特约评述

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基于化学品生物合成的嗜甲烷菌人工细胞构建及应用进展

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摘要：由于来源广泛且储量丰富，甲烷被认为是极具应用潜力的下一代生物碳源。嗜甲烷菌是一种分离自富含甲烷环境中的革兰氏阴性细菌，其体内含有独特的甲烷单加氧酶能够让这类微生物以甲烷为唯一碳源和能源进行生长、代谢与产物合成。作为一种重要的工业微生物，嗜甲烷菌在甲烷生物转化利用、温室气体减排和“碳中和”策略开发方面具有重要意义。近年来，随着嗜甲烷菌基因编辑方法、代谢路径调控、生物元件挖掘等菌种构建工具和策略的不断开发，嗜甲烷菌人工细胞可高效转化甲烷生物合成多种大宗化学品和生物燃料。本文围绕遗传改造工具、甲烷碳流调控、异源途径表达和代谢节点累积等方面的研究进展，概述了构建嗜甲烷菌人工细胞的方法和提高甲烷同化效率的策略。同时介绍了基因组学、转录组学、代谢组学等组学研究方法在调控嗜甲烷菌底盘碳代谢流向和通量中的应用。最后，结合生物转化甲烷合成酸类、萜类、醇类等化学品的研究，分析并展望了嗜甲烷菌工业化应用所面临的挑战和机遇。

关键词：甲烷；嗜甲烷菌；细胞工厂；构建策略；化学品生物合成

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Progress in construction and applications of methanotrophic cell factory for chemicals biosynthesis

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Abstract: Methane has been considered as a potential carbon source in industrial biotechnology because of its abundance, sustainability, high reducibility, and microbial availability. The biological conversion of methane into chemicals or fuels does not only reduce greenhouse gas emissions, but also substitute food-based substrates used in bio-manufacturing. Methanotrophs are gram-negative bacteria, and most are isolated from methane-plentiful environments. Owing to the presence of the methane monooxygenase, methanotrophs constitute a unique group of microbes. As an

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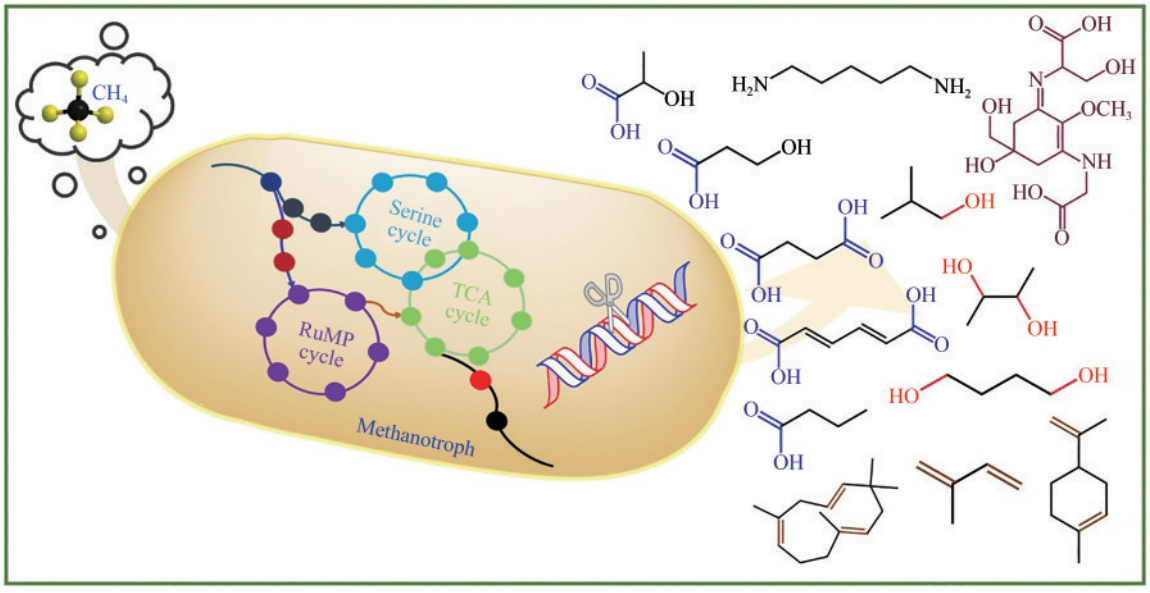
important industrially-promising microorganism with the characteristics of robust and anti-contamination ability, methanotrophs capable of growing with methane as the sole energy and carbon source play a significant role in carbon-neutral society by replacing petroleum-based products with biosynthesized products. Therefore, studies on methanotrophs for the biological conversion of methane have attracted extensive attention in recent years. With the rapid development of genetic manipulations tools and strategies for metabolically-engineered methanotrophs construction, including gene editing methods, regulation of metabolic pathways, and bio-elements mining, methanotrophic cell factories have been employed to efficiently convert methane into a variety of bulk chemicals and biofuels. In this review, biosynthetic technologies related to bioconversion of under-utilized methane ranging from fundamental understanding, systematic analysis, metabolic engineering to bio-product production are introduced. The genetic manipulations tools of methanotrophs, the approaches of methanotrophic cell factory construction, and the enhancement of methane assimilation efficiency are summarized from the aspects including the research progress of genetic engineering of methanotrophs, the regulation of methane carbon flux, the overexpression of heterologous pathway genes, and the accumulation of metabolic intermediates. Besides, the applications of genomics, transcriptomics, metabolomics, and metabolic modeling have been also deployed to facilitate the methane metabolism in methanotrophs chassis. Finally, given the strategy of ‘waste-to-value’ production, the challenges and opportunities for methane bioconversion by methanotrophs are also discussed and prospected based on industrial applications in terms of the research progress in the biosynthesis of methane-based acids, terpenes, alcohols, and other chemicals.



