及降低碳源流向生物量积累的系统改造,将脂肪酸的产量提高至33.4 g/L。但是,胞质中的柠檬酸形成异柠檬酸后产生乙醛酸,柠檬酸合酶(malate synthase, MLS)利用乙醛酸和乙酰辅酶A生成苹果酸进入过氧化物酶体,造成乙酰辅酶A分流的问题。Chen等人^[9]进一步将乙醛酸分流途径中的MLS1和CIT2敲除,避免乙酰辅酶A的代谢流量损失,将 α -檀香烯产量提高了4倍。酿酒酵母细胞质中的丙酮酸脱羧酶支路合成乙酰辅酶A时,生成CO₂副产物损失碳源。

1.3 构建外源乙酰辅酶途径提高乙酰辅酶A合成效率

磷酸转酮酶(phosphoketolase, PK)和磷酸转乙酰酶(phosphotransacetylase, PTA)途径存在于酵母和乳酸菌中,参与异性乳糖发酵。PK以5-磷酸木酮糖为底物,将无机磷酸盐等转化为乙酰基-磷酸,后者进一步被PTA催化生成乙酰辅酶A^[10]。整个反应消耗1分子葡萄糖和1分子ATP,产生3分子乙酰辅酶A、2分子H₂O。该反应无CO₂副产物生成,可提高碳源利用率。Evans和Ratlidge^[11]在木糖发酵产乙醇酵母TMB3001c菌株中组合内源的D-木糖-5-磷酸/D-果糖-6-磷酸的PK基因和枯草芽孢杆菌(*Bacillus subtilis*)来源的PTA将木酮糖-5磷酸转化为乙酰辅酶A,减少木糖醇的形成并提高木糖的利用率。进一步过表达痢疾变形虫(*Entamoeba histolytica*)来源的adh2基因和敲除NADPH-依赖的ald6基因策略减少乙酸对木糖发酵的抑制,将乙醇的产量提高20%、木糖的发酵率提高40%^[12]。乙酰辅酶A是酿酒酵母胞内甲羟戊酸途径的直接前体,关联这两个合成途径可提高代谢途径流量和能量利用效率。Meadows等人^[13]表达肠膜明串珠菌(*Leuconostoc mesenteroides*)来源的PK(xPK)和克氏梭菌(*Clostridium kluyveri*)来源的PTA酶重构乙酰辅酶A的合成途径,提高碳源利用效率。同时,引入腺苷脱氨酶(Adenosine deaminase, ADA; 乙醛脱氢酶

酰基化酶)催化乙醛产乙酰辅酶A和NADH, 减少ATP消耗. 进一步, 构建NADH-依赖的HMG-CoA还原酶(3-hydroxy-3-methyl glutaryl coenzyme A reductase, HMGR), 利用ADA催化产生的NADH. 采用关联乙酰辅酶A-甲羟戊酸合成途径策略重构的法尼烯合成菌株在以葡萄糖为碳源时, 将产量提高了25%并减少了75%的氧气消耗. 在200000 L的发酵体系中, 法尼烯的产量达到103 g/L^[13]. 这表明, 通过化学计量分析从头设计的乙酰辅酶A合成途径减少ATP的消耗和碳源的损失.

1.4 乙酰辅酶A合成途径改造策略总结

理性设计酿酒酵母胞内乙酰辅酶A的合成通路,可最优化地供给天然产物的合成前体乙酰辅酶A(图1)。PK/PTA-ADA途径对C₆的利用率最高,避免产生CO₂造成碳源溢流。敲除乙酰辅酶A的竞争途径基因*MLS*和*CIT2*可积累乙酰辅酶A供给合成途径。同时,表达异源柠檬酸裂解酶将线粒体中的乙酰辅酶A代谢产物转化为胞质中的乙酰辅酶A。最近研究中, Liu等人^[14]比较了不同乙酰辅酶A合成途径对β-香树脂醇合成的影响,其中上述的PK/PTA-ADA途径将单位葡萄糖的产率提高

至0.296 g β -香树脂醇/1 g葡萄糖. 优化后的乙酰辅酶A途径平衡了氧化还原力、降低了ATP的消耗. 因此, 在PK/PTA-ADA途径的基础上理性设计乙酰辅酶合成途径, 可进一步提高酿酒酵母合成乙酰辅酶A的效率.

