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我国产甲烷古菌研究进展与展望

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摘　要：产甲烷古菌广泛分布在湿地、水稻田、动物瘤胃、油藏、海洋和热液等缺氧环境，在全球碳素循环、气候变化和清洁能源生产等领域发挥着重要作用，一直是国内外的研究热点。本文简要回顾了我国产甲烷古菌的研究进展，重点阐述了产甲烷古菌的资源与分类、生理生化、分子生物学、生态学功能和应用等方面的研究进展，并展望了产甲烷古菌的未来研究趋势。

关键词：产甲烷古菌；生物学功能；碳循环

Methanogen research in China: current status and prospective

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Abstract: Methanogens are widely distributed in anaerobic environments, such as wetlands, paddy fields, animal rumens, oil reservoirs, oceans, and hydrothermal vents. They play critical

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roles in global carbon cycling, climate change, and clean energy production, making them a hot research topic both domestically and internationally. This article briefly reviews the research progress of methanogens in China, focusing on their resources and taxonomy, physiology and biochemistry, molecular biology, ecological roles, and applications. The future research trends of methanogens are also highlighted.

Keywords: methanogen; biological function; carbon cycle

产甲烷古菌被认为是地球上最早的生命形式之一，是研究生命起源与进化的重要模式物种[1]。全球每年约有一半以上的 CH_4 是由产甲烷古菌在缺氧环境中形成的[2]。另一方面，CH_4 是主要的温室气体之一，在 20 年尺度范围内，CH_4 的增温效应是 CO_2 的 84 倍[3]。因此，产甲烷古菌对全球气候变化也有重要影响。同时，产甲烷古菌产生的 CH_4 可以替代化石能源、补充能源供给、减少化石燃料的使用和减轻环境污染[4]。因此深入理解产甲烷古菌的微生物学机制和生态学功能，有助于认识生命进化过程、揭示碳循环机制，并指导开发绿色可再生能源。

1 资源与分类

产甲烷古菌对氧气敏感、培养条件苛刻，分离培养难度较大。1933 年，Stephenson 等从英国乌斯河的污泥中分离出第一株利用甲酸产甲烷的产甲烷古菌纯培养物[5]。1980 年，我国学者周孟津等从猪粪发酵液中分离获得甲烷八叠球菌(*Methanosarcina*)的纯培养物，它具有代谢甲醇、甲胺、乙酸盐和 H_2/CO_2 产甲烷的能力，实现了我国产甲烷古菌分离培养的突破[6]。随后，我国学者先后从沼气池、反应器、湿地、水稻土等环境中分离出多株产甲烷古菌(附表 1，数据已提交至国家微生物科学数据中心，编号为 NMDCX0000196)，但是一直没有新物种的报道。直到 2005 年，东秀珠团队才从厌氧消化器中分离鉴定出第一个新种 *Methanobacterium beijingense* sp. nov. (表 1)[7]。2007 年，本团队从胜利油田分离鉴定了甲基营养型产甲烷古菌新属(*Methermicoccus shengliensis* gen. nov., sp. nov.)，并提出新科 *Methermicoccaceae* fam. nov.，实现了我国在产甲烷古菌资源分类从新种到新科的跨越[8]。截至 2022 年 11 月，有效发表的产甲烷古菌共有 171 个种，分属于 38 属、17 科和 8 目中。其中我国科学家共提出 1 个新科、1 个新属和 14 个新种(表 1)[7-19]。

过去认为产甲烷古菌仅分布在古菌域的广古菌门(*Euryarchaeota*)。2015 年，国外学者利用宏基因组技术，发现 *Candidatus* Bathyarchaeota 类群中 BA1 和 BA2 具有利用 H_2 还原甲基化合物产甲烷的代谢潜力[20]，首次提出产甲烷古菌可能也分布在广古菌门以外的分支上。截至目前，国内外多项研究从 *Ca*. Bathyarchaeota、*Ca*. Korarchaeota、*Ca*. Nezhaarchaeota、*Thermoproteota*、*Ca*. Thermoplasmatota 和 *Ca*. Verstraetearchaeota 等类群中发现 25 个未培养产甲烷古菌新物种(新属及以上分类单元) (表 2)[20-30]。其中我国科学家作为主要贡献者，参与了 11 个新属的提出，包括 *Ca*. Nezhaarchaeota 这个新门。本团队首次通过培养实验，证明了 *Ca*. Verstraetearchaeota 古菌具有氢依赖的甲醇和甲氨还原产甲烷能力(预印版 https://doi.org/10.21203/rs.3.rs-2501667/v1)。