Keywords: methane; methanotrophs; cell factory; construction strategy; chemical biosynthesis

作为一种无色、无味、无毒的可燃性气体，甲烷（CH_4）是目前发现的最简单、能量密度最大的烷烃（热值为55 MJ/kg）[1]，也是仅次于二氧化碳（CO_2）的第二大温室气体源头[2-3]。近期，Feldman等[4]通过实验和观测首次证实了甲烷排放增长与全球变暖加速之间的正向关系。当前大部分甲烷排放来自于能源开采（天然气和石油）、牲畜饲养、垃圾填埋场等人类活动[5-6]。随着人口增长导致的肉类食品和能源需求的不断提高，甲烷的排放量还将持续上涨，这将对节能减排和“碳中和”等国家战略造成一定冲击。而作为天然气和沼气的主要组成部分，甲烷也具有储量丰富、

价格低廉、碳原子还原性强、微生物可利用等优势，是一种极具潜力的碳源[7]。嗜甲烷菌是一种能够以甲烷作为唯一碳源和能源进行生长和代谢的微生物，这类菌群在缓解大气中甲烷排放方面发挥着重要作用[8]。近年来，围绕嗜甲烷菌开展甲烷生物转化利用的研究得到了国内外广泛的关注[9-18]。

由于抗污染能力强，且可在温和生长条件下实现甲烷的快速氧化和同化代谢，嗜甲烷菌已成为理想的甲烷生物利用工程菌改造宿主，研究证实开发和构建嗜甲烷菌细胞工厂具有重要的科学价值和研究意义[1, 13, 19-21]。到目前为止，用于化学品和生物燃料合成的微生物多以糖类作为发酵底物，考虑到其原料价格不稳定，开发廉价、非食品的碳源已成为生物制造领域中亟待解决的重要课题之一[22]。研究发现，当甲烷被用作底物生产还原性化学品时，能够提供更多的电子，可有效促进目标产物得率和产量[23-24]。利用前沿合成生物技术对嗜甲烷菌的代谢网络进行理性设计和改造，已实现生物转化甲烷合成多种平台化合物。由此可见，通过开发甲烷生物转化技术，不但可以促进甲烷有效利用和减少温室气体排放量，还能够为建立循环经济模式提供一种全新的研发策略和实践方向[13]。

本文主要介绍了嗜甲烷菌人工细胞构建的相关研究进展，重点分析了嗜甲烷菌代谢工程改造策略和化学品合成相关研究成果。最后结合在生物制造领域的应用前景，探讨了嗜甲烷菌人工细胞构建和改造等方面所面临的挑战和机遇。

1　嗜甲烷菌及其改造策略

分离自富含甲烷环境中的嗜甲烷菌是一种天然的甲烷利用菌株。虽然研究发现嗜甲烷菌可在厌氧条件下利用甲烷生长，但是绝大多数可人工培养的嗜甲烷菌均需在好氧环境中完成甲烷的氧化过程[25-26]。因此本文主要围绕好氧性嗜甲烷菌的相关研究展开一系列讨论和分析。如图 1 所示，嗜甲烷菌体内独有的甲烷单加氧酶（methane monooxygenases，MMO）可在胞内将甲烷转换为甲醇，随后通过内源代谢途径完成碳同化[28]。目