2 甲羟戊酸途径的代谢工程改造

酿酒酵母体内的甲羟戊酸途径是萜类化合物的主要合成路线, 其中HMG-CoA合成酶(HMG-CoA synthetase, HMGS)和是合成甲羟戊酸的两个关键限速步骤(图2)。筛选异源催化效率高的HMGS和HMGR酶, 可增强酿酒酵母内源甲羟戊酸合成途径代谢流。Tsuruta等人^[15]将酿酒酵母来源的HMGS和tHMGR(N端氨基酸序列截断的HMGR)分别替换为金黄色葡萄球菌(*Staphylococcus aureus*)来源的HMGS(mvaS)和HMGR(mvaA), 将紫穗槐二烯的产量提高至25 g/L。蛋白工程改造粪肠球菌(*Enterococcus faecalis*)来源的HMGS(EfMvas)获得EfMvas(A110G)突变体, 通过提高催化反应过渡态的稳定性将反应速率提高了140倍^[16]。Yang等人^[17]在途径中引入EfMvas(A110G)酶将异戊二烯产量提高至6.3 g/L。

Figure 1 The metab engineering of acetyl-CoA synthesis pathway in *S. cerevisiae*. Blue line, optimized pathway; orange line, adopted heterologous pathway; red line, knock-out pathway

Figure 2 Biosynthesis of terpene from mevalonate pathway. Red font, adopted heterologous pathway; orange line, nature product pathway; orange font, nature product. IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; ERG10, MvaE, acetoacetyl-CoA ligase; HMGS, MvaS (A110G), 3-hydroxy-3-methylglutaryl-CoA synthetase; tHMGR, MvaE, 3-hydroxy-3-methylglutaryl-CoA reductase; ERG12, mevalonate kinase; ERG8, phosphomevalonate kinase; MVD1, mevalonate pyrophosphate decarboxylase; IDI, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase; ERG20, farnesyl pyrophosphate synthetase; ERG20*, ERG20 (F96W, N127W); FS, farnesene synthetase; CrtE, geranylgeranyl pyrophosphate synthetase

5-phosphate decarboxylase, MVD)催化合成异戊烯基焦磷酸(isopentenyl diphosphate, IPP)前体。在IPP异构酶(IPP isomerase, IDI1)的作用下, IPP被转化为异构体二甲基烯丙基焦磷酸(dimethylallyl diphosphate, DMAPP)。法尼基焦磷酸合成酶(farnesyl pyrophosphate synthase, FPPS)催化IPP及其异构体DMAPP合成GPP和FPP产物(图2)。最终, FPPS酶缩合IPP和DMAPP合成GPP^[18]。但是, 在真核微生物体内FPPS酶进一步催化GPP合成菌体生长所必需的麦角固醇前体。因此, FPPS酶对底物的亲和力及反应速率决定了代谢流量的分布。Ignea等人^[19]在酿酒酵母来源的FPPS酶Erg20中引入F96W和N127W突变, 增加Erg20(F96W, N127W)突变体对GPP底物的亲和位阻。最终将积累的GPP引入松桧烯合成途径, 并将产量提高了10.4倍。以FPP为底物可合成倍半萜和三萜等^[20]。表达致伤埃希菌(*Escherichia vulneris*)来源的香叶酰香叶酰合成酶CrE催化FPP合成GGPP代谢产物^[21], 用于合成类胡萝卜素、番茄红素、虾青素、玉米黄质和紫杉醇等天然萜类产物^[22,23]。高产萜类化合物的菌株中IPP和DMAPP来源于乙酰辅酶A, 过量消耗乙酰辅酶A前体是限制萜类化合物产率和细胞生长的关键因素。部分古细菌和植物中存在以异戊烯醇为底物的IPP合成途径^[24]。因此, 引入非乙酰辅酶A为底物的IPP和DMAPP途径, 可进一步提高甲羟戊酸途径的代谢流量。Luo等人^[25]在解脂耶氏酵母*Y. lipolytica*中引入拟南芥*A. thaliana*来源异戊烯磷酸激酶(isopentenyl phosphate kinase, IPK)和*S. cerevisiae*来源的胆碱激酶(choline kinase, CHK)构建异戊烯醇利用途径经合成IPP。通过添加异戊烯醇将IPP和DMAPP含量提高了15.7倍, 最终将番茄红素产量提高至4.2 g/L。