表 **1** 我国学者分离鉴定的产甲烷古菌新物种

Table 1 Novel methanogenic taxon isolated and identified by Chinese scholars

Isolate species	Substrate	Morphology (μm)	Optimal growth conditions		Growth factor	Source	Genome	16S rRNA	Reference
			pH	*T*/°C					
Methanobacterium beijingense 8-2^{T}	H_2/CO_2, formate	Rod, 0.4–0.5×3–5	7.2	37	Nd	Anaerobic digesters	Nd	AY350742	[7]
Methanobacterium flexile GH^{T}	H_2/CO_2, formate	Rod, 0.3–0.5×2–5	7.0–7.5	35–38	YE	Gahai lake	Nd	EU333914	[9]
Methanobacterium movens TS-2^{T}	H_2/CO_2	Rod, 0.3–0.5×2–5	7.2–7.5	35–38	YE	Tuosu lake	Nd	EU366499	[9]
Methanocella conradii HZ254^{T}	H_2/CO_2	Rod, 0.2–0.3×1.4–2.8	6.8	55	Nd	Rice field soil located in Hangzhou	GCA_000251105.1	CP003243	[10]
Methanococcoides orientis LMO-1^{T}	Methanol, methylamine, dimethylamine and trimethylamine	Irregular cocci, 1.0–2.0	7.0–7.5	30–35	Nd	East China Sea sediment	GCA_021184045.1	NR_177426.1	[11]
Methanoculleus hydrogenitrophicus HC^{T}	H_2/CO_2	Irregular cocci, 0.8–2.0	6.6	37	Nd	Zoige wetland	Nd	FJ977567	[12]
Methanoculleus receptaculi ZC-2^{T}	H_2/CO_2, formate	Cocci, 0.8–1.7	7.5–7.8	50–55	Nd	Shengli oilfield	Nd	DQ787476	[13]
Methanoculleus sediminis S3Fa^{T}	H_2/CO_2, formate	Cocci, 0.5–1.0	7.1	37	Nd	Mud volcano	GCA_001017125.1	JXOJ01000002	[14]
Methanofollis fontis FWC-SCC2^{T}	H_2/CO_2, formate	Cocci, 0.8–1.2	6.7–7.0	37	Nd	Deep-sea sediments	GCA_004297185.1	MG437305	[15]
Methanolobus halotolerans SY-01^{T}	Methanol	Cocci, 0.8–1.0	8.0	30	YE	Sediment of saline Lake Tus	GCA_004745425.1	MG437307	[16]
Methanosaeta harundinacea 8Ac^{T}	Acetate	Rod, 0.8–1.0×3–5	7.2–7.6	34–37	YE, Trp	Anaerobic sludge blanket reactor	GCA_000235565.1	AY817738	[17]
Methanospirillum psychrodurum X-18^{T}	H_2/CO_2	Rod, 0.4–0.5×11–62	7.0	25	YE	Soil of the Madoi wetland	Nd	KF153052	[18]
Methanothermobacter crinale Tm2^{T}	H_2/CO_2	Slightly curved rods, 0.3–0.5×2.2–5.9	6.9	65	Nd	Production water of oil reservoirs	Nd	HQ283273	[19]
Methermicoccus shengliensis ZC-1^{T}	Methanol, methylamine and trimethylamine	Cocci, 0.7–1.0	6.0–6.5	65	SF, CoM	Oil-production water	GCA_000711905.1	JONQ01000007	[8]

YE: Yeast extract; Trp: Tryptone; SF: Sludge fluids; CoM: Coenzyme M; Nd: Not report.

表 2　新型潜在产甲烷古菌

Table 2　Novel candidate methanogenic archaea

Phylum	Genus	Metabolic type	Source	Research group	Reference
Ca. Bathyarchaeota[a]	BA1 and BA2	MM	Coal-bed methane	TYSON GW	[20]
Euryarchaeota	*Ca*. Methanohalarchaeum	MM	Salt lake	KOONIN EV	[31]
	Ca. Methanofastidiosum	MM	Anaerobic digestion reactor	LIU WT	[32]
	Ca. Methanoliparum	ALM	Petroleum sample	LI WJ[c]	[33]
	Ca. Methanolliviera	ALM	Oil seeps	LI WJ[c]	[33]
	Ca. Methanoflorens	HM	Rice, wetland, sediment, permafrost	TYSON GW	[34]
	Ca. Methanomixotrophus	MM	Oil reservoir	MU BZ[c]	[35]
Ca. Geoarchaeota[b]	Cren_UShs and Cren_JZhs	HM&AM	Hot spring	ZHANG T[c]	[21]
Ca. Korarchaeota[a]	*Ca*. Methanodesulfokores	MM	Hot spring	MCKAY LJ	[22]
Ca. Nezhaarchaeota[b]	WYZ-LMO7 and WYZ-LMO8	HM	Hot spring	WANG FP and XIAO X[c]	[23]
Ca. Thermoplasmatota	*Ca*. Methanomethylophilus	MM	Gut	BRUGÈRE JF	[24]
	Ca. Methanoplasma	MM	Gut	BRUNEA A	[25]
	Ca. Methanogranum	MM	Anaerobic sludge	IINO T	[26]
	Ca. Methanospyradousia	MM	Gut	HILDEBRAND F	[27]
Thermoproteota	*Ca*. Methylarchaeum	MM	Hot spring	HOU LJ[c]	[28]
	Ca. Methanotowutia	MM	Lake	HOU LJ[c]	[28]
	Ca. Methanoinsularis	MM	Harbour	HOU LJ[c]	[28]
	Ca. Methanoporticola	MM	Harbour	HOU LJ[c]	[28]
Ca. Verstraetearchaeota[a]	*Ca*. Methanomethylicus	MM	Anaerobic digestion reactor	TYSON GW	[29]
	Ca. Methanosuratincola	MM	Coalbed methane well	TYSON GW	[29]
	Ca. Methanohydrogenicus	HM	Yellowstone national park	QUAKE SR	[30]
	Ca. Methanomedium	HM	Yellowstone national park	QUAKE SR	[30]