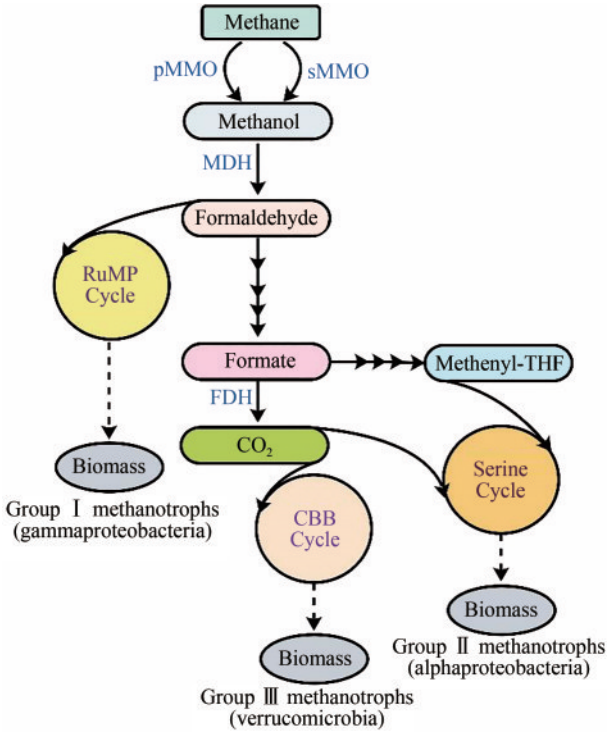


图 1　嗜甲烷菌中甲烷的代谢途径[8, 27]

Fig. 1　The pathways of methane metabolism in methanotrophs[8, 27]

pMMO—particulate methane monooxygenase; sMMO—soluble methane monooxygenase; MDH—methanol dehydrogenase; FDH—formate dehydrogenase

前，根据甲烷同化途径的不同，好氧性嗜甲烷菌主要被分为Group Ⅰ、Group Ⅱ和Group Ⅲ[29]，其分别通过单磷酸核酮糖（ribulose monophosphate，RuMP）循环、丝氨酸循环（serine cycle）和卡尔文循环（Calvin-Benson-Bassham cycle， CBB）实现甲烷的生物转化。上述3种同化过程已在前期文章中进行了详细的介绍[1, 9, 18, 25, 30]，本文不再赘述。

遗传改造工具在嗜甲烷菌人工细胞构建研究中发挥着重要作用。目前基于嗜甲烷菌报道的基因操作工具主要包括质粒载体和基因转移方法。质粒载体主要用于基因复制、基因表达和基因敲除的相关工作，而基因转移方法多用于将质粒或外源基因转入嗜甲烷菌体内，包括接合转移和电穿孔。现阶段通过优化的接合转移和电穿孔方法，研究人员在嗜甲烷菌体内已成功实现了基因缺失、整合和位点特异性重组等多种遗传操作[20]。此外，研究人员基于嗜甲烷菌体系还成功开发了人工诱变技术[31]、适应性进化[32]、基因表达模块和元件优化（启动子和其他调节元件）[33]以及前沿基因编辑[10]等人工细胞构建方法[34-35]。在探索镧系元素开关对甲醇脱氢酶mxaF调节功能的研究中，Groom等[31]通过化学诱变（亚硝基胍）方法，基于*Methylotuvimicrobium buryatense* 5GB1C菌株成功筛选出镧元素无法抑制mxaF启动子的突变体。Lee等[32]基于*Methylomonas* sp. DH-1通过适应性进化筛选出一株耐乳酸菌株JHM80，用于提高工程菌株的乳酸耐受性和产量。Puri[34]等报道了*M. buryatense* 5GB1S中不同表达强度的启动子元件。Garg等[33]通过模块化组装策略，进一步优化了利用甲烷合成乳酸的*M. buryatense* 5GB1人工细胞。表1列出了嗜甲烷菌遗传改造工具。

近期，CRISPR/Cas9基因编辑系统也被证实可在嗜甲烷菌体系中应用。Tapscott等[42]在*M. capsulatus* Bath体内利用CRISPR/Cas9实现了sMMO的敲除及GFP蛋白的修饰。此外，Liu等[10]利用苯丙氨酰tRNA合酶编码基因*phzS*^{AG}，在*M. buryatense* 5GB1C体系中开发了一种基因无痕敲除方法，有效提升了基因敲除效率。以上这些基因操作方法的建立进一步拓宽了嗜甲烷菌在甲烷转化中的应用，使嗜甲烷菌成为具有工业应用前景的模式菌株[14]，也为实现甲烷基化学品的高效生物合成打通了路径（表2）。

当前，基于化学品生物合成的嗜甲烷菌人工细胞的改造工作主要围绕关键代谢节点（丙酮酸和乙酰辅酶A）累积和甲烷同化过程（RuMP循环、丝氨酸循环）理性改造开展，通过有效地调控甲烷碳通量走向，实现碳转化效率的提高和目标产物的高效合成。如图2所示，RuMP循环主要通过核酮糖-5-磷酸（ribulose-5-phosphate， Ru5P）利用甲醛以实现甲烷的同化[13]。其中，磷酸转酮酶（phosphoketolase， PKT）途径产生的赤藓糖4-磷酸（E4P）或甘油-3-磷酸（GAP）重新排列，能够实现Ru5P的再生，从而促进Ru5P对甲烷的同化效率[20, 61]。通过磷酸转酮途径增加甲烷代谢的碳通量被认为是一种十分有效的代谢工程改造方法，这一途径的重构已使得多种微生物人工细胞