改造和优化酿酒酵母中的脂肪酸代谢途径可以获得各种脂肪酸及其衍生物, 如脂肪醇、脂肪酸甲酯、脂肪酸乙酯、烯烃和羟基脂肪酸, 它们可作为生产生物能源、润滑油、塑料和其他工业制品的原材料^[26]。酿酒酵母中脂肪酸的合成主要在细胞质中进行, 由脂肪酸合酶 I (Fatty acid synthase I, FAS I) 介导合成 C₁₆和C₁₈的长链饱和脂肪酸。FAS I 是Fas1和Fas2两个亚基组成的 $\alpha 6\beta 6$ 异聚体。两个亚基包含了脂肪酸合成需要的关键酶, 包括乙酰基转移酶(acetyl transferase, AT)、烯酰还原酶(enoyl reductase, ER)、脱水酶(dehydratase, DH)、丙二酰/棕榈酰转移酶(malonyl/palmitoyl

transferase, MPT)、酰基载体蛋白(acyl carrier protein, ACP)、酮基还原酶(ketoacyl reductase, KR)、酮基合成酶(ketoacyl synthase, KS)和甲硫氨酸转移酶(phosphopantetheine, PPT)^[27]. FAS I合成的脂肪酸在内质网延伸后参与细胞的多种生理活动.

目前, 酿酒酵母中脂肪酸代谢的改造主要集中在FAS I系统介导的合成途径(图3). 乙酰辅酶A是脂肪酸合成的初始单位, 丙二酰辅酶A是脂肪酸合成的二碳供应单位. 乙酰辅酶A羧化酶(acetyl-CoA carboxylase, ACC1)催化乙酰辅酶A合成丙二酰辅酶A, 这一步反应

图3 改造酿酒酵母的脂肪酸代谢途径产脂肪酸及其衍生物. 黑色实线代表酿酒酵母中的代谢通路, 黑色虚线代表抑制的代谢通路, 红色实线代表改造的代谢通路, 绿色实线代表上调的代谢通路. FAA, 脂酰辅酶A合成酶; PXA1, 过氧化物酶体ABC转运蛋白; POX1, 脂酰辅酶A氧化酶1; PEX11, 过氧化物酶体膜蛋白11; ANT1, 腺嘌呤核苷酸转运蛋白1; TE, 硫脂酶; FAS I, I型脂肪酸合酶; r-BOX, β -氧化的逆反应途径

Figure 3 Production of fatty acid and derivatives from fatty acid synthesis pathway in *S. cerevisiae*. Black line, intrinsic metabolic pathway in *S. cerevisiae*; black dotted-line, the repressed metabolic pathway; red line, engineered metabolic pathway; green line, up-regulated metabolic pathway. FAA, fatty acyl-CoA synthetase; PXA1, peroxisomal ABC-transporter; POX1, fatty acyl-CoA oxidase 1; PEX11, peroxisomal membrane protein 11; ANT1, peroxisomal adenine nucleotide transporter; TE, acyl-ACP thioesterase; FAS I, I type fatty acid synthase; r-BOX, reversal beta-oxidation pathway

是脂肪酸合成途径的限速步骤。超表达内源的ACC1以及ACC1突变均可以提高丙二酰辅酶A的浓度,进而提高脂肪酸的产量。将酿酒酵母线粒体中的乙酰辅酶A羧化酶Hfa1在胞液中表达也可提高脂肪酸的产量^[28,29]。由于酵母中的FAS I结构复杂不易进行编辑,异源表达其他物种的FAS有利于改造脂肪酸合酶的灵活性。Eriksen等人^[30]将放线菌中的4种FAS I在酿酒酵母突变体*fasI*△中表达可以互补突变体的生长缓慢表型,并产生和野生型有相同组分的脂肪酸。而通过将人类的脂肪酸合酶hFAS与不同的硫脂酶在酿酒酵母中表达可以获得不同长度的游离脂肪酸。Leber等人^[31]在酵母中同时表达来自人类的hFAS△TE、枯草芽孢杆菌来源的PPT和兔来源的TE II,将短中链脂肪酸的产量提高64倍,其中C₈产量达到82 mg/L, C₆和C₁₀的总量达111 mg/L。除了表达不同来源的脂肪酸合酶,对酿酒酵母内源的脂肪酸合酶进行蛋白工程改造,以及调控催化亚基的底物选择性和催化效率可以获得不同碳链长度的短链或中链脂肪酸。Gajewski等人^[32]将FAS I中KS、AT和MPT结构域中的活性位点进行点突变,将C₆和C₈脂肪酸总产量提高至464 mg/L。同时,构建MPT(R1834K)突变体产245 mg/L C₈脂肪酸(占85.9%),构建AT(I306A)/KS(G1250S)突变体产71.5 mg/L C₆脂肪酸(占78.2%)^[32]。短/中链脂肪酸的疏水和低pH性质对酿酒酵母细胞有产物毒性,这是限制这类脂肪酸合成的瓶颈问题。在最新的研究中,Zhu等人^[33]通过定向进化和适应性进化的策略分别获得了对C₁₀和C₈脂肪酸耐受性提高的膜转运蛋白Tpo1突变体,结合其他工程改造策略将胞外中链脂肪酸产量提高至1000 mg/L以上。