[a]: Recently GTDB has reclassified it as a subordinate class of *Thermoproteota*; [b]: GTDB has recently reclassified it as an order of *Thermoproteota*; [c]: Chinese. AM: Acetoclastic methanogensis; HM: Hydrogentrophic methanogensis; MM: Methylotrophic methanogensis; ALM: Alkylotrophic methanogensis.

2　产甲烷途径

产甲烷古菌底物谱简单，一般认为其只能利用少数的 C1 和 C2 化合物生长。根据底物谱，产甲烷古菌的产甲烷途径可分为 CO_2 还原途径 (hydrogenotrophic)、乙酸发酵途径 (acetoclastic)和甲基裂解途径(methylotrophic)[36-37]。进行 CO_2 还原途径的产甲烷古菌可以利用 H_2 作为电子供体将 CO_2 还原为 CH_4；而采用乙酸发酵途径的产甲烷古菌可以将乙酸分解成 CH_4 和 CO_2；甲基裂解途径的产甲烷古菌可以将简单的甲基类化合物降解产生 CH_4 和 CO_2，还有一类特殊的甲基营养型产甲烷古菌需要 H_2 提供电子还原甲基类物质产甲烷[38]。*Methanosarcina* 是目前已被证实唯一可以同时利用 CO_2、乙酸和简单甲基类化合物产甲烷的古菌。而一直认为仅能利用乙酸产甲烷的 *Methanosaeta* (*Methanothrix*)，被发现可以利用种间直接电子

传递获得的电子还原 CO_2 产甲烷[39]。2016 年，日本科学家发现 *M. shengliensis* 具有直接降解煤炭中的甲氧基化合物产甲烷的功能，提出了第 4 条产甲烷途径——甲氧基营养型产甲烷途径(methoxylotrophic)[40]。2022 年，本团队联合深圳大学李猛和德国马普海洋所 Gunter Wegener 等团队，通过稳定碳同位素示踪培养、宏基因组和宏转录组测序及高分辨质谱分析，证实了一种新型的产甲烷古菌 *Ca*. Methanoliparum 能够直接降解 C13 及以上正构烷烃、C19 及以上长链烷基环己烷和烷基苯产甲烷，提出并证实了第 5 条产甲烷途径——烷基型产甲烷途径(alkylotrophic)[41]。

3 分子生物学研究

目前国外构建的产甲烷古菌的遗传操作系统有 *Methanococcus*、*Methanosarcina* 和 *Methanothermobacter* 属的古菌[42-46]。2022 年，我国东秀珠团队在国外研究的基础上开发了 *Methanococcus maripaludis* 的 CRISPR-Cas9 基因操作体系，实现了对产甲烷古菌的靶向基因编辑[47]。东秀珠团队在产甲烷古菌分子机制方面也开展了大量研究，他们在 *Methanolobus psychrophilus* R15 中发现了 mRNA 的加工现象，打破了原核生物“mRNA 不加工”的传统认知[48]。mRNA 加工在产甲烷古菌适应低温环境时起着重要作用，拥有更长 mRNA 的甲醇甲基转移酶和乙酸激酶具有更好的低温稳定性[49-50]。同时他们阐明了 *M. psychrophilus* 中 rRNA 操纵子(16S-tRNAAla-23S-tRNACys-5S)是如何加工成熟的，完善了对古菌中 rRNA 成熟过程的认知[51]；发现了古菌第一个冷休克蛋白 TRAM 通过 RNA 伴侣作用，在基因组水平上调控基因表达，从而保证菌株在低温胁迫下能正常转录和生长[52-53]；发现了古生菌中第一个通用转录终止因子——保守核糖核酸酶 aCPSF1，并阐明了终止机制，提出 aCPSF1 蛋白可以作为新的系统发育分子标志物，用于古菌的分类研究[54-55]。最新的研究结果表明，产甲烷古菌依赖 aCPSF1 转录终止模式与真核生物 RNA 聚合酶 II 终止机制相似，为“古菌是真核生物的进化起源”提供了新的证据[56]。此外，我国科学家在产甲烷古菌的膜脂研究中也作出了贡献，2022 年，曾芝瑞团队通过在 *M. maripaludis* 中异源表达 GDGT 合成基因，首次鉴定出产甲烷古菌中参与 GDGT 生物合成的关键蛋白——四醚合成酶(tetraether synthase, Tes)[57]。2023 年，司同团队通过在酿酒酵母(*Saccharomyces cerevisiae*)中异源表达来自 *Methanosarcina acetivorans* 的合成膜脂关键基因 G1PDH (glycerol-1-phosphate dehydrogenase)、GGGPS (geranylgeranyl pyrophosphate synthase) 和 DGGGPS [(S)-2,3-diO-geranylgeranylglyceryl phosphate synthase]，成功合成了具备古菌醚键特征的极性脂，首次在真核模式生物中实现了古菌特征细胞膜脂的异源合成[58]。以上研究填补了产甲烷古菌膜脂合成研究领域的空白，为认识和研究产甲烷古菌的膜脂合成奠定了基础。