表1 嗜甲烷菌遗传改造工具

Tab. 1 The genetic tool used for metabolic engineering of methanotrophs

功能	质粒	筛选标记	菌株	参考文献
基因复制	pBHR1	Km, Cm	*Methylomonas* sp. 16a	[36]
	pAWP78	Km	*M. buryatense* 5G	[34]
基因表达	pAWP89	Km	*M. buryatense* 5G	[34]
	pCAH01:emGFP	Km	*M. buryatense* 5G	[37]
基因敲除	pK18mobsacB	Km, SacB	*Methylococcus capsulatus* (Bath)	[38]
	pCM184	Gm, SacB	*Methylomicrobium alcaliphilum* 20Z	[39]
基因转移技术				
接合转移	pRK2013	Km	*Methylomonas* sp. 16a	[40]
	pBHR1	Km	*Methylomonas* sp. 16a	[40]
电转化	pAWP89（线性化）	Km	*M. buryatense* 5G	[41]
	pheS^{AG}（线性化DNA片段）	Km	*M. buryatense* 5G	[10]

表2 嗜甲烷菌人工细胞转化甲烷合成化学品和生物燃料

Tab. 2 Biosynthesis of various chemicals and biofuels from methane by methanotrophic cell factories

菌株	产物种类	代谢途径/前体	产物	产量	参考文献
M. buryatense 5GB1C[43]	有机酸	丙酮酸	L-乳酸	800 mg/L	[37]
	有机酸	丙酮酸	L-乳酸	600 mg/L	[33]
	有机酸	乙酰辅酶A	丁烯酸	70 mg/L	[44]
	有机酸	乙酰辅酶A	丁酸	40 mg/L	[44]
	有机酸	莽草酸途径	黏糠酸	12.4 mg/L	[45]
	有机酸	乙酰辅酶A	脂肪酸	111 mg/g DCW	[46]
M. alcaliphilum 20Z[47]	生物醇	丙酮酸	2,3-丁二醇	86.2 mg/L	[14]
	生物醇	丙酮酸	2,3-丁二醇	361.3 mg/L	[39]
	有机酸	乙酰辅酶A	3-羟基丙酸	196.3 mg/L	[39]
	有机酸	莽草酸途径	黏糠酸	0.75 mg/L	[45]
	萜类	MEP 途径	α-蛇麻烯	0.75 mg/g DCW	[48]
	—	TCA循环	腐胺	98.08 mg/L	[49]
	有机酸	丙酮酸	乳酸	0.027 g/(g DCW·h)	[50]
	—	RuMP 循环	Shinorine	31 mg/L	[39]
M. capsulatus Bath[51]	有机酸	莽草酸途径	黏糠酸	1.0 mg/L	[45]
	萜类	MEP 途径	异戊二烯	10 mg/L	[52]
	生物醇	TCA 循环	1,4-丁二醇	—	[53]
	生物醇	丙酮酸	异丙醇	220 mg/L①	[54]
	生物醇	丙酮酸	异丙醇	1 mg/L	[54]
Methylomonas sp. DH-1[55]	有机酸	TCA 循环	琥珀酸	195 mg/L	[56]
	有机酸	丙酮酸	D-乳酸	1190 mg/L	[32]
Methylosinus trichosporium OB3b[57]	有机酸	乙酰辅酶A	3-羟基丙酸	60.6 mg/L	[15]
	—	TCA 循环	尸胺	283.6 mg/L	[58]
Methylomonas sp. 16a[36]	萜类	MEP 途径	柠檬烯	0.5 mg/L	[59]
	萜类	MEP 途径	法尼烯	—	[20]
	萜类	MEP 途径	虾青素	2.4 mg/g DCW	[60]
	萜类	MEP 途径	虾青素	2.0 mg/g DCW	[36]

①supply of 2-ketoisovaleric acid.

Note：MEP—methylerythritol 4-phosphate pathway；TCA—tricarboxylic acid；DCW—dry cell weight.