采用β-氧化途径中发挥作用的硫解酶、水解酶、还原酶和脱氢酶构成基本运行模块,以乙酰辅酶A为二碳供应单位在酿酒酵母中构建β-氧化逆反应途径,从而获得不同碳链长度的短中链脂肪酸及衍生物^[34,35](图3)。另一方面,通过敲除在β-氧化过程中起作用的关键酶POX1、FAA2和PXA1可以显著提高长链脂肪酸的积累,而敲除FAA2、ANT1和PEX11则可以明显提高中链脂肪酸的产量^[36]。在获得高产量的游离脂肪酸和脂酰CoA的情况下,通过超表达异源的蜡酯合成酶WSs、脂肪酸还原酶FAR、AAR和ADO可以将脂酰CoA分别催化为脂肪酸酯、脂肪醇和烷烃。而超表达CAR和ADH则可以将游离脂肪酸催化为脂肪醇。在酿酒酵母中超表达mFAR1、OLE1和ACC1^{S659A, S1157A}、同时抑制其他的支路途径,可以将脂肪醇的产量提高

到1.2 g/L,发酵罐产量高达6 g/L^[29]。虽然利用酿酒酵母已经可以将长链脂肪酸、脂肪醇和脂肪酸酯产量提高到1~10 g/L,但是短中链的脂肪酸及其衍生物产量依然很低,还需要继续改善该途径以提高短中链脂肪酸及其衍生物的产量。

4 结论与展望

近年来,基于对酿酒酵母的机理研究和合成生物工具的发掘,酿酒酵母逐渐被开发为绿色制造的微生物细胞工厂,并实现了从实验室研发向产品工业化生产的发展。加州大学的Jay D. Keasling团队^[40]在2006年通过合成生物学方法利用酿酒酵母工业化生产了治疗疟疾的青蒿素,这是利用合成生物学方法实现转化应用的典范。随着对酿酒酵母代谢机理的理解,协调酿酒酵母的中心代谢和异源合成途径的代谢流平衡,可提高产量、产率和转化率。Amyris公司(2016年)利用酿酒酵母产法尼烯,生产规模达到200000 L发酵体系。但是,仍然有超过95%由酿酒酵母合成的天然产物无法实现工业化应用。原因是酿酒酵母对其中心代谢与异源途径的代谢网络、代谢流量的动态调控未知,导致异源代谢途径的合成效率低。仅限于代谢途径的工程改造策略,限制了酿酒酵母的开发应用。因此,结合对酿酒酵母的基础生理代谢和遗传的研究,有助于酿酒酵母细胞工厂的开发。近年来,对酵母的工具化改造越来越多样化,Dai等人^[37]通过对酿酒酵母的适应性进化,筛选到RNA聚II的中介突变体MED2*^{432Y},该突变体可消除高浓度葡萄糖积累引发的乙醇的Crabtree效应。酿酒酵母合成基因组研究,精简了酿酒酵母的冗余基因组序列,将染色的数量缩小至1~2条^[38,39]。Luo等人^[41]构建的SCAMBLE系统可以快速地对整条染色体进行重排和重组,进而极大地增加基因组的多样性。近期,将酵母改造成自养型菌株的研究也得到了长足进展,Gassler等人^[42]利用合成生物学的技术路线,将毕赤酵母(*Pichia pastoris*)的甲醇同化吸收途径改造为依赖Calvin-Benson-Bassham循环的二氧化碳固定途径。这一策略成功地实现了酵母细胞以二氧化碳作为唯一碳源生长。因此,未来可利用酿酒的染色体部件定制人工合成的代谢途径基因簇序列,构建不同的代谢模块并对代谢模块的表达水平进行定量控制,获得利用包括光能与电能在内的其他清洁能源,并通过固定二氧化碳来生产高附加值化工品的酿酒酵母细胞工厂。