4 生态分布

4.1 湿地

湿地甲烷是大气中甲烷的主要来源，约占总排放的 30%–50%[59]，产甲烷古菌是湿地甲烷排放的主要贡献者。青藏高原湿地和红树林湿地是我国湿地甲烷研究的主要对象，不同地区湿地中产甲烷古菌类型存在差异。青藏高原若尔盖高寒湿地以乙酸发酵产甲烷途径为主，伴随着 CO_2 还原途径[60]。另有研究显示，甲基营养型产甲烷古菌对若尔盖湿地产甲烷的贡献同样不容忽视[61]。在西藏高海拔湿地土壤中，氢营养型产甲烷古菌则成为主要类群[62]。与青藏高原湿地不同，红树林湿地中 SO_4^{2-} 含量较高，存在大量

硫酸盐还原菌,它们对乙酸的亲和力远高于乙酸营养型产甲烷古菌[63]。因此在红树林湿地以氢营养型和甲基营养型产甲烷古菌为主,但不同地区的产甲烷古菌类群有一定差异。例如：在深圳福田红树林生态系统中,氢营养型和甲基营养型的产甲烷途径是主要的产甲烷代谢方式[64]。而在海南东寨港红树林沉积物中,氢营养型产甲烷古菌为主要的产甲烷菌类群[65-66]。温度、盐度、地理位置、有机碳浓度、导电碳材料和周围植被类型等均会影响产甲烷古菌群落结构。在10–30 °C条件下，乙酸营养型产甲烷古菌占主导，而在45 °C条件下，氢营养型产甲烷古菌占主导地位[67]。氢营养型产甲烷古菌在盐湖、水底沉积物和高浓度有机碳环境中占优势[68-70],非盐湖、湿地浅层土和低浓度有机碳环境中乙酸营养型产甲烷古菌占优势[68-70]。导电碳材料促进了乙酸发酵产甲烷过程[71]。在刚毛荸荠土壤中甲基营养型产甲烷途径为主要贡献者,木里苔草土壤中乙酸产甲烷代谢途径占优势[72]。毛果草沼泽主要以氢营养型产甲烷菌为主,而黄叶草沼泽以氢营养型和乙酸营养型产甲烷菌为主[73]，这与植物细胞壁降解成不同的产甲烷前体物质有关[74]。尽管产甲烷古菌是湿地甲烷的主要贡献者,但湿地产甲烷潜力与产甲烷古菌数量无显著相关性[70]。有机碳浓度、降水和温度等是影响湿地甲烷排放量的重要因素,研究表明湿地产甲烷潜力主要由其中的溶解有机碳浓度决定[70]，降水导致的湿地扩张加剧了甲烷排放[75]，全球气温每升高1.5–2 °C，湿地100年全球变暖潜力上升57%[76]。越来越多的研究表明，湿地甲烷排放正在加剧[76-77],对全球碳排放控制目标构成威胁。现有湿地产甲烷相关研究为制定节能减排目标提供了科学依据。

4.2 水稻田

水稻田也是大气甲烷的重要排放源，据颜晓元团队在2009年的报道，全球稻田CH_4排放量约为25.6 Tg/a，占全球甲烷排放量的10%–25%[78]。国内关于水稻土中产甲烷古菌的研究最早可以追溯到1993年，钱泽澍团队采用改进的Hungate厌氧技术，结合稀释培养计数法(the most probable number, MPN)和滚管法测定了沉水水稻土中产甲烷古菌的数量，表明产甲烷古菌在根表生存而非在植物根内[79]。随着测序技术的发展，国内外多个研究发现产甲烷古菌未培养类群rice cluster I (RC-I)在水稻田中广泛存在[80-85]。陆雅海团队利用稳定同位素探针技术(stable isotope probe, SIP)证实RC-I在水稻根际甲烷形成过程中起关键作用[86]。同时他们发现RC-I在水稻田中具有抗氧胁迫的优势[87-88]。2012年，他们分离报道了RC-I类群的第3个新种*Methanocella conradii* sp. nov..[10]。