中的乙酰辅酶A积累量显著提高，为乙酰辅酶A衍生化学品的生物合成提供了可行的工程改造策略[62-65]。以 *M. buryatense* 5G 为例，Henard 等[61]研究发现该菌株通过RuMP途径可将6分子甲醛转化为2分子的乙酰辅酶A，而利用RuMP-PKT途径可将6分子甲醛转化为3分子的乙酰辅酶A。同时，RuMP-PKT途径能够避免源于糖酵解（Embden-Meyerhof-Parnas， EMP）途径中丙酮酸脱羧造成的ATP消耗，从而在不损失碳和能量的情况下实现乙酰辅酶A的合成[20]。此外，嗜甲烷菌中丝氨酸循环与乙基丙二酰辅酶（Ethylmalonyl-CoA，EMC）途径及三羧酸循环紧密相连，丝氨酸循环产生的乙酰辅酶A可被整合到乙基丙二酰辅酶通路中用于乙醛酸的再生和细胞产物PHB（poly-β-hydroxybutyrate）的生物合成[23]。作为关键中间代谢物和乙酰辅酶A合成前体，丙酮酸合成效率也是甲烷基化学品合成的关键瓶颈。目前敲除其竞争途径是提高丙酮酸累积浓度的最有效、最简单的方式之一[20]。有研究发现，嗜甲烷菌中的丙酮酸和GAP的产量分别是乙酰辅酶A的150倍和30倍，而在大肠杆菌中乙酰辅酶A的产量远高于丙酮酸和GAP[20，66-67]。这可能是由于嗜甲烷菌中内源MEP途径代谢通量较强导致丙酮酸和GAP倾向于缩合形成脱氧-D-木酮糖5-磷酸（deoxy-D-xylulose 5-phosphate， DXP），从而减少了丙酮酸向乙酰辅酶A合成过程的碳流。

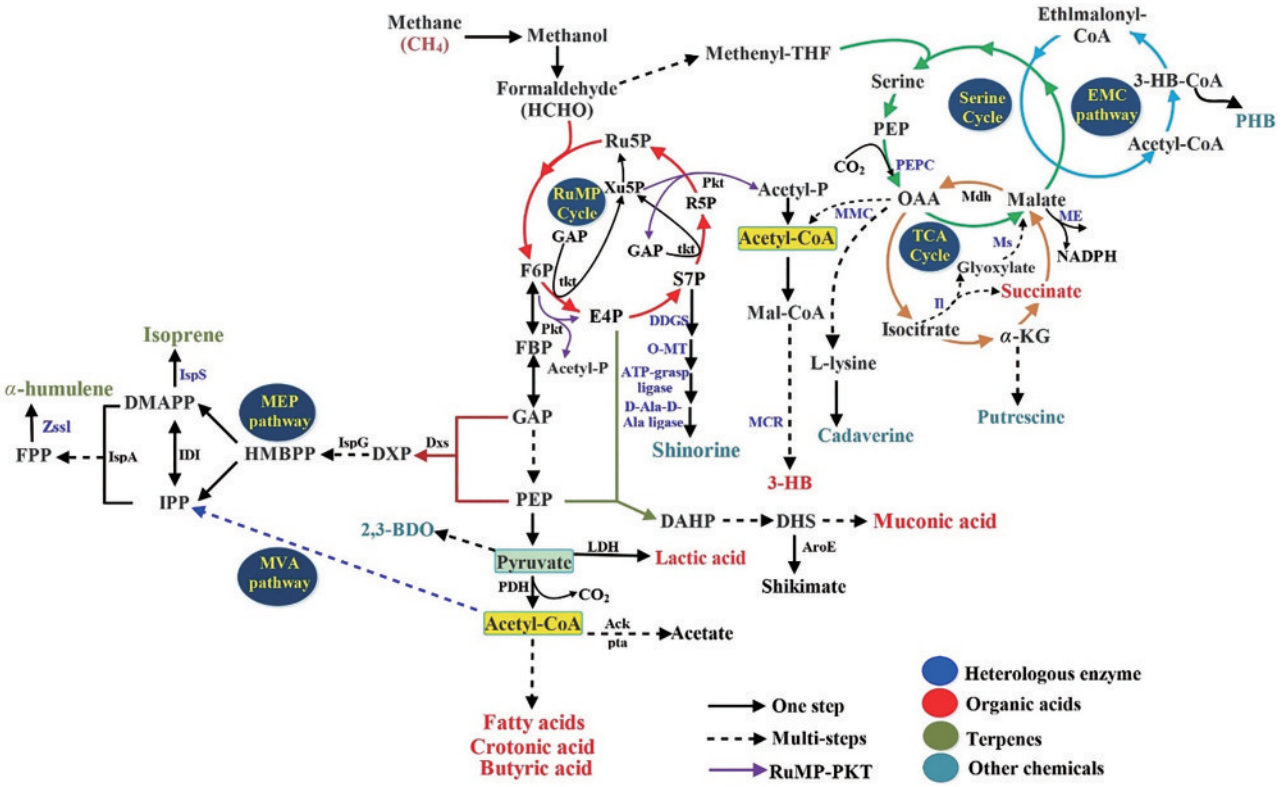


图2 嗜甲烷菌转化甲烷合成化学品和燃料的代谢途径[27]