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Summary for “代谢工程改造酿酒酵母底盘细胞”

Metabolic engineering of *Saccharomyces cerevisiae* chassis

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Saccharomyces cerevisiae acts as a bio-foundry for producing natural products. *S. cerevisiae*'s intrinsic pathways highlight microbial cell factories to produce pharmaceuticals, food additive, and fine chemicals. For high-titer, yield, and conversion of target compounds, the critical point is that fine-tuning and optimizing intracellular metabolic flux in the yeast cell. Acetyl-CoA is the fundamental precursor for central metabolism and heterologous pathway. In comparison, acetyl-CoA compartmentalizes in mitochondria, nucleus, peroxisome subcellular, and cytosol. Most of the pathway enzymes are expressed in *S. cerevisiae* cytosol. So, we summarized the fine-tuning strategies of acetyl-CoA synthesis in cytosol matrix. The new acetyl-CoA pathway (phosphoketolase, phosphotransacetylase, and acetaldehyde dehydrogenase, PK/PTA-ADA) shows a high conversion ratio from the carbon source. The PK/PTA-ADA pathway presents improved redox balance, limited ATP requirement, and reduced carbon loss to CO₂. Moreover, the heterologous ATP-dependent citrate lyase precisely converts mitochondria metabolism into acetyl-CoA. Knock-out acetyl-CoA depletion pathways retain high acetyl-CoA titer in the cytosol. The continuous metabolic engineering on PK/PTA-ADA pathway is expected for higher acetyl-CoA titer.

The Mevalonate pathway has been engineered to produce terpenoids in *S. cerevisiae*. The exogenous HMG-CoA synthase and HMG-CoA reductase boost mevalonate pathway from acetyl-CoA. Further, the diversified terpenoids are generated from C₅ unite Isopentenyl diphosphate and its' isomer dimethylallyl diphosphate. Intrinsic farnesyl diphosphate synthase (Erg20) shows both dimethylallyltransferase (geranyl diphosphate, C₁₀ product) and geranyltransferase (farnesyl diphosphate, C₁₅ product) activity. The F96W/N127W mutations in Erg20 caused steric hindrance for geranyl diphosphate substrate. So, geranyl diphosphate is accumulated to produce C₁₀ terpenoids or meroterpenoids. Moreover, geranylgeranyl diphosphate synthase (CrtE) convert farnesyl diphosphate (C₁₅) into geranylgeranyl diphosphate (C₂₀) for carotene, lycopene, astaxanthin, and taxol, etc. Interestingly, the novel isopentenol utilization pathway has been constructed in *E. coli* and *Y. lipolytica* to supply IPP and IMAPP precursors from isopentanol. This alternative pathway could relieve the acetyl-CoA supply burden in the mevalonate pathway.

Fatty acid synthase (FAS) produces fatty acid with specific carbon length and derivatives from acetyl-CoA and malonyl-CoA. Acetyl-CoA carboxylase (ACC1) catalyzes acetyl-CoA to produce malonyl-CoA, which is utilized as the carbon unite in fatty acid pathway. Overexpression of ACC1 and mitochondria located isoenzyme Hfa1 significantly improves fatty acid production. FAS in *S. cerevisiae* is an α6β6 heterodimer protein and these catalytic domains are engineered to produce specific fatty acids. Directed mutations in ketoacyl synthase (KS), acetyltransferase (AT), and malonyl/palmitoyl transferase (MPT) domains engage FAS to generate high-yield short/medium fatty acid (C₆ and C₈). Furthermore, fatty acid reductase and acyl-ACP reductase convert fatty acid or fatty acyl-ACP to fatty aldehydes, respectively. Aldehyde deformylating oxygenase produces alkanes from fatty aldehydes substrate. Significantly, fatty acid-derived bio hydrocarbons showed closets properties to petroleum fuel.

We reviewed the metabolic strategies of acetyl-CoA synthesis, the novel mevalonate pathway and fatty acid synthesis. These strategies may be valuable for producing high-yield terpene and fatty acid derivatives in *Saccharomyces cerevisiae*.

***Saccharomyces cerevisiae*, synthetic biology, acetyl-CoA, terpenoid, fatty acid**

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