环境因子会影响产甲烷古菌群落的结构和活性，间接影响甲烷排放。陆雅海团队在这一方面做了大量研究，他们发现采用间歇性排水模式可以显著降低了产甲烷古菌活性，减少甲烷的产生[85]。排水间隔时间的长短也会影响CH_4排放量，与长循环排水模式相比，在短循环模式下CH_4排放显著降低[89]。不同的温度、水稻品种和植物残体也会影响甲烷产量。温度从15 °C升高到45 °C；CH_4的产量增加[90]；杂交稻根际可溶性CH_4浓度显著低于籼稻和粳稻[91]，而秸秆残渣比根残渣产生更多的CH_4[90]。在水稻土中添加生物炭也会影响甲烷的生成，且不同来源的生物炭对生成甲烷的影响有差异。历红波团队发现来自稻草的生物炭促进了细菌与铁矿物之间的电子传递，导致铁还原菌的丰度增加，铁还原菌与产甲烷古菌竞争，从而降低CH_4的排放[92]。而朱永官团队发现来自稻草和粪肥的生物炭可以促进甲烷生成，而来自木屑的生物炭则对甲烷生成无显著影响[93]。进一步

的研究发现不同类型的生物炭对产甲烷的不同影响可能是由于氧化还原活性或充放电能力的差异，生物炭表面的官能团(主要是醌类)在促进甲烷生成中发挥了重要作用[93]。这些研究结果为调控土壤甲烷排放提供了理论指导。

4.3 动物瘤胃

反刍动物在瘤胃中产生了大量的 CH_4，这也是大气甲烷的重要来源。据统计，反刍动物产生的甲烷占总全球甲烷排放量的 15%[94]。反刍动物瘤胃内的 CH_4 主要由氢营养和甲基营养型产甲烷古菌产生，*Methanobrevibacter* 和 *Methanomassiliicoccus* 是其中主要的产甲烷类群[95-97]。研究表明，抑制甲烷生成和提高动物生产力之间存在联系[98]。抑制产甲烷菌可以使瘤胃中的氢最大限度地流向挥发性脂肪酸的生成，将更多的能量导向宿主，提高反刍动物的生产效率，减弱对环境的影响[99]。改变饲料的组成是一种减少甲烷排放的有效策略。在饲料中添加富马酸盐、椰子油、硝酸盐、茶皂素和大豆油等物质可以有效抑制产甲烷古菌活性，减少甲烷排放[100-104]。国外学者发现了一种效果更显著的瘤胃产甲烷菌抑制剂——3-硝基丙醇(3-nitropropanol, 3-NOP)[105]，但它也会导致氢积累，造成能量浪费。因此，我国科学家提出 3-NOP 与富马酸盐联合使用，可减轻奶牛体内 H_2 的积累，增强对甲烷生成的抑制作用[106]。以上结果为产甲烷古菌与动物营养之间的关系提供了新思路，为调控反刍动物的甲烷排放奠定了基础。

4.4 油藏

油藏也是产甲烷古菌的一类重要生境，产甲烷古菌在原油降解和生物气形成过程中发挥着重要作用。石油烃是油藏的主要组成部分，在产甲烷条件下，石油烃主要通过富马酸加成机制被激活[107-108]，这个过程主要由细菌完成，产甲烷古菌主要负责利用烷烃降解后生成的小分子物质产生甲烷。本团队最新的研究表明，存在一类直接降解烷烃产甲烷的古菌——*Ca.* Methanoliparum[41]。油藏中产甲烷古菌营养类型丰富，乙酸营养型、氢营养型、甲基营养型、甲氧基型和烷基型产甲烷古菌均在油藏中存在。不同温度下油藏环境中占主导的产甲烷古菌不一致。高温油藏中氢营养型产甲烷古菌占主导[109]，而低温油藏中，则同时由氢营养型(如 *Methanoculleus*)和乙酸营养型 *Methanothrix* 占主导地位[110]。外来物的添加也会导致油藏中产甲烷途径的改变，如加入针铁矿会促使油藏中主导产甲烷古菌从氢营养型转变为乙酸营养型[111]。培养时间甚至也会影响产甲烷古菌群落结构，陆雅海团队发现随着培养时间的增长，优势产甲烷古菌从乙酸营养型向氢营养型转变[112]。牟伯中团队发现在降解 C15–C20 正构烷烃的富集培养物中，由 *Ca.* Methanomethyliaceae 介导的氢依赖的甲基营养型产甲烷途径发挥了重要作用[113]。本课题组从胜利油田分离出的 *Methermicoccus shengliensis* ZC-1^{T}[8]被发现可以降解甲氧基化合物产甲烷[40]。此外，本课题组还发现烷基型产甲烷古菌 *Ca. Methanoliparum* 在油藏中也广泛存在[41]。以上研究结果表明，油藏是一个巨大的产甲烷古菌资源库，可能存在许多目前尚未发现的具有产甲烷功能的古菌。

4.5 其他特殊生境

海洋和热液等特殊生境中同样存在产甲烷古菌。海洋中以甲基裂解产甲烷途径为主[114]。热液中以 CO_2 还原产甲烷途径为主[115]。目前已经从海洋和热液分离出 *Methanothermus fervidus*[116]、*Methanococcus aeolicus*[117]和 *Methanomicrobium paynteri*[118]等多个产甲烷古菌。海洋和热液这些特殊的生态环境还存在大量未培养的产甲烷古菌。如 2015 年，国外学者