Fig. 2 Metabolic pathways for the production of chemicals and fuels by methanotrophs using methane[27]

(F6P—Fructose-6-phosphate; FBP—Fructose bisphosphate; DHAP—Dihydroxyacetone phosphate; Xu5P—Xylulose-5-phosphate; Ru5P—Ribulose-5-phosphate; R5P—Ribose-5-phosphate; GAP—Glyceraldehyde-3-phosphate; OAA—Oxobutanedioic acid; 3HB-CoA—3-hydrobutylryl-CoA; E4P—Erythrose 4-phosphate; S7P—Sedoheptulose-7-phosphate; DAHP—Dihydroxyacetone phosphate; DHS—3-dehydroshikimate; HMBPP—1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate; DMAPP—Dimethylallyl Pyrophosphate; IPP—Isopentenyl pyrophosphate; PEP—Phosphoenolpyruvic acid; FPP—Farnesyl Pyrophosphate; MVA—Mevalonate pathway)

2 基于化学品合成的嗜甲烷菌人工细胞的构建

2.1 有机酸类化学品

有机酸类化学品作为天然产物，在食品、制药和生物基材料工业中应用广泛[68-72]。研究人员基于丙酮酸、乙酰辅酶A以及磷酸烯醇式丙酮酸（phosphoenolpyruvic acid，PEP）等甲烷代谢中间产物构建的嗜甲烷菌人工细胞已成功将甲烷转化为C_3～C_{18}不同链长的有机酸类化学品（表2）。乳酸（lactic acid）在嗜甲烷菌中的生物合成已在途径构建、模块筛选以及培养优化等方面均取得了重要进展[33]，这主要是得益于应用合成生物学模块化思路完成了嗜甲烷菌合成乳酸模块的代谢路径和表达水平的优化。近期，Lee等[32]基于适应性进化策略获得乳酸耐受菌株*Methylomonas* sp. DH-1 JHM80，通过基因组测序发现该菌株启动子区域突变引发了弱酸耐受调节子（weak acid tolerance regulator，WatR）表达的上调。随后，该研究通过定向精准调控*watR*基因的表达水平，使乳酸产量提高到1.19 g/L。嗜甲烷菌*M. trichosporium* OB3b中具有独特的乙基丙二酰辅酶途径，能够利用3分子乙酰辅酶A同化1分子CO_2和1分子HCO_3^-，从而积累2分子苹果酸[73-74]。Nguyen等[15]基于该菌株中特有的丝氨酸循环，通过引入非产氧光合菌*Chloroflexus aurantiacus*中的还原酶（malonyl-CoA reductase，MCR），构建了3-羟基丙酸（3-hydroxypropionic acid，3HP）合成模块，并通过过表达苹果酸酶（malic enzyme，ME）转化苹果酸实现了NADPH再生，为MCR催化过程提供了充足的还原力，进而有效提高了3HP的产量。同时，为了避

免乙酰辅酶A羧化成丙二酰辅酶A的限速步骤，该研究将来源于费氏丙酸杆菌（*Propionibacterium freudenreichii*）中的甲基丙二酰羧基转移酶（methylmalonyl-CoA carboxyltransferase，MMC）和磷酸烯醇式丙酮酸羧激酶（phosphoenolpyruvate carboxylase，PEPC）共表达用以构建乙酰辅酶A羧化旁路[15, 74-75]。此外，Garg等[44]利用反向β-氧化途径成功在*M. buryatense* 5GB1C内构建了异源丁烯酸（crotonic acid）合成途径，并在此基础上进一步优化了异源基因表达的核糖体结合位点和启动子、敲除磷酸转乙酰酶基因*pta*以及过表达乙酰辅酶A合成酶将乙酸再循环为乙酰辅酶A，这些改造策略使该嗜甲烷菌人工细胞可以同时积累丁烯酸（70 mg/L）和丁酸（40 mg/L）[44, 76-77]，为甲烷基C_4羧酸的合成提供了有效的策略。同样，为了提高琥珀酸（succinic acid）产量，Nguyen等[56]通过引入异源异柠檬酸裂合酶（isocitrate lyase，Il）和苹果酸合酶（malate synthase，Ms）用以分流乙醛酸并补充TCA循环中的苹果酸盐，从而成功将嗜甲烷菌丝氨酸代谢途径中产生的草酸乙酸和乙酰辅酶A用于合成琥珀酸（图2）。

近期，Henard等[45]围绕莽草酸途径，利用^{13}C代谢分析建立的基因尺度模型（genome-scale metabolic models，GSMs）预测了*M. alcaliphilum* 20Z合成黏糠酸的竞争途径。虽然基于模型分析结果敲除了丙酮酸脱氢酶（pyruvate dehydrogenase，Pdh）和莽草酸脱氢酶（shikimate dehydrogenase，Skd）编码基因*AroE*，但黏糠酸的产量仅1.5 mg /g DCW，为预测产量值的6.5%。这可能是由于*M. alcaliphilum* 20Z中存在莽草酸合成的替代途径或Skd的同工酶，敲除竞争途径并未能充分地将碳流转向黏糠酸。值得一提的是，基因测序技术及组学（基因组学、转录组学、蛋白质组、代谢组）技术也为深入理解嗜甲烷菌的生理学基础、细胞代谢和反应过程提供了新的方法[46, 51, 67, 77-83]。在嗜甲烷菌中，由于中心代谢途径与细胞内膜合成相关联，因而能够积累大量的长链脂肪酸（fatty acids）。Demidenko等[46]利用转录组分析发现*M. buryatense* 5GB1菌株中脂肪酸降解、乙酰辅酶A和丙二酰辅酶A供给水平是提高脂肪酸产量的瓶颈，在敲除醋酸激酶和过表达乙酰辅酶A羧化酶后，脂肪酸的积累量较原始菌株提高了20%。而Fei等[84]则在上述研究的基础上进一步敲除糖原、蔗糖-6-磷酸等竞争路径基因，最终在高密度发酵条件下将长链脂肪酸的产率提高了3倍。上述研究成果充分说明利用组学数据进而确立代谢改造靶点，可在深入认识嗜甲烷菌的代谢系统及生化反应机制机理的同时，有效提高目标产物和合成效率。

2.2 萜类化学品

虽然在嗜甲烷菌体内有部分甲基赤藓糖醇-4-磷酸（methylerythritol-4-phosphate，MEP）途径或甲羟戊酸（mevalonate，MVA）途径，但考虑到MEP途径拥有较高的IPP的理论合成率，甲烷基萜类化学品主要通过改造和优化MEP途径实现[11, 20, 67]。Leonard等[52]为了强化IspS催化活性，将葛根（*Pueraria montana*）中的IspS经密码子优化后，利用MDH强启动子在*M. capsulatus* Bath中表达并首次获得了10 mg/L异戊二烯。而Donaldson等[85]试图利用异源IspS以及异戊烯基焦磷酸异构酶（isopentenyl diphosphate isomerase，IDI）双基因提高异戊二烯合成效率，但遗憾的是异戊二烯的产量仅为0.056 mg/L，这主要是由于双基因表达效率及催化活性限制造成了该合成模块较低的碳通量。整体来看，现阶段嗜甲烷菌合成异戊二烯的研究主要集中于合成途径构建，节点累积和辅因子及能量供给平衡等代谢改造策略的研究相对较少。

除了异戊二烯，柠檬烯（limonene）和法尼烯（Farnesene）等萜类化合物也是重要的精细化学品、香料、药品的合成前体[86]。DiCosimo等[59]利用pR58载体，将异源柠檬烯合成酶（limonene synthase）在*Methylomonas* sp.16a菌株中表达，首次实现在嗜甲烷菌中累积0.5 mg/L柠檬烯。而Nguyen等[48]则基于*M. alcaliphilum* 20Z菌株中MEP途径的中间代谢物法尼焦磷酸（Farnesyl pyrophosphate，FPP），通过引入异源蛇麻烯合成酶（humulene synthase）Zssl[48, 87]，成功实现了蛇麻烯的从头合成。围绕过表达合成关键酶（IspA、IspG及Dxs）、重新定向EMP途径、NADPH辅因

子再生、电子供应强化、基因尺度模型等策略，该研究首次对萜类化学品的合成途径进行了系统的理性代谢工程改造和优化，最终将蛇麻烯产量提高到0.75 mg/g DCW，达到出发菌株的18.8倍[67, 81]（图2）。角黄素（canthaxanthin）、虾青素（astaxanthin）和番茄红素（lycopene）[88]等类胡萝卜素产物也可在嗜甲烷菌中实现生物合成[88]，但产量仍有待进一步提高。