在海洋中发现非广古菌门的深古菌也具有潜在的产甲烷能力[20]，2019 年，王风平和肖湘团队也从瓜伊马斯盆地热液甲烷渗漏区发现的一个非广古菌门的产甲烷古菌，并将其以古代神话的“哪吒”(Nezhaarchaeota)命名[23]，特殊生境中产甲烷古菌多样性研究，对揭示碳素循环的生物地球化学过程有重要意义。

5 产甲烷古菌的进化

早期研究认为产甲烷古菌起源于广古菌门(*Euryarchaeota*)[119]，但近年来，宏基因组学研究发现产甲烷古菌广泛分布在广古菌门外，推测产甲烷古菌起源于 *Euryarchaeota*、TACK 和 Asgard 古菌的共同祖先[120]。最新的研究提出了这样的假说：在现存古菌的共同祖先(last archaea common ancestor, LACA)出现之前，由甲基化合物产生甲烷的反应已经存在——那可能是一种由非蛋白结构的辅酶 F_{430} 或类似的以一价镍为核心的催化反应。当 LACA 出现之时，它已经能够进行多种由蛋白(Mcr 及其同系物)催化的产甲烷反应[121]。另一方面，关于产甲烷古菌的原始祖先是 CO_2 还原型还是甲基营养型仍存在争议。国外有学者认为产甲烷古菌起源于一种利用 Wood-Ljungdahl 途径还原 CO_2 的生物[30]。也有观点认为产甲烷古菌的祖先可能是一种依赖氢的甲基营养性产甲烷古菌[33]。王风平团队提出甲基营养产甲烷古菌是产甲烷古菌祖先[120]。此外，产甲烷古菌一直被认为是一种严格厌氧菌，但在一些有氧环境中检测到了产甲烷古菌，基于抗氧基因含量聚类分析发现，产甲烷古菌可以分为第I类和第II类产甲烷古菌，它们分别在大氧化事件之前和大氧化事件时进化，后者的抗氧化特性可能有利于产甲烷古菌抗氧能力的进化[122]。

6 互营代谢产甲烷

有机物厌氧降解产甲烷,需要厌氧细菌与产甲烷古菌相互依赖和协作。有机物厌氧降解的中间代谢产物如挥发性脂肪酸,进一步降解产生乙酸和氢气的过程,在标准状态下的吉布斯自由能(ΔG^0)为正值，无法自发进行(表 3)。当产甲烷古菌消耗这些 H_2 并使其维持在较低水平(可低至 Pa 级别)时，脂肪酸等氧化产氢反应的ΔG变为负值，反应可自发进行[123]。这种通过种间氢转移进行的互营代谢,是有机物厌氧降解产甲烷的关键步骤。H_2 本质是一种电子载体。研究发现，甲酸可以替代 H_2 作为电子载体[124-125]。由于技术手段的限制，种间 H_2 和甲酸转移在互营氧化产甲烷过程中的相对贡献一直不清楚。东秀珠等发现互营细菌 *Syntrophospora bryantii* 只能通过种间甲酸转移与产甲烷古菌 *Methanospirillum hungatei* 互营降解丁酸产甲烷[126]。此外，还发现了种间甲酸转移对于互营丙酸氧化的重要性[127]。陆雅海团队也证明了二元培养物中 *Pelotomaculum thermopropionicum* 和 *M. conradii* 通过种间甲酸转移来代谢丙酸产甲烷[128]。汤岳琴团队发现温度是影响 2 种电子传递方式的重要因素,中温条件下丙酸互营产甲烷过程主要依赖于种间甲酸转移，而高温条件下则更倾向于种间氢转移[129]。电子亦可经厌氧细菌细胞膜上的氧化还原蛋白，直接传递给产甲烷古菌，即种间直接电子传递(direct electron transport between species, DIET)[39]。2012 年，刘芳华发现 *Geobacter metallireducens* 可将胞内氧化乙醇产生的电子通过 DIET 传递给产甲烷古菌 *Methanosarcina barkeri*[130]。2015 年陆雅海团队发现水稻田土壤中存在 DIET 介导的互营丁酸产甲烷过程，*Geobacteraceae* 在其中扮演着重要

表 3 互营有机物降解产甲烷过程中的吉布斯自由能变化[156]

Table 3 Gibbs free energy changes of syntrophic degradation of organic compounds[156]

Substrates		Reactions	ΔG^0 (kJ/mol)	Reference
Anaerobic oxidation				
Short chain fatty acids	CH_3COOH	$CH_3COO^-+H^++2H_2O \rightarrow 2CO_2+4H_2$	95	[157]
	CH_3CH_2COOH	$CH_3CH_2COO^-+2H_2O \rightarrow CH_3COO^-+CO_2+3H_2$	72	
	$C_4H_8O_2$	$C_4H_7O_2^-+2H_2O \rightarrow 2CH_3COO^-+H^++2H_2$	49	
Long chain fatty acids	$C_{18}H_{32}O_2$	$C_{18}H_{31}O_2^-+16H_2O \rightarrow 9CH_3COO^-+14H_2+8H^+$	272	[158]
	$C_{18}H_{34}O_2$	$C_{18}H_{33}O_2^-+16H_2O \rightarrow 9CH_3COO^-+15H_2+8H^+$	338	
	$C_{18}H_{36}O_2$	$C_{18}H_{35}O_2^-+16H_2O \rightarrow 9CH_3COO^-+16H_2+8H^+$	404	
	$C_{16}H_{32}O_2$	$C_{16}H_{31}O_2^-+14H_2O \rightarrow 8CH_3COO^-+14H_2+7H^+$	353	
Lactate	$CH_3CH(OH)COOH$	$CH_3CH(OH)COO^-+2H_2O \rightarrow CH_3COO^-+2H_2+H^++HCO_3^-$	−4	[159]
Alcohol	CH_3CH_2OH	$CH_3CH_2OH+H_2O \rightarrow CH_3COO^-+2H_2+H^+$	9	[160]
Amino acid	$C_3H_7NO_2$	$C_3H_7NO_2+2H_2O \rightarrow CH_3COO^-+2H_2+CO_2+NH_4^+$	10	[123]
Alkane	$C_{16}H_{34}$	$4C_{16}H_{34}+64H_2O \rightarrow 32CH_3COO^-+68H_2+32H^+$	471	[161]
Aromatics	C_6H_5COOH	$4C_6H_5COO^-+6H_2O \rightarrow 3CH_3COO^-+CO_2+2H^++3H_2$	50	[123]
	C_6H_6O	$C_6H_6O+5H_2O \rightarrow 3CH_3COO^-+3H^++2H_2$	10	
Methanogenesis				
H_2		$4H_2+CO_2 \rightarrow CH_4+2H_2O$	−131	[123]
HCOOH		$4HCOOH+H_2O \rightarrow CH_4+3HCO_3^-$	−130	[159]

作用[131]。然而，具有 DIET 功能的物种多样性认识非常有限，细菌主要为 *Desulfobacula*、*Desulfobacterium*、*Deferribacter*、*Geobacter*、*Geoalkalibacter*、*Thauera*、*Syntrophus* 和 *Pseudomonas* 等属[132]；产甲烷古菌均属于 *Methanosarcinales*，包括 *Methanothrix harundinacea* 8Ac[39](由我国东秀珠团队分离[17])、*Methanothrix soehngenii*[133]、*M. barkeri* DSM 800[134]和 *Methanosarcina mazei*[135]。直到 2020 年，刘芳华团队发现专性甲酸营养型产甲烷古菌 *Methanobacterium electrotrophus* YSL 能与 *G. metallireducens* 建立基于 DIET 的互营代谢[136]，首次报道了 *Methanosarcinales* 外的产甲烷古菌具有 DIET 能力。目前的研究认为 DIET 主要依赖于导电菌毛 Pili 和细胞色素 C，而导电物质可在一定程度上代替 Pili 和细胞色素 C[137-138]。DIET 电子传递机制的导电菌毛论和细胞色素论存在一定的争议。一些研究认为导电菌毛对于 DIET 至关重要[39,139]。周顺贵团队研究发现 *Geobacter* 导电菌毛的组装依赖于一种新型分子伴侣蛋白 Spc，为在微生物界中筛选导电菌毛提供了新标准[140]。然而，敲除菌毛(Δ*pil*B)的 *G. metallireducens* GS-*ΔpilB* 和 *G. sulfurreducens* PCA-*ΔpilB* 之间依然能形成 DIET 互作关系，表明细胞色素 C 与 DIET 具有更直接的关系[141]。随后该团队利用原位光谱电化学技术进一步证实了细胞色素 C 在 DIET 中的重要作用[142]。最近他们又证实了细胞色素形成的纳米导线才是胞外电子传递的关键[143]。由此可见，对微生物的 DIET 机制尚需深入研究。

7 产甲烷古菌的应用

7.1 沼气发酵

沼气发酵是产甲烷古菌重要的应用领域之一，其不仅可以提供清洁能源，还减少了废弃物污染及其产生的无序甲烷排放，沼肥还田利

用可以改善耕地质量，增加土壤碳汇。我国对沼气发酵的应用可追溯到 18 世纪 80 年代[144]。在环境问题日益突出，化石能源越发短缺的大环境下，沼气发酵已被广泛应用于农业、工业以及市政废水废物的排放处理中[144-149]。产甲烷古菌作为沼气发酵的最终执行者，其生物强化作用对调控沼气发酵微生物，提升沼气发酵效率及稳定性具有显著效果。研究表明，添加产甲烷古菌 *M. barkeri* 可使高氨氮厌氧污泥的甲烷产量提升 59.7%[150]。此外，产甲烷菌系的强化作用亦可提升厌氧消化的反应负荷[151]，改善对高 C/N 比原料厌氧消化效率[152]，提高对高氨氮原料的耐受能力及甲烷产量[153-155]。据农业农村部不完全统计，截至 2021 年底，全国累计建成户用沼气池 2 000 多万户，各类沼气工程近 10 万处。沼气发酵是实现农业碳中和的重要途径。

7.2 残余油气化

传统原油开采技术的开采率不足 50%，大部分原油仍然残留在地下[162]。近年来，科学家们提出一种利用产甲烷菌群转化残余油为甲烷的方式，以提高原油开采率，延长油藏寿命[163]。牟伯中团队已经在实验室中成功构建了以细菌 *Smithella* 与产甲烷古菌互营作用降解正十六烷烃至正二十烷烃的产甲烷群落体系[107]。本课题组也构建了一个互营降解十六烷烃产甲烷体系[164]。但是这些原油气化体系均存在着培养周期长、转化效率低等缺点。最近，本团队发现了一种够独立降解长链烷基烃的新型产甲烷古菌，它能显著缩短从烷烃到甲烷的时间[41]，这一发现为微生物驱油促使残余油气化打开了一扇窗口。但另一方面，也必须认识到，微生物驱油的理论基础尚不完善，从实验室到现场还需要大量的基础研究。

7.3 光能产甲烷

产甲烷古菌利用光电子还原 CO_2 产甲烷，是实现 CO_2 捕获与甲烷化利用的重要途径之一。周顺贵团队利用古菌产甲烷代谢的高效选择性和半导体的光捕获性，构建的 *M. barkeri*-CdS 生物-非生物混合光电化学系统，实现了太阳能驱动的 CO_2 或 CO 还原产 CH_4[165-166]。改进的 *M. barkeri*-NiCu@CdS 系统产甲烷反应速率提升至(79.38±2.83) μmol/(g_{cat}·h)，远高于已知的生物-非生物混合系统以及光催化系统，为太阳能驱动的 CO_2 的能源化利用开辟了新道路[167]。最近，他们提出了“微生物全细胞光敏剂”的概念通过构建了厌氧光合微生物与产甲烷古菌的共培养体系(*Rhodopseudomonas palustris*-*M. barkeri*)实现了光能驱动的 CO_2 还原产甲烷[168]。

7.4 非产甲烷功能

产甲烷古菌除了产甲烷功能外，还具有非产甲烷功能，例如经遗传改造后的 *M. maripaludis* 具有利用 CO_2 产聚 β-羟基丁酸(polyhydroxybutyrate, PHB)的功能[169]。这表明产甲烷古菌具有固碳生成高值化合物的应用潜力，但国内鲜有这方面研究的报道。

8 展望

(1) 新型产甲烷古菌的分离培养。利用未培养组学的代谢途径预测，结合培养组、高通量分选和定向分离等技术，在特异性序列引导下进行分离获得纯培养物或者高丰度培养物。

(2) 产甲烷代谢的分子机制研究。选择代表性的产甲烷古菌物种，编辑改造产甲烷遗传操作体系，开展产甲烷代谢机理和调控机制研究。

(3) 产甲烷古菌与环境互作。结合同位素标记、原位模拟培养、宏组学测序和关键代谢产物分析，系统评估产甲烷古菌在不同环境中的种群结构与演替，以及生态功能，揭示产甲烷古菌在碳素循环中的生物地球化学作用。

(4) 甲烷生成的示范研究：产甲烷代谢(沼气

发酵)是有机废弃物无害化、资源化利用的核心纽带，开发提升沼气发酵效率和速率的新菌种、新工艺和新装备，提高沼气发酵技术的商业价值；开发利用新型的产甲烷技术，如电转化、光催化生物产甲烷等新技术的中试研发，提高产甲烷古菌开发利用的新场景，推动产业化发展。

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承磊，博士，农业农村部成都沼气科学研究所研究员/博导，中国厌氧微生物菌种保藏管理中心主任。一直从事厌氧微生物资源与利用研究。开发了厌氧微生物长期保藏和高通量筛选平台，建立了国内最大的厌氧微生物模式物种保藏中心(保藏量近900种)；提出了厌氧微生物新目新科等高等级分类单元11个；发现并证实了产甲烷古菌直接降解长链烷基烃产甲烷，而不需要通过传统的互营代谢，并提出了第五条产甲烷代谢途径——烷基型产甲烷代谢。先后主持国家自然基金、国家高技术研究发展计划重点项目和国家重大专项子课题等国家级课题。目前，主要通过厌氧微生物培养组、未培养组、稳定同位素示踪、生化与分子生物学、有机地球化学等多学科交叉的理论和方法，开展枯竭油藏残余原油降解产甲烷过程与机理研究。在*Applied and Environmental Microbiology*、*International Journal of Systematic and Evolutionary Microbiology*、*the ISME Journal*、*Nature*和《微生物学报》等期刊发表第一/通讯作者(含共同)论文40余篇。