2.3 其他化学品

随着可持续发展战略的提出，生物醇类物质作为生物能源的前体，近年来备受工业界和科学界的关注[54, 89-90]。研究人员结合基因尺度模型i20ZR-BDO的分析数据，预测了2,3-丁二醇（2,3-butanediol，BDO）合成过程的改造靶点，在累计敲除竞争途径中的*ldh*、*ack*和*mdh*等基因后，将BDO浓度提升到86.2 mg/L[14]。为充分发挥基因尺度模型在嗜甲烷菌人工细胞设计、构建及优化中的重要作用，Nguyen研究组又建立了基于尸胺（cadaverine）和腐胺（putrescine）生物合成的基因尺度模型，预测了关键代谢节点和终端产物的代谢工程改造靶点。通过过表达目的基因、敲除竞争途径或强化产物分泌效率，有效地提高了碳通量，并最终分别将尸胺和腐胺的产量提高到283.64 mg/L[14]和98.08 mg/L[49]。此外，与大肠杆菌及酿酒酵母相比，嗜甲烷菌在RuMP途径代谢中更具优势。研究人员通过将异源2-脱甲基4-脱氧葡萄糖醇合酶（2-demethyl 4-deoxygadusol synthase，DDGS）、氧甲基转移酶（*O*-methyltransferase，OMT）、ATP-连接酶（ATP-grasp ligase）和丙氨酸连接酶（D-Ala-D-Ala ligase）整合到*M. alcaliphilum* 20Z中，成功设计并合成了具有防晒功能的类菌胞素氨基酸Shinorine[91, 92]。与此同时，研究人员基于铜、钙等微量元素对MMO及MDH活性的影响机理，在微生物培养过程添加上述微量元素并利用操纵子计算器（operon calculator）进一步优化了Shinorine合成模块，将产量提高到31 mg/L[39, 78, 93]（图2）。上述基于系统生物学及合成生物学的代谢工程策略已广泛应用于嗜甲烷菌人工细胞的构建和优化。

3 总结与展望

随着全球甲烷供应量的增加，特别是天然气和沼气产量的提高[94-96]，人们对甲烷的生物转化利用产生了更广泛关注。近年来，由于具有相对高效的甲烷固定途径以及能够实现高密度发酵，嗜甲烷菌作为甲烷转化人工细胞在化学品合成中表现出了良好的应用前景[37, 97]。利用其特有的甲烷氧化和同化系统，结合前沿的基因编辑工具及代谢途径调控策略，目前已开发了不同类型和功能的嗜甲烷工程菌用于生物合成化学品，并展现出了独特的优势[15, 67, 98-99]。虽然通过代谢工程改造已实现多种甲烷基化学品生物合成，但目前嗜甲烷菌人工细胞相关研究仍然处在关键共性基础技术的研发阶段，在设计、构建和优化等方面仍然面临很多挑战[67, 82, 98, 100-101]。

尽管基于嗜甲烷菌工业菌株已开发出多种基因操作工具，但相较于大肠杆菌和酿酒酵母等模式菌株，嗜甲烷菌中可用于人工细胞构建的生物技术仍十分有限，而针对外源基因在嗜甲烷菌人工细胞内稳定表达的基因转化方法也亟需进行拓展。此外，嗜甲烷菌中电子传递系统、生物固氮/固硫机制、磷酸盐代谢调控等关键科学问题的研究也有待探究。未来相关工作需进一步提高嗜甲烷菌基因尺度模型预测的准确性，并基于对代谢机制机理的探索，有针对性地改造和优化化学品合成路径。同时，结合各类组学分析数据，从系统生物学角度加深对嗜甲烷菌细胞行为的认识，明确嗜甲烷菌特异性基因表达调控机制、甲烷代谢过程的酶活性以及代谢流分布，为高效关键基因元件挖掘和人工细胞构建提供理论指导，为甲烷的高效生物利用和化学品合成提供全新的研究思路和工业应用。

符号说明

BDO —— 2,3-丁二醇
CBB —— 卡尔文循环
DCW —— 细胞干重
DDGS —— 2-脱甲基4-脱氧葡萄糖醇合酶
DXP —— 脱氧-D-木酮糖5-磷酸
E4P —— 赤藓糖4-磷酸

EMC——乙基丙二酰辅酶
EMP——糖酵解途径
FPP——法尼焦磷酸
GAP——甘油-3-磷酸
GSMs——基因尺度模型
3HP——3-羟基丙酸
IDI——异戊烯基焦磷酸异构酶
Il——异源异柠檬酸裂合酶
MCR——丙二酰基辅酶A还原酶
MDH——甲醇脱氢酶
ME——苹果酸酶
MEP——甲基赤藓糖醇4-磷酸
MMC——甲基丙二酰羰基转移酶
MMO——甲烷单加氧酶
Ms——苹果酸合酶
MVA——甲羟戊酸
OMT——氧甲基转移酶
Pdh——丙酮酸脱氢酶
PEP——磷酸烯醇式丙酮酸
PEPC——磷酸烯醇式丙酮酸羧激酶
PHB——聚羟基丁酸
PKT——磷酸转酮酶
R5P——核糖-5-磷酸
Ru5P——核酮糖-5-磷酸
RuMP——单磷酸核酮糖
Skd——莽草酸脱氢酶
WatR——弱酸耐受调节子